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The rachitic tooth: A histological examination

Lori D'Ortenzio [a, *], Isabelle Ribot [b], Emeline Raguin [b], Annabelle Schattmann [a], Benoit Bertrand [c, d], Bonnie Kahlon [a], Megan Brickley [a]

[a] Department of Anthropology, McMaster University, Chester New Hall Rm. 517, 1280 Main Street West, Hamilton, Ontario, L8S 4L9, Canada
[b] Facultés des arts et des sciences, Département d'Anthropologie, Pavillon Lionel-Groulx, 3150, rue Jean Brillant Bureau C-3068, Montreal, Quebec, H3C 3J7, Canada
[c] Communauté d'Agglomération du Douaisis, Direction de l'Archéologie, Laboratoire d'Analyses et de Recherche, 227, rue Jean Perrin, 59500, Douai, France
[d] Lille University, Unité de Taphonomie Médico-Légale, Université, Lille 2 Droit et Santé, rue André Verhaeghe, 59000, Lille, France

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ABSTRACT

Diagnosing previous episodes of vitamin D deficiency is particularly challenging due to the subtle changes retained in the skeleton. This study investigates whether abnormal mineralisation in tooth dentin can be observed in archaeological individuals with past vitamin D deficiency. Methods taken from the clinical literature were used, where defects in tooth dentin of those with deficiency have been identified. SEM and histological analysis of tooth dentin were utilized to diagnose vitamin D deficiency in adult and juvenile skeletal remains in individuals who recovered from a period of deficiency. Archaeological skeletons were from St. Matthew and St. Marie, Quebec (1771–1860), and St. Jacques, France (1225–1798). The objective was to determine if interglobular dentin could be observed in individuals with skeletal evidence of vitamin D deficiency. A differential diagnosis revealed that the only conditions that cause mineralisation defects are those that disrupt vitamin D, calcium, and phosphorous pathways, with nutritional rickets being the most common cause. Results found that all of the archaeological individuals (6/6) who showed skeletal evidence of past deficiency displayed the formation of interglobular dentin (spaces) due to unfused calcospherites, whereas interglobular dentin was absent in modern healthy controls (n = 3). We propose that a temporary inhibition of dentin growth leads to modification of calcospherite shape and size, resulting in characteristic interglobular spaces in individuals with deficiency. Although further research is needed, we conclude that systemic mineralisation problems of individuals with deficiency may cause dentin mineralisation to stop or falter, preventing further dentin growth and fusion. Dentin has the potential to enable past episodes of vitamin D deficiency to be recognized in cases where skeletal indicators are not clear.

1. Introduction

Healthy development of the skeleton requires homeostatic control of mineral metabolism including calcium and phosphate. Vitamin D plays a vital role in the absorption of calcium and phosphate and a deficiency in vitamin D triggers the body to release hormones that leads to loss of those minerals from hard tissues, resulting in inadequate bone and tooth mineralisation. The term rickets is used to describe lack of mineralisation at growth plates (Pettifor, 2003), and softening and weakening of bones in children due to inadequate mineralisation (Foster et al., 2014). Rickets is caused by a number of factors that affect vitamin D metabolism (see Table 1), but an important cause relates to inadequate synthesis of vitamin D due to insufficient exposure of skin to ultraviolet sunlight and/or deficiency in foods containing vitamin D (Brickley and Ives, 2008:77). This type of deficiency is currently referred to as nutritional rickets (Pettifor, 2003). Paleopathological research has examined bone to identify cases of rickets in infants and children (e.g., Ortner and Mays, 1998; Mays et al., 2006; Veselka et al., 2013), but identification of residual deformities in adults caused by previous vitamin D deficiency has remained challenging as few of the subtle morphological changes survive in the adult skeleton (Brickley et al., 2010).

As tooth mineralisation occurs through comparable processes to skeletal mineralisation, teeth are susceptible to the same failures as

* Corresponding author.
E-mail addresses: dortenl@mcmaster.ca (L. D'Ortenzio), i.ribot@umontreal.ca (I. Ribot), benoit.bertrand@univ-lille2.fr (B. Bertrand).

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Table 1
Summary of the conditions associated with mineralisation defects.

Condition	Prevalence of condition in a given population	Source
Nutritional rickets	Cases have been reported from all regions of the world, but prevalence varies from: 0.018% in South Asia and Southern Denmark 0.059% in Africa 0.085% in the Middle East	Thacher et al. (2006:10); Beck-Nielsen et al. (2009:160) Robinson et al. (2006)
Hereditary, vitamin-D-dependent rickets, Type I, Type II, Type III combined	0.00062% in Southern Denmark	Beck-Nielsen et al. (2009:160)
Hereditary, vitamin-D-resistant rickets (VDRR)	0.00005% in Southern Denmark	Beck-Nielsen et al. (2009:160)
Fibroblast growth factor 23 (FGF23)	Rare condition, prevalence rates unavailable	
Hypophosphatemia	São Paulo pediatric hospital for critically ill children: 0.00050% of hospital admissions	Santana e Meneses et al. (2009)
Autosomal dominant hypophosphatemic rickets	0.00057% in Southern Denmark	Francis et al. (1997)
X-linked hypophosphatemic rickets		Beck-Nielsen et al. (2009:160)
Tumour-induced osteomalacia	Caused by a number of rare neoplastic conditions, prevalence rates unavailable	
Renal tubular disorders	Prevalence rates unavailable	
Hypophosphatasia	Hereditary condition, prevalence rates unavailable	
Fibrogenesis imperfecta ossium	Hereditary condition, prevalence rates unavailable	

Conditions considered taken from Brickley and Ives, 2008:88, Table 5.4. Note: Prevalence rates based on hospital records.

bone under metabolic disturbance related to vitamin D deficiency (Foster et al., 2014). We hypothesize that abnormal mineralisation in tooth dentin can be observed as interglobular dentin (spaces) in archaeological individuals who have experienced a period of vitamin D deficiency. Interglobular dentin occurs when mineralisation processes have slowed down or stopped resulting in calcospherites (calcium salts) that do not fully coalesce, leaving identifiable spaces in dentin (Mellanby, 1934; Seow et al., 1989). This study utilizes scanning electron microscopy (SEM) and histological techniques on teeth from archaeological skeletal remains with clear skeletal evidence of past deficiency to investigate the presence or absence of interglobular dentin. Methods used were taken from the clinical literature where defects in tooth dentin linked to vitamin D deficiency have been identified (e.g., Shellis, 1983; Seow et al., 1989; Vital et al., 2012; Linglart et al., 2014). This study investigates whether dentin reflects biomineralisation processes in the human body and so acts as a biomarker for pathological conditions, such as nutritional rickets, that lead to mineralisation defects at the histological level.

1.1. Vitamin D deficiency in bone

It is estimated that 1 billion people worldwide have vitamin D deficiency (Holick, 2007). A recent survey in the United Kingdom showed that more than 50% of the adult population have insufficient levels of vitamin D and that 16% have severe deficiency (16,000 out of 100,000), particularly during the winter and spring months (Pearce and Cheetham, 2010). The increasing prevalence of disorders linked to vitamin D deficiency is illustrated by the growing number of children treated with rickets each year (Pal and Shaw, 2001). Histological analysis on bone biopsies from German adults ($n = 675$) showed that 25.63% of individuals manifested mineralisation defects related to osteomalacia (Priemel et al., 2010). This investigation undertaken in Germany, where fortification of food is not permitted, demonstrates that low serum 25(OH) D levels are associated with high levels of mineralisation defects.

Paleopathologists primarily use macroscopic lesions observed in skeletal remains to diagnose cases of vitamin D deficiency. Rickets manifests as skeletal bending, defects of the growth plate and flaring of the metaphysis, but once vitamin D is obtained recovery is anticipated as the bone turns over and remodels (healed rickets). Remodelling and growth of bone will return growth plates to normal and all but the most severe bowing deformities can be lost. Despite progress in the identification of rickets in archaeological bone, there remain problems in the recognition of adults who have experienced vitamin D deficiency. Hess (1930) suggested that in untreated cases, only 10–25% of rickets cases result in visible leg deformity. Once healed, it is difficult to observe slight bowing deformities, particularly in adults (Brickley et al., 2010). A number of features visible in active rickets may still be observable in the adult skeleton, but none of these are pathognomic for rickets. Diagnosing deficiency in adults is challenging due to the subtlety of changes retained in the long bones and we suggest that techniques using dentin will increase the identification of deficiency in the many cases where skeletal features are inconclusive.

1.2. Tooth dentin formation and vitamin D deficiency

Mineralisation of the dentin matrix does not occur until the formation of predentin and this zone is infiltrated with collagen fibers embedded in ground substance (Bevelander and Nakahara, 1966; Hillson, 2002:185). As mineralisation occurs, the fibers condense and thicken in areas adjacent to the odontoblast process. Fibers continue to invade the developing dentin matrix and granular masses in an advancing wave of mineralisation, progressing from the dentin-enamel junction to the pulp chamber (Bevelander and Nakahara, 1966). Secondary dentin formation continues in both the crown and the root throughout the life of the tooth.

Similar to mineralisation defects seen in bone, vitamin D deficiency can interrupt normal dentin deposition. Animal studies have shown that disruption of the vitamin D pathway decreases the mineralisation of bones and has a negative impact on teeth (e.g., Cohen et al., 1976; Berdal et al., 1987). A study of mice with vitamin D deficiency showed both a reduction of dentin mineralisation and early enamel hypermineralisation. It was concluded that vitamin D likely plays a role in tooth mineralisation and appears to indirectly regulate dentin mineralisation (Zhang et al., 2009).

Dentin in healthy individuals has normal matrix formation that appears homogeneous without interglobular spaces and displays complete fusion of calcospherites (tiny round spheres containing calcium salts) (Isokawa et al., 1963). Dentin is formed and calcified slowly and for this reason interglobular spaces are absent or infrequent in individuals with optimum nutritional conditions (Isokawa et al., 1963). When an individual has vitamin D deficiency,

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some calcospherites do not grow sufficiently (failure to fuse) and leave a poorly mineralised patch of matrix (Vital et al., 2012).

Animal studies have found a clear association between vitamin D deficiency and interglobular dentin. Using beagle dogs, Mellanby (1928, 1934) demonstrated an association between vitamin D deficient diets and an increase in frequency and prominence of both interglobular spaces and enamel hypoplasia. Yoshiki and Yanagisawa (1974) examined dentin in rats made rachitic by sunlight deprivation and a diet deficient in calcium and vitamin D and found that mineralisation of dentin was irregular and periodic with the predentin wider than that of the control group.

Clinically, interglobular dentin is present only in association with conditions that result in mineralisation defects due to a disruption in the pathway of either vitamin D, phosphate or calcium. Dentists view interglobular spaces as a defect in mineralisation, not a defect in matrix formation (Chiego, 2014:10), and vitamin D deficiency is considered to be pathognomic for this disease (McDonnell et al., 1997; Chaussain-Miller et al., 2003; Souza et al., 2010, 2013; Vital et al., 2012). Seow et al. (1989) conducted histological examinations on patient's teeth who had been diagnosed with familial hypophosphatemia, an inherited disease that causes the development of rickets due to the decreased renal reabsorption of phosphate. Patients presented with the identical symptoms seen in nutritional rickets (i.e., bowed leg bones). Although odontoblast function is normal, hypophosphatemia leads to poorly mineralised dentin with areas of interglobular dentin (Seow et al., 1989). Histological findings from individuals with vitamin D-resistant rickets include marked globular dentin where the entire dentin mineralisation is abnormal with large non-mineralised interlobular spaces between non-merged calcospherites (Seow et al., 1984, 1987; 1989; Seow and Latham, 1986; Seeto and Seow, 1991; Tumen and Yavuz, 2009; Vital et al., 2012).

While many of the reported cases of interglobular dentin are from rare causes of vitamin D deficiency, as these attract significant clinical attention, cases have also been reported in nutritional rickets. McDonnell et al. (1997) reported a case of nutritional rickets in a 2-year-old child from Canada. Histological examination revealed irregularity of the dentin-predentin border, and interglobular dentin in the deciduous mandibular and maxillary first molars, as well as the central and lateral incisors.

1.3. Interglobular dentin in association with vitamin D deficiency

The presence of interglobular dentin appears to be directly linked to a deficiency in vitamin D or associated conditions. A differential diagnosis of conditions associated with mineralisation defects in teeth was conducted to determine if disorders other than a vitamin D deficiency or vitamin D related conditions could be responsible for the presence of interglobular dentin. Table 1 presents conditions associated with the presence of interglobular dentin, Table 2 (in Supplementary Data) displays other pathological conditions and nutritional deficiencies investigated and determined not to produce interglobular dentin. There are a number of causes of vitamin D deficiency (see discussion in Brickley et al., 2014), but the main cause is nutritional; lack of exposure of skin to effective sunlight and/or lack of foods containing vitamin D (or consumption of foods with high phytate levels). Of the conditions presented in Table 1, nutritional rickets is the most commonly occurring (Holick, 2007), while hereditary vitamin D related conditions were too rare to have notable prevalence rates. As expected, all genetic causes of vitamin D deficiency (vitamin D-resistant and vitamin D-dependant rickets) exhibited interglobular dentin. Phosphorus and calcium deficiencies (which affect dentin formation) were also found to have interglobular dentin. Endocrine disorders such as hypothyroidism, hypoparathyroidism were investigated and it was found that these vitamin D related conditions showed hypomineralisation (spaces) in dentin. Various other vitamin deficiencies (A, C, and E) were investigated and interglobular dentin was found to be absent. Additionally, magnesium deficiency (which causes enamel hypoplasia, and pulp calcification) did not produce interglobular dentin. Other possible aetiologies of mineralisation defects, such as fluorosis, liver disease, and gastrointestinal malabsorption were ruled out as causing interglobular dentin (Avery, 2002; Panov and Krasteva, 2011; Rashid et al., 2011). Only in cases where insufficient vitamin D affects the regulation of calcium and phosphate homeostasis did dentin display the distinctive marbled or bubbled appearance due to disturbed calcospherite fusion.

2. Materials and methods

Twelve teeth from six archaeological individuals with clear evidence of rickets were collected. Teeth from three known healthy modern individuals (HIREB ethics approval 14–670-T) were also collected to act as control teeth. All individuals and methods are summarized in Table 2. Archaeological skeletal remains from three sites were examined, Saint-Matthew (n = 1), Saint-Marie (n = 1), and Saint Jacques (n = 4). Sites were chosen because they were known to have cases of rickets and individuals who survived periods of childhood vitamin D deficiency. Macroscopic examination was completed on skeletons classified as 'marked' to 'severe' for deformities associated with vitamin D deficiency, using the criteria set out in Brickley and Ives (2008), followed by SEM and histological analysis of selected teeth.

Skeletons 15A-S36 and 2E4, originated from two well-defined Euro-Quebecois cemeteries that represent key historic sites in the Saint Lawrence Valley, Canada. The first individual (15A-S36) was obtained from Saint-Matthew cemetery, Quebec City (1771–1860), known as "Protestant burying ground", and was the first official Anglican and Presbyterian cemetery in Quebec City, located just on the outskirts of the fortifications (Noppen, 1987; Cloutier, 2000; Simoneau, 2003). To date, various bioarchaeological studies on health and/or diet have been completed on these skeletal remains (e.g., Arpin, 2006; Perron, 2006; Morland, 2010; Ribot et al., 2010, 2016; Caron, 2013). The second individual was a 3-year old child (±12 months, determined using Ubelaker's (1989:55–70) dental development and long bone length) from Saint-Marie, Quebec City (1748–1878). Individuals buried in the Saint-Marie cemetery were descendants of settlers, from other regions in Quebec (e.g., Côte-de-Beaupré) or France.

Tooth samples were collected from four individuals from Saint-Jacques cemetery, France, in conjunction with Laboratoire d'Analyses Physiques et de Caractérisation des Matériaux, Communauté d'Agglomération du Douaisis, and analyzed at McMaster University. Saint Jacques's Church (A.D. 1225–1798) was discovered and excavated by the Communauté d'Agglomération du Douaisis, Direction de l'Archéologie Préventive from May to December 2007.

2.1. Macroscopic examination

Skeletons from Quebec were evaluated to identify a clear case of past vitamin D deficiency in the adult and child who had teeth available. A differential diagnosis was conducted for indicators of vitamin D deficiency on skeletons 15A-S36 and 2E4 using Mays et al. (2006) and Brickley et al. (2010). Paleopathological examination for the St. Jacques skeletons (SJ 384, SJ 562, SJ 892, SJ 970) was conducted by William Devriendt, and the diagnosis of vitamin D deficiency was agreed upon by MB (Fig. 1a-b). The combination of morphological features in the bones helped identify evidence of past vitamin D deficiency. For example, skeleton 15A-S36 had

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Table 2
Description of individuals.

Identifier	Tooth type	Description	Type of analysis
M59	Permanent RM^1	Modern healthy juvenile (age 14)	Histological
TT3	Deciduous RM_2	Modern healthy juvenile (age 10)	SEM, Histological
TT1	Permanent RM^3	Modern healthy adult (age 19)	SEM, Histological
2E4	Deciduous RM^1	Archaeological juvenile with past rickets (age~3) (Saint-Marie, Quebec)	SEM, Histological
15A-S36	Permanent RM^1, RM^2, LM^3	Archaeological adult with past rickets (Saint-Matthew, Quebec)	SEM, Histological
SJ 384	Permanent RM_1, RM_2	Archaeological adult with past rickets (Saint Jacques, France)	Histological
SJ 562	Permanent LM_2, RC^1	Archaeological adult with past rickets (Saint Jacques, France)	Histological
SJ 892	Permanent RC_1, LM_3	Archaeological adult with past rickets (Saint Jacques, France)	Histological
SJ 970	Permanent LM^1, RM^3	Archaeological adult with past rickets (Saint Jacques, France)	Histological

SEM = scanning electron microscope.

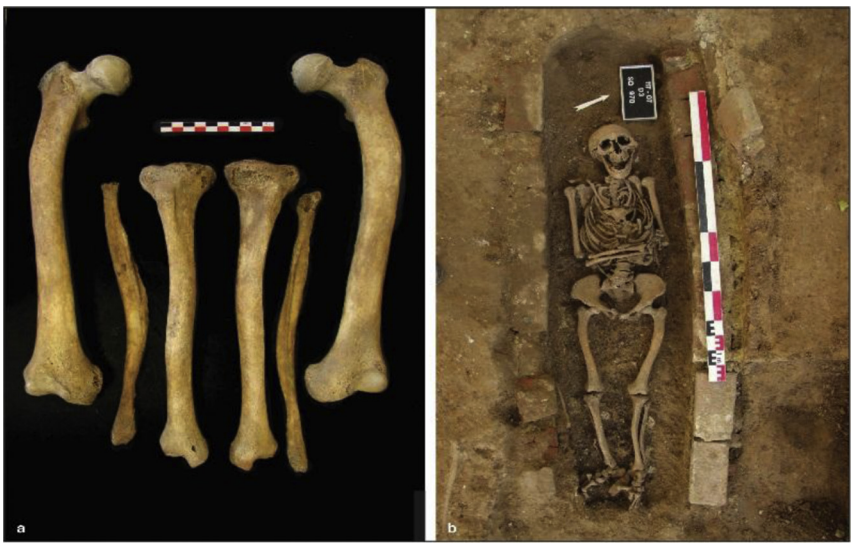

Fig. 1. a) Femora, tibiae, and fibulae for skeleton SJ 970 from Saint Jacques, France, with clear-cut case of rickets; Note: Bowing of all leg bones; **b**) Skeleton SJ 970 in situ.

bowing of the tibias and fibulas and angulation of the sacrum was greater than expected (Fig. 2a-c). Skeleton 2E4 showed signs of past rickets such as bilateral bowing of leg bones and flaring of the distal metaphyses of the femora and tibiae (Fig. 3a-b). Growth plates appeared normal and evaluation of radiographs determined that 2E4 was a healed case of deficiency.

2.2. SEM and histological analysis

Both SEM and histological methods have been used clinically to examine interglobular dentin with good results (e.g., Seow et al., 1989; Vital et al., 2012). For the first four samples, both methods were employed in this study. Histological examination was found to be quicker and easier to use and gave excellent results (nothing was

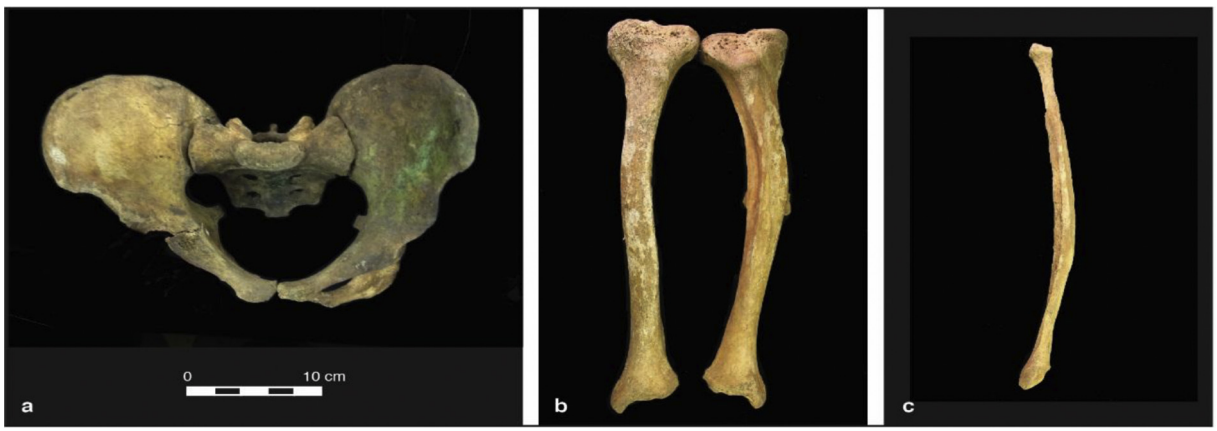

Fig. 2. a) 15A-S36 (adult with past rickets) with medio-lateral curvature of ischium and pubic symphysis associated with past rickets; **b**) Medio-lateral bending of tibiae; **c**) Fibula with lateral bowing. Note: Same scale for all images.

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Fig. 3. **a**) Medial view of femora of 2E4 (juvenile with past rickets) showing shaft curvature; **b**) Medial view of both tibiae showing shaft curvature associated with rickets. Note: Same scale for both images.

seen using SEM that could not be observed with histology), therefore only histological analysis was conducted on the remaining 11 teeth.

Following Saunders' et al. (2007) procedures, archaeological and control tooth samples were embedded in a chemical-setting resin (SPURR for SEM analysis, Epo-Thin for thin sections), and sectioned in a buccolingual direction into 3 mm blocks with a precision diamond wafering saw (Buehler IsoMet 1000). The samples were lapped and polished to remove saw marks with a Buehler MiniMet grinder-polisher and lapped using 400, 600, 1200 grit paper and a texmet pad with 3 μm diamond polish, followed by 1 μm diamond polish on a microcloth pad. The polished samples were ultrasonicated for 15 min in distilled water. For SEM analysis, the 3 mm block was mounted onto an aluminum stub with carbon tape and sputter-coated with platinum. The sample sections were examined under a scanning electron microscope (JEOL JSM-6610LV). Using backscattered electron imaging (BEC), images were taken at 250x to 5000x magnification at 15 kV, using working distances of 10–11 mm. For microscopic analysis, the previously embedded SEM sections were further thin sectioned and mounted on glass microscope slides, lapped and polished using the above method. Thin-sections were imaged using a Nikon DsR:1 camera attached to an Olympus BX51 digital microscope, (100x magnification).

2.3. Scoring system used to grade the severity of interglobular dentin

Scoring of interglobular dentin was performed in order to develop a link between interglobular dentin severity and the severity of deficiency experienced by the individual. Being the first to record the severity of interglobular dentin in animals and in British children, Mellanby (1934:38) employed symbols to represent the severity of spaces in dentin, ranging from No S (no interglobular spaces) to S++ (severe interglobular spaces). More recently, Seow et al. (1989:204–205) scored the severity of interglobular dentin on patients with vitamin D resistant rickets using Grades I-III. For Grade I, interglobular dentin was less than 50% of the total dentin thickness, with small interglobular spaces and Grade II was more than 50%, but did not cover the entire dentin thickness. Grade III interglobular dentin extended throughout the entire thickness of dentin and the interglobular spaces were large. These scoring systems work well for clinical studies but for the purposes of paleopathogical examination we combined Mellanby (1934) and Seow et al.'s (1989) scoring system to grade the severity and the relative amount of interglobular dentin (Table 3). The system incorporated symbols similar to Mellanby (1934) and Seow et al.'s (1989) percentages, but was refined to divide the estimation percentages further in order to better quantify the amount of interglobular dentin observed. Histological grading was established by estimating the percentage of interglobular dentin present in the region of interest relative to the surrounding normal dentin (Molnar and Ward, 1975). Interglobular dentin was compared relative to normal dentin observed in the field of view in the microscope eyepiece. Interglobular dentin was estimated based on the percent of field covered at 100x magnification, using an eye piece reticle; a grid with 0.1 mm squares. In Grade 1, the amount of interglobular dentin was less than 25% relative to the surrounding normal dentin, with small interglobular spaces, indicating that the mineralisation defect was mild. Grade 3 was the most severe with interglobular dentin covering over 75% of the region of interest, relative to the normal dentin, accompanied by large spaces appearing as bubbles or scallops running across the dentin tubules in the dentin matrix. For examples of the scoring system, see Fig. 4.

2.4. Approximate age of when an episode of vitamin D deficiency may have occurred

Dental age estimation was based upon the rate of development and calcification of tooth buds and the progressive sequence of their eruption. Using Moorrees et al.'s (1963) technique of assessing the dental age according to the degree of calcification observed in permanent teeth. The age at which an episode of past vitamin deficiency may have occurred was approximated by assessing the location of interglobular dentin in the tooth (Fig. 5, Table 4). As dentin is secreted ~4–6 μm per day in permanent teeth, the first 1 mm under the crown of a first molar represents approximately 12 months to 1.5 years of age (Moorrees et al., 1963; Hillson, 2002; Beaumont et al., 2013). Whereas dentin closer to the pulp horn may represent an age of 2 years (Moorrees et al., 1963; Hillson, 2002; Eerkens et al., 2011). Five out of six individuals had two to

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Table 3
Scoring system for IGD (interglobular dentin).

Grade	Grade 0	Grade 1	Grade 2	Grade 3
Interglobular spaces	Normal: no interglobular spaces	Minimal interglobular spaces (IGD-)	Moderate interglobular spaces (IGD+)	Large interglobular spaces (IGD++)
Description	Dentin is homogeneous, interglobular dentin is absent.	Interglobular spaces present but small; spaces are <25% relative to surrounding normal dentin.	Interglobular spaces moderately large and more numerous than Grade 1; spaces are 25–50% relative to surrounding normal dentin.	Interglobular spaces are large and very numerous with a clear scalloped or bubbled appearance; spaces are >75% relative to surrounding normal dentin.
Defect in Dentin Mineralisation	Absent	Mild	Moderate	Severe

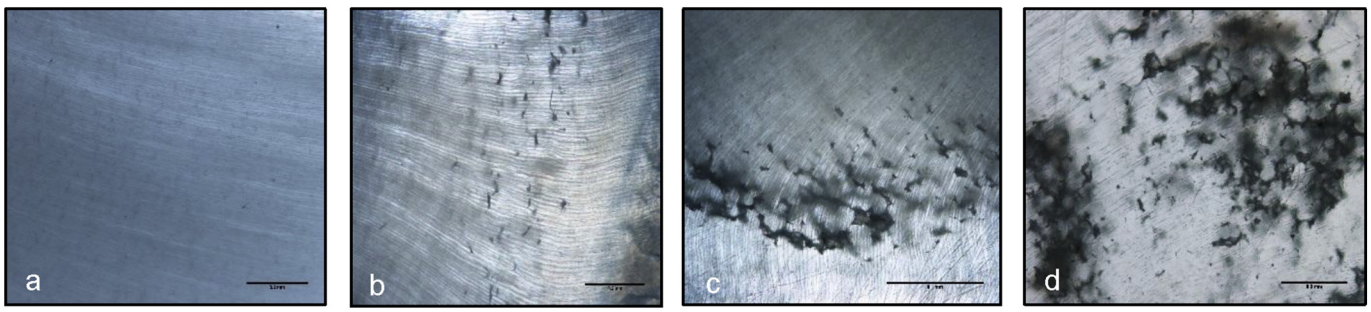

Fig. 4. **a**) Example of Grade 0, note homogeneous appearance of dentin; **b**) Grade 1, interglobular dentin <25%; **c**) Grade 2, interglobular dentin 25–50%; **d**) Grade 3, interglobular dentin >75%.

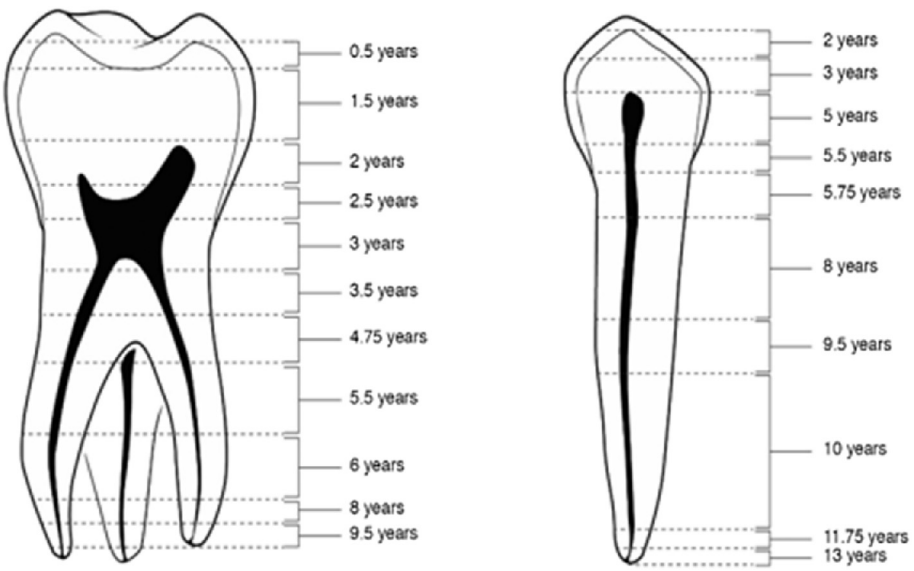

Fig. 5. Diagram of a first molar and a canine showing the approximate ages of mineralisation of dentin (Moorrees et al., 1963). Note: Degree of mineralisation in developing teeth can be affected by sex and the differing dental maturity of maxillary and mandibular dentition.

three teeth available that form at different age sequences and the location of interglobular dentin relative to crown enamel, pulp chamber, dentin-enamel junction, and the root of the tooth was noted. The location of the interglobular dentin was correlated with Moorrees et al.'s (1963) tooth development stages that gives an approximate age at which mineralisation occurs. Age at which an episode of vitamin D deficiency may have occurred was estimated by using the timing of crown inception, dentin and pulp chamber completion, and apical root closure (Fig. 5). Note that timing of tooth formation is approximate as dentin grows in concentric cones not the horizontal layers depicted in Fig. 5 (Eerkens et al., 2011).

3. Results

3.1. SEM results

SEM analysis of four individuals revealed that the controls (TT1 and TT3) exhibited normal dentin formation (Fig. 6a–c). The dentin was scored as a Grade 0 as it appeared homogeneous with evenly distributed dentin tubules that were continuous and regular, as expected for healthy individuals. Conversely, the two individuals determined to have had rickets displayed abnormal dentin. For example, the adult from Saint-Matthew, Quebec (15A-S36) scored

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Table 4
Summary of interglobular scores and approximate age of vitamin D deficiency.

Identifier	Tooth type	Interglobular dentin score	Approximate age of vitamin D deficiency episodes (years)
M59 (modern control)	RM^1	Grade 0	No deficiency
TT3 (modern control)	RM_2	Grade 0	No deficiency
TT1 (modern control)	RM_3	Grade 0	No deficiency
2E4 (St. Marie individual with past rickets)	RM^1	Grade 2	1 episode at 2 years
15A-S36 (St. Matthew individual with past rickets)	RM^1	Grade 3,	2 episodes 1.5–2 years
	RM^1	Roots Grade 2	[a]1 episode at 5.5 years
	RM^2	Grade 2	[a]5–6 years
	LM^3	Grade 3	1 episode at 12.5 years
SJ 384 (St. Jacques individual with past rickets)	RM_1	Grade 3	1 episode at 1.5 years
	RM^1	Roots Grade 2	[b]1 episode at 6 years
	RM_2	Grade 2	[b]6.5–11 years
SJ 562 (St. Jacques individual with past rickets)	RC_1	Grade 3	4–6 years
	RC^1	Grade 3	[c]1 episode at 4–6 years
	LM_2	Grade 2	[c]6.5 years
SJ 892 (St. Jacques individual with past rickets)	RC_1	Grade 3	1 episode at 4 years
	LM_3	Grade 0	No deficiency
SJ 970 (St. Jacques individual with past rickets)	LM^1	Grade 3	1 episode at 1.5 years
	RM^3	Grade 1	1 episode at 12.5 years

[a] Both vitamin D deficiency events represent the same episode of deficiency as formation of RM^1 root overlaps with formation of RM^2.
[b] Both vitamin D deficiency events represent the same episode of deficiency as formation of RM_2 overlaps with root formation of RM_1.
[c] Both vitamin D deficiency events represent the same episode of deficiency as formation of RC^1 overlaps with formation of LM_2.

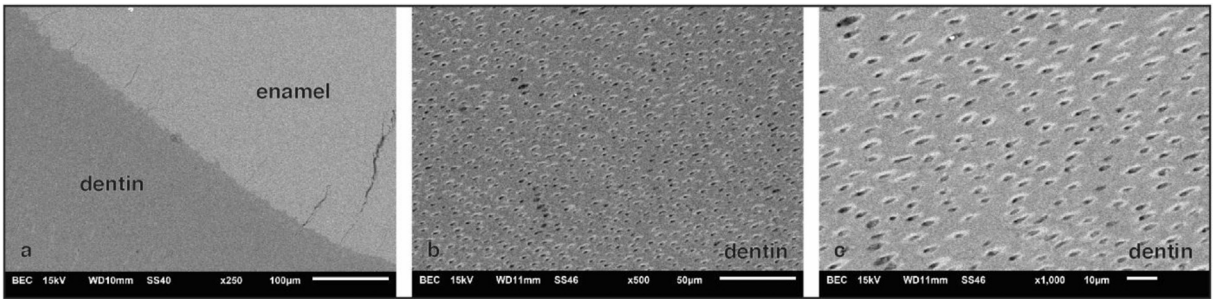

Fig. 6. **a**) SEM image of normal dentin (Grade 0) observed in control TT1, (healthy adult) (250x magnification); **b**) Dentin tubules with homogeneous appearance (500x magnification); **c**) Dentin tubules again showing homogeneous appearance (1000x magnification).

Grade 3 (severe) for the presence of interglobular dentin in the right maxillary first molar and left mandibular 3rd molar, while 2E4 scored a grade of 2 (moderate) in the right maxillary first molar. As shown in Fig. 7a–c and Fig. 8a–c, there were differences in the degree of dentin fusion, characterized by a large number of non-merged calcospherites separated by irregular zones of non-mineralised interglobular dentin.

3.2. Histological results

Disturbances in dentin mineralisation were absent in the three controls (e.g., Fig. 9b, d), but present in the six individuals with previous episodes of rickets in which histological assessment was undertaken using thin sections (e.g., Fig. 9a, c). All histological images are available in Table 1, Supplementary Data A accompanied by grades of severity for interglobular dentin. Interglobular spaces representing unfused calcospherites, were clearly observed in at least one tooth for all of the individuals with previous episodes of rickets (n = 6). All adult individuals with rickets had Grade 2 to Grade 3 interglobular severity in two or three teeth. Only one tooth (out of 12), a mandibular third molar from SJ 892, had Grade 0 indicating an absence of vitamin D deficiency. The relative

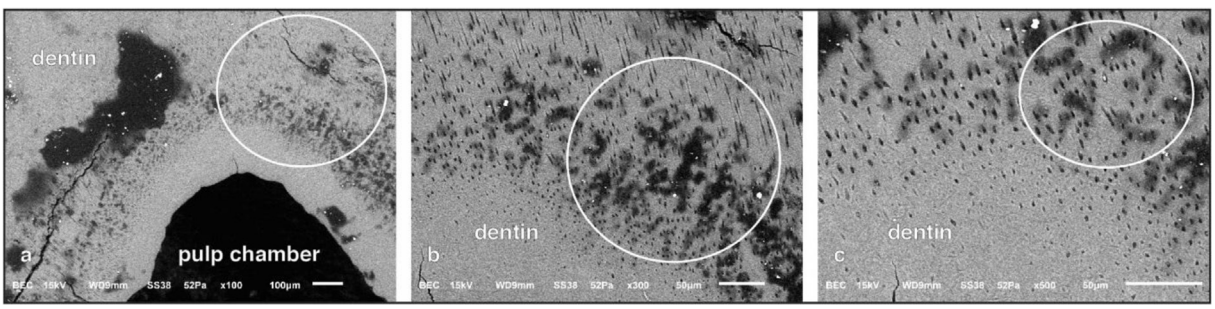

Fig. 7. **a**) SEM image of interglobular dentin (Grade 3 severity) observed in 15A-S36; adult individual with skeletal evidence of past rickets, 100x magnification; **b**) 300x magnification; **c**) 500x magnification. Note: Uneven dentin with interglobular spaces representing absence of mineralisation (white circles). Black areas are calcospherites that have failed to fuse.

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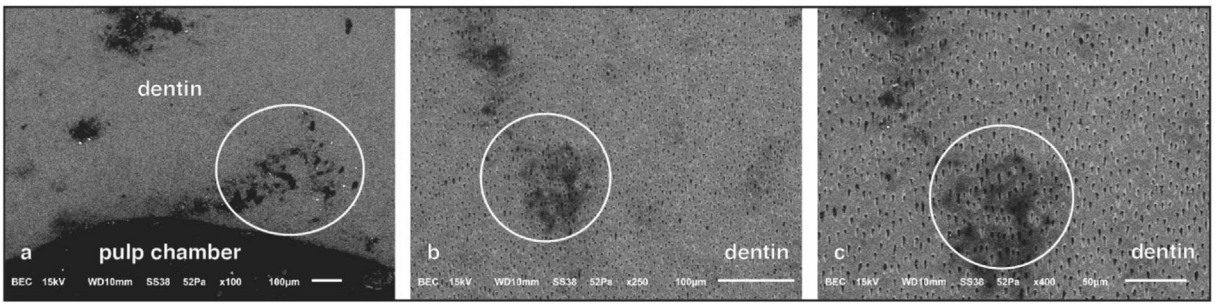

Fig. 8. **a**) SEM image of interglobular dentin (Grade 2 severity) observed in 2E4 juvenile with evidence of past rickets, 100x magnification; **b**) 250x magnification; **c**) 400x magnification. Note: Patches of uneven dentin growth representing a cessation of mineralisation and black areas of calcospherites that have failed to fuse (white circles).

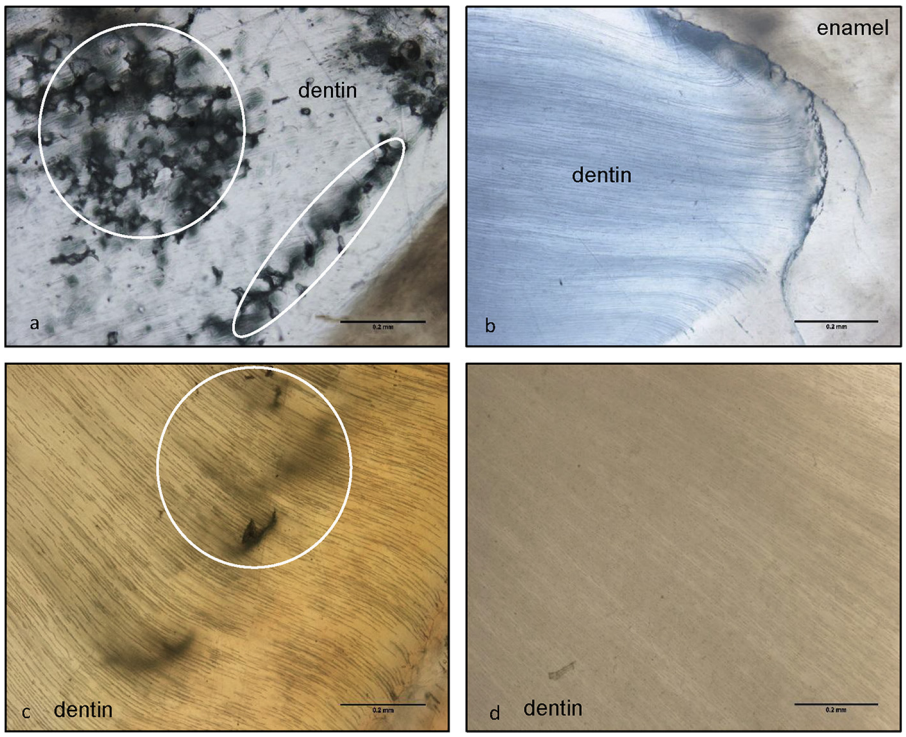

Fig. 9. **a**) Histological image of dentin for 15A-S36 (adult with past deficiency, Grade 3 interglobular severity); **b**) TT1 (adult control, Grade 0 interglobular severity); **c**) 2E4 (juvenile with past rickets, Grade 2 interglobular severity); **d**) TT3 (juvenile control, Grade 0 interglobular severity), 100x magnification. Note: Black areas (marbled) are calcospherites that have failed to fuse. The main concentrations are indicated by white circles. The modern controls exhibit homogeneous dentin matrix.

amount of interglobular dentin varied between the adults with rickets, ranging from pronounced unfused calcospherites to less pronounced calcospherites. The enamel looked normal, as were the dentino-enamel junctions and cementum.

3.3. Grades of severity of interglobular dentin

Table 4 summarizes the interglobular dentin scores. All modern healthy control individuals scored a Grade 0 (normal) for interglobular spaces (n = 3). The archaeological individuals with past vitamin D deficiency scored between Grade 1 and Grade 3 for interglobular severity. All archaeological individuals (n = 6) had at least one tooth that received a score of Grade 3 for interglobular severity, most of whom had two or three teeth with varying grades of interglobular dentin (Table 1, Supplementary Data).

3.4. Approximate age of when an episode of vitamin D deficiency may have occurred

Table 4 displays the approximate ages of a vitamin D deficiency using interglobular dentin location and Moorrees et al. (1963) tooth development and calcification sequences. Ages of deficiency are variable for some individuals (1.5–11 years old) as it depends on the timing of formation of the tooth sampled. For example, maxillary first molars start to form in utero while maxillary third molars begin to form between 7 and 10 years (Hillson, 2002:123). Histological images of individual 15A-S36's molars revealed that there may have been up to four episodes of deficiency (see Fig. 10). Large calcospherites were observed directly under the crown of the tooth and a second area of less pronounced calcospherites were found above the pulp horn in the maxillary first molar. This suggests at least two episodes of deficiency while the first molar was forming; one occurring soon after the crown formed at 1.5 years and a subsequent episode at 2 years (Moorrees et al., 1963; Hillson, 2002).

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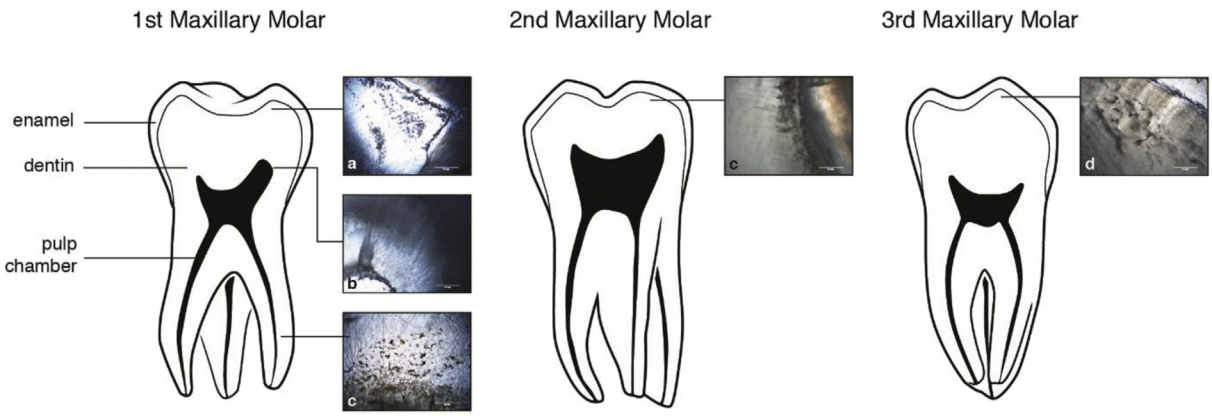

Fig. 10. The four episodes of vitamin D deficiency that occurred during the development of the 1st– 3rd molars for archaeological individual 15A-S36 are illustrated; **a**) Episode 1 (age 1.5 years); **b**) Episode 2 (age 2 years) **c**) Episode 3 (age 5.5–6 years); **d**) Episode 4 (age 12.5 years), 100x magnification. Note: Episode 3 occurs concurrently in the root of the first molar and the crown of the second molar.

Interglobular dentin was observed in both the buccal and lingual roots of the first molar (see Supplementary Data A, Table 1 for images). Root formation of the maxillary first molar overlaps with the formation of the maxillary second molar between 5 and 6 years, subsequently both teeth exhibited an area of interglobular dentin (both Grade 2), indicating a third episode of vitamin D deficiency. The interglobular dentin found in the maxillary third molar from the same individual suggests a fourth episode of deficiency (Fig. 10). This episode may have occurred later during adolescence, one that was associated with the timing of the angulation of the sacrum, as the sacrum fuses at puberty (~12 + years) (Scheuer and Black, 2004:209).

4. Discussion

This study found that abnormal mineralisation, manifested as interglobular dentin, could be observed in archaeological individuals with clear evidence of past vitamin D deficiency using scanning electron microscope (SEM) and histological analysis. SEM and histological examination of tooth dentin revealed evidence of morphological changes associated with a deficiency, because unlike bone, dentin is not remodelled, but continues to be laid down slowly throughout life in permanent teeth by odontoblasts located on the wall of the pulp chamber. The rate of dentin secretion in a permanent tooth is relatively consistent (~4–6 μm per day in the crown, ~1.3–1.5 μm per day in the root) (Dean and Scandrett, 1995). As vitamin D binds with vitamin D receptors, a deficiency inhibits the proliferation of certain cell types such as odontoblasts, which decreases mineralisation resulting in the formation of interglobular dentin (Zhang et al., 209). The archaeological skeletons, who showed marked skeletal evidence of past rickets, displayed the formation of interglobular dentin (spaces) within their teeth. Rachitic dentin is characterized by the presence of a large number of calcospherites separated by irregular zones of interglobular dentin and this investigation has shown that the mineralisation defects are observable histologically (Fig. 9a, c, Table 1, Supplementary Data), and are likely correlated with the manifestation of rickets in an affected individual. During normal dentin mineralisation, calcospherites are formed from centres of mineral seeding (Seeto and Seow, 1991). Local mechanisms promote mineral deposition around the seeds permitting calcospherites to grow uniformly by mineral accretion until they contact other calcospherites (Shellis, 1983; Couve, 1987). SEM and histological analysis revealed that individuals with evidence of past rickets exhibited zones where calcospherites fusion was absent, while healthy controls (TT1, TT3, M59) had calcospherites that were so well fused that the boundaries were indistinct (Fig. 6a–c, 9b, d).

Animal studies have shown that rickets can be produced by diets low in calcium and vitamin D resulting in mineralisation defects in both bone and teeth (e.g., Mellanby, 1928; Howe et al., 1940). Similar to the mineralisation defects seen in bone, the disruption of the vitamin D pathway leads to inadequate levels of calcium and phosphate causing an increase in interglobular dentin in teeth (Shellis, 1983; Seow et al., 1989; Limeback et al., 1992; Zhang et al., 2009). Interglobular dentin is likely to be found in many histological tooth sections as prevalence rates of vitamin D deficiency indicate that deficiency is quite common in the current population. Priemel et al.'s (2010) study found that up to 25% of individuals examined via bone biopsy had evidence of a deficiency ($n = 675$). Rickets has also begun to be identified in a range of past contexts (e.g., Pettifor, 2003; Brickley and Ives, 2008:134–150). Clinically, interglobular dentin has been recognized in case reports for nutritional vitamin D deficiency and for hereditary deficiencies as being pathognomic for the disorder (e.g., Seeto and Seow, 1991; McDonnell et al., 1997; Chaussain-Miller et al., 2003; Linglart et al., 2014). While clinical studies tend to investigate rare genetic types of deficiency, the indicators of deficiency are the same as in nutritional deficiency because the human body is affected systemically (Foster et al., 2014). Endocrinologically, the human body reacts in a limited way to vitamin D deficiency. Consequently the mineralisation defects of the deficient skeleton are the same regardless of whether the cause was nutritional or hereditary (Foster et al., 2014). This research found that given the prevalence of rickets worldwide, the most likely cause of mineralisation defects is nutritional rickets (Pearce and Cheetham, 2010, Table 1). This further suggests that tooth sections containing interglobular dentin originate from individuals who have experienced past deficiency.

Current medical therapy for genetic causes of vitamin D deficiency requires administering vitamin D or intensive oral calcium/phosphate therapy (Malloy and Feldman, 2010). Standards of care for genetic deficiency aim to improve skeletal mineralisation and will in some cases provide improvement to dentin mineralisation (Foster et al., 2014). Vital et al. (2012) noted through SEM observation that upon administering phosphate treatment during childhood on a patient with hypophosphatemia, the third molar showed regions where the calcospherites fused during treatment. The patient discontinued treatment and regions of unfused calcospherites were subsequently observed. In the past, without

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available medical treatment, individuals with genetic causes of deficiency would not recover. There are reported cases of rare genetic causes of vitamin D deficiency in the past (e.g., Formicola, 1995), and in the future findings from the current study should enable genetic cases to be determined using histological examination.

Other conditions associated with vitamin D deficiency, such as tumour induced osteomalacia or renal tubular disorders (Holick and Chen, 2008), could result in mineralisation defects in teeth, but they are so rare that little data are currently available on the prevalence of these disorders. Mineralisation defects in the teeth of patients with renal failure were not even recognized until 1983 and were found in cases where a kidney transplant was necessary to sustain life (Clark and Wysocki, 1988). In the past, if defects did occur it would likely be very close to the time of death of the individual, and the periods of recovery and return to normal mineralisation observed in the current study would be absent. In past communities, children with these types of conditions would probably not have lived sufficiently long for mineralisation defects to occur. Unlike genetic causes or rare conditions of deficiency, one may see fluctuating regions of interglobular dentin with nutritional rickets as individuals were likely to have periods of deficiency followed by periods of recovery. For example, SJ 892 who had interglobular dentin present in the canine (age 4), but was absent in the third molar may have recovered from a deficiency by the time the third molar was forming (age ~12.5 years).

Vitamin D deficiency during gestation affects deciduous teeth, whereas during early childhood it affects permanent teeth. Consequently, a deficiency at a given time period affect various teeth differently. For example, if a deficiency occurs in newborns, primary tooth crown formation and initial mineralisation of permanent first molars are affected. A deficiency at age 5–6 years disrupts the mineralisation in the roots of permanent first molars as well as the crown region of the permanent second molars. This was demonstrated by the presence of Grade 2 interglobular dentin in the roots of the first molar and under the crown of the second molars for archaeological individuals 15A-S36, SJ 384 (Table 4). By correlating the age at which a deficiency occurred, it was possible to determine that three individuals had more than one episode of deficiency (15A-S36, SJ 384, SJ 970). Multiple episodes of deficiency would be impossible to accurately assess from macroscopic examination even when clear skeletal changes are present. Mays et al. (2006), using careful macroscopic and radiological assessment, were able to show that some juveniles from St. Martin's Birmingham had multiple episodes of deficiency. However, histological examination of dentin could provide clear information on cyclical and repeat episodes of deficiency. As discussed by Brickley et al. (2014), individuals who have experienced one episode are likely to be vulnerable to experiencing further episodes.

All skeletons examined in this study had distinct interglobular spaces in their dentin associated with marked bowing deformities in the leg bones ($n = 6$). At the very slight end of the spectrum, it may be impossible to correctly link skeletal changes to vitamin D deficiency due to individual skeletal variation. Individual variation in femoral curvature is influenced by factors such as body weight, activity, and ancestry, all of which exhibit varying degrees of bowing that can be mistaken for deficiency (Gilbert, 1976). It is also possible to miss cases of deficiency where skeletal evidence is too subtle. Where bony changes remodel leaving no evidence of previous rickets, the changes in teeth are permanent and remain as evidence of the disease process (Wolfe, 1935). We advocate that a histological analysis of dentin completed concurrently with skeletal analysis will further aid in diagnosing a deficiency, particularly when skeletal evidence is ambiguous.

Areas of future research could involve investigation of adults with osteomalacia to determine if interglobular dentin is present and if there is a correlation between severity of osteomalacia and the severity of interglobular dentin. As secondary dentin is formed slowly throughout life, there exists the possibility that in severe longstanding cases of osteomalacia that interglobular dentin may be observed. While this study analyzed permanent teeth, deciduous teeth could provide valuable information related to the intrauterine environment of mothers with vitamin D deficiency. The presence of mineralisation defects in dentin can contribute further to the Barker hypothesis, which asserts that stressors early in an individual's life have negative health consequences later in life (Barker, 1997; Armelagos et al., 2009). Paterson and Ayoub (2014) reviewed published reports of congenital rickets and found that maternal deficiency led to significant bone impairment in the fetus. Wolfe's (1935) case report clearly noted mineralisation defects in the deciduous teeth of children and the developing teeth of stillborn infants whose mothers were markedly deficient in calcium or had osteomalacia during pregnancy.

The data suggest that human dentin can reflect periods of vitamin D deficiency, which are known to interfere with systemic mineralisation processes. Although, additional investigation needs to be conducted, current research shows that impaired mineralisation of the microstructures in tooth dentin is suitable for studying vitamin D deficiency. The features observed histologically in dentin appear to develop in various age groups during periods of deficiency and this offers novel insights into dentinogenesis under rachitic conditions.

5. Conclusions

Skeletal indicators of vitamin D deficiency may be slight or easily missed once the condition has healed, this preliminary research demonstrates that the recognition of deficiency may be observable in tooth dentin based on characteristic dental manifestations. The results of this study are in line with the clinical literature (e.g., Seow and Latham, 1986; Seeto and Seow, 1991; Vital et al., 2012; Linglart et al., 2014), that show that the pathological processes of vitamin D deficiency present as clear demarcation between interglobular (non-mineralised) and normal (mineralised) dentin, seen on SEM and histological images. The systemic mineralisation problems of the rachitic skeleton may cause dentin mineralisation to stop or falter, preventing further dentin growth and fusion, resulting in a lag between predentin matrix synthesis and its mineralisation into mature dentin. Histological analysis shows promise in the diagnosis of archaeological individuals with vitamin D deficiency and warrants further investigation, particularly in relation to different age groups. The tooth is a valuable tissue to study vitamin D deficiency, especially where skeletal changes are very subtle, and the techniques outlined in this study have the potential to provide improved recognition of archaeological individuals who have experienced vitamin D deficiency.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jas.2016.06.006.

References

Armelagos, G.J., Goodman, A.H., Harper, K.N., Blakey, M.L., 2009. Enamel hypoplasia and early mortality: bioarchaeological support for the Barker hypothesis. Evol. Anthropol. 18, 261–271.
Arpin, C., 2006. Sépultures du cimetière St-Matthew. Étude sur les critères paléodémographiques et la représentativité d'une collection d'ossements témoignant de la présence protestante à Québec entre 1771 et 1860. Master's thesis. Laval University, Québec City.
Avery, J.K., 2002. Development of teeth and supporting structures. In: Oral Development and Histology, third ed. Thieme Medical Publishers, New York.
Barker, D.J.P., 1997. Maternal nutrition, fetal nutrition, and disease in later life. Nutrition 13 (9), 807–813.
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 (2), 277–295.
Beck-Nielsen, S.S., Brock-Jacobsen, B., Gram, J., Brixen, K., Jensen, T.K., 2009. Incidence and prevalence of nutritional and hereditary rickets in southern Denmark. Eur. J. Endocrinol. 160, 491–497.
Berdal, A., Balmain, N., Cuisinier-Gleizes, P., Mathieu, H., 1987. Histology and microradiography of early post-natal molar tooth development in vitamin-D deficient rats. Arch. Oral Biol. 32 (7), 493–498.
Bevelander, G., Nakahara, H., 1966. The formation and mineralisation of dentin. Anat Rec 1560 (3), 303–323.
Brickley, M.B., Ives, R., 2008. The Bioarchaeology of Metabolic Bone Disease. Academic Press, San Diego.
Brickley, M.B., Mays, S., Ives, R., 2010. Evaluation and interpretation of residual rickets deformities in adults. Int. J. Osteoarchaeol 20 (1), 54–66.
Brickley, M.B., Moffat, T., Watamaniuk, L., 2014. Biocultural perspectives of vitamin D deficiency in the past. J. Anthropol. Archaeol. 36, 48–59.
Caron, D., 2013. Essai de détection de processus migratoires à travers les isotopes de strontium et d'oxygène: étude des restes humains du cimetière Saint-Matthew (Québec, 1771–1860). Master's thesis. University of Montréal.
Chaussain-Miller, C., Sinding, C., Wolikow, M., Lasfargues, J.J., Godeau, G., Garabedian, M., 2003. Dental abnormalities in patients with familial hypophosphatemic vitamin D-resistant rickets: prevention by early treatment with 1-hydroxyvitamin D. J. Pediatr. 142 (3), 324–331.
Chiego Jr., D.J., 2014. Essentials of Oral Histology and Embryology: a Clinical Approach, 4rth edition. Elsevier Mosby, St. Louis Missouri.
Clark, D.B., Wysocki, G.P., 1988. Dentin in chronic renal failure: an ultrastructural study. J. Oral Pathol. Med. 17 (2), 60–69.
Cloutier, C., 2000. Tombeaux, cercueils, caveaux et linceuls: les témoins archéologiques au dernier repos. Cimetière St-Matthew. Ministère de la Culture et des Communications du Québec (Unpublished Archaeological Report).
Cohen, S., Becker, G.L., Origin, 1976. Diagnosis and treatment of the dental manifestations of vitamin D-resistant rickets: review of the literature and report of case. J. Am. Dent. Assoc. 92 (1), 120–129.
Couve, E., 1987. Changes in predentin thickness and mineralisation front configuration in developing human premolars. Acta Anat. 130 (4), 324–328.
Dean, M.C., Scandrett, A.E., 1995. Rates of dentine mineralization in permanent human teeth. Int. J. Osteoarchaeol 5 (4), 349–358.
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 (11), 3101–3111.
Formicola, V., 1995. X-linked hypophosphatemic rickets: a probable upper Paleolithic case. Am. J. Phys. Anthropol. 98, 403–409.
Foster, B.L., Nociti, F.H., Somerman, M.J., 2014. The rachitic tooth. Endocr. Rev. 35 (1), 1–34.
Francis, F., Strom, T.M., Hennig, S., BoddrichA, Lorenz B., Brandau, O., Mohnike, K.L., Michele Cagnoli, M., Steffens, C., Klages, S., Borzym, K., Pohl, T., Oudet, C., Econs, M.J., Rowe, P.S.N., Reinhardt, R., Meitinger, T., Lehrach, H., 1997. Genomic organization of the human *PEX* gene mutated in X-linked dominant hypophosphatemic rickets. Genome Res. 7, 573–585.
Gilbert, M.B., 1976. Anterior femoral curvature: its probable basis and utility as a criterion of racial assessment. Am. J. Phys. Anthropol. 45 (3), 601–604.
Hess, A.F., 1930. Rickets, Including Osteomalacia and Tetany. Kimpton, London.
Hillson, S., 2002. Dental Anthropology. Cambridge University Press, UK.
Holick, M.F., 2007. Vitamin D deficiency. N. Engl. J. Med. 357 (3), 266–281.
Holick, M.F., Chen, T.C., 2008. Vitamin D deficiency: a worldwide problem with health consequences. Am. J. Clin. Nutr. 87 (Suppl. I.), 1080S–1086S.
Howe, P.R., Wesson, L.G., Boyle, P.E., Wolbach, S.B., 1940. Low calcium rickets in the guinea pig. Exp. Biol. Med. 45 (1), 298–301.
Isokawa, S., Kosakai, T., Kajiyama, S., 1963. Interglobular dentin in the deciduous tooth. J. Dent. Res. 42 (3), 831–834.
Limeback, H., Schlumbohm, C., Sen, A., Nikiforuk, 1992. The effects of hypocalcemia/hypophosphatemia on porcine bone and dental hard tissues in an inherited form of Type 1 pseudo-vitamin D deficiency rickets. J. Dent. Res. 71 (2), 346–352.
Linglart, A., Biosse-Duplan, M., Briot, K., Chaussain, C., Esterle, L., Guillaume-Czitrom, S., Kamenicky, P., Nevoux, J., Prié, D., Rothenbuhler, A., Wicart, P., Harvengt, P., 2014. Therapeutic management of hypophosphamic rickets from infancy to adulthood. Endocr. Connect. 3, R13–R30.
Malloy, P.J., Feldman, D., 2010. Genetic disorders and defect in vitamin D action. Endocrinol. Metab. Clin. North Am. 39 (2), 333–346.
Mays, S., Brickley, M., Ives, R., 2006. Skeletal manifestations of rickets in infants and young children in historic population from England. Am. J. Phys. Anthropol. 129 (3), 362–374.
McDonnell, D., Derkson, G., Zhang, L., Hlady, J., 1997. Nutritional rickets in a 2-year-old child: case report. Pediatr. Dent. 19 (2), 127–130.
Mellanby, M., 1928. The influence of diet on the structure of teeth. Physiol. Rev. 8, 545–577.
Mellanby, M., 1934. Diet and the Teeth: an Experimental Study. Part III, the Effect of Diet on Dental Structure and Disease in Man. Medical Research Council, Special Report Series, No. I9I. His Majesty's Stationery Office, London.
Molnar, S., Ward, S.C., 1975. Mineral metabolism and microstructural defects in primate teeth. Am. J. Phys. Anthropol. 43 (1), 3–17.
Moorrees, C.F.A., Fanning, E.A., Hunt Jr., E.E., 1963. Age variation of formation stages for ten permanent teeth. J. Dent. Res. 42 (6), 1490–1502.
Morland, F., 2010. Nutrition and Health: Paleochemical and Paleopathological Studies Population Exhumed the Protestant Cemetery of St. Matthew. University of Montreal, Quebec City, Canada, pp. 1771–1860. Master's thesis.
Noppen, L., 1987. Un quartier en sursis? Cap aux Diamants 3 (1), 5–7.
Ortner, D.J., Mays, S., 1998. Dry-bone manifestations of rickets in infancy and early childhood. Int. J. Osteoarchaeol 8 (1), 45–55.
Pal, B.R., Shaw, N.J., 2001. Rickets resurgence in the United Kingdom: improving 4 antenatal management in Asians. J. Pediatr. 139, 337–338.
Panov, V., Krasteva, A., 2011. Oral health issues in patients with liver disease. J. IMAB - Annu. Proc Sci. Pap. 17 (2), 140–142. http://dx.doi.org/10.5272/jimab.2011172.140.
Paterson, C.R., Ayoub, D., 2014. Congenital rickets due to vitamin D deficiency in the mothers. Clin. Nutr. 34, 793–798.
Pearce, S.H., Cheetham, T.D., 2010. Diagnosis and management of vitamin D deficiency. BMJ 340, 142–147.
Perron, J.S., 2006. Les marqueurs osseux d'activité physique: une étude des restes humains du cimetière St-Matthew à Québec (XVIIIème et XIXème siècles). Laval University, Québec City. Master's thesis.
Pettifor, J.M., 2003. Nutritional rickets. In: Glorieux, F.H., Pettifor, J.M., Jüppner, H. (Eds.), Pediatric Bone. Biology and Diseases. Academic Press, San Diego, pp. 541–565.
Priemel, M., von Domarus, C., Orla Klatte, T., Kessler, S., Schlie, J., Meier, S., Proksch, N., Pastor, F., Netter, C., Streichert, T., Püschel, K., Michael Amling, M., 2010. Bone mineralisation defects and vitamin D deficiency: histomorphometric analysis of iliac crest bone biopsies and circulating 25-hydroxyvitamin D in 675 patients. J. Bone Min. Res. 25 (2), 305–312.
Rashid, M., Zarkadas, M., Anca, A., Limeback, H., 2011. Oral manifestations of Celiac disease: a clinical guide for dentists. J. Can. Dent. Assoc. 77 (39), 1–6.
Ribot, I., Morland, F., Boisjoli, M.-E., 2010. La bioarchéologie humaine, à la frontière entre le 'social' et le 'biologique': état de santé et démographie de populations historiques québécoises. Paléo Québec 34, 27–54.
Ribot I, Morland F, Desrosiers E. In press. Ce que la composition chimique des ossements humains nous apprend sur la nutrition. In Archéologie Montréalaise, Balac AM, Bélanger F, Lavergne MG, Morin MC (eds). Recherches Amérindiennes au Québec: Montreal. p.287.
Robinson, P.D., Högler, W., Craig, M.E., Cf, Verge, Walker, J.L., Piper, A.C., Woodhead, H.J., Cowell, C.T., Ambler, G.R., 2006. The re-emerging burden of rickets: a decade of experience from Sydney. Arch. Dis. Child. 91, 564–568.
Santana e Meneses, J.F., Pons Leite, H., de Carvalho, W.B., Lopes, E., 2009. Hypophosphatemia in critically ill children: prevalence and associated risk factors. Pediatr. Crit. Care Med. 10 (2), 234–238.
Saunders, S.R., Chan, A.H.W., Kahlon, B., Kluge, H.F., 2007. Sexual dimorphism of the dental tissues in human permanent mandibular canines and third molars. Am. J. Phys. Anthropol. 133 (1), 735–740.
Scheuer, L., Black, S., 2004. The Juvenile Skeleton. Elsevier Academic Press, United Kingdom.
Seeto, E., Seow, K.W., 1991. Scanning electron microscopic analysis of dentin in vitamin D-resistant rickets-assessment of mineralisation and correlation with clinical findings. Pediatr. Dent. 13 (1), 43–48.
Seow, K.W., Brown, J.P., Tudehope, D.A., O'Callaghan, M., 1984. Dental defects in the deciduous dentition of premature infants with low birth weight and neonatal rickets. Pediatr. Dent. 6 (2), 88–92.
Seow, K.W., Latham, S.C., 1986. The spectrum of dental manifestations in vitamin D-resistant rickets: implications for management. Pediatr. Dent. 8 (3), 245–250.

Seow, K.W., Humphrys, C., Tudehope, D.I., 1987. Increased prevalence of developmental dental defects in low birth-weight, prematurely born children: a controlled study. Pediatr. Dent. 9 (3), 221–225.
Seow, K.W., Romaniuk, K., Sclavos, S., 1989. Micromorphologic features of dentin in vitamin D-resistant rickets: correlation with clinical grading of severity. Pediatr. Dent. 11 (3), 203–208.
Shellis, R.P., 1983. Structural organization of calcospherites in normal and rachitic human dentin. Arch. Oral Biol. 28 (1), 85–95.
Simoneau, D., 2003. Église et cimetière Saint-Matthew, rapport de surveillance archéologique. Ministère de la Culture et des Communications du Québec, Division design et patrimoine, Centre de développement économique et urbain, Ville de Québec (Unpublished Archaeological Report).
Souza, M.A., Soares Junior, L.A., Santos, M.A., Vaisbick, M.H., 2010. Dental abnormalities and oral health in patients with hypophosphatemic rickets. Clin. (Sao Paulo) 65 (10), 1023–1026.
Souza, A.P., Kobauashi, T.Y., Lourenco Neto, N., Silva, S.M.B., Machado, M.A.A., Oliveira, T.M., 2013. Dental manifestations of patient with vitamin D-resistant rickets. J. Appl. Oral Sci. 21 (6), 601–606.
Thacher, T.D., Fischer, P.R., Strand, M.A., Pettifor, J.M., 2006. Nutritional rickets around the world: causes and future directions. Ann. Trop. Paediatr. 26, 1–16.
Tumen, E.C., Yavuz, Atakul F., 2009. Types of rickets, dental and histologic findings: review of the literature. Pesq bras odontoped clin integr. João Pessoa 9 (2), 241–246.
Ubelaker, D.H., 1989. The estimation of age at death from immature human bone. In: Işcan, M.Y. (Ed.), Age Markers in the Human Skeleton. Charles C. Thomas, Springfield, IL.
Veselka, B., Hoogland, M.L.P., Waters-Rist, A.L., 2013. Rural rickets: vitamin D deficiency in a post-Medieval farming community from the Netherlands. Int. J. Osteoarchaeol. http://dx.doi.org/10.1002/oa.2329 online.
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 mineralisation. Bone 50 (4), 989–997.
Wolfe, J.J., 1935. Teeth in fetal rickets. Am J Dis Child 49 (4), 905–911.
Yoshiki, S., Yanagisawa, T., 1974. The role of vitamin D in the mineralisation of dentin in rats made rachitic by a diet low in calcium and deficient in vitamin D. Calcif. Tissue Res. 15 (1), 295–302.
Zhang, X., Rahemtulla, F., Zhang, P., Beck, P., Thomas, H.F., 2009. Different enamel and dentin mineralisation observed in VDR deficient mouse model. Arch. Oral Biol. 54 (4), 299–305.

Further reading

Amaral, T.H.A.D., Guerra, C.D.S., Bombonato-Prado, K.F., Garcia de Paula e Silva, F.W., De Queiroz, A.M., 2008. Tooth pigmentation caused by bilirubin: a case report and histological evaluation. Special Care Dent. 28 (6), 254–257.
Berman, M., Edwards, L.F., Kitchin, P.C., 1939. Effect of artificially induced hyperpyrexia on tooth structure of the rabbit. P Soc. Exp. Biol. Med. 41, 113–115.
Bevelander, G., Bernstein, J.G., 1940. The effect of artificially induced fever on the structure of the developing teeth of the rat. J. Dent. Res. 19 (2), 155–163.
Boyce, B.F., Path, M.R.C., Prime, S.S., Halls, D., Johnston, E., Critchlow, H., MacDonald, D.G., Path, M.R.C., Junor, B.J.R., 1986. Does osteomalacia contribute to development of oral complications of oxalosis? Oral Surg. 61 (3), 272–277.
De Coster, P.J., 2012. Dentin disorders: anomalies of dentin formation and structure. Endod. Top. 21, 41–61.
Hasegawa, K., Higuchi, Y., Yamashita, M., TanakaH, 2015. Japanese familial case with metaphyseal dysplasia, Schmid Type caused by the p.T555P mutation in the *COL10A1* gene. Clin. Pediatr. Endocrinol. 24 (1), 33–36.
Irving, J.T., 1940. The influence of diets low in magnesium upon the histological appearance of the incisor tooth of the rat. J. Physiol. 99, 8–17.
Kuijpers, M.H., Van De Kooij, A.J., Slootweg, P.J., 1996. The rat incisor in toxicologic pathology. Lab. Anim. Path 24 (3), 346–360.
Pitt, M.J., 1995. Rickets and osteomalacia. In: Resnick, D. (Ed.), Diagnosis of Bone and Joint Disorder, third ed. Saunders, Philadelphia, pp. 1885–1922.

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