TOOTH TALES: WHAT INTERNAL DENTAL STUCTURES REVEAL ABOUT VITAMIN D DEFICIENCY AND AGE ESTIMATION

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By

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LAY ABSTRACT

Teeth record life events and the three papers in this thesis use dental structures to provide methodological foundations to evaluate the occurrence and severity of vitamin D deficiency in early life. The potential long-term consequences of such events are investigated through accurate recognition of older adults. Vitamin D regulates skeletal health by mediating calcium absorption and phosphorous homeostasis and deficiency is recognised as an important health concern. Accurate identification of older adults is also a widely recognised problem in skeletal studies. Age-at-death estimation in older individuals was calculated and the exploration of abnormal pulp chamber shape and mineralisation defects in tooth dentin was done to determine vitamin D status in both younger and older individuals. This research established that internal dental structures enables past episodes of vitamin D deficiency to be recognized in cases where skeletal indicators are not clear and permits increased precision in age-at-death estimations in the older individual.

ABSTRACT

Exploration of the internal structures of teeth is complex and has the potential to add greatly to existing information about the lifecourse of archaeological individuals, but has yet to realize its full interpretative value as an avenue of bioarchaeological inquiry. This thesis consists of three papers that focus on the potential for internal dental structures to provide important information on chronological age, and physiological alterations linked to vitamin D deficiency.

The first paper used SEM, microscopic imaging, and histological investigation of tooth dentin to determine the presence of mineralisation defects, observed as interglobular dentin (IGD) (spaces following incremental lines) in living (with known medical history) and archaeological individuals with clear healed rickets. This paper demonstrated that incremental bands of IGD are indicative of vitamin D deficiency.

The second paper expands identification of those with deficiency by quantifying morphological changes in pulp chambers of living and archaeological individuals. Pulp chambers were radiographed, evaluated histologically, and measured. Those with evidence of past vitamin D deficiency displayed constricted or chair shaped pulp horns. This radiographic technique provides a non-destructive tool to identify individuals that experienced childhood vitamin D deficiency.

The role vitamin D plays in the development of IGD over the lifecourse requires that accurate age estimates be conducted on older as well as younger adults. The third paper used a new version of pulp/tooth area ratios to provide an accurate estimation of age-at-death in older adults (50+). ImageJ software was used to calculate areas on sectioned teeth and results provided a mean absolute error (MAE) of ±3.9 years in older adults.

The results described in this thesis contribute to broader topics of discussion in anthropology, such as investigating health and metabolic disease in human populations, and adds to the ongoing discussion and evaluation of age-at-death techniques used to extend our ability to study the lifecourse of archaeological individuals.

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"I've learned as time passes, all the things you're afraid of will come and they will go, and you will be alright"
~Stevie Nicks~

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Statement of Academic Achievement

I am the main contributor to the three articles that comprise this thesis. Chapter 2, 'The rachitic tooth: A histological examination' is a co-authored paper published in the Journal of Archaeological Science, and Chapter 3 'The rachitic tooth: The use of radiographs as a screening technique' is a co-authored paper that is published in the International Journal of Paleopathology. Chapter 4 consists of a co-authored paper entitled 'Age estimation in older adults: Use of pulp/tooth ratios calculated from tooth sections**'** that has been accepted for publication in the American Journal of Physical Anthropology: Brief Communications. I am the first author for all three papers, conducting all laboratory work, measurements, and analyses, with input from my co-authors. I wrote the first draft, prepared the figures and tables, with the exception of four diagrams, and collaborated with co-authors for subsequent feedback and revisions.

CHAPTER 1: INTRODUCTION

An important aspect of bioarchaeological research is the ability to elucidate the lives of individuals from the past. By developing methodologies that utilize teeth to aid in the identification of conditions such as vitamin D deficiency, bioarchaeologists can reconstruct past disease patterns and illuminate individual life histories. Vitamin D deficiency is related to nutritional deficiencies that affect mineralisation processes in bone and tooth dentin. The mineralisation of dentin and bone occur through comparable processes; therefore, tooth dentin is susceptible to the same failures as bone during metabolic disturbance related to vitamin D deficiency (Boskey et al., 1997; Foster et al., 2014). To date, limited research has been conducted using the internal structures of teeth to investigate childhood vitamin D deficiency, although it is widely recognized that there are considerable problems in assessing deficiency using skeletal evidence (Brickley and Ives, 2008; Brickley et al., 2010). The first part of this thesis uses the analysis of tooth dentin microstructures to assess information on both the severity of a deficient episode, and the age at which a past episode occurred. Research undertaken demonstrated that dental microstructures have considerable potential to shed light on vitamin D deficiency in past communities.

Accurate adult ageing is integral to our understanding of morbidity and mortality in the past and provides part of an essential biological profile for bioarchaeologists; however, our current ability to estimate the age of older adults is confounded by methodological limitations. This thesis also demonstrates the utility of tooth dentin to estimate age-at-death of older adult individuals in skeletal samples. To date it has been

impossible to accurately assess the long-term health consequences of the occurrence of vitamin D deficiency in infancy and childhood. To address these issues, this thesis utilized four different methods; scanning electron microscopy (SEM), histology, radiography, and pulp/tooth area ratios to estimate age-at-death in the older adult and to identify vitamin D deficiency.

Advantages of teeth

Throughout life teeth incorporate characteristics that provide valuable data for bioarchaeological studies, and these are retained due to lack of remodelling. Teeth provide information about dietary patterns, genetic relationships, and geographic origins that help to elucidate the social, economic, and biological status of past populations (Rohland and Hofreiter, 2007; Prowse, 2011; Beaumont et al., 2013). The range of analytical techniques now available to aid in reconstructing ancient life is diverse and expanding. These techniques encompass dental paleopathology, stable isotope analysis, biomonitoring of trace elements, dental microwear analysis, and studies on macroscopic tooth wear (e.g., Molnar 1971; Lukacs, 1989; Zaichick et al., 1999). Teeth are durable, evolutionarily conservative, yet adaptable (Smith et al., 1997). Bone also incorporates valuable information, but changes associated with modelling and remodelling mean that with time much information will be lost. Lesser structural integrity in bone also mean that bones frequently undergo diagenetic alternation in burial contexts, whereas dentition maintains its integrity and so teeth may be the only biological tissue sufficiently well preserved for scientific analysis. While tooth enamel is harder and more highly

mineralised than dentin, enamel derives much of its strength and ability to withstand mechanical loading from the underlying dentin (Beaumont et al., 2013). Teeth are of paramount importance for use in this thesis as the internal anatomy, such as dentin (where the earliest dentin formed reflects the intrauterine environment), and pulp chamber shape, are preserved over the lifecourse, permitting analyses to be conducted that reveals information about the life of an individual from birth to adulthood.

Microscopically, dentin is comprised of intertubular dentin that is formed during odontogensis (tooth formation), and dentin tubules that contain the odontoblastic processes (Hillson, 2002). Primary dentin consists of all dentin that is formed until completion of the root, and dentin deposited after root completion is referred to as secondary dentin (Schroeder, 1991; Hillson, 2002). The composition of dentin is comparable to bone; the cells that compose dentin, the odontoblasts, remain active forming new secondary dentin throughout life. Due to this, one can explore whether vitamin D deficiency will affect or alter the microstructure of dentin, similar to mineralisation defects found in bone (see Bonucci et al., 1969).

The use of dentin contributes to discourse on classifying vitamin D deficiency as a systemic condition, one that affects a number of tissues or the body as a whole. Bone and dentin are similar in that they are both mineralised tissue that are comparable in composition and the mechanisms of their formation (Huang et al., 2008). They are both composed of an organic matrix rich in collagen and a mineral consisting of carbonate hydroxyapatite crystals (Vital et al., 2012). Primarily, osteoblasts (bone) and odontoblasts (dentin) secrete cells that function as the matrix of these tissues. Although parallels can be

drawn between bone and dentin, these tissues also display several differences. Bone is a dynamic tissue, continuously remodelled, whereas dentin does not turnover, although secondary dentin does form. Bone formation occurs through the production of matrix by osteoblasts under the control of local growth factors (Boskey et al., 1997). Conversely, dentinogenesis (dentin formation) is a continuous process of matrix deposition throughout the life of the tooth, where secondary dentin is secreted after tooth eruption at a rate of approximately 0.4 μm/day (Solheim, 1992). Both bone and dentin reflect the biomineralisation process in both physiological and pathological conditions and this thesis explores how tooth dentin can reflect pathological mineralisation during vitamin D deficiency.

The permanent human dentition provides a time record of approximately 18 years, from before birth until the late teens when the third molar root is completed. The formation processes of teeth are crucial to meaningful interpretation of histological and radiological analysis in studies of individuals who lived in the past. It is not just their macroscopic formation but formation on the microscopic scale that must be understood to assess how this relates to deficiency and age-at-death estimations. Ultimately, the use of teeth makes it possible to investigate the full range of ages that cover critical periods of development of past individuals, enabling a nuanced understanding of age-at-death patterns and vitamin D deficiency in the past.

An additional advantage of the use of teeth is that the deposition of secondary dentin in the pulpal chamber continues with increasing age, which was the rationale for use of secondary dentin deposition for age-at-death age estimation in the older

archaeological adult (Bodecker, 1925; Solheim, 1992). As teeth are sensitive indicators of human biological processes, they provide a permanent record of critical events in childhood such as episodes of stress or malnutrition (e.g., enamel hypoplasia). Traditionally, enamel is the tissue of choice for most dental anthropological studies, but dentin is gaining recognition with the advent of isotopic studies (e.g., Fuller et al., 2003; Beaumont et al., 2013). Research undertaken for this thesis found that dentin can provide specific information on a condition that cause disruptions in mineralisation of dentin and indicates that nutritional rickets is a relatively common in both past and present communities. Teeth are ideal for exploration into the lifecourse of individuals who lived in the past, as they maintain their integrity; are readily available across populations; are suitable biomarkers for exposure to certain conditions, and can be measured with accuracy.

Vitamin D deficiency

Vitamin D is a pro-hormone that is synthesized in the skin by sunlight. The human body can fulfill its vitamin D requirements by either exposure to the sun for enough time to produce adequate amounts or by ingesting food containing vitamin D (Holick 2004, 2008). Vitamin D controls calcium and phosphate absorption in the small intestine and interacts with parathyroid hormone to mediate skeletal and tooth mineralisation and maintain calcium homeostasis in the blood stream (Kulie et al., 2009). Vitamin D is manufactured in the skin through exposure to ultra-violet light (UV) B rays. UVB energy converts the metabolically inactive pro-hormone D_3 and transports it to the liver where it

is hydroxylated into the active form 25-hydroxyvitamin D_3 (Holick, 2007). A subsequent conversion occurs converting 25-hydroxyvitamin D_3 to 1,25-dihydroxyvitamin D in the kidneys, which produces the active form of circulating vitamin D (Brickley and Ives, 2008; Holick, 2007, 2008). UVB rays are present only during midday at higher latitudes and do not penetrate clouds. The time needed to produce adequate vitamin D from the skin depends on the strength of the UVB rays (i.e., correlated with latitude of residence), length of time spent in the sun, and the amount of pigment in the skin (Holick, 2006). The half-life of vitamin D is approximately three weeks, which emphasizes the need for frequent replenishment of the body's supply (Kulie et al., 2009). Vitamin D can be also acquired from a limited number of natural dietary sources. Oily fish (e.g., salmon and tuna) and foods such as egg yolks contain vitamin D, but aside from cod liver oil the amount that can be acquired is still low (Brickley and Ives, 2008).

Rickets and osteomalacia are typical manifestations of severe vitamin D deficiency. Clinically the term rickets is used to describe defects in endochondral mineralisation, and is specific to juveniles (Brickley and Ives, 2008:91; Mayo Clinic, 2017), while osteomalacia is used for defective mineralisation of osteoid on pre-existing bone surfaces (Brickley and Ives, 2008:91). Technically juveniles, therefore experience both rickets and osteomalacia. In paleopathology the term rickets is, however commonly used to refer to lesions linked to defective mineralisation of bone resulting from deficiency of vitamin D, calcium or phosphorous, and the term osteomalacia is used to refer to such lesions in adults. In adulthood bone continues to remodel and if deficiency persists for any length of time the osteoid content relative to mineralised tissue can

develop to the extent that pathological fractures and eventually bowing deformities occur (Brickley et al., 2005; 2007). Nutritional rickets is caused by a number of factors that affect vitamin D, calcium, and phosphate metabolism; 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). Mineralisation defects in the body can be influenced by an imbalance of circulating calcium and phosphate, which is regulated by vitamin D. Vitamin D deficiency limits the body's ability to absorb calcium and phosphorus in the intestines resulting in metabolic bone disease and other chronic illness (Brickley et al., 2014). In this thesis, vitamin D deficiency will be defined as a low concentration of vitamin D that can cause pathological changes in the skeleton and in dental structures such as tooth dentin. This will include the concept that a prolonged lack of vitamin D also affects the absorption of calcium and phosphorous in the intestines resulting in mineralisation defects in the body.

It is estimated that 1 billion people worldwide have vitamin D deficiency (Holick, 2007). Once thought to be eradicated in the 20th century, vitamin D deficiency has seen a resurgence in many countries in Europe and North American. Vitamin D deficiency continues to be a problem among infants in many communities, especially among exclusively breast-fed infants, and infants and children of darker skinned immigrants living at higher latitudes (e.g., infants and mothers from the Middle East), and those who avoid direct sunlight because of the risk of developing skin cancer (Pettifor, 2004). Deficiency is widely recognized in many developing countries including some located in subtropical regions such as Nigeria, Yemen, and Bangladesh (Pettifor, 2004). Vitamin D

deficiency remains a cause of rickets where social and religious customs (e.g., clothing that covers most of the body), and/or climatic conditions often prevent adequate ultraviolet sunlight exposure. Another factor that contributes to vitamin D deficiency is observed in those with predominantly cereal-based diets, as high levels of phytate in cereal grains bind to calcium preventing proper absorption in the body (Brickley and Ives, 2008:84).

Diagnosing vitamin D deficiency in paleopathology

Traditionally, 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., 2015), but identification of residual deformities in adults caused by previous vitamin D deficiency remains challenging as few of the subtle morphological changes seen in childhood survive in the adult skeleton (Brickley et al., 2010). Paleopathologists predominantly use macroscopic lesions observed in skeletal remains to diagnose cases of vitamin D deficiency. Most cases of rickets are caused by severe vitamin D deficiency that occur during periods of rapid skeletal growth, whereas less severe deficiency or cases of deficiency that occur during periods of slower growth, for example during adulthood, will not result in marked skeletal changes, making diagnosis difficult (Brickley et al., 2010). Despite progress in the identification of rickets in archaeological bone, there remain difficulties in recognizing adults who experienced vitamin D deficiency. Brickley et al. (2014) state that individuals who have experienced an episode of deficiency have a higher chance of experiencing further episodes. However, even when deficiency is

identified, how can we determine how many episodes have occurred from examination of the skeleton? It has been suggested that in untreated cases, only 10-25% of rickets cases result in visible leg deformity in adult individuals (Hess, 1930). Diagnosing deficiency in adults is also challenging due to the wide range of normal individual variation. This research explores novel investigative techniques using tooth dentin and pulp chamber morphology to increase the identification of deficiency in the many cases where skeletal features are inconclusive. The next sections will highlight why the internal structures of dentition are useful for assessing physiological processes such as vitamin D deficiency.

Interglobular dentin (IGD)

Conditions that disrupt vitamin D, calcium, and phosphate pathways cause systemic mineralisation defects in teeth that are referred to as incremental interglobular dentin (used interchangeably as IGD or IIGD). IGD is observed as clear bands of bubble-like spaces that follow incremental lines within the dentin matrix. Given that vitamin D deficiency causes mineralisation defects in bone, and tooth dentin is structurally and chemically similar to bone, the hypothesis tested in the first paper of this thesis is that individuals with vitamin D deficiency will have interglobular dentin in their teeth. 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). Using beagle dogs, Mellanby (1928, 1934) demonstrated an association between vitamin D deficient diets and/or lack of sunlight exposure and an increase in frequency and prominence of both interglobular spaces in dentin and enamel hypoplasia on enamel.

During this time, it was thought that a range of metabolic factors, such as a deficiency in vitamin A could have caused defects in the dentition, however, Mellanby (1934) clearly showed that vitamin D was responsible for the calcifying process in teeth and alveolar bone, whereas vitamin A controlled the development of the gingival epithelium, which became overgrown in dogs lacking vitamin A. A study of mice with vitamin D deficiency showed both reduced dentin mineralisation and early enamel hypermineralisation leading to the conclusion that vitamin D likely played a key role in tooth mineralisation and appeared to indirectly regulate dentin mineralisation (Zhang et al., 2009). Dentin in individuals without vitamin D deficiency displays normal matrix formation that appears homogeneous without interglobular spaces and shows 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, some calcospherites do not grow sufficiently (failure to fuse) and leave a poorly mineralised patch of matrix, recognized microscopically as interglobular dentin (Vital et al., 2012).

Dentists view interglobular spaces as a defect in mineralisation, not a defect in matrix formation (Chiego, 2014:10), and IGD is considered by dentists to be pathognomonic of vitamin D deficiency (e.g., McDonnell et al., 1997; Vital et al., 2012). Seow et al. (1989), in one of the first studies to report the association between interglobular dentin and genetic cases of vitamin D deficiency, conducted histological examinations on patients' 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). Vitamin D-resistant rickets is an X-linked form of rickets (or osteomalacia) that differs from nutritional rickets in that ingestion of vitamin D is ineffective (Litman et al., 1957). Histological findings from individuals with vitamin D-resistant rickets included marked IGD where the entire dentin mineralisation was abnormal with large non-mineralised interglobular spaces between non-merged calcospherites (Seow et al., 1989). Many of the reported cases of IGD are from rare hereditary causes of disruption to the calcium and phosphorous pathways due to vitamin D deficiency; however, a case of nutritional rickets has been reported by McDonnell et al. (1997) in a 2-year-old child from Canada. Histological examination revealed interglobular dentin in the deciduous mandibular and maxillary first molars.

Pulp chamber shape morphology in association with vitamin D deficiency

Clinical studies have demonstrated that conditions, such as vitamin D deficiency, lead to the development of a number of abnormalities in the internal dental structure (e.g., Seow and Latham, 1986). For a full description of the dental abnormalities that can be observed radiographically, see Table 1 in Chapter 3. Teeth in individuals with vitamin D deficiency often display enlarged pulp chambers, wide predentin, marked IGD (indicative of a mineralisation defect in dentin), and uneven, constricted (narrow) pulp horns that extend from the pulp toward the enamel (Batra et al., 2006). Many of the other dental abnormalities such as dental abscesses or enamel hypoplasias have a number of other

possible causes, therefore the second paper focuses on pulp horn morphology for investigation of vitamin D deficiency, as changes in pulp horns appear to be only caused by conditions linked to disruption of the vitamin D, calcium and phosphorous pathways.

Using tooth dentin to age older adults

Adult age-at-death is one of the most fundamental characteristics for developing a biological profile on a skeleton as it aids in the formulation of a life history for an individual and is an integral part of investigating vitamin D deficiency in paleopathological studies. Techniques used to derive age-at-death need to reliably produce consistent results to permit age-at-death in skeletal samples from different time periods and populations. The age of juveniles is estimated through patterns of epiphyseal fusion and development of the dentition where the timing of these processes varies only slightly between the sexes (Scheuer and Black, 2004). Once an individual reaches skeletal maturity, adult age estimation becomes increasingly less precise as it is accomplished by placing individuals in different age categories based on evidence of degeneration. Physical ageing proceeds at an unpredictable rate, which can vary significantly between individuals and populations and depends on a multitude of factors such as genetics, behaviour differences, nutrition, health, and one's interaction with the environment (Kemkes-Grottenthaler, 2002; Falys and Lewis, 2011). The process of ageing causes degeneration of the joints that increase over time, however it is the many unknown aspects of an individual's lifestyle that influence the skeletal markers that may appear more delayed or advanced in relation to their chronological age (Cox, 2000). This results

in two problems: 1) the age indicators of current methods are often too narrow (e.g., ages 18-19, Todd, 1920), too broad (e.g., ages 25-83, phase 5 in Brooks and Suchey, 1990), or simply too vague (e.g., ages 50+), and 2) while age-at-death methods are successful when applied to the collections from which they were developed, they often fail to work as well on other collections (Bedford et al., 1993; Bocquet-Appel and Masset, 1996; Merritt, 2013).

Difficulties in adult age estimations have been identified in all adult ageing methods including problems with applying the techniques and interpreting the results (e.g., Saunders et al., 1992) and inter-population inconsistencies (Hoppa, 2000). Using skeletal ageing methods produces large age ranges that tend to overlap one another, and several ageing methods display the final phase as an open-ended interval for older individuals (i.e., 50+) (Boldsen et al., 2002). For example, the Suchey-Brooks (1990) pubic symphysis method has an average age range for the six phases of ±34.8 for females and ±28.8 for males, with the greatest ranges for phases III-VI for both sexes (Brooks and Suchey, 1990). Skeletal ageing methods also have a tendency to consistently overestimate age in younger individuals while underestimating age in older individuals, resulting in estimated ages that are closer to the mean age of the reference sample used rather than the actual chronological age (Aykroyd et al., 1997).

Inaccuracies and bias confound adult age-at-death estimations and while we can confidently age juvenile individuals, current problems with ageing methods for older individuals require evaluation of the applicability of using other methods such as those involving dentin. As the process of dentin apposition results in the reduction in the size of

the pulp cavity due to formation of secondary dentin associated with increased age, it could be utilized for age estimation. Evaluation of pulp chamber size may offer a way forward to more accurately assign individuals into this important 50+ age group.

Conceptual Framework

The conceptual framework underlying the three papers in this thesis draws on the lifecourse perspective, which views the process of life through the context of a culturally defined sequence of age categories that individuals are normally expected to pass through as they progress from birth to death (Elder et al., 2003; Prowse, 2011; Agarwal, 2016). Conceptions of the lifecourse perspective include a multidisciplinary paradigm for the study of individual's lives and is useful in bioarchaeology for investigating the interrelationships between biological health at different age phases. The lifecourse approach can be viewed as transitions through different life stages that are marked by biological and social changes (Prowse, 2011; Agarwal, 2016), and in which the long-term effects of a condition on later health can be observed during gestation, childhood, adolescence, young adulthood, and later adult life.

Internal dental structures are ideal for use in the application of a lifecourse approach to vitamin D deficiency, as abnormalities observed in dentin and pulp chambers are permanent in nature and with new technologies can be measured with accuracy. They reflect common experiences and potential social determinants - the economic and social conditions among a population that influence individual and group health status

(Heilmann et al., 2015). They are suitable as a biological marker for exposure to risk factors across the lifecourse.

The formation of dental structures in permanent teeth takes place over a time window starting in utero (~3 months after conception) and spans the first years of life up to age ~18 with specific developmental timing dependent on tooth type (Reid and Dean, 2006). Dentin is formed incrementally and cyclically during tooth development. Disturbances occurring during the development of dentin result in visible mineralisation defects and misshapen pulp chambers. The microstructure of dentin, therefore provides a record of not only the timing of tooth development, but also the age at which a mineralisation disturbance occurred due to early life experiences. Of interest from a lifecourse perspective is that IGD is a systemic condition that can affect several teeth at the same time, depending on their developmental stage, making it possible to deduce the timing and severity of the deficiency.

The first paper established a technique that allowed age of deficiency to be estimated during the development of the dentition permitting deficiency to be diagnosed from in utero to 18 years. The second paper developed a screening technique to determine past deficiency and to narrow down the age at which it occurred using a non-destructive technique. Both of these techniques provided a way to look back at the health of an individual during childhood. More accurate assessment of adult age-at-death will allow the question of frailty in cases of vitamin D deficiency to be investigated within the Developmental Origins of Health and Disease framework (DOHaD). This framework demonstrates the importance of early life experiences on chronic disease risk later in life

(Uauy et al., 2011) and investigates how environmental factors during human development interact with genetic variation to alter the capacity of the body to cope with its environment later in life (Gluckman and Hanson, 2006).

If age-at-death estimations can be improved from the fourth decade to the eighth, it will be possible to map vitamin D status amongst older adults, who currently demonstrate an alarmingly high level of deficiency in various regions of the world (Mitchell et al., 2015). As with younger adults, a significant portion of older adults are not obtaining adequate vitamin D and/or calcium making deficiency highly prevalent among seniors. Older individuals are prone to develop vitamin D deficiency due to various risk factors including decreased dietary intake, diminished sunlight exposure, reduced skin thickness, impaired intestinal absorption, and impaired hydroxylation in the liver and kidneys (Janssen et al., 2002). Being able to accurately age these individuals would assist in improved characterization of the long-term health consequences of childhood vitamin D deficiency. It is possible that childhood deficiency resulted in compromised bone quantity in these individuals, which is why their skeletons were poorly preserved and could not be accurately aged. For this reason, deficiency may lead to this group being removed from analysis (Brickley and Buckberry, 2015).

Using a lifecourse perspective provides a theoretical basis for internal dental structures to aid in age-at-death estimations that could ultimately identify deficiency in all age groups. Application of the aging technique presented in this thesis would also enable the number and severity of episodes of deficiency in the 0 to18-year period to be seen and to be able to assess possible long-term consequences in terms of survival. This will

improve the recognition of individuals who have reached a much older age and will enhance further research on life expectancy and longevity of those with vitamin D deficiency.

Estimating the age-at-death of skeletons from archaeological and forensic contexts is also essential, but often exceedingly difficult. In medico-legal investigations, age is a crucial part of the individual identification process. Age-at-death is a fundamental aspect of investigating the mortality characteristics and disease experience of past populations (Milner and Boldsen, 2012). While individual skeletons are of interest, paleodemographic studies focus on understanding the characteristics of past populations as inferred from examinations of a large number of skeletons (Hoppa, 2002). Ultimately, bioarchaeologists try to look at what life was like in a particular time and region, and how it may have differed from the experiences of temporally, geographically, or culturally distinct groups of individuals (Milner and Boldsen, 2012). Interpretations made from skeletal remains concerning social organization, social status, and ritual practices in ancient societies are also hindered without accurate age-at-death estimations. Therefore, important to the effective use of the lifecourse approach is our ability to age skeletons from all stages of life.

The use of internal dental structures was specifically chosen for investigation over the lifecourse. Once formed, tooth dentin represents an enduring archive that offers the opportunity to reach back into the past and examine information about vitamin D deficiency and age estimation during the entire period of dentin development.

Research Questions

This thesis aimed to develop methodological techniques for histological and radiological analysis, to interpret abnormalities found in tooth dentin and pulp chambers, and to enable the tracing of age-at-death patterns linked to older individuals. One goal in using the lifecourse perspective is to characterize the interplay of biological variables found in internal dental structures to enable a window into an aspect of the health of individuals who lived in the past.

To address what can be learned about the presence or absence of vitamin D deficiency and adult age estimation in individuals who lived in the past, I used modern individuals as a comparative sample. The use of modern individuals of known age was fundamental for the third paper and they also played an important role in the other two papers. Using internal dental structures, the following questions represent the research focus of this thesis:

1) The first research question investigated whether it was possible to identify episodes of vitamin D deficiency in the teeth of archaeological individuals. The clinical dental literature refers to indicators of vitamin D deficiency in various scattered articles, but I have pieced the material together and methodically tested the claims that had been made. Based on the idea that abnormal mineralisation in tooth dentin can be observed as interglobular dentin (spaces) in individuals who have experienced a period of vitamin D deficiency, I hypothesized that deficiency occurring during the development of the dentition could be observed in tooth dentin. Using scanning electron microscopy (SEM) and histological techniques on archaeological teeth with clear skeletal evidence of past

deficiency, I evaluated if the presence or absence of interglobular dentin could be linked with skeletal evidence of vitamin D deficiency. Did individuals with clear skeletal indicators of vitamin D deficiency consistently exhibit the presence of interglobular dentin (IGD) and was it possible to determine the age of deficiency from the location of IGD in the tooth? Conversely, was there an absence of IGD in healthy controls?

2) The second research question involved determining the potential of developing a screening technique to detect vitamin D deficiency in teeth without the need to do histological analysis on every tooth sample. I proposed that the abnormal mineralisation related to vitamin D deficiency in childhood could be detected through pulp chamber shape changes in permanent molars (specifically changes to pulp horns) and explored the potential to quantify such morphological observations. I investigated whether radiological examination of pulp chambers in permanent molars could aid in determining the timing of deficiency in individuals who exhibited pulp changes in their molars. Could changes in the shape of the pulp chamber during the formation of permanent molars be used as a screening technique, providing a non-destructive means of identifying individuals who experienced past episodes of deficiency?

3) The third research question explored the possibility of extending our knowledge of events that occur throughout the lifecourse of individuals using the size of pulp chambers to estimate adult age-at-death. Could histological sections of pulp chambers provide a more accurate means of determining age among older adults? Using single-rooted permanent teeth, I calculated the age of both modern and archaeological individuals at the terminal end of the lifecourse. I specifically targeted the category

labelled as 'old' (defined as 50+ years). Developing a new version of pulp/tooth area calculations I investigated the chronological age of older archaeological individuals, to determine the feasibility of narrowing age ranges for those in the 50+ category.

Through these analyses, I determined whether dentin reflects systemic biomineralisation processes in the human body and if it acts as a biomarker for pathological conditions, particularly vitamin D deficiency. I also explored related questions such as at what approximate age did deficiency occur and was it possible to determine the severity of deficiency? It is important to find dental methodologies that can help in the identification of vitamin D deficiency, particularly in cases where skeletons are not well preserved. The potential significance arises because of the important biocultural information that can be inferred from definitive diagnosis of the condition.

Thesis Format

This thesis follows a 'sandwich' format that includes an introduction, three papers and a discussion and conclusions chapter. The theme that connects the papers in this thesis is the unique analyses of the internal structures of teeth (i.e., dentin and pulp chambers) to reveal information about individuals who lived in the past. There is some necessary overlap between the chapters with regard to literature describing previous dental research, as well as descriptions of some of the techniques and statistical applications employed for the analysis of interglobular dentin. Chapters 1 and 5 are the introduction, and conclusions, respectively. Chapter 2 consists of a paper that has been published in the Journal of Archaeological Science (2016, 74: 152-163), and Chapter 3 is

a paper that is published in The International Journal of Paleopathology. Chapter 4 has been accepted for publication in The American Journal of Physical Anthropology: Brief Communications. The following is a brief summary of each of the papers, their objectives; methods utilized, and author contributions.

Chapter 2: Paper 1: The rachitic tooth: A histological examination.

Authors: Lori D'Ortenzio, Isabelle Ribot, Emeline Raguin, Annabelle Schattmann, Benoit Bertrand, Bonnie Kahlon, and Megan Brickley.

Chapter 2 represents the foundational work devised to diagnose previous episodes of vitamin D deficiency using tooth histology. Methods taken from the clinical literature were used, where defects in tooth dentin of those with deficiency had been identified. These methods, outlined in the paper, included SEM and histological analysis of tooth dentin in adult and juvenile skeletal remains in individuals who had recovered from a period of deficiency. Archaeological samples were from St. Matthew and St. Marie, Quebec, Canada (1771-1860), and St. Jacques, France (1225-1798). The objective was to determine if interglobular dentin (IGD) could be observed in individuals with skeletal evidence of vitamin D deficiency. A differential diagnosis was conducted and revealed that the only conditions that cause mineralisation defects in dentin are those that disrupt vitamin D, calcium, and phosphorous pathways, with nutritional rickets being the most common cause. By correlating the age at which a deficiency occurred, it was possible to determine multiple episodes of deficiency that would likely have been missed from macroscopic examination, even in cases where clear skeletal changes had been present.

I performed all SEM and histological analysis and B. Kahlon provided assistance with the production of thin-sections of the tooth specimens. I developed the scoring system used to score the severity of IGD observed in dentin and devised a way to approximate the age at which a detected deficiency occurred utilizing tooth development standards by Moorrees et al. (1963). Collections of teeth from skeletons were provided by I. Ribot, B. Bertrand, and M. Brickley. Teeth from three known healthy modern individuals (HIREB ethics approval 14-670-T) were also collected to act as control teeth. I wrote the first draft of the paper and all co-authors reviewed subsequent drafts to provide feedback.

Chapter 3: Paper 2: The rachitic tooth: The use of radiographs as a screening technique.

Authors: Lori D'Ortenzio, Isabelle Ribot, Bonnie Kahlon, Benoit Bertrand, Emmy Bocaege, Emeline Raguin, Annabelle Schattmann, and Megan Brickley.

Chapter 3 presents a method to determine possible cases of vitamin D deficiency in archaeological individuals by observing the quantifiable morphology of their pulp chambers through radiological analysis. The goal was to create a method to screen individuals for further histological examination of interglobular dentin (IGD), to avoid the necessity of thin-sectioning every tooth sample, and to provide basic information where destructive work was not possible. To quantify pulp horn and pulp chamber morphology, radiographs from both living and archaeological teeth were taken and measurements of pulp horns and pulp chambers were conducted. I used molars from 29 modern individuals, four with known medical and dental records to establish that shape changes in pulp chambers could be observed radiologically and/or histologically in those with past

deficiency (HIREB ethics approval 2246). Three archaeological groups had molar teeth that were radiographed and selected teeth were further evaluated histologically for the presence of IGD. One group had clear skeletal evidence of rickets from St. Matthew, Quebec, and St. Jacques, France (n=5); a second group from Bastion des Ursulines, Quebec (n=6), had slight skeletal indicators; and a third group from St. Antoine and Pointe-aux-Trembles (n=10) lacked both skeletal and radiological evidence of deficiency. The use of permanent molars made it possible to investigate a wide range of ages (~1.4-12.5 years) that covered critical periods of development and the radiographs provided a non-destructive aid in the diagnosis of individuals with vitamin D deficiency.

I performed the evaluation and measurements on all radiograph images of pulp chamber shapes from the three groups, representing 5 five sites from Quebec and France. I also conducted the radiograph imaging on teeth from individuals from St. Matthew and Bastion des Ursulines, Quebec, with the help of M. Brickley and A. Schattmann. B. Bertrand conducted the radiograph imaging on the St. Jacques, France, individuals and E. Jennings radiographed individuals from St. Antoine and Pointe-aux-Trembles, Quebec. I conducted and assessed all histological analyses and B. Kahlon made the thin-sections. I created the diagram for Figure 2 and M. Brickley produced Figure 1 in the manuscript and Figure 1 in the Supplementary Data B. I wrote the first draft of the paper and did the subsequent revisions informed by feedback from co-authors.

Chapter 4: Paper 3: Age estimation in older adults: Use of pulp/tooth ratios calculated from tooth sections

Authors: Lori D'Ortenzio, Tracy Prowse, Michael Inskip, Bonnie Kahlon, and Megan Brickley.

Chapter 4 presents data from a newly developed method that uses pulp/tooth area ratios from tooth sections to calculate the age of older archaeological individuals. The first goal was to compare age-at-death estimates from dental radiographs with those derived from tooth sections of modern teeth. This tests the hypothesis that histological images of secondary dentin deposition will provide a more accurate means of estimating age-at-death for adults over the age of 50 years. For the second goal, I then applied this method to previously sectioned teeth of individuals from the 19th century St. Thomas' Church Cemetery, Belleville, skeletal sample to determine if this method could be successfully used on archaeological skeletal material.

I completed all laboratory work, which included sectioning the modern teeth. I imaged all of the sectioned teeth and performed all pulp/tooth area ratio calculations. I wrote the first draft of the paper, created the figures, tables, and images, while the co-authors provided editorial support.

Chapter 5: Discussion and Conclusions

This thesis explores the internal structures of teeth in novel ways to address ongoing methodological challenges in discerning vitamin D deficiency and in estimating age-at-death in archaeological individuals. As it is not always possible to diagnose vitamin D deficiency by assessing bowing deformities in long bones, the principal

contribution of this thesis is the use of IGD to detect vitamin D deficiency in the teeth of individuals from past communities. A significant contribution of this thesis is the determination of the approximate severity and age at which an episode of vitamin D deficiency may have occurred. This research also contributes to the study of health and disease in older individuals by developing a technique for age-at-death calculations for older individuals (50+), as there are a whole range of methodological issues that have interfered with the ability to age the older individual. This thesis opens up several potential avenues for future research, such as: 1) focusing on the identification of children with vitamin D deficiency using both histological and radiograph procedures on deciduous teeth, and 2) refining the pulp/tooth area ratio technique for ageing older adults using different tooth types and applying the method to additional communities.

References

Aykroyd, R.G., Lucy, D., Pollard, A.M., Solheim, T. 1997. Regression analysis in adult age estimation. Am J Phys Anthropol, 104, 259-265.

Agarwal, S.C. 2016. Bone morphologies and histories: Life course approaches in bioarchaeology. Am J Phys Anthropol, 159 (S61), S130-S149.

Armelagos, G.J., Goodman, A H., Harper, K.N., Blakey, M.L. 2009. Enamel hypoplasia and early mortality: Bioarcheological support for the Barker hypothesis. Evol Anthropol: Issues, News, and Reviews, 18 (6), 261-271.

Batra, P., Tejani, Z., Mars, M. 2006. X-linked hypophosphatemia: Dental and histologic findings. J Can Dent Assoc,72, 69–72.

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.

Bedford, M.E., Russell, K.F, Lovejoy, C.O., Meindl, R.S., Simpson, S.W., Stuart-Macadam, P.L. 1993. Test of the multifactorial aging method using skeletons with known ages-at-death from the Grant Collection, Am J Phys Anthropol, 91 (3), 287 297.

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.

Bocquet-Appel, J., Masset C. 1996. Paleodemography: Expectancy and false hope. Am J Phys Anthropol, 99 (4), 571–584.

Bodecker, C.F., 1925. A consideration of some of the changes in the teeth from young to old age. Dent Cosmos, 67 (6), 543-549.

Boldsen, J.L, Milner, G.R., Konigsberg, L.W, Wood, J.W. 2002. Transition analysis: A new method for estimating age from skeletons. In: Hoppa R.D., Vaupel J.W., (eds). Paleodemography: age distributions from skeletal samples. Cambridge: Cambridge University Press. Pp. 73-106.

Bonucci, E., Denys-Matrajt, H., Tun-Chot, S., Hioco, D.J. 1969. Bone structure in osteomalacia, with special reference to ultrastructure. J. Bone Joint Surg, 51, 511-528.

Boskey, A.L., Boyan, B.D., Schwartz, Z. 1997. Matrix vesicles promote mineralization in a gelatin gel. Calcif Tissue Int, 60, 309–315.

Brickley, M.B., Mays, S., Ives, R. 2005. Skeletal manifestations of vitamin D deficiency osteomalacia in documented historical collections. Int J Osteoarchaeol, 15 (6), 389-403.

Brickley, M., Mays, S., Ives, R. 2007. An investigation of skeletal indicators of vitamin D deficiency in adults: Effective markers for interpreting past living conditions and pollution levels in 18th and 19th century Birmingham, England. Am J Phys Anthropol, 132 (1), 67-79.

Brickley, M., Ives, R. 2008. The bioarchaeology of metabolic bone disease. San Diego: Academic Press.

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. and Buckberry, J. 2015. Picking up the pieces: Utilizing the diagnostic potential of poorly preserved remains. Int J Paleopathol, 8, 51-54.

Brickley, M.B., Moffat, T., Watamaniuk, L. 2014. Biocultural perspectives of vitamin D deficiency in the past. J Anthropol Arch, 36, 48-59.

Brooks, S.T., Suchey, J.M. 1990. Skeletal age determination based on the os pubis: A comparison of the Acsádi-Nemeskéri and Suchey-Brooks methods. J Hum Evol, 5, 227-238.

Chiego, Jr. D.J. 2014. Essentials of oral histology and embryology: A clinical approach. 4th edition. Saint-Louis Missouri: Elsevier Mosby.

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.

Cox, M. 2000. Ageing adults from the skeleton. In: Cox, M., Mays, S. (Eds.), Human osteology archaeology and forensic science. Greenwich Medical Media: London. Pp. 61–81.

Elder, G.H., Kirkpatrick Johnson, M., Crosnoe, R. 2003. In: Mortimer, J.T., Shanahan, M.J., (eds.). The emergence and development of life course theory. Handbook of the life course. Minneapolis, U.S. Springer. Pp. 3–19.

Falys, C.G. and Lewis, M.E., 2011. Proposing a way forward: a review of standardisation in the use of age categories and ageing techniques in osteological analysis (2004–2009). Int. J. Osteoarchaeol, 21 (6), 704-716.

Foster, B.L., Nociti, F.H., Somerman, M.J. 2014. The rachitic tooth. Endocr Rev, 35 (1), 1-34.

Fuller, B.T., Richards, M.P., Mays, S.A. 2003. Stable carbon and nitrogen isotope variations in tooth dentine serial sections from Wharram Percy. J Archaeol Sci, 30 (12), 1673-1684.

Gluckman, P., and Hanson, M. 2006. The developmental origins of health and disease: An overview. In: Gluckman, P., and Hanson, M., (Eds), Developmental origins of health and disease. Cambridge: Cambridge University Press. Pp.1-5.

Heilmann, A., Tsakos, G., Watt, R.G., 2015. Oral health over the life course. In: A life course perspective on health trajectories and transitions. Springer International Publishing. Pp. 39-59.

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

Hillson, S. 2002. Dental anthropology. Cambridge: Cambridge University Press.

Holick, M.F. 2004. Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular disease. Am J Clin Nutr, 80 (6, suppl), 1678S-1688S.

Holick, M.F. 2006. Resurrection of vitamin D deficiency and rickets. J Clin Invest, 116 (8), 2062-2072.

Holick, M.F. 2007. Vitamin D deficiency. N Engl J Med, 357 (3), 266-281.

Holick, M.F. 2008. Vitamin 1 D: A D-lightful health perspective. Nutrition Rev, 66, S182-S194.

Hoppa, R. 2000. Population variation in osteological aging criteria: An example from the pubic symphysis. Am J Phys Anthropol, 111, 185–191.

Hoppa, R. 2002. Paleodemography: looking back and thinking ahead. In: Hoppa R., Vaupel. J.W., (Eds), Paleodemography: Age distributions from skeletal samples. Cambridge, Cambridge University Press. Pp.9-28.

Huang, B., Sun, Y., Maciejewska, I., Qin, D., Peng, T., McIntyre, B. 2008. Distribution of SIBLING proteins in the organic and inorganic phases of rat dentin and bone. Eur J Oral Sci, 116:104–112.

Isokawa, S., Kosakai, T., Kajiyama, S. 1963. Interglobular dentin in the deciduous tooth. J Dent Res, 42, (3), 831-834.

Janssen, H.C., Samson, M.M. and Verhaar, H.J. 2002. Vitamin D deficiency, muscle function, and falls in elderly people. Am J Clin Nut, 75 (4), 611-615.

Jones, G., Strugnell, S.A. and DeLuca, H.F. 1998. Current understanding of the molecular actions of vitamin D. Physiol Rev, 78 (4), 1193-1231.

Kulie, T., Groff, A., Redmer, J., Hounshell, J., Schrager, S. 2009. Vitamin D: An evidence-based review. J Am Board Family Med, 22 (6), 698-706.

Kemkes-Grottenthaler, A. 2002. Aging through the ages: Historical perspectives on age indicator methods. In: Hoppa R., Vaupel. J.W., (Eds), Paleodemography: Age distributions from skeletal samples. Cambridge: Cambridge University Press. Pp.48-72.

Litman, N.N., Ulstrom, R.A. and Westin, W.W. 1957. Vitamin D resistant rickets. California Med, 86 (4), 248-253.

Lukacs, J.R. 1989. Dental paleopathology: methods for reconstructing dietary patterns. In: Lukacs, J.R., (Ed), Reconstruction of life from the skeleton. Allen R Liss Inc: New York. Pp. 261.

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.

Mayo Clinic. 2017. Rickets, symptoms and causes. Electronic document. https://www.mayoclinic.org/diseases-conditions/rickets/symptoms-causes/syc-20351943.

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.

Merritt, C. 2013. Testing the accuracy of adult skeletal age estimation methods: Original methods versus revised and newer methods. Explorat Anthropol, 12 (1), 102-119.

Milner, G.R. and Boldsen, J.L. 2012. Transition analysis: A validation study with known-age modern American skeletons. Am J Phys Anthropol, 148 (1), 98-110.

Mitchell, P.J., Cooper, C., Dawson-Hughes, B., Gordon, C.M. and Rizzoli, R., 2015. Life-course approach to nutrition. Osteoporosis Int, 26 (12), 2723-2742.

Molnar, S. 1971. Human tooth wear, tooth function and cultural variability. Am J Phys Anthropol, 34 (2), 175-189.

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.

Ortner, D.J, Mays, S. 1998. Dry-bone manifestations of rickets in infancy and early childhood. Int J Osteoarchaeol, 8 (1), 45-55.

Paine, R.R., Boldsen, J.L. 2002. Linking age-at-death distributions and ancient population dynamics: A case study. In: Hoppa R.D., Vaupel, J.W., (Eds.), Paleodemography: age distributions from skeletal samples. Cambridge: Cambridge University Press. Pp 169-180.

Pettifor, J.M. 2004. Nutritional rickets: Deficiency of vitamin D, calcium, or both?. Am J Clin Nutr, 80 (6), 1725S-1729S.

Prowse, T.L. 2011. Diet and dental health through the life course in Roman Italy. In: Agarwal S.C., Glencross, B.A., (Eds.), Social bioarchaeology. West Sussex, U.K: Wiley-Blackwell Publishing Ltd. Pp. 410-437.

Reid, D.J., Dean, M.C. 2006. Variation in modern human enamel formation times. J Hum Evol, 50 (3), 329–346.

Rogers, T. 1991. Sex determination and age estimation: Skeletal evidence from Saint-Thomas' Cemetery Belleville, Ontario. Master's Thesis. Department of Anthropology, McMaster University.

Rohland, N. and Hofreiter, M. 2007. Ancient DNA extraction from bones and teeth. Nature Protocols, 2 (7), 1756-1764.

Saunders, S., Fitzgerald, C., Rogers, T., Dudar, C., McKillop, H. 1992. A test of several methods of skeletal age estimation using a documented archaeological sample. Can Soc Forensic Sci J, 25, 97‑111.

Scheuer, L. and Black, S., 2004. The Juvenile Skeleton. Oxford, U.K. Academic Press.

Schroeder, H.E., 1991 Oral structure biology: embryology, structure, and function of normal hard and soft tissues of the oral cavity and temporomandibular Joints. G. Germany: Thieme Verlag.

Scott, G.R., Turner, C.G. 1988. Dental anthropology. Ann Rev Anthropol, 17 (1), 99-126.

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, 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.

Smith, P., Gomorri, J.M., Spitz, S., Becker, J. 1997. Model for the examination of evolutionary trends in tooth development. Am J Phys Anthropol, 102 (2), 283-294.

Sofaer, J., 2011. Towards a social bioarchaeology of age. In: Agarwal S.C., Glencross, B.A., (Eds.), Social bioarchaeology. West Sussex, U.K: Wiley-Blackwell Publishing Ltd. Pp. 283-311.

Solheim, T. 1992. Amount of secondary dentin as an indicator of age. Europ J Oral Sci, 100 (4), 193-199.

Todd, T.W. 1920. Age changes in the pubic bone. I. The male white pubis. Am J Phys Anthropol, 3 (3), 285-334.

Uauy, R., Kain, J., Corvalan, C. 2011. How can the Developmental origins of health and disease (DOHaD) hypothesis contribute to improving health in developing countries?. Am J Clin Nutr, 94 (6 Suppl), 1759S-1764S.

Veselka, B., Hoogland, M.L., Waters-Rist, A.L. 2015. Rural rickets: Vitamin D deficiency in a post-medieval farming community from the Netherlands. Int J Osteoarchaeol, 25 (5), 665-675.

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.

Zaichick, V., Ovchjarenko, N. and Zaichick, S. 1999. In vivo energy dispersive X-ray fluorescence for measuring the content of essential and toxic trace elements in teeth. App Rad Isotopes, 50 (2), 283-293.

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.

CHAPTER 2: THE RACHITIC TOOTH: A HISTOLOGICAL EXAMINATION

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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.

Keywords: vitamin D deficiency, interglobular dentin, nutritional rickets

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 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 (Belelander 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, 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 Yangisawa

(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; Vital et al., 2012; Souza et al., 2013). Seow et al. (1989) conducted histological examinations on patients' 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 et al., 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.

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	Sãn Paulo pediatric hospital for critically ill children: 0.00050% of hospital admissions	Santana e Meneses et al. (2009)
Autosomal dominant hypophosphatemic rickets X-linked hypophosphatemic rickets	0.00057% in Southern Denmark	Francis et al. (2016) 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 medical records.

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 a "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; Caron, 2013; Ribot et al., in press). 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 analysed 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.

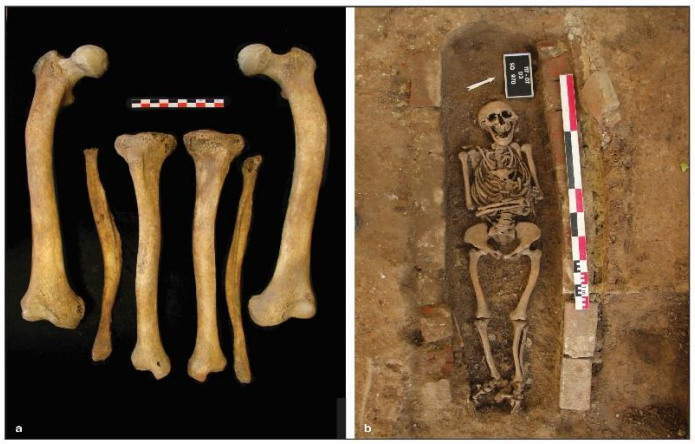
Table 2: Description of individuals.

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

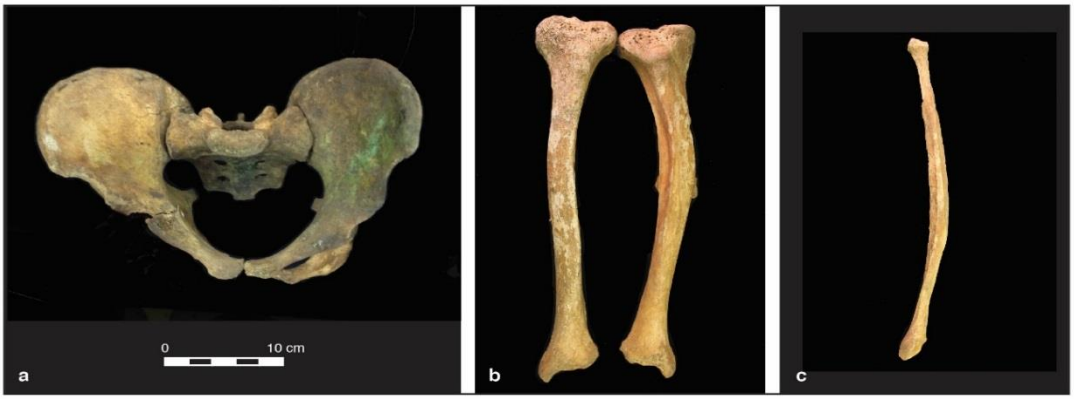
SEM=scanning electron microscope.

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 (Figs. 1a-b). The combination of morphological features in the bones helped identify evidence of past vitamin D deficiency. For example, skeleton 15A-S36 had bowing of the tibias and fibulas and angulation of the sacrum was greater than expected (Figs. 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 (Figs. 3a-b). Growth plates appeared normal and evaluation of radiographs determined that 2E4 was a healed case of deficiency.



Figs. 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.



Figs. 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.

Figs. 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.

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 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.'s (2007) procedures, archeological 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 3mm 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 micron diamond polish, followed by 1 micron diamond polish on a microcloth pad. The polished samples were ultrasonicated for 15 minutes in distilled water. For SEM analysis, the 3mm 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 15kV, 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 a Olympus BX51 digital microscope, (100x magnification).

2.3 Scoring system used to grade the severity of interglobular dentin

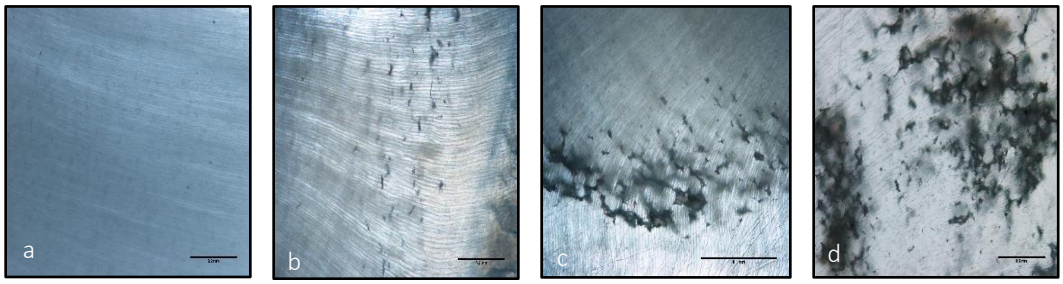
Scoring of interglobular dentin was performed in order to develop a link between interglobular dentin severity and the severity of deficiency experienced by the individual. Being the first to record the severity of interglobular dentin in animals and in British

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 4). 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 0.1mm 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.

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



Figs. 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%.

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 5). 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 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).

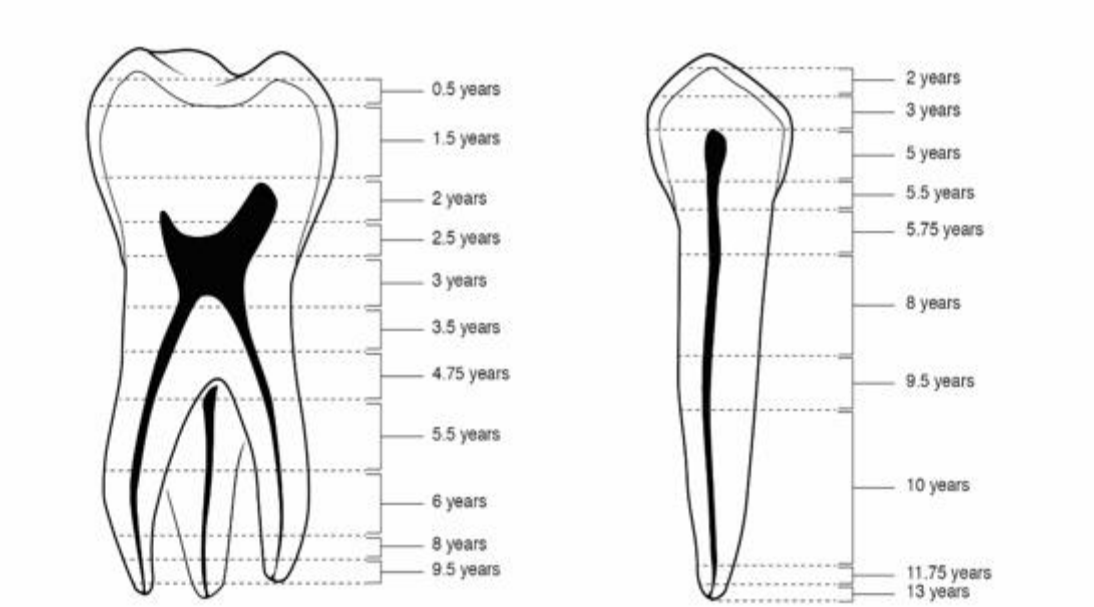


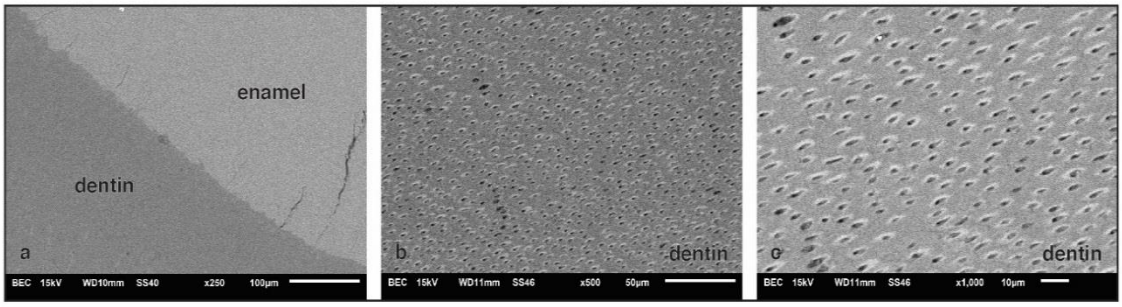
Fig. 5. Diagram of a first molar and a canine showing the approximate ages of mineralisation of dentin (Moorrees et al., 1963). Note: Degree of mineralisation in developing teeth can be affected by sex and the differing dental maturity of maxillary and mandibular dentition.

3. Results

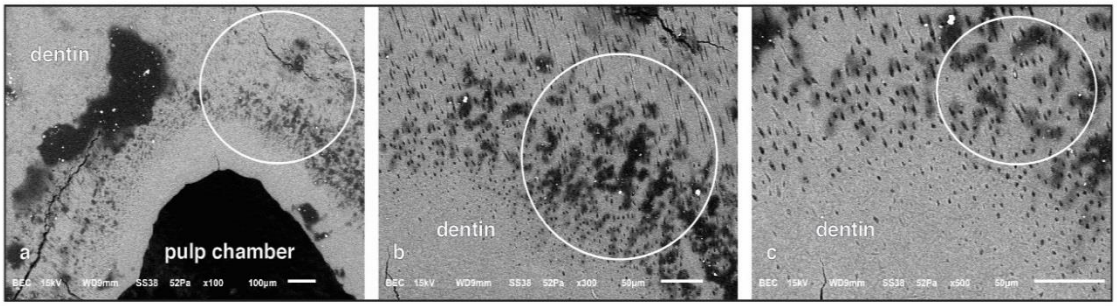
3.1 SEM results

SEM analysis of four individuals revealed that the controls (TT1 and TT3) exhibited normal dentin formation (Figs. 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 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 Figs. 7a-c and Figs.

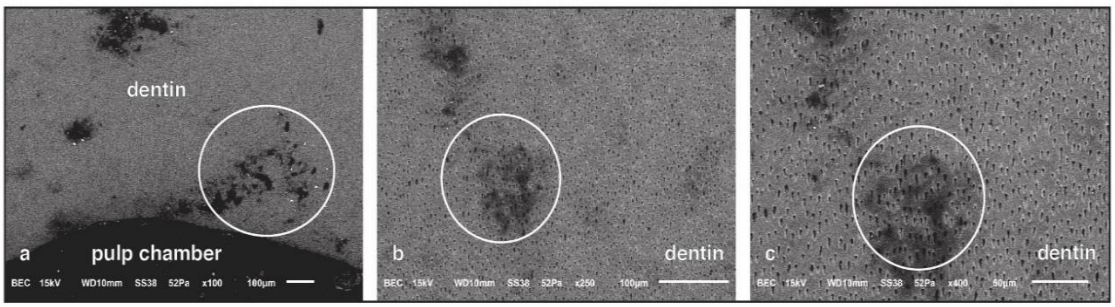
8a-c, there were differences in the degree of dentin fusion, characterized by a large number of non-merged calcospherites separated by irregular zones of non-mineralised interglobular dentin.



Figs. 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).



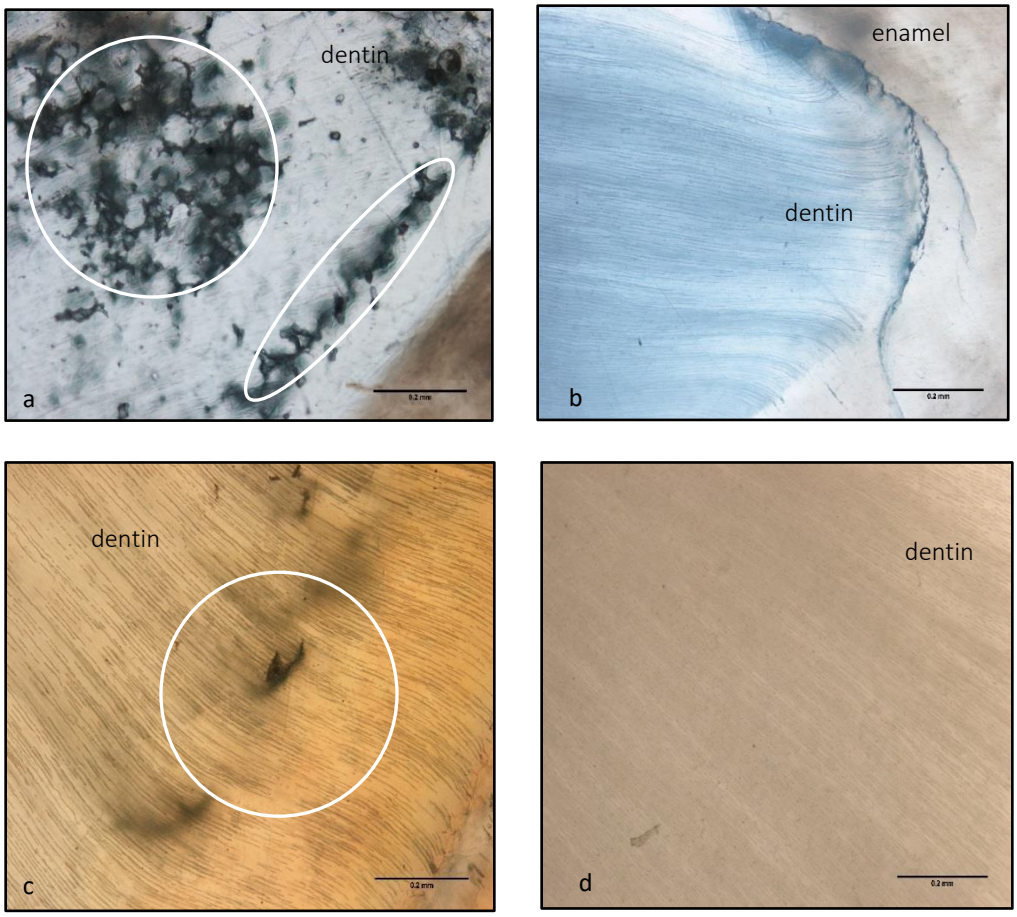
Figs. 7a) SEM image of interglobular dentin (Grade 3 severity) observed in 15A-S36; adult individual with skeletal evidence of past rickets, 100x magnification; **b)** 300x magnification; **c)** 500x magnification. Note: Uneven dentin with interglobular spaces representing absence of mineralisation (white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



Figs. 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).

3.2. Histological Results

Disturbances in dentin mineralisation were absent in the three controls (e.g., Figs. 9b, d), but present in the six individuals with previous episodes of rickets in which histological assessment was undertaken using thin sections (e.g., Figs. 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 (4/5) 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 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.



Figs. 9a) 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.

3.3 Grades of severity of interglobular dentin

Table 5 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).

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	*1 episode at 5.5 years
	RM^2	Grade 2	*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	**1 episode at 6 years
	RM_2	Grade 2	**6.5-11 years
SJ 562 (St. Jacques individual with past rickets)	RC^1	Grade 3 RC_1	Grade 3 ***1 episode at 4-6 years
	LM_2	Grade 2	***6.5 years

Identifier	Tooth Type	Interglobular Dentin Score	Approximate Age of Vitamin D Deficiency Episodes (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

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

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

Table 5 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 7-10 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). 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 5-6 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).

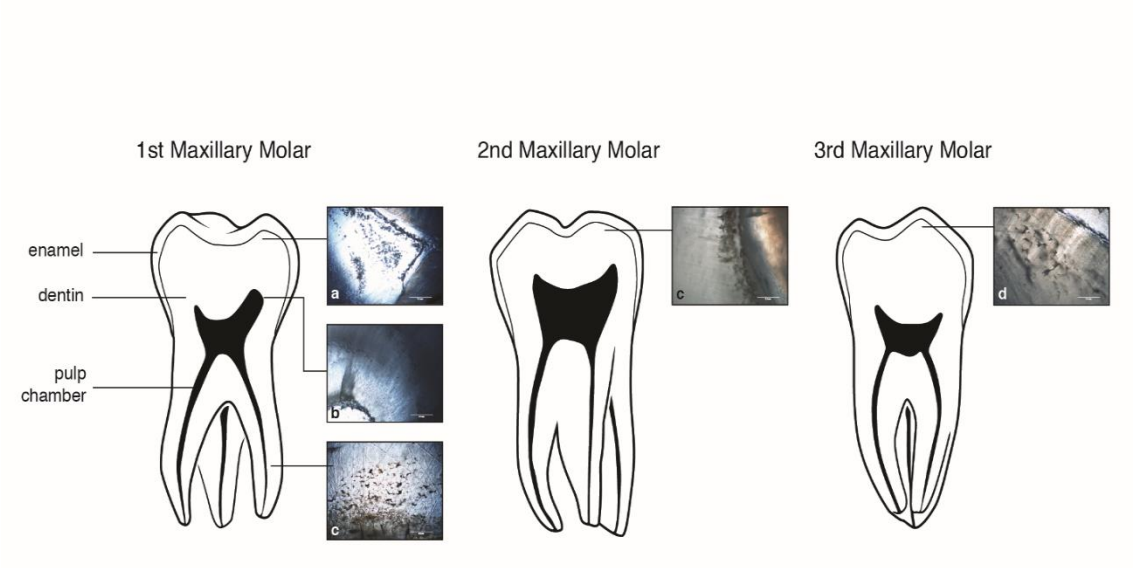


Fig. 10. The four episodes of vitamin D deficiency that occurred during the development of the first – 3rd molars for archaeological individual 15A-S36 are illustrated; **a)** Episode 1 (age 1.5 years); **b)** Episode 2 (age 2 years) **c)** Episode 3 (age 5.5-6 years); **d)** Episode 4 (age 12.5 years),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

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, secondary 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 (Figs. 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 (Figs. 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 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 and SJ 384 (Table 5). 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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References

Amaral THAD, Guerra CDS, Bombonato-Prado KF, Garcia de Paula e Silva FW, De Queiroz A M. 2008. Tooth pigmentation caused by bilirubin: a case report and histological evaluation. Special Care in Dentistry 28(6):254-257.

Armelagos GJ, Goodman AH, Harper KN, Blakey ML. 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 JK. 2002. Development of teeth and supporting structures. In: Oral Development and Histology (3rd edition). New York: Thieme Medical Publishers.

Barker DJP. 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 SS, Brock-Jacobsen B, Gram J, Brixen K, Jensen TK. 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.

Berman M, Edwards LF, Kitchin PC. 1939. Effect of artificially induced hyperpyrexia on tooth structure of the rabbit. P Soc Exp Biol Med 41:113-115.

Bevelander G, Bernstein JG. 1940. The effect of artificially induced fever on the structure of the developing teeth of the rat. J Dent Res 19(2):155-163.

Bevelander G, Nakahara H. 1966. The formation and mineralisation of dentin. The Anat Rec 1560(3):303-323.

Brickley MB, Ives R. 2008. The Bioarchaeology of metabolic bone disease. San Diego: Academic Press.

Brickley MB, Mays S, Ives R. 2010. Evaluation and interpretation of residual rickets deformities in adults. Int J Osteoarchaeol 20(1): 54-66.

Brickley MB, Moffat T, Watamaniuk L. 2014. Biocultural perspectives of vitamin D deficiency in the past. J Anthropol Archaeol 36:48–59.

Boyce BF, Path MRC, Prime SS, Halls D, Johnston E, Critchlow H, MacDonald DG, Path MRC, Junor BJR. 1986. Does osteomalacia contribute to development of oral complications of oxalosis? Oral Surg 61(3):272-277.

Brothwell D. 1981. Digging up bones. Ithaca: Cornell University Press.

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 JJ, 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):324–31.

Chen L, Liu H, Sun W, Bai X, Karaplis AC, Goltzman D, Miao D. 2011. Fibroblast growth factor 23 overexpression impacts negatively on dentin mineralisation and dentinogenesis in mice. Clin Exp Pharmacol P (38):395-402.

Chiego Jr. DJ. 2014. Essentials of oral histology and embryology: a clinical approach. 4rth edition. St. Louis Missouri: Elsevier Mosby.

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 GL, 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 MC, Scandrett AE. 1995. Rates of dentine mineralization in permanent human teeth. Int J Osteoarchaeol 5(4):349-358.

De Coster PJ. 2012. Dentin disorders: anomalies of dentin formation and structure. Endod Topics 21:41-61.

Direction de l'Archéologie de la Communauté d'Agglomération du Douaisis. 2006. Vie et mort à la collégiale Saint-Amé: 950-1797. Douai: Communauté d'Agglomération du Douaisis.

Eerkens JW, Berget AG, Bartelink EJ. 2011. Estimating weaning and early childhood diet from serial micro-samples of dentin collagen. J Archaeol Sci 38(11);3101-3111.

Ferguson HW, Hartles RL. 1964. The effect of vitamin D on the dentine of the incisor teeth and on the alveolar bone of young rats maintained on diets deficient in calcium or phosphorus. Arch Oral Biol (9):447-460.

Formicola V. 1995. X-linked hypophosphatemic rickets: a probable upper Paleolithic case. Am J Phys Anthropol 98:403-409.

Foster BL, Nociti FH, Somerman MJ. 2014. The rachitic tooth. Endocr Rev 35(1):1-34.

Francis F, Strom TM, Hennig S, BoddrichA, Lorenz B, Brandau O, Mohnike KL, Michele Cagnoli M, Steffens C, Klages S, Borzym K, Pohl T, Oudet C, Econs MJ, Rowe PSN, 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 MB. 1976. Anterior femoral curvature: its probable basis and utility as a criterion of racial assessment. Am J Phys Anthropol 45(3):601-604.

Gomes MF, Albernaz Camargo AM, Alves Sampaio T, Aparecida M, Graziozi OC, Costa Armond M. 2002. Oral manifestations of Albright hereditary osteodystrophy: a case report. Rev Hosp Clin Fac Med S. Paulo 57(4):161-166.

Harrison M, Odell EW, Path MRC Path, Sheehy EC. 1999. Dental findings in Lowe syndrome. Pediatr Dent 21(7):425-428.

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.

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

Hillson S. 2002. Dental anthropology. UK: Cambridge University Press.

Hochberg Z. 2003. Vitamin D and rickets. Endocr Dev. Basel Karger. Vol 6. pp 1–13.

Holick MF. 2007. Vitamin D deficiency. N Engl J Med 357(3):266-281.

Holick MF, Chen TC. 2008. Vitamin D deficiency: a worldwide problem with health consequences. Am J Clin Nutr 87(suppl):1080S-1086S.

Howe PR, Wesson LG, Boyle PE, Wolbach SB. 1940. Low calcium rickets in the guinea pig. Exp Biol Med 45(1):298-301.

Irving JT. 1940. The influence of diets low in magnesium upon the histological appearance of the incisor tooth of the rat. J Physiol 99:8-17.

Isokawa S, Kosakai T, Kajiyama S. 1963. Interglobular dentin in the deciduous tooth. J Dent Res 42(3):831-834.

Kuijpers MH, Van De Kooij AJ, Slootweg PJ. 1996. The rat incisor in toxicologic pathology. Lab Anim Path 24(3):346-360.

Larocque R. 1999. Deaths at an early age in the city of Quebec, 17th -19th centuries. Can Bull Med Hist 16(2):341-361.

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, Prie´ D, Rothenbuhler A, Wicart P, Harvengt P. 2014. Therapeutic management of hypophosphamic rickets from infancy to adulthood. Endocr Connect 3:R13-R30.

Liu H, Guo J, Wang L, Chen N, Karaplis A, Goltzman D, Miao D. 2009. Distinctive anabolic roles of 1,25-dihydroxyvitamin D3 and parathyroid hormone in teeth and mandible versus long bones. J Endocrinol 203:203-213.

Malloy PJ, 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 SC. 1975. Mineral metabolism and microstructural defects in primate teeth. Am J Phys Anthropol 43(1): 3-17.

Moorrees CFA, Fanning EA, Hunt Jr. EE. 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, Quebec City, Canada (1771-1860). Master's thesis, University of Montreal.

Noppen L. 1987. Un quartier en sursis? Cap-aux-Diamants 3(1):5-7.

Ortner DJ, Mays S. 1998. Dry-bone manifestations of rickets in infancy and early childhood. Int J Osteoarchaeol 8(1):45-55.

Pal BR, Shaw NJ. 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. Journal of IMAB - Annual Proceeding (Scientific Papers) 17(2):140-142. DOI: 10.5272/jimab.2011172.140.

Paterson CR, Ayoub D. 2014. Congenital rickets due to vitamin D deficiency in the mothers. Clin Nutr 34:793-798.

Pearce SH, Cheetham TD. 2010. Diagnosis and management of vitamin D deficiency. BMJ 340:142-147.

Pereira CM, Roberto de Andrade C, Agustín Vargas P, Della Coletta R, Paes de Almeida O, Ajudarte Lopes. 2004. Dental alterations associated with X-Linked hypophosphatemic rickets. J Endodont 30(4):241-245.

Perron JS. 2006. Les marqueurs osseux d'activité physique: une étude des restes humains du cimetière St-Matthew à Québec (XVIIIème et XIXème siècles). Master's thesis, Laval University, Québec City.

Pettifor JM. 2003. Nutritional rickets. In: Glorieux FH, Pettifor JM, Jüppner H, (eds.) Pediatric Bone. Biology and Diseases. San Diego: Academic Press. pp. 541-565.

Pitt MJ. 1995. Rickets and osteomalacia. In: Diagnosis of bone and joint disorder. Resnick D, (ed), 3rd edition. Philadelphia: Saunders. pp. 1885-1922.

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 Miner 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: Montréal.

Robinson PD, Högler W, Craig ME, Verge Cf, Walker JL, Piper AC, Woodhead HJ, Cowell CT, Ambler GR. 2006. The re-emerging burden of rickets: a decade of experience from Sydney. Arch Dis Child 91:564–568.

Santana e Meneses JF, Pons Leite H, de Carvalho WB, Lopes Jr, E. 2009. Hypophosphatemia in critically ill children: prevalence and associated risk factors. Pediatr Crit Care Med 10(2)234.238.

Saunders SR, Chan AHW, Kahlon B, Kluge HF. 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. Oxford, U.K: Elsevier Academic Press.

Seeto E, Seow KW. 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 KW, Brown JP, Tudehope DA, 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 KW, Latham SC. 1986. The spectrum of dental manifestations in vitamin D-resistant rickets: implications for management. Pediatr Dent 8(3):245-250.

Seow KW, Humphrys C, Tudehope DI. 1987. Increased prevalence of developmental dental defects in low birth-weight, prematurely born children: a controlled study. Pediatr Dent 9(3):221-225.

Seow KW, 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.

Seow KW. 1997. Effects of preterm birth on oral growth and development. Aust Dent J 42(2):85-91.

Shellis RP. 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 MA, Soares Junior LA, Santos MA, Vaisbick MH. 2010. Dental abnormalities and oral health in patients with hypophosphatemic rickets. Clinics (Sao Paulo) 65(10):1023-1026.

Souza AP, Kobauashi TY, Lourenco Neto N, Silva SMB, Machado MAA., Oliveira TM. 2013. Dental manifestations of patient with vitamin D-resistant rickets. J Appl Oral Sci 21(6):601-606.

Thacher TD, Fischer PR, Strand MA, Pettifor JM. 2006. Nutritional rickets around the world: causes and future directions. Ann Trop Paediatr 26:1-16.

Tumen EC, 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 DH. 1989. The estimation of age-at-death from immature human bone. In: Age markers in the human skeleton. Işcan MY, ed. Springfield, IL: Charles C. Thomas.

Veselka B, Hoogland MLP, Waters-Rist AL. 2013. Rural rickets: vitamin D deficiency in a post-Medieval farming community from the Netherlands. Int J Osteoarchaeol, online, DOI:10.1002/oa.2329.

Vital SO, Gaucher C, Bardet C, Rowe PS, George A, Linglart A, Chaussain C. 2012. Tooth dentin defects reflect genetic disorders affecting bone mineralisation. Bone 50(4):989-997.

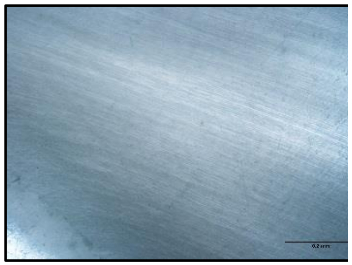
Wolfe JJ. 1935. Teeth in fetal rickets. Am J Dis Child 49(4):905-911.

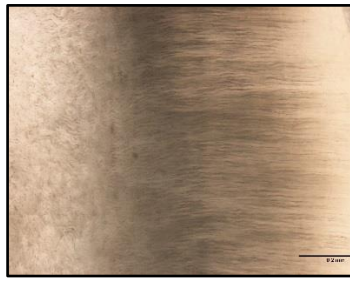
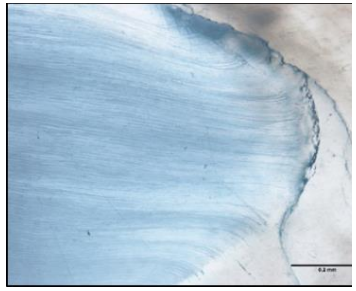
Yoshiki S, Yanagisawa T. 1974. The role of vitamin D in the mineralisation of dentin in rats made rachitic by a diet low in calcium and deficient in vitamin D. Calcif Tissue Res 15(1):295-302.

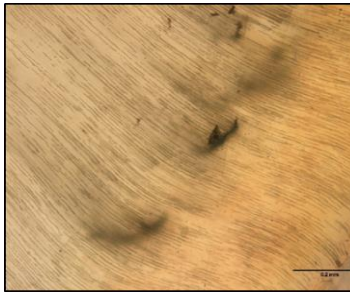
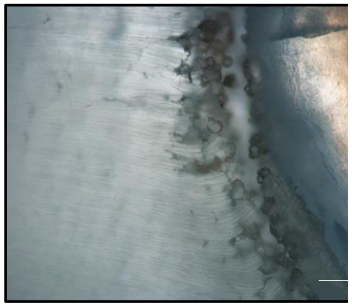
Zhang X, Rahemtulla F, Zhang P, Beck P, Thomas HF. 2009. Different enamel and dentin mineralisation observed in VDR deficient mouse model. Arch Oral Biol 54(4):299-305.

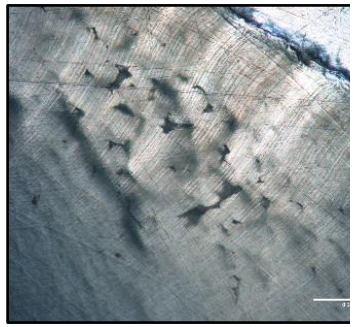
Supplementary Data

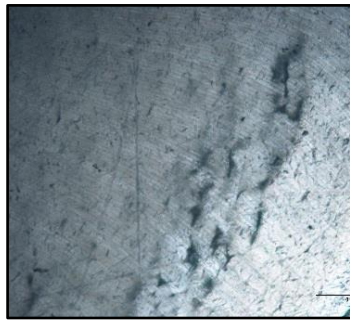
Table 1: Summary of histological results, interglobular score, and age of vitamin D deficiency.

Identifier	Tooth Type	Individual's Description	Interglobular dentin: Unfused calcospherites (Present/Absent)	Histological Image	Interglobular Dentin (IGD) Score	Approximate Age Ranges of Vitamin D Deficiency
M59	RM^1	Modern healthy female juvenile (age 14).	Normal: unfused calcospherites absent.		Grade 0	No deficiency
TT3	RM_2	Modern healthy female juvenile (age 10).	Normal: unfused calcospherites absent.		Grade 0	No deficiency

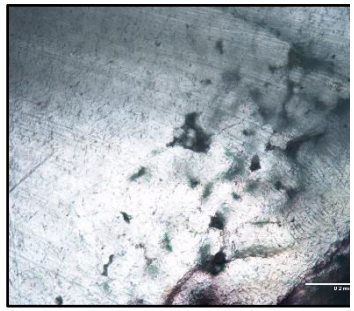
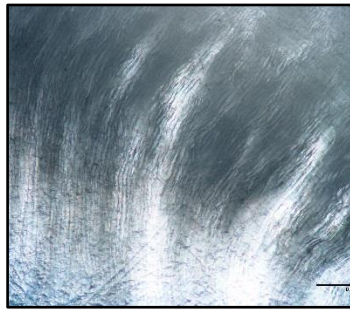
Identifier	Tooth Type	Individual's Description	Interglobular dentin: Unfused calcospherites (Present/Absent)	Histological Image	Interglobular Dentin (IGD) Score	Approximate Age Ranges of Vitamin D Deficiency
TT3	RM_2 Roots		Roots are normal: unfused calcospherites absent. Location is in the apical third of the lingual and buccal roots adjacent to the cementum.		Grade 0	No deficiency
TT1	RM_3	Modern healthy adult (age 19).	Normal: unfused calcospherites absent.		Grade 0	No deficiency

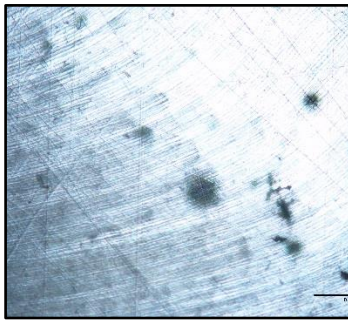
Identifier	Tooth Type	Individual's Description	Interglobular dentin: Unfused calcospherites (Present/Absent)	Histological Image	Interglobular Dentin (IGD) Score	Approximate Age Ranges of Vitamin D Deficiency
2E4	RM[1]	St. Marie (Quebec) archaeological juvenile with past rickets (age~3).	Unfused calcospherites present under crown (root not fully developed).		Grade 2	2 years
15A-S36	RM[2]	St. Matthew (Quebec) archaeological male adult with rickets (age 23).	Pronounced unfused calcospherites under crown enamel.		Grade 2	5-6 years

Identifier	Tooth Type	Individual's Description	Interglobular dentin: Unfused calcospherites (Present/Absent)	Histological Image	Interglobular Dentin (IGD) Score	Approximate Age Ranges of Vitamin D Deficiency
15A-S36	LM^3	St. Matthew (Quebec) archaeological male adult with rickets (age 23).	Very wide pulp chamber. Very pronounced unfused calcospherites under both dentin horns.		Grade 3	A fourth episode at ~12.5 years
SJ 384	RM_1	St. Jacques (France) archaeological female adult with rickets (age 40+).	Very pronounced unfused calcospherites under crown enamel.		Grade 3	1.5 years

Identifier	Tooth Type	Individual's Description	Interglobular dentin: Unfused calcospherites (Present/Absent)	Histological Image	Interglobular Dentin (IGD) Score	Approximate Age Ranges of Vitamin D Deficiency
SJ 384	RM_1 Roots		Less pronounced calcospherites on both buccal and lingual roots located in the apical third of the roots (middle to lower third of the roots).		Grade 2	A second episode occurring in the root at 6 years. Overlaps with RM_2 formation (below).
SJ 562	RC^1	St. Jacques (France) archaeological female adult with rickets (age 40-58).	Very pronounced unfused calcospherites under pulp horns and running right to DEJ.		Grade 3	4-6 years

Identifier	Tooth Type	Individual's Description	Interglobular dentin: Unfused calcospherites (Present/Absent)	Histological Image	Interglobular Dentin (IGD) Score	Approximate Age Ranges of Vitamin D Deficiency
SJ 892	LM_3	St. Jacques (France) archaeological female adult with rickets (age 40-58).	A few tiny unfused calcospherites, not clear or large enough to be diagnostic.		Grade 0	No deficiency
SJ 892	RC_1	St. Jacques (France) archaeological female adult with rickets (age 40-58).	Very pronounced unfused calcospherites under pulp horns.		Grade 3	4 years

Identifier	Tooth Type	Individual's Description	Interglobular dentin: Unfused calcospherites (Present/Absent)	Histological Image	Interglobular Dentin (IGD) Score	Approximate Age Ranges of Vitamin D Deficiency
SJ 970	LM[1]	St. Jacques (France) archaeological female adult with rickets (age 45-56)	Very pronounced unfused calcospherites, particularly in the dentin horn.		Grade 3	1.5 years
SJ 970	LM[1] Roots		Roots are normal: unfused calcospherites absent. Location is in the apical third of the lingual and buccal roots adjacent to the cementum.		Grade 0	No deficiency

Identifier	Tooth Type	Individual's Description	Interglobular dentin: Unfused calcospherites (Present/Absent)	Histological Image	Interglobular Dentin (IGD) Score	Approximate Age Ranges of Vitamin D Deficiency
SJ 970	RM^3	St. Jacques (France) archaeological female adult with rickets (age 45-56).	Less pronounced calcospherites but still present, spherical anomalies, appears to have areas where dentin matrix is sparse.		Grade 1	12.5 years

Sources: Moorrees et al. (1963) for age ranges; Mellanby (1934:47) and Seow et al. (1989:204-205) for IGD scores.

Table 2: Summary of conditions not associated with interglobular dentin.

Condition	Features	Source
Fever	Rat incisors show a linear band of irregular dentin tubules coinciding with dilation of blood vessels in pulp chamber.	Berman et al. (1939); Bevelander and Bernstein (1940)
Vitamin A deficiency (hypovitaminosis A)	Excessive osteodentin deposition (bonelike inclusions in dentin) or insufficient dentin deposition coinciding with hypoplastic enamel or absence of enamel formation. Rat incisors show increased thickness of enamel and reduced thickness of cementum.	Avery (2002:142-150); Kuijpers et al. (1996:350)
Vitamin C deficiency	Causes bone, dentin, and cementum deposition to cease; dentin tubules become irregular, reduced, and spindle-like vascular inclusions may develop.	Avery (2002:142-150)
Vitamin E deficiency (hypovitaminosis E)	Degeneration of enamel replaced by fibrous connective tissue.	Kuijpers et al. (1996:350)
Mg deficiency	Rat incisors showed zone of stratified dentin coinciding with enamel atrophy, pulp calcification, and hypoplasia	Kuijpers et al. (1996:350); Irving (1940)
Tetracycline	Enamel stained brown to gray; enamel can be absent; mineralisation defects in enamel only; affected dentin may show faint bands. Rat incisors show enamel defects only	Avery (2002:142-150); Kuijpers et al. (1996:350)

Condition	Features	Source
Fluorosis	Brown staining mottled and pitted enamel coinciding with hypomineralised dentin with increased globular spaces. Rat incisors show disturbed dentin mineralisation, enamel hypoplasia, pulp stone formation.	Avery (2002:142-150); Kuijpers et al. (1996:350)
Dentinogenesis imperfecta 1. Type 1 2. Type II 3. Type III	Soft blue-brown translucent teeth (opalescent teeth); shortened teeth from attrition; erosion of the crown; pulp chambers gradually obliterate over time. Alternating areas of tubular and atubular dentin; areas of normal dentin and areas with scarce or enlarged tubules; inclusion of large blood vessels in dentin mass.	De Coster (2012)
Dentin dysplasia 1. Type I 2. Type II	Normal enamel formation; short, blunt roots; varying degree of pulp chamber obliteration; thistle tube deformity in dentin; pulp stones.	De Coster (2012)
Gastrointestinal malabsorption (e.g., Celiac disease)	Enamel has colour defects, brown or yellow; horizontal grooves, shallow pits to large vertical pits.	Pitt (1995); Rashid et al. (2011)
Liver disease	Increase in dental caries and plaque; green staining (circular stripes) in dentin seen with end-stage liver disease; enamel hypoplasia.	Pitt (1995); Panov and Krasteva (2011); Amaral et al. (2008)
Aluminum Toxicity	Root resorption and oxalate crystals present in pulp cavity. Aluminum is a dark line near cementum; dentin looks normal.	Pitt (1995); Boyce et al. (1986)

Condition	Features	Source
Metaphyseal Chondrodysplasia (Type Schmid)	N/A Genetic cause that affects collagen; symptoms: coxa vara, bowed leg, short stature, short limbs.	Pitt (1995); Hasegawa (2015)

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CHAPTER 3: THE RACHITIC TOOTH: THE USE OF RADIOGRAPHS AS A SCREENING TECHNIQUE

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Abstract

This study investigates morphological changes in pulp chambers of living and archaeological individuals with past vitamin D deficiency. Living individuals (n=29), four with detailed medical and dental records and three groups of archaeological individuals (n=25) were radiographed; selected individuals were further evaluated histologically for the presence of incremental interglobular dentin (IIGD), indicative of deficiency (28 living; 17 archaeological). Measurements of pulp horns/chambers from radiographs were conducted to quantify morphological observations. One group had clear skeletal evidence of rickets from St. Matthew, Quebec (n=1) and St. Jacques, France (n=4); a second group had slight skeletal indicators from Bastion des Ursulines, Quebec (n=6); and a third group lacked both skeletal and radiological evidence of deficiency from St. Antoine (n=6) and Pointe-aux-Trembles (n=4). Results showed archaeological individuals with clear and slight skeletal evidence of past deficiency displayed constricted or chair shaped pulp horns. Living individuals with deficiency exhibited similar pulp chamber morphology. Radiographic pulp horn/chamber measurements corroborated morphological findings and significant differences were found in pulp horn/chamber measurements between those with and without deficiency. Results suggest that radiograph assessment of teeth can be used as a screening technique to elucidate patterns of deficiency and select individuals for microCT or histological assessment.

Keywords: vitamin D deficiency; rickets; dentin; dental radiographs

1. Introduction

Vitamin D deficiency has emerged as a significant public health problem in many communities (Holick, 2006). Identifying the number of individuals who may have experienced vitamin D deficiency has significant potential to further our understanding of the range of factors that may have compromised the health of people in the past (Brickley et al., 2017; Brickley et al., 2014). Identification of individuals who may have experienced past deficiency based on skeletal changes is difficult as few adults retain clear skeletal changes associated with rickets (Hess, 1930). Disturbances in metabolism are reflected in the microstructure of developing tooth dentin (Arana-Chavez and Massa, 2004; Foster et al., 2014) and one consequence of vitamin D deficiency is the presence of mineralisation defects in teeth. Recently it has been demonstrated that conditions that disrupt vitamin D, calcium, and phosphate pathways cause systemic mineralisation defects in teeth known as incremental interglobular dentin (IIGD) (D'Ortenzio et al., 2016), which is observed as clear bands of bubble-like spaces that follow incremental lines within the dentin matrix (Noyes, 1921).

Clinical studies have demonstrated that vitamin D deficiency from nutritional and genetic causes produce morphological changes in dental structures that can be detected radiologically (e.g., Seow and Latham, 1986; McDonnell et al., 1997). We hypothesised that abnormal mineralisation related to vitamin D deficiency in childhood can be detected radographically in both living and archaeological individuals through quantifiable pulp chamber changes in permanent molars. Teeth from known living controls and three groups of archaeological individuals were radiographed to evaluate if there were

measurable differences in the pulp horns and pulp chambers in those with deficiency; histological analysis confirmed the presence or absence of incremental interglobular dentin (IIGD). Radiographic imaging provides a useful screening tool, enabling an inexpensive non-destructive means of identifying individuals who experienced past episodes of deficiency. We focused on permanent dentition as dentin in these teeth provides a record from early childhood to young adulthood (dentin formation is present in the 1st permanent molar before birth and the 3rd permanent molar ends at ~18 years), permitting the development of a chronological profile of deficiency throughout an individual's early life.

1.1. Background

Dental pulp consists of connective tissue derived from mesenchyme (embryonic connective tissue) cells and is localised within the pulp chamber and root canals of the tooth (Avery, 2002:190). The pulp contains cells that provide nutritive, sensory, and defensive functions and permits preservation of vitality of the tooth. Dental pulp is divided into two components: 1) the odontoblasts, which are the cells responsible for the production and maintenance of predentin and dentin, and 2) blood vessels and nerves. Deciduous teeth are fast forming, as many secretory odontoblasts are active at one time (Dean, 2016); permanent teeth are slower to form as fewer odontoblasts are simultaneously active. Due to their greater size and slower rate of formation, it takes approximately 3-6 years for the completion of dentin in the crowns of each permanent tooth, during which time endocrine and nutritional factors can influence enamel and dentin formation (Schour and Massler, 1940; Hillson, 2002:123). Similar to the failure of

osteoid mineralisation in bone (Foster et al., 2014), systemic factors, such as conditions causing rickets, can also influence the internal environment of the tooth leading to an absence of secondary dentin formation, producing distinguishable morphological changes to the pulp chamber.

Clinical studies have demonstrated that conditions that disrupt vitamin D, calcium, and phosphate pathways lead to the development of a number of abnormalities of the dentition and surrounding structures and these are listed in Table 1. Many of the dental abnormalities have a number of possible causes and so for this study pulp horn morphology was selected for investigation because changes in pulp horns are only caused by conditions linked to mineralisation defects (D'Ortenzio et al., 2016; Table 1).

Table 1: Dental abnormalities of vitamin D deficiency observed radiographically.

Dental abnormality	**Conditions that disrupt vitamin D, phosphate, calcium pathways**	**Source**	**Other causes of dental abnormality**	**Source**
Enlarged pulp chambers*	Vitamin D-resistant hypophosphatemic rickets	Seow (1984); Seow and Latham (1986)	Odontogenesis imperfecta (rare hereditary disease)	Cahuana et al. (2005)
	Nutritional rickets	McDonnell et al. (1997); Davit-Béal et al. (2014)		
High, constricted (narrow) pulp horns extending into the dentin-enamel junction	Vitamin D-resistant hypophosphatemic rickets; Vitamin D-dependent rickets	Harris and Sullivan (1960); Hernández and Laguna (2013)	-------	
	Nutritional rickets	McDonnell et al. (1997); Galhotra et al. (2015)		
Short roots	Vitamin D–dependent rickets	Zambrano et al. (2003); Souza et al. (2010; 2013)	Dentin dysplasia (rare genetic disease)	Arana-Chavez and Massa (2004)
Poorly defined lamina dura	Vitamin D-resistant hypophosphatemic rickets; Vitamin D–dependent rickets; Hyperparathyroidism	Zambrano et al. (2003); Pereira et al. (2004)	Dental abscess; Giant cell granuloma	Chapman et al. (2013)
			Osteoporosis; Old age	Worth (1963:181)
	Nutritional rickets	McDonnell et al. (1997)		
Apical radiolucency (transparency)	Vitamin D–dependent rickets	Zambrano et al. (2003)	Cysts; Benign and Malignant lesions; Infection	Razavi et al. (2015)

Dental abscesses	Vitamin D-resistant hypophosphatemic rickets	Chaussain-Miller et al. (2007); Douyere et al (2009); Beltes and Zachou (2012)	Infection	Robertson and Smith (2009)
	Nutritional rickets	McDonnell et al. (1997); Davit-Béal et al. (2014)		
Radiolucency region near the dentin-enamel junction	Vitamin D-resistant hypophosphatemic rickets	Seow and Latham (1986); Pereira et al. (2004)	Pre-eruptive intracoronal resorption (lesion in the crown dentin)	Ari (2014)
Enamel hypoplasia	Vitamin D–resistant rickets	Seow and Latham (1986); Tumen et al. (2009)	Hereditary conditions; Trauma; Systemic metabolic stress; for full review see Goodman and Rose (1990)	Armelagos et al. (2009); Anthonappa and King (2015)
	Nutritional rickets	Davit-Béal et al. (2014); Galhortra et al. (2015)		
Normal but thin enamel	Vitamin D-resistant hypophosphatemic rickets	Hernández and Laguna (2013)	Dentinogenesis Imperfecta	Arana-Chavez and Massa (2004)
	Nutritional rickets	McDonnell et al. (1997); Davit-Béal et al. (2014)		
Increased incidence of carious lesions	Vitamin D-resistant hypophosphatemic rickets	Pereira et al. (2004); Chaussain-Miller et al. (2007)	Diet; Poor oral hygiene, for full review see Selwitz et al. (2007)	Hillson (2002:269-284)
	Nutritional rickets	Davit-Béal et al. (2014); Galhotra et al. (2015)		

Missing or unerupted teeth (agenesis)	Vitamin D-resistant hypophosphatemic rickets Nutritional rickets	Pereira et al. (2004); Rathore et al. (2013) Galhotra et al. (2015)	Idiopathic Hypoparathyroidism (rare decrease in blood calcium); Cysts; Osteomyelitis	Worth (1963:184-197)

*Notes: enlarged pulp chambers are different from taurodontism, which is an apical elongation of the pulp chamber that results in shortened roots associated with Neanderthal dentition, amelogenesis imperfecta, and Down's syndrome (Tulensalo et al., 1989). -----No other known cause.

2. Materials

Teeth from living individuals were collected to act as controls (n=29). Four individuals provided medical histories and bitewing dental radiographs, (three supplied blood serum 25(OH)D levels), of those, three were diagnosed with previous vitamin D deficiency (KT1, KT2, KT3), and one had no previous deficiency (TT1). KT3 was diagnosed with osteopenia. Permanent molars from twenty-five other living individuals were collected for radiographic and histological analysis to provide a comparative sample (1^{st} molars: n=8; 2^{nd} molars: n =7; 3^{rd} molars: n=10) (HIREB ethics approval 2246). These individuals (n=25) had no pre-existing medical condition, however they may have experienced asymptomatic nutritional vitamin D deficiency, therefore histological analysis was undertaken to check for IIGD. Table 2 presents all individuals in the study sample.

Radiological and/or histological data were also evaluated from three different groups of archaeological skeletons classified as 'slight' to 'marked' for deformities associated with previous vitamin D deficiency. Teeth with carious lesions and/or severe attrition were avoided where possible. Based on information in Table 1, when excluding individuals with carious lesions, there is a higher chance that individuals with previous episodes of deficiency may also be excluded. The three archeological groups consisted of individuals of European ancestry, therefore the criterion described in Brickley et al. (2010) were used. The first archaeological group from Saint Matthew, Quebec (n=1) and Saint Jacques, France (n=4) had severe skeletal deformity from healed rickets and histological evidence of at least one systemic episode of IIGD. Radiographs were

conducted on the permanent molars of these individuals whose skeletal deformities were severe.

The site of Bastion des Ursulines, Quebec City, curated at the Canadian Museum of History, Ottawa, was chosen as the second group, because some individuals buried at this site will have spent their childhoods in conditions that were conducive to the development of rickets. Cases of rickets have been reported from Euro-Quebecois cemeteries (e.g., Larocque, 1999; Morland and Ribot, 2010), and findings by D'Ortenzio et al. (2016) showed that some individuals in these communities experienced multiple episodes of deficiency. Macroscopic examination of bowing defects was conducted on 32 adults. Ten were chosen for further radiographic analysis of the dentition, six with skeletal indicators of a deficiency and four with none observed (Table 2).

To evaluate how many individuals might have had deficiency and recovered, but may be missed when radiographic techniques are used, a third group was selected to investigate individuals who lacked clear skeletal and dental radiographic indicators of vitamin D deficiency. These individuals from Saint Antoine (n=6), and Pointe-aux-Trembles (n=4), Montreal were buried in Euro-Quebecois cemeteries (Table 2). Individuals were first screened for an absence of skeletal deformity indicating healed rickets, 10 were identified, and permanent molars were radiographed to confirm that no morphological changes were present in the pulp chambers. To establish how many cases of deficiency were missed using radiographs, histological analysis was conducted on this set of individuals.

Table 2: Description of individuals in study sample.

Geographical Location	ID	Age (Years)	Serum Level (nmol/L)	Past Deficiency	Molars Radiographed	Molar used Histological Analysis
North America (Living individuals)	KT1	18	31	✓	U & L 1st, 2nd,3rd	RM^3
	TT1	19	127	×	U & L 1st, 2nd,3rd	RM_3
	KT2	42	--	✓	U & L 1st, 2nd,3rd	×
	KT3	46	21	✓	U & L 1st, 2nd,3rd	Rm_1
	M12	89	--	×	LM^1	LM^1
	M13	58	--	×	LM^1	LM^1
	M16	42	--	×	RM_1	RM_1
	M17	57	--	×	LM^1	LM^1
	M18	58	--	×	RM^1	RM^1
	M19	21	--	×	LM^1	LM^1
	M111	48	--	×	RM^1	RM^1
	M22	64	--	×	LM_2	LM_2
	M24	37	--	×	RM_2	RM_2
	M25	56	--	×	LM_2	LM_2
	M27	32	--	×	LM^2	LM^2
	M28	27	--	×	RM_2	RM_2
	M29	20	--	×	RM_2	RM_2
	M32	18	--	×	RM_3	RM_3
	M33	21	--	×	LM_3	LM_3
	M34	26	--	×	RM_3	RM_3
	M35	26	--	×	LM_3	LM_3
	M36	25	--	×	LM^3	LM^3
	M37	32	--	×	LM^3	LM^3
	M38	18	--	×	RM^3	RM^3
	M39	27	--	×	RM^3	RM^3
	M310	30	--	×	LM_3	LM_3
	M11	64	--	✓	LM_1	LM_1
	M210	42	--	✓	LM_2	LM_2
	M31	24	--	✓	LM^3	LM^3
Bastion des Ursulines, Quebec (1746-1747)	19G37-M02	18-21	--	×	U & L 1st, 2nd, 3rd	×
	19G37-E03	20-24	--	✓	U & L 1st, 2nd, 3rd	RM^1
	19G37-M01	25-29	--	✓	U & L 1st, 2nd, 3rd	×
	19G37-M03	18-22	--	✓	L 1st, 2nd, 3rd	RI^1
	39G6-B2	N/A	--	✓	U & L 1st, 2nd, 3rd	×
	19G37-F05	28-34	--	×	RM^1	×
	19G37-N01	18-21	--	✓	L 1st, 2nd, 3rd	×

	19G41-D01	45-54	--	✓	U & L 1st, 2nd, 3rd	×
	19G35-H01	20-24	--	×	RM^1	×
	19G37-F04	20-24	--	×	U & L 1st, 2nd, 3rd	×
Saint. Matthew, Quebec (1771-1860)	15A-S36	~23	--	✓	RM^2, RM^3	RM^1, RM^2, RM^3
Saint Antoine, Quebec (1799-1854)	STA 18K 55	50+	--	×	LM^1	LM^1
	STA 25C 55	17-25	--	×	L 1st, 2nd, 3rd	RM^1
	STA 25A 53	20-34	--	×	RM_1, RM_2, LM_1, LM_2	LM_1
	STA 22A511	17-25	--	×	RM_2, RM_3, LM_3	RM_2
	STA 25C520	16-25	--	×	RM_1, RM_2, RM_3	RM_3
	STA 25C518	35-49	--	✓	RM_1, LM_1	RM_1
Pointe-aux-Trembles, Quebec (1709-1843)	PAT 7A9513	16-25	--	×	U & L 1st, 2nd, 3rd	RM^1
	PAT 7A9546	35-49	--	×	LM_1	LM_1
	PAT 7A11 560	25-34	--	×	RM_3, LM_3	RM_3
	PAT 7A11 561	19-26	--	×	RM^2, LM^2	LM^2
Saint Jacques, France (A.D. 1225-1798)	SJ 384	40+	--	✓	U & L 1st, 2nd, 3rd	RM_1, RM_2
	SJ 562	40-58	--	✓	U & L 1st, 2nd, 3rd	LM_2, RC^1
	SJ 892	40-58	--	✓	U & L 1st, 2nd, 3rd	LM_3, RC_1
	SJ 970	45-56	--	✓	U & L 1st, 2nd, 3rd	LM^1, RM^3

Notes: U = maxillary; L = mandibular.
Age for living individuals was the age that the radiograph was obtained. Age at death for archaeological individuals was estimated using auricular surface (Lovejoy et al., 1985), pubic symphysis (Brooks and Suchey, 1990), and cementochronology (Naji et al., 2016). -- Information not available.

3. Methods

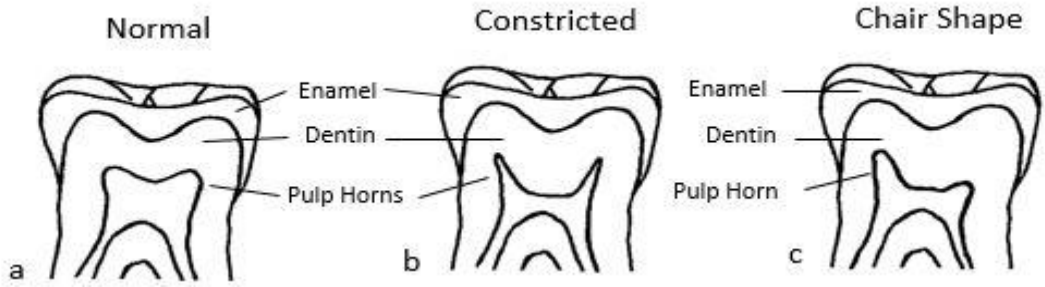
3.1. Radiographs to observe pulp horn and pulp chamber morphology

To observe pulp horn and pulp chamber morphology, radiographic images were taken on living and archaeological permanent molars (n=50) and bitewing radiographs supplied by dentists were analysed for four additional living individuals (Table 2). Four locations using two different types of radiological equipment were used for archaeological individuals (see Table 1 in Supplemental Data A for locations and specifications). Single permanent molars and/or mandibles and maxillae containing molars were placed on the imaging plate at a distance from the source ranging from 20cm to 150cm, depending on the radiograph set-up. Clinical radiographs were obtained in a standardised orientation (buccolingual direction) that clearly showed the pulp chambers. To ensure consistency and facilitate comparison with clinical cases, archaeological dentition was positioned as perpendicular to the X-ray beam as possible in order to obtain the intersection of the X-ray beam in a buccolingual orientation (Worth, 1963:3). The number of pulses (exposure time) was selected (~6-29 pulses). Images were then converted to TIFF files and saved to a database.

3.2. Blind test

To establish if other researchers could use the radiograph instructions produced for identification of individuals with a previous episode of vitamin D deficiency a blind test was conducted using radiographs of archaeological individuals with slight to marked skeletal indicators of vitamin D deficiency and presence or absence of IIGD was confirmed by histological analysis (Saint Matthew, Saint Jacques, Bastion des Ursulines).

See Supplementary Data B for further information. Known clinical images were also used. Twelve radiographs of permanent molars from individuals with and without deficiency were examined by six participants. Participants were instructed to examine the pulp chamber, paying close attention to pulp horns that were normal (evenly matched); uneven (referred to as chair shaped); or constricted (narrow), that could indicate deficiency (see Figs. 1a-c). Participants answered yes: clear evidence of uneven or constricted pulp horns; no: appears normal and evenly matched; possible yes: could have morphological changes, but not definitive; or undecided: not sure. See Supplementary Data B, Table 3 for detailed instructions, results, and examples from the blind test.



Figs. 1a-c: Diagram of different pulp chamber shapes in a generic permanent molar, **a)** normal: evenly matched pulp horns; **b)** constricted: high, narrow pulp horns; **c)** chair shape: pulp horns are uneven resembling a chair. Can be used for all three molars, recognising that the 3rd molar is more variable.

To aid in determining potential ages of deficiency reflected by changes to the morphology of the pulp chamber, Table 3 displays the approximate timing of pulp chamber initiation in the three types of permanent molar. For pulp horn shape changes to arise, deficiency has to occur during the process of pulp formation. Third molars will show considerably more variation in size, contour, and number of roots than 1st and 2nd molars (Ahmed, 2012). Root formation was excluded, as this feature does not influence

pulp chamber initiation. Evaluation of dental formation allows us to determine the approximate age when pulp changes arose and subsequently provides key information as to the age at which a deficiency occurred.

Table 3: Approximate age of pulp chamber initiation in permanent molars.

Tooth type (mandibular)	**Pulp chamber initiation**	
1st Permanent Molars	Male 1.5-2 years	Female 1.4-2 years
2nd Permanent Molars	Male 5-6.5 years	Female 4.5-6.5 years
3rd Permanent Molars	Male 10-12.5 years	Female 9.5-12 years

Note: Approximate age of initiation for pulp chambers as described in Moorrees et al. (1963:1492). Stage Crc (crown complete). Average age of mandibular molars is given.

3.3. Pulp horn and pulp chamber measurements taken from radiographs for living and archeological individuals

To quantify pulp horn and pulp chamber morphology, radiographs from both living and archaeological individuals (Table 2) were taken and measurements of pulp horns and pulp chambers were conducted blind to ensure unbiased calculations. Radiographs were imported into the image editing software program ImageJ to perform linear measurements. The scale from the radiograph images was used to calibrate the images imported into ImageJ. Using the Straight-Line Tool in ImageJ, the scale for each image was calibrated to the radiograph scale, where 1mm equaled ~7 pixels. Pulp horn height (PH1-h and PH2-h), width (PH1-w and PH2-w), and pulp chamber height (PC-h) were measured using the Straight-Line Tool where measurements were automatically derived for pulp horns and pulp chambers (Fig. 2). Ratios were calculated for pulp horn heights (PH-h Ratio) to observe any differences between those with and without vitamin

D deficiency (see Supplementary C for all measurements). The ratios were calculated to reduce the effect of external tooth size and the type of tooth used as pulp horns are proportional relative to the whole tooth. Kvaal et al.'s (1995) study did not find significant differences in measurements between teeth from the left or right side of the maxilla or mandible, therefore, teeth from both sides were used in this study. Student's t tests (two-tailed) were conducted in a Microsoft Excel program that compared the five measurements taken from the pulp horns and pulp chambers of living and archaeological individuals with vitamin D deficiency to those without. Student's t tests were also conducted to determine any significant differences in measurements between tooth types and between teeth on the left versus right side of the maxillae and mandible. To assess whether a dental restoration affected the shape of the pulp chamber, measurements were compared on KT3's (diagnosed with deficiency, Table 2) right (dental restoration) and left (no dental restoration) mandibular 1st molars. Bitewing dental radiographs were also obtained for KT3 for a 7-year period to determine if measurements of pulp chambers changed over time due to secondary or tertiary dentin formation.

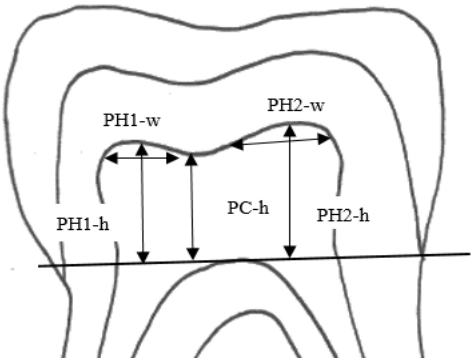


Fig. 2. Diagram of measurements taken on a generic permanent molar. PH1-h and PH2-h are pulp horn heights; PH1-w and PH2-w are pulp horn widths; PC-H is pulp chamber height (modified from Zilberman and Smith, 2001).

3.4. Histological analysis

Archaeological individuals who exhibited a number of dental and skeletal features associated with vitamin D deficiency were selected for histological analysis (n=17). Living controls (n=28), with and without deficiency, were also evaluated histologically to confirm the presence or absence of incremental interglobular dentin (IIGD). Histological analysis was undertaken to directly observe pulp chamber changes in individuals with deficiency, to determine presence/absence and severity of IIGD, and to estimate the age at which deficiency occurred (Table 2). Age of deficiency, based on location of IIGD in dentin, was determined using Moorreess' et al. (1963) developmental standards, and severity of deficiency was graded according to the scoring system described in D'Ortenzio et al. (2016). For example, for Grade 1, the amount of IIGD is less than 25% relative to the surrounding normal dentin, with small interglobular spaces, indicating a mild mineralisation defect. Grade 3 is the most severe with IIGD covering over 75% of the region of interest, accompanied by large spaces that follow the incremental lines in the dentin (D'Ortenzio et al., 2016).

Teeth were sectioned to expose the pulp chamber from the buccal side of the tooth to be consistent with the direction of the X-ray beam using a precision diamond wafering saw (Buehler IsoMet 1000) (Saunders et al., 2007). 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 5 minutes in distilled water and glued to a microscope slide that adhered using a UV

activated adhesive. For microscopic analysis, the thin-sections were imaged using a Nikon DsR:1 camera attached to an Olympus BX51 digital microscope, (40x to 100x magnification).

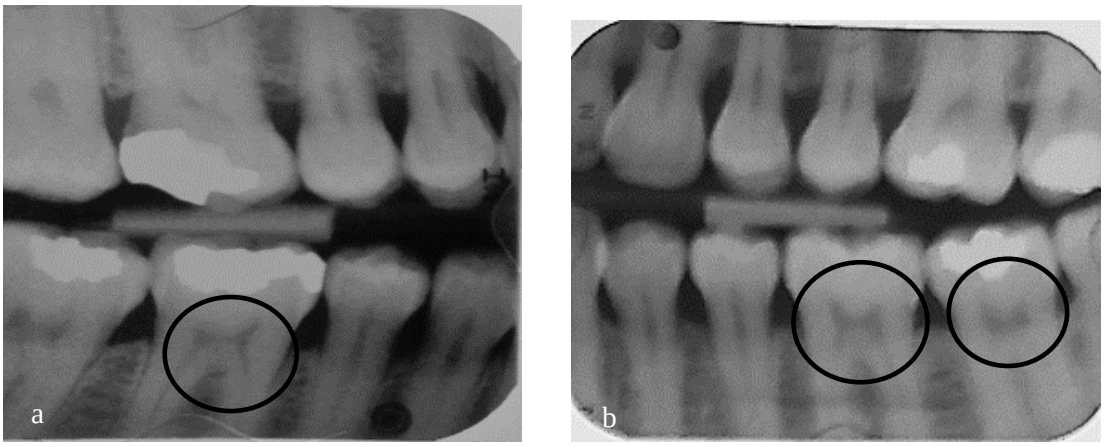
4. Results

4.1. Radiographs observing morphology of pulp horns and pulp chambers for known living controls

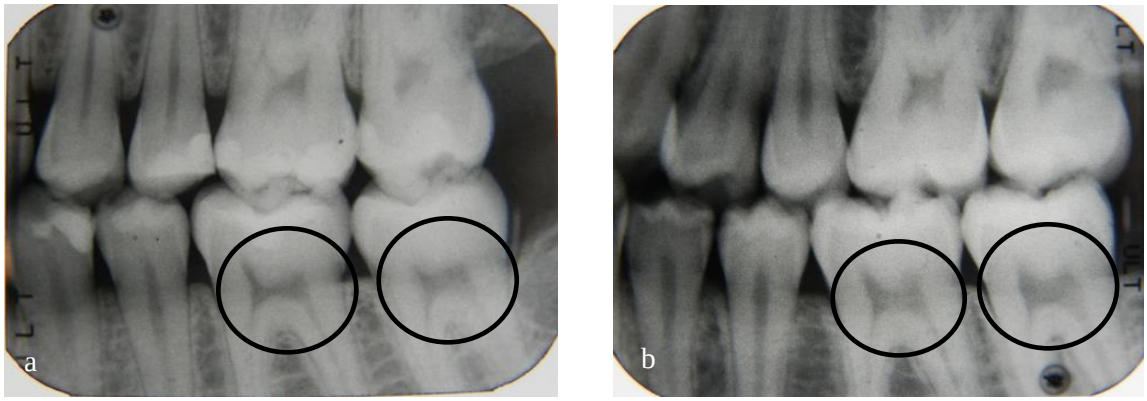
Permanent molars of living indiviudals (n=25) were radiographed (Table 2) to determine pulp horn and pulp chamber shape in those potentially lacking deficiency. Of these, three individuals (M11, M210, M31) displayed chair shaped pulp chambers, indicative of vitamin D deficiency and further histological analysis confirmed the presence of IIGD (see histological results below). Using Table 3, we approximated the ages of deficiency, based on location of IIGD, which ranged for each individual; M11 (LM_1) was 1.4-2 years; M210 (LM_2) was 4.5-6.5 years; and M31 (LM^3) was 9.5-12.5 years. Note that deficiency could have occurred earlier in M210 and M31 as we only had their 2nd and 3rd molars available for histological analysis.

We also examined bitewing radiographs of three living individuals with clinically diagnosed vitamin D deficiency; one diagnosed with rickets at age 4 (KT2), and two with asymptomatic deficiency diagnosed using serum 25(OH)D results (Table 2). All three individuals showed changes to the pulp horns, with the mandibular 1st molar showing the most marked change suggesting deficiency at ~ 1.8-2.6 years (Figs. 3a-b, 4a). In KT2, poor image quality due to overexposure prevented a definitive statement being made on the 2nd molar. The second mandibular molar for both KT1 and KT3 showed slight

changes indicating deficiency continued. A right deciduous mandibular first molar from KT3 showed there was no deficiency from 14.5-17 weeks in utero to 5.5 months old (Hillson 2002), however bitewing radiographs showed chair-shaped 1st mandibular molars (Fig. 3a-b). Histological analysis on KT1's extracted 3rd molar, which begins to form at approximately 7-10 years (Hillson, 2002:123), confirmed the radiographic results (Fig. 4a); the presence of Grade 2 IIGD under the crown and the mesiobuccal cusp, which is the first cusp to form in the maxillary 3rd molar and could overlap with the formation of the pulp chamber in the second molar (Reid and Dean, 2006). Bitewing radiographs of individual TT1 (Fig. 4b), who lacked deficiency, did not display constricted or chair shaped pulp horns in any molars; the pulp horns were even and regular. Histological analysis of the 3rd molar confirmed the absence of IIGD (Grade 0) from ages ~7-19 years.



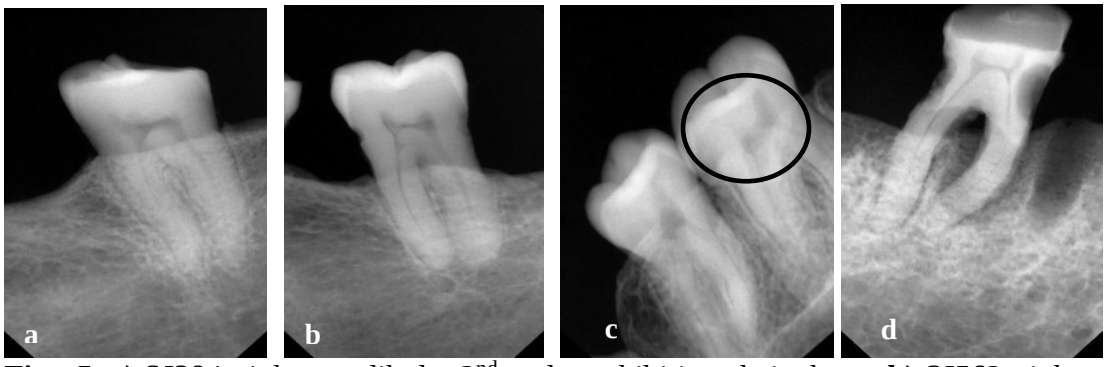
Figs. 3a) Radiograph of KT3 (low in vitamin D), exhibits chair shaped pulp chamber in permanent mandibular right 1st molar (black circle); **b)** chair shape exhibited in permanent mandibular left 1st and 2nd molars (black circles).



Figs. 4a) Radiograph of KT1 (low in vitamin D) showing chair shaped, constricted pulp horns in the left 1st mandibualr molar and a chair shaped 2nd mandibular molar (black circles); **b)** radiograph of TT1 (normal vitamin D level) showing regular, even pulp horns in the left mandibualar 1st and 2nd molars (black circles).

4.2. Radiographs observing morphology of pulp horns and pulp chambers for archaeological individuals

Table 5 presents pulp horn shape viewed histologically and radiographically, severity of IIGD grades, pulp horn/chamber measurements, and approximate timing of deficiency for the subsampled living and archaeological individuals (for a full description of all individuals, including tooth type analysed, see Supplemental Data A, Table 2). Radiographic images of molars from the first archaeological group (Saint Matthew, Saint Jacques), had marked skeletal evidence of vitamin D deficiency and were on the severe end of a deficiency spectrum. The maxillary 1st permanent molar of individual 15A-S36, from Saint Matthew, Quebec, revealed prominent constricted pulp horns. The Saint Jacques dentition showed that all individuals (n=4) exhibited chair shape, constricted pulp horns in all maxillary and mandibular molars available, with pulp horn extensions towards the occlusal aspect of permanent teeth (Figs. 5a-d).



Figs. 5. a) SJ384, right mandibular 2nd molar exhibiting chair shape; **b)** SJ562, right mandibular 2nd molar exhibits constriction and a slight chair shape; **c)** SJ892, left mandibular 2nd and 3rd molars, 2nd molar exhibiting chair shape (black circle); **d)** SJ970, right mandibular 1st molar with carious lesion and occlusal wear exhibits constricted pulp horns.

Radiographs of group two (Bastion des Ursulines, n=10) consisted of cases with slight deformity of long bones revealed the presence of observable chair shaped pulp horns in five out of six individuals with bowing deformities. The exception was 19G37-M03 who had skeletal indicators of deficiency, but dental radiographs did not display constricted or chair shaped pulp horns. In the individuals where skeletal bowing was absent (n=4), pulp horns appeared evenly matched, without constrictions.

4.3. Results of radiological and histological examination to determine how many vitamin D deficient cases were missed

Group three (Saint Antoine, Pointe-aux-Trembles) consisted of archaeological individuals who displayed no macroscopic skeletal indicators of past deficiency or characteristic pulp horn shape changes observed on radiograph. However, these individuals were from communities in which vitamin D deficiency was known to be present. Histological analysis was conducted to determine how many of these individuals had clear bands of IIGD suggestive of vitamin D deficiency (Table 2, Supplemental Data

A). One individual (STA 25C 518) had chair shaped pulp horns in their right mandibular 1st molar observed radiographically also had Grade 1+ IIGD. Histological analysis showed that the other individuals (7/9) had no IIGD (as anticipated); however, the remaining two had Grade 1 IIGD (minimal interglobular spaces). One individual from Saint Antoine, Quebec (STA 18K 55) had only one molar available for analysis. The radiograph angle was not exactly perpendicular to the X-ray beam, which made it difficult to discern pulp horn shape.

Of the living individuals (n=25) used as controls, 3 displayed chair shaped pulp horns radiographically and histological analysis showed that M11 had Grade 1.5-2 IIGD, whereas M210 and M31 exhibited Grade 2 IIGD, indicating that these individuals experienced moderate past vitamin D deficiency. The remaining 22 individuals did not display chair shape pulp horns and scored a Grade 0 for IIGD, indicative of an absence of deficiency (Fig. 6a-d).

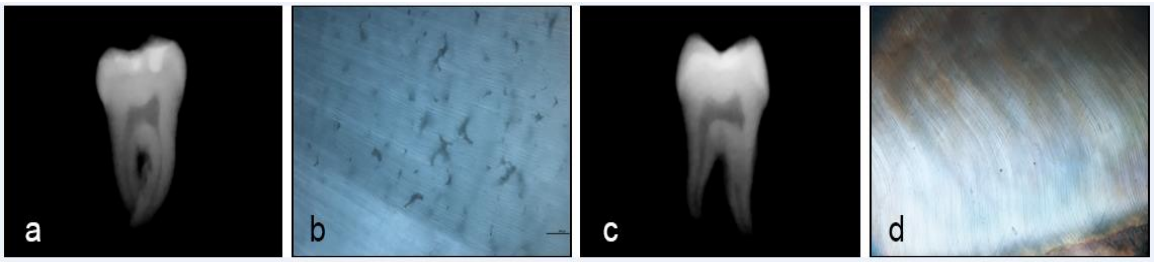


Fig.6. a) M210 radiograph showing chair shape pulp horns in LM_1; **b)** M210 exhibits Grade 2 IIGD (crescent shaped spaces), indicative of moderate deficiency; **c)** M13 radiograph showing even pulp horns in LM^1; **d)** M13 exhibits Grade 0 IIGD (no spaces), indicating absence of deficiency. Histology 100x magnification.

4.4. Results of blind test

The blind test revealed an accuracy of 75% among participants for selecting the correct answer (yes: misshapen pulp horns detected; or no: none detected). Results obtained for each case varied, ranging from 17% correct (case 3) to 100% correct (cases 2, 4, 6, 11, 12). A review of the radiological images demonstrated that positive results were clearly linked to the quality and proper angulation of the radiograph observed. Evaluating the data indicated that differences in individual results were due to the positioning of the teeth on the radiograph plate. All cases that received 100% correct scores involved just one or two teeth with good magnification and positioning (see Table 3, Supplementary Data B). Positioning of teeth at a right angle to the X-ray beam is critical for good results, but once that has been accomplished, observation of pulp chambers can be completed with relative ease. The results show that with properly aligned radiographs, pulp chamber abnormalities, indicative of deficiency, were consistently identified by observers.

4.5. Pulp horn and pulp chamber measurements taken from radiographs for living and archaeological individuals

Table 4 presents the mean values for pulp horn/chamber measurements taken on living and archaeological individuals (n=154) (for all measurements see Supplementary Data C). Table 5 displays measurements for selected individuals. There were no significant differences between measurements of teeth from archaeological individuals versus living individuals in both those with deficiency or those without. Measurements on pulp horn widths showed that pulp horns that were <1 mm were associated with those with vitamin D deficiency, whereas those with widths >1 mm, were without deficiency.

Students t tests determined that differences between pulp horn widths on living and archaeological individuals with deficiency versus those without were highly significant (living individuals p=0.014; archaeological individuals p= 0.018). Pulp horn heights were compared and differences were also highly significant between living and archaeological individuals with and those without deficiency (p= 0.007, p= 0.015, respectively). There were no significant differences in measurements of teeth from the left or right side of the maxilla or mandible (p= 0.11-0.96) or between different tooth types (p= 0.06-0.67) for all individuals tested. Ratios conducted to compare pulp horn heights for all individuals found that those without deficiency had an approximate 1:1 ratio, whereas those with deficiency had close to a 2:1 ratio (Table 4). Pulp chamber heights ranged from 0.8-6.1 mm with archaeological individuals with deficiency having the narrowest pulp chamber height (0.3-2.9 mm) (Table 4).

Evaluation of pulp chamber measurements of teeth with dental restorations versus those without to assess possible pulp chamber shape change in KT3 showed that although there were slight differences between LM_1 (no restoration) and RM_1 (restoration) widths (e.g., PH2-w: 0.3 mm for RM_1; 0.5 mm for LM_1), these differences were not significant and the pulp horn height ratios (differences in height between both pulp horns) were identical (1.64:1) in both molars. More work is required to determine the full effect of dental restoration on pulp chamber measurements. Changes in the amount of secondary and/or tertiary dentin were not detectable over the 7-year time period in KT3's tooth with the dental restoration. The right mandibular 1st molar (dental restoration) was re-measured for each year and results showed that pulp horn/chamber measurements were almost

identical over the seven-year period with fluctuations of only ±0.01-0.03 mm for all measurements taken (Supplemental Data D).

Table 4: Summary of mean values for measurements taken on permanent molars observed on radiograph.

Measurement (mm)	N, Mean, SD	Modern: no deficiency	Modern: with deficiency	Archaeological: no deficiency	Archaeological: with deficiency
PH1-h	N	14	34	44	62
	Mean	2.9	4.4*	2.6	2.7
	SD	0.8	1.9	0.9	1
	Range	1.6-4.9	2.5-8.6	1.4-4.7	1.2-4.3
PH2-h	Mean	2.6	2.7	2.5	1.6*
	SD	0.9	1.3	0.9	0.8
	Range	1.4-3.7	0.9-4.9	1.2-4.9	0.5-3.5
PC-h	Mean	1.8	2.1	2.0	1.2
	SD	0.7	1.5	0.9	0.7
	Range	0.9-3.0	0.8-6.1	0.8-3.9	0.3-2.9
PH1-w	Mean	1.2	0.7*	1.1	0.5*
	SD	0.2	0.2	0.2	0.2
	Range	1-1.7	0.4-1.1	0.9-1.5	0.2-1.2
PH2-w	Mean	1.3	0.6*	1.1	0.5*
	SD	0.3	0.2	0.2	0.3
	Range	1-1.9	0.3-0.9	1-1.4	0.1-1
PH-h Ratio		1.1:1	1.8:1	1.1:1	1.8:1

*Note: N is the number of molars measured on radiograph. Ph1-h = pulp horn 1 height; Ph2-h = pulp horn 2 height; PC-h = pulp chamber height; PH1-w = pulp horn 1 width; Ph2-w = pulp horn 2 width; PH-h Ratio = ratio between pulp horn heights.
* P value < 0.01

Table 5: Summary of radiographic measurements and histological morphological data for selected individuals.

IIGD severity score	Identifier	Age (years)	Past deficiency*	Histological pulp horn/chamber shape	Radiological pulp chamber measurements (mm) of permanent molar	Approximate age of episode (yrs.) (Moorrees et al., 1963)
Grade 3	15A-S36	~23	✓	RM^1: constricted	RM^2: PH1-w: 0.61; PH2-w: 0.51; PH-h Ratio= 1.75:1	2 episodes between 1.5-2
	SJ 970	45-56	✓	LM^1: constricted; chair shape	LM^1: PH1-w: 0.27; PH2-w: 0.44; PH-h Ratio= 1.92:1	1.5
Grade 2	KT1	18	✓	RM^3: moderately constricted: chair shape	RM^1: PH1-w: 0.61; PH2-w: 0.8; PH-h Ratio= 2.85:1	10.5
	SJ 562	40-58	✓	LM^1: severely constricted	LM^1: PH1-w: 0.12; PH2-w: 0.14; PH-h Ratio= 1.38:1	1.5-2
Grade 1+	19G37-M03	N/A	✓	N/A: single root (RI^1)	**RM_1: normal and even; multiple carious lesions obscured view	5.5-6.5
	STA 25C 518	35-49	✓	RM_1: moderately constricted; chair shape	RM_1: PH1-w: 0.71; PH2-w: 0.81; PH-h Ratio= 1.59:1	1.5-3
Grade 1	PAT 7A9 513	16-25	✓	RM^1: normal and even	RM^1: PH1-w: 1.06; PH2-w: 0.97; PH-h Ratio= 1.04:1	1.5-2
Grade 0	TT1	19	×	RM_3: normal and even	RM_2: PH1-w: 1.11; PH2-w: 1.17; PH-h Ratio= 1.02:1	--
	KT3	44	✓	-- Rm_1 (deciduous tooth)	**RM_1: PH1-w: 0.54; PH2-w: 0.3; PH-h Ratio= 1.64:1	--
	STA 25A 53	20-34	×	LM^1: normal and even	LM^1: PH1-w:1.01; PH2-w: 1.02; PH-h Ratio= 1.01:1	--

Notes: Ages as set out in note for Table 3. *Deficiency diagnosed by blood serum levels (living individuals), skeletal changes, and presence/absence of IIGD in archaeological individuals. Full histological and radiological data available in Supplementary Data Table 2. Details on positioning covered in Supplementary Data B. **Observed RM_1 on radiograph as this forms near the same time as RI^1 and roots of Rm_1 (Moorrees et al., 1963).
PH1-w= pulp horn 1 width; PH2= pulp horn 2 width; PH-h Ratio= ratio between pulp horn heights.

5. Discussion

Radiographic imaging was specifically chosen for this preliminary study, as this technology is widely available, is non-destructive, and produced consistent results using a variety of different settings/exposure times, with the caveat that the positioning of the teeth on the radiograph plate is correct. The archaeological individuals in the first group (Saint Matthew, Saint Jacques), who displayed marked skeletal evidence of past rickets all (5/5) exhibited the formation of constricted and chair shaped pulp horns. These individuals with marked evidence of past rickets revealed extensions of the pulp horns into the occlusal edges of permanent molars (Figs. 5a-d), and all had a Grade 1+ to 3 IIGD severity score for deficiency. Figure 4b displays a radiograph of pulp chambers for an individual known to have healthy 25(OH)D levels (TT1); this individual exhibited evenly matched pulp horns. Among individuals who had experienced severe rickets, indicated by marked skeletal deformity, the pulp horns were less similar in length (chair shaped) and/or constricted.

Histological results in both living and archeological individuals appear to support the concept that a certain severity in the threshold of deficiency is required before morphological changes develop in the pulp chamber. The data suggest that the level of disruption to the mineralisation of a tooth that occurs with Grade 1 IIGD is insufficient to affect the shape of the pulp chamber during development. For example, an individual from Point-aux-Trembles (PAT 7A9 513), had no changes to their pulp chambers when viewed radiologically, but had Grade 1 IIGD in their 1st molar that formed between ~1.5 to 2-3 years. Conversely, two individuals from Saint Jacques (SJ 384, SJ 970), also had

episodes of deficiency starting at ~1.5 years, but both showed clear radiological dental features even though their episodes of deficiency were shorter. Their severity score was Grade 3 (Supplementary Data A, Table 2). This indicates that it is not only the timing of deficiency, but the severity of deficiency that causes pulp chamber shape changes. The data indicate that an IIGD severity score of approximately a Grade 2 and above must be reached before alterations in permanent molar pulp chambers are observed (Supplemental Data A, Table 2). Grade 2 severity consists of interglobular spaces that are moderately large and more numerous than Grade 1 (D'Ortenzio et al., 2016).

Clinical cases of vitamin D deficiency reviewed in Roberts and Brickley (in press) indicate that the development of skeletal features is strongly linked to rates of skeletal growth. Once growth stops, as demonstrated by our data from living individuals and cases reviewed by Roberts and Brickley (in press), serum levels likely need to be much lower before skeletal changes develop that would allow paleopathologists to diagnose vitamin D deficiency. Dentin from the right deciduous mandibular first molar from KT3 (blood serum level 21 nmol/L) (Table 2), showed there was no deficiency from 14.5-17 weeks in utero to 5.5 months old (Hillson 2002), therefore deficiency developed at a later age. It is likely that osteopenia in KT3 is associated with the serum 25(OH)D result, and deficiency may have been present for a while. It is generally accepted that serum 25(OH)D >50mnol/L are required for skeletal health and there are links to low bone mineral density (BMD) (Peterlik, 2012; Gallagher 2013). Dental changes to pulp chambers have appeared in individuals with blood serum levels of 31 nmol/L (e.g., KT1), suggesting that a

threshold for severity of deficiency needs to be met before pulp chamber alteration occurs.

Although severe and long-standing vitamin D deficiency can produce clear skeletal changes, similar to those observed in group one (Saint Matthew, Saint Jacques), many individuals who experience deficiency will not be identified from paleopathological investigation of skeletal changes, particularly individuals who survive to adulthood (Hess, 1930; Brickley et al., 2010). To determine if radiograph analysis of pulp chamber shape could be used to identify individuals who had experienced rickets during childhood using a collection where skeletal changes (i.e., bowing of leg bones) were not marked, the second group of individuals from Bastion des Ursulines, Quebec was used. For individuals who exhibited bowing deformities (n=6), dental radiographs revealed constricted, and/or chair shaped pulp horns in permanent molars in five out of six cases. However, one individual (19G37-M03), had skeletal changes, but did not have a positive dental radiological result for the three mandibular molars. Due to extensive carious lesion damage on the molars, an incisor was used for histological analysis. Evidence of Grade 1+ IIGD was observed histologically in a maxillary incisor (see Table 5). Timing of deficiency in this individual occurred later than the period in which the pulp chamber was forming in the permanent molars. The IIGD observed in the incisor that was formed at ~5.5-6.5 years, whereas pulp chamber formation is initiated in the 1st molar at an earlier age (~1.5-2 years).

It is not conclusive that the negative controls did not experience vitamin D deficiency, as they may have had slight deficiency but lacked skeletal and radiographic

indicators. This appears to be the case with two individuals from the third group of individuals (Saint Antoine, Pointe-aux-Trembles) who had no skeletal indicators of deficiency and lacked morphological changes to the pulp horns on radiograph. Histological analysis revealed Grade 1 IIGD (mild), suggesting that some individuals who experienced deficiency that caused very slight mineralisation defects may not develop morphological changes in the pulp chamber and would be missed in radiological screening.

Examination of dental radiographs were useful to evaluate if there were measurable differences in the pulp horns and pulp chambers of those with or without past vitamin D deficiency. We demonstrated that measurements on maxillary and mandibular 1^{st}, 2^{nd}, and 3^{rd} molars were not difficult to perform and significant differences were found in pulp horn widths and for the ratios between pulp horn heights in those with deficiency versus those without (Table 4) (Supplemental Data C). Measurements on pulp horn widths showed that pulp horns labelled morphologically as 'constricted' were <1 mm and were associated with those with vitamin D deficiency, whereas those labelled 'normal' were >1 mm, were found on those without deficiency. Those without deficiency had an approximate 1:1 ratio for pulp horn height, whereas those with deficiency had close to a 2:1 ratio. This signifies that the 'chair shape' observed on radiograph in those with deficiency was validated by the measurements, where one pulp horn was shorter relative to the other. As anticipated, measurements of pulp horn widths and heights taken on living individuals with deficiency were similar to archaeological individuals with deficiency and vise versa, further validating the trends found (Table 4). This was also

evidenced in the living individuals (n=25) used as controls, where 3 individuals displayed chair shaped pulp horns radiographically and histologically they had Grade 1.5-2 IIGD (M11, M210, M31), indicative of moderate asymptomatic vitamin D deficiency (Fig. 6a-d). Pulp chamber widths showed that all 3 individuals had constricted pulp horns (<1 mm) and the ratio between pulp horn heights ranged from 1.5-1.8:1, which is higher than the 1.2:1 ratio found in the living individuals without deficiency.

Teeth with dental restoration were avoided where possible, as this may influence the shape of the pulp horns by producing reparative (tertiary) dentin adjacent to the irritated zone of a tooth (Stanley et al., 1966; Kraus et al., 1969). One individual (KT3) had radiographs that showed a dental restoration in the right mandibular 1st molar (Fig. 3a), but lacked a dental restoration in the left mandibular 1st molar (Fig. 3b), suggesting that the chair shape observed could have been the result of the restoration. Measurements found that differences between the two were not significant indicating that the dental restoration did not affect the pulp chamber shape in KT3 through subsequent growth of tertiary dentin. This individual likely received prompt dental care before the carious lesion was able to progress to the dentin layer. Similarly, radiographs of KT3 were taken in progression over a seven-year period and pulp chambers were measured to determine potential growth of secondary and/or tertiary dentin. Results showed that pulp horn/chamber measurements were indistinguishable over the seven-year period with fluctuations of only ±0.01-0.03 mm for all measurements taken (Supplemental Data D), suggesting that in KT3, there was little to no secondary/tertiary dentin growth, as we would have observed a measurable reduction in the pulp chamber.

Radiological examination of pulp chambers can aid in determining the timing of deficiency in individuals who have pulp changes in their 1st molars (Table 3), however continual dentin deposition reduces the size of the pulp chamber with age, due to recurrent secretion of dentinal matrix by odontoblasts (Solheim, 1992; Kvaal et al., 1995; Goldberg, 2014). This may affect our ability to observe pulp horn shape in very old individuals, as the chamber condenses in size over time altering the original pulp horn shape. Examination of KT2 and KT3's pulp chamber shape (living adults diagnosed with evidence of deficiency), showed pulp chamber changes in permanent molars observed radiographically at age 42 and 46 years. M12 was an 89-year-old living individual without deficiency who exhibited normal even pulp horns and a measurable pulp chamber in the left maxillary 1st molar demonstrating that radiographic examination of pulp chamber shape could still be useful for middle to old-aged adults. However, further work is required to determine how long such features might remain consistently visible for very old-aged adults because the pulp chamber shape can be obliterated with age, making it difficult to observe radiographically.

The exact mechanisms for morphological changes in the pulp chamber are not fully understood, but are likely linked to severity of deficiency. As the pulp chamber and dentin are part of the same functional unit, the pulpodentinal complex (Dean, 2016), we hypothesize that in cases of vitamin D deficiency, there are periods of non-mineralisation in dentin affecting the shape of the pulpal margin during tooth development. Absence of dentin mineralisation could result in a less homogeneous or uneven circumpulpal zone directly affecting pulp horn shape. Another explanation relates to the growth of

calcospherites (calcium salts) in dentin. In normal dentin, the calcospherites continue to grow uniformly, in all directions during tooth formation until contact is made with other calcospherites, forming a homogeneous matrix (Shellis, 1983; Dean, 2016). A lack of dentin mineralisation leads to modification of the calcospherite shape and size and calcospherites close to the pulp chamber that could alter the normal formation of pulp horns in individuals with deficiency. Pulp chamber changes linked to vitamin D, calcium, and phosphate imbalance could potentially initiate an immune response from the dental pulp. Studies have shown that odontoblasts initiate an immune and/or inflammatory response to injury, microbial infiltration, and systemic disease (Yu and Abbott, 2007). Absence of mineralisation could disrupt the odontoblast layer surrounding the pulp chamber and initiate chemotactic (cell movement) signals that cause intercellular spaces to become filled with fluid and proteins, similar to that of an inflammatory response (Kraus et al., 1969:184; Avery, 2002:206). The fluid-filled intercellular spaces are unable to communicate due to a decrease in permeability of pulpal and dentin cells, thus preventing further formation of the pulp horns (Avery, 2002:208).

Although tooth formation differs from bone formation, tooth mineralisation is susceptible to similar failures as bone during vitamin D deficient episodes (Vital et al., 2012; Foster et al., 2014). We propose that loss of vitamin D signaling directly affects dental cell functions that stop the mineralisation process and subsequently affect the shape of the pulp horns. As the pulp chamber and pulp horns form the bulk of the tooth, incomplete mineralisation potentially compromises pulp horn shape. Our preliminary work suggests that the deficiency needs to occur during the initiation of the pulp chamber

formation, but if this happens, then it is severity of deficiency that results in pulp chamber changes. Additional work using clinical radiographs and associated medical data will further understanding of the pathogenesis of pulp tissue to obtain detailed insight into the formation of dentin and pulp chambers in individuals with vitamin D deficiency. Further studies are needed to explore questions such as whether there is a differential sensitivity to deficiency in different tooth types as changes appear most marked in the 1st molar and this could be partly linked to speed of development. Preliminary results from our investigation suggest that radiograph assessment could be used as a screening method to elucidate patterns of deficiency in communities and aid in the selection of individuals for further histological or microCT assessment.

6. Conclusions

The results of this study indicate that individuals with measurable morphological changes to their pulp chamber observed radiographically will have experienced a condition that causes mineralisation defects. To the best of our knowledge, only conditions linked to vitamin D, calcium, or phosphate imbalance cause these types of pulp chamber changes linked to abnormal mineralisation (see D'Ortenzio et al., 2016). Pulp shape abnormalities could be the first stage of evidence for a previously undiagnosed vitamin D deficiency. Our study indicates that severity of a deficient episode needs to be approximately Grade 2 or greater to exhibit marked pulp chamber changes, as less severe deficiency will not influence the formation of the pulp chamber. The use of permanent molars makes it possible to investigate a wide range of ages (~1.5-12.5 years)

that cover critical periods of development of past individuals, enabling a nuanced understanding of patterns of deficiency in the past. Radiographs permit measurements to be taken to corroborate morphological observations and provide a non-destructive aid in the diagnosis of individuals with vitamin D deficiency, particularly where skeletal changes are very subtle. The techniques outlined in this study also have the potential to provide a useful tool to aid in the selection of individuals for microCT or histological assessment. Importantly, the technique can be used on both modern and archaeological individuals, thus addressing the call made by Wright and Yoder (2003) for stronger interaction between modern health research and paleopathology. Radiograph screening of teeth enables better interpretations of health data from past societies and provide a time depth to inform current health debates on vitamin D deficiency in present communities.

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References

Ahmed, H.M., 2012. Management of third molar teeth from an endodontic perspective. Eur. J. Gen. Dent. 1, 148-60

Anthonappa, R.P., and King, N.M., 2015. Enamel defects in the permanent dentition: prevalence and etiology. In: Drummond, B.K., Kilpatrick N., (eds.), Planning and care for children and adolescents with dental enamel defects, Berlin Heidelberg: Springer, pp. 15-30.

Arana-Chavez, V.E. and Massa, L.F., 2004. Odontoblasts: the cells forming and maintaining dentine. Int. J. Biochem. Cell Bio. 36(8), 1367-1373.

Ari, T., 2014. Management of "hidden caries": a case of severe pre-eruptive intracoronal resorption. J. Can. Dent. Assoc. 80, e59.

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.

Avery, J.K., 2002. Development of teeth and supporting structures. In: Avery, J.K. (ed.), Oral Development and Histology (3rd edition). New York: Thieme Medical Publishers.

Beltes, C., Zachou, E., 2012. Endodontic management in a patient with vitamin D resistant rickets. J. Endodont. 38 (2), 255-258.

Brickley, M., D'Ortenzio, L., Kahlon, B., Schattmann, A., Ribot I., Raguin, E., Bertrand, B., 2017. Ancient vitamin D deficiency: long term trends. Current Anthropol. 58 (3), 420- 427.

Brickley, M., 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., and Watamaniuk, L., 2014. Biocultural perspectives of vitamin D deficiency in the past. J. Anthropol. Archaeol. 36, 48-59.

Brooks, S. and Suchey, J.M., 1990. Skeletal age determination based on the os pubis: a comparison of the Acsádi-Nemeskéri and Suchey-Brooks methods. Hum. Evol. 5 (3), 227-238.

Brothwell, D., 1981. Digging up bones. Ithaca: Cornell University Press.

Cahuana, A., González, Y., Palma, C. 2005. Clinical management of regional

odontodysplasia. Ped. Dent., 27 (1), 34-39.

Chapman, M.N., Nadgir, R.N., Akman, A.S., Saito, N., Sekiya, K., Kaneda, T., Sakai, O. 2013. Periapical lucency around the tooth: radiologic evaluation and differential diagnosis. Radiographics 33(1), E15-E32.

Chaussain-Miller, C., Sinding, C., Wolikow, M., Lasfargues, J.J., Godeau, G., Garabedian, M., 2007. Dental abnormalities in patients with familial hypophosphatemic vitamin D-resistant rickets: prevention by early treatment with 1-hydroxyvitamin D. J. Pediatr. 142 (3), 324–31.

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.

Davit-Béal, T., Gabay, J., Antoniolli, P., Masle-Farquhar, J., Wolikow, M., 2014. Dental complications of rickets in early childhood: Case report on two young girls. Pediatrics 133 (4), 1077-1081.

Dean, C., 2016. How the microstructure of dentine can contribute to reconstructing developing dentitions and the lives of hominoids and hominins. C. R. Palevol. 16 (5), 557-571.

Dhir, P., David, C.M., Keerthi, G., 2014. Radiographic manifestations of systemic diseases in jawbones: a systematic review. Asian Pac. J. Health Sci. 1 (2), 120-130.

Douyere, D., Joseph, C., Gaucher, C., Chaussain, C., Courson, F., 2009. Familial hypophatemic vitamin D-resistant rickets-prevention of spontaneous dental abscesses on primary teeth: a case report. Oral Surg. Oral Med. 107 (4), 525-530.

Foster, B.L., Nociti, F.H., Somerman, M.J., 2014. The rachitic tooth. Endocr. Rev. 35 (1), 1-34.

Galhotra, V., Ahluwalia, P., Jodhka, S., Sethi, S., Gambhir, R.S., 2015. Effect of nutritional rickets on dental development in north Indian children: a prospective study. J. Pediatr. Dent. 3 (3), 88-91.

Gallagher, J.C., 2013. Vitamin D insufficiency and deficiency. In: Rosen, T.M., (ed.). Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism, ASBMR. 8th edition. Ames: Wiley-Blackwell, pp. 624-631.

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.

Goldberg, M., 2014. The dental pulp. Biology, pathology, and regenerative therapies. Paris: Springer.

Goodman, A.H., and Rose, J.C. 1990. Assessment of systemic physiological perturbations from dental enamel hypoplasias and associated histological structures. Am. J. Phys. Anthropol. 33 (S11), 59-110.

Harris, R., Sullivan, H.R., 1960. Dental sequelae in deciduous dentition in vitamin D resistant rickets: case report. Aust. Dent. J. 5 (4), 200-203.

Hernández, G.G., Laguna, F.B., 2013. Dental characteristics of hypophosphatemic rickets. Case report. Revista Odontológica Mexicana 17 (2), 101-108.

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

Hillson, S., 2002. Dental anthropology. Cambridge: Cambridge University Press.

Holick, M.F., 2006. Resurrection of vitamin D deficiency and rickets. J. Clin. Investig. 116 (8), 2062-2072.

Kraus, B.S., Jordan R.E., Abrams, L., 1969. Dental anatomy and occlusion. Baltimore: Williams and Wilkins.

Kvaal, S.I., Kalltveit, K.M., Thomsen, I.O., Solheim, T., 1995. Age estimation of adults from dental radiographs. Forensic Sci. Int. 74 (3), 175-85.

Larocque, R., 1999. De la curiosité médicale aux petits maux de tous les jours. Le parcours du simple au complexe de la paléopathologie. Paléo-Québec 29, 9-21.

Lovejoy, C.O., Meindl, R.S., Pryzbeck, T.R., Mensforth, R.P., 1985. Chronological metamorphosis of the auricular surface of the ilium: a new method for the determination of adult skeletal age at death. Am. J. Phys. Anthrop. 68 (1), 15-28.

MacDonald, D.G., Boyle, I.T., 1980. In: J.H., Mason D.K., (Eds), Oral Manifestation of Systemic Diseases. Jones London: W B Saunders Company, pp. 616-659.

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.

Morland, F., Ribot I., 2010. Health and nutrition: a paleopathological and paleochemical study of the historical Euro-Québécois population from the protestant St-Matthew cemetery. Am. J. Phys. Anthrop. (Suppl.) 50, 172-173.

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.

Naji, S., Colard, T., Blondiaux, J., Bertrand, B., d'Incau, E., Bocquet-Appel, J. P., 2016. Cementochronology, to cut or not to cut?. Int. J. Paleopathol. 15, 113-119.

Noyes, F. B., & Thomas, N. G. 1921. A text-book of dental histology and embryology, including laboratory directions. London: Lea & Febiger.

Pereira, C.M., de Andrade, C.R., Vargas, P.A., Della Coletta, R., de Almeida, O.P., Lopes, M.A., 2004. Dental alterations associated with X-linked hypophosphatemic rickets. J Endod. 30 (4), 241-245.

Peterlik, M., 2012. Vitamin D insufficiency and chronic diseases: hype and reality. Food Funct. 3, 784–794.

Rathore, R., Nalawade, T.M., Pateel, D., Mallikarjuna, R., 2013. Oral manifestations of vitamin D resistant rickets in orthopantomogram. BMJ Case Reports. 1-2, doi:10.1136/bcr-2012-008318.

Razavi, S.M., Kiani, S., Khalesi, S., 2013. Periapical lesions: a review of clinical, radiographic and histopathologic features. Avicenna J. Dent. Res. 7 (1), e19435, 1-7.

Reid, D.J., Dean, M.C., 2006. Variation in living human enamel formation times. J. Hum. Evol. 50, 329-346.

Roberts, C., Brickley, M., in press. Infectious and metabolic diseases: a synergistic relationship. In: Biological Anthropology of the Human Skeleton, Katzenberg, A., Grauer, A. (3rd edition). Wiley: New Jersey. Accepted by editors.

Robertson, D., Smith, A.J., 2009. The microbiology of the acute dental abscess. J. Med. Microbiology 58 (2), 155-162.

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 3rd molars. Am. J. Phys. Anthropol. 133 (1), 735-740.

Schour, I., Massler M., 1940. Studies in tooth development: the growth pattern of human teeth. J. Am. Dent. Ass. 27, 1918-1931.

Seeto, E., Seow, K., 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.

Selwitz, R.H., Ismail, A.I., and Pitts, N.B., 2007. Dental caries. Lancet 369 (9555), 51-59.

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, W.K., 1984. X-linked hypophosphataemic vitamin D-resistant rickets. Aust. Dent. J. 29 (6), 371-377.

Shellis RP., 1983. Structural organization of calcospherites in normal and rachitic human dentin. Arch. Oral. Biol. 28 (1):85-95.

Solheim T., 1992. Amount of secondary dentine as an indicator of age. Scand. J. Dent. Res. 100 (4):193-199.

Souza, M.A., Soares, Junior, L.A., Santos, M.A., Vaisbick, M.H., 2010. Dental abnormalities and oral health in patients with hypophosphatemic rickets. Clinics (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.

Stanley, H.R., White, C.L., McCray, L., 1966. The rate of tertiary (reparative) dentine formation in the human tooth. Oral Surg. Oral Med. Oral Pathol. 21 (2), 180-189.

Stanley, H.R., Pereira, E., Spiegel, C., Broom, M. Schultz., 1983. The detection and prevalence of reactive and physiologic sclerotic dentin, reparative dentin and dead tracts beneath various types of dental lesions according to tooth surface and age. J. Oral Path. Med. 12 (4), 257-289.

Tulensalo, T., Ranta, Kataja, M., 1989. Reliability in estimating taurodontism of permanent molars from orthopantomograms. Community Dent. Oral 17 (5), 258-262.

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.

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.

Whaites, W., 2003. Essentials of dental radiography and radiology (3rd edition). Elsevier Science: London, UK.

Worth, H.M., 1963. Principles and practice of oral radiologic interpretation. Chicago: Year Book Medical Publishers Inc.

Wright, L.E., Yoder, C.J. 2003. Recent progress in bioarchaeology: Approaches to the Osteological Paradox. J. Archaeol. Res. 11 (1), 43-70.

Yu, C., Abbott, P.V., 2007. An overview of the dental pulp: its functions and responses to injury. Aust. Dent. 52 (s1), S4-S6.

Zambrano, M., Nikitakis, N. G., Sanchez-Quevedo, M. C., Sauk, J. J., Sedano, H., Rivera, H., 2003. Oral and dental manifestations of vitamin D-dependent rickets type I: report of a pediatric case. Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod. 95 (6), 705-709.

Zilberman, U., and Smith, P., 2001. Sex-and age-related differences in primary and secondary dentin formation. Adv. Dent. Res. 15 (1), 42-45.

Supplementary Data A

Table 1: Radiograph locations and specifications.

Location	Radiograph machine	Meters from source	Pulses/Photon energy
a) McMaster Anthropology Department, Hamilton, Ontario	Vidisco FlashX Pro*	120 cm from the source to x-ray plate	20-29 pulses, equates to 1.394 seconds exposure time; photon energy of 150 kVp
b) Unité de Taphonomie Médico-Légale, Université Lille 2 Droit et Santé, Lille	Nomad Pro handheld X-ray generator (Dental Nomad) associated with an X-Pod Wireless Dental Digital X-Ray Sensor (MyRay)	20 cm from the source to x-ray plate; focal spot, 0.4 mm	60 kV true DC, Anode Current, 2.3 mA, exposure time range, 0.01 - 1.00 seconds
c) Canadian Museum of History, Ottawa, Ontario	Vidisco FlashX Pro*	120 cm from the source to x-ray plate	20-29 pulses, equates to 1.0250 seconds exposure time; photon energy of 150 kVp
d) Laboratory of Ecomorphology and Paleoanthropology, University of Montreal	Vidisco FlashX Pro	51-70 cm from source to the x-ray plate	6-10 pulses; exposure time 0.25 seconds

*Same radiograph equipment.

Table 2: Summary of IIGD severity scores; pulp chamber shape seen histologically and radiologically; and approximate age of episode.

IIGD severity score	Identifier	Age (years)	Past deficiency*	Histological pulp chamber shape	Radiological pulp chamber shape of permanent molars	Approximate age of episode (yrs.) (Moorrees et al., 1963)
Grade 3	15A-S36	~23	✓	RM^1: constricted	N/A	2 episodes between 1.5-2
	15A-S36	~23	✓	LM^3: pulp horns completely flat	LM^3: pulp horns flat	12.5
	SJ 384	40+	✓	RM_1: severely constricted	RM_1: severely constricted	1.5
	SJ 562	40-58	✓	-- single root (RC^1)	**RM^1: constricted	1.5
	SJ 892	40-58	✓	-- single root (RC_1)	**RM_1: chair shape	4
	SJ 970	45-56	✓	LM^1: constricted, chair shape	LM^1: constricted	1.5
Grade 2	19G37-E03	--	✓	RM^1: severely constricted	RM^1: severely constricted	1.5-2

	15A-S36	~23	✓	RM^2: severe constriction	RM^2: severely constricted	5-6
	SJ 384	40+	✓	RM_2: severely constricted	RM_2: severely constricted	5-6
	SJ 562	40-58	✓	LM_2: moderately constricted	LM^1: severely constricted	1.5-3
	KT1	18	✓	RM^3: moderately constricted, chair shape	RM^3: moderately constricted	10.5
Grade 1+	19G37-M03	--	✓	--single root (RI^1)	**RM_1: normal and even; multiple carious lesions obscured view	5.5-6.5
	STA 25C 518	35-49	✓	RM_1: moderately constricted	RM_1: chair shape	1.5-3
Grade 1	SJ 970	45-56	✓	RM^3: moderately constricted	RM^3: moderately constricted (possibly due to occlusal wear)	12.5
	STA 18K 55	50+	✓	LM^1: normal and even	N/A: only one molar (LM^1) available for radiographs	1.5-3
	PAT 7A9 513	16-25	✓	RM^1: normal and even	RM^1: normal, even	1.5-2
	STA 25C 520	16-25	✓	RM_3: normal and even	RM_3: normal, even	11.5

Grade 0	TT1	19	×	RM_3: normal and even	RM_3: normal, even
	KT3	44	✓	-- Rm_1 (deciduous tooth)	**RM_1: chair shape
	STA 25C 55	17-25	×	RM^1: normal and even	RM^1: normal, even
	STA 25A 53	20-34	×	LM^1: normal and even	LM^1: normal, even
	PAT 7A9 546	35-49	×	LM_1: normal and even	N/A: pulp horns difficult to observe
	STA 22A 511	17-25	×	RM_2: normal and even	RM_2: normal, even
	PAT 7A9 561	19-26	×	LM^2: normal and even	LM^2: normal, even
	SJ 892	40-58	×	LM_3: normal and even	LM_3: normal, even
	PAT 7A9 560	25-34	×	RM_3: normal and even	RM_3: normal, even

*Deficiency diagnosed by blood serum levels (living individuals), skeletal changes, and presence/absence of IIGD. **Observed RM_1 on radiograph as this forms near the same time as RI^1, RC^1, RC_1, and roots of Rm_1 (deciduous tooth) (Moorrees et al., 1963).
-- Information not available.

Supplementary Data B

Instructions for Blind Radiograph Test

Instructions for radiographic observation of vitamin D deficiency in permanent molars:

Presented here are instructions on morphological changes seen in pulp horns observed radiographically in permanent molars of individuals with/without deficiency, followed by detailed examples.

1) Focusing on permanent molars in the radiographs, Fig. 1 is a diagram of features of a generic molar. The pulp chamber in the circle is where we need to look and the arrows are pointing to the pulp horns that may be normal (evenly matched) in healthy individuals, or constricted (narrow), or chair shape in cases of deficiency.

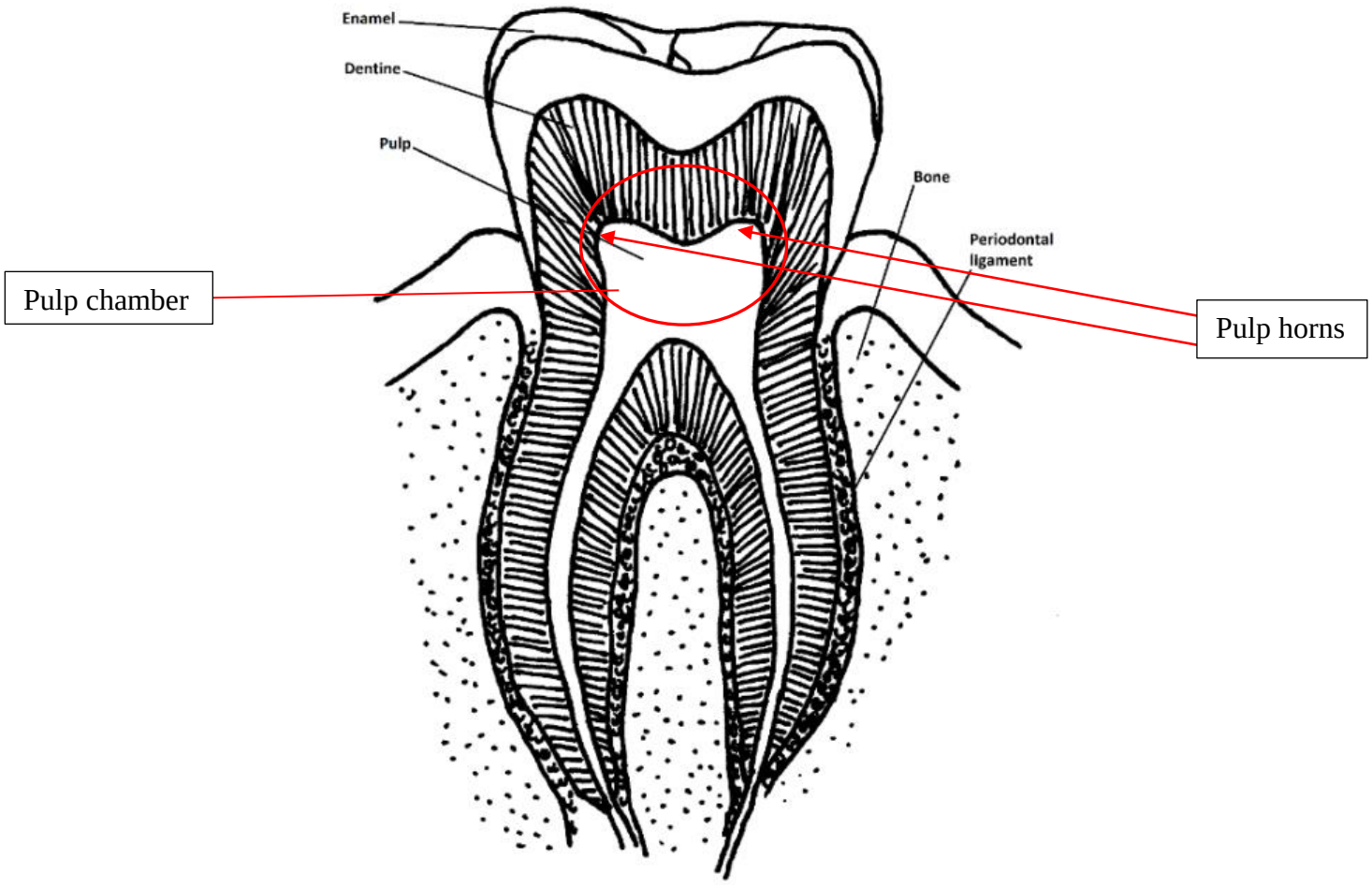


Fig. 1. Diagram of the internal structures of a generic molar showing the location of the pulp horns and pulp chamber.

2) Longitudinally sectioned teeth showing examples of individuals with and without deficiency (Figs. 2a, b).

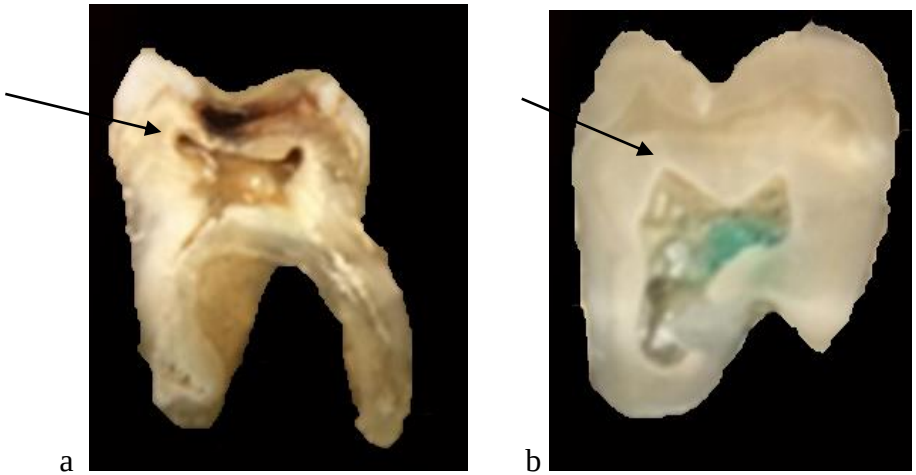


Fig. 2a) St. Matthew, Quebec (15A-S36), adult (age ~23) identified with having four different episodes of rickets through presence of IIGD observed through histological analysis. Pulp horns are constricted and chair shaped; **b)** Healthy living individual lacking deficiency (TT1, age 19) has evenly shaped horns that are matched in height. Black arrows indicate the constricted pulp horns in image a and the evenly shaped (not constricted) pulp horns in image b.

3) Below are radiographs showing examples of an individual with deficiency (a) and a healthy individual (b).

The deficient individual may exhibit the following:

a) Main features are constricted (narrow), and/or chair shape (uneven) pulp horns in the individual with deficiency (a) compared to the evenly matched pulp horns in the healthy individual (b).

b) There may be a flattening of the region between the two pulp horns in deficient individuals (a), whereas in healthy individuals (b) the area is curved, this can be observed in Fig. 2a above.

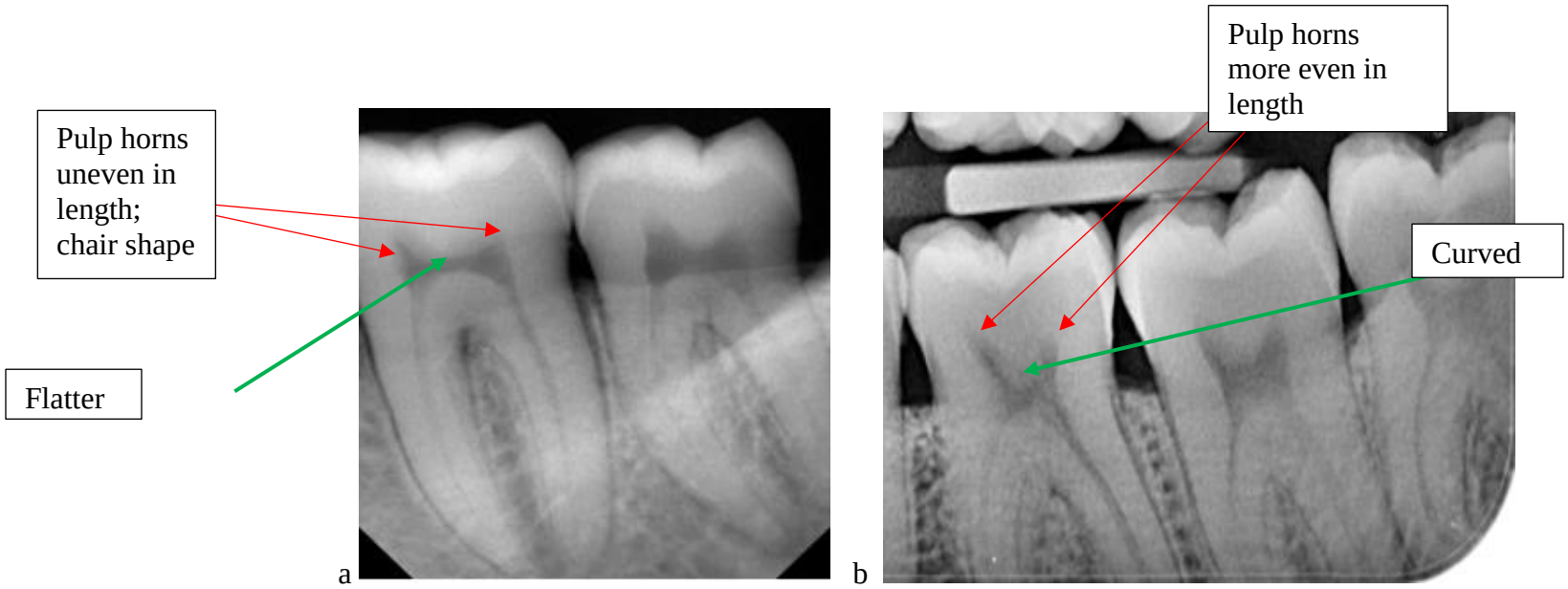


Fig 3a) Radiograph of St. Jacques adult individual (SJ892, age 40-58 years) identified as having past rickets through presence of IIGD observed during histological analysis. Pulp horns are chair shaped; **b)** Radiograph of normal pulp horns on a healthy individual (provided by Rice et al., 2015).

4) Problems you may encounter while observing pulp chambers on radiograph:

Other than the typical problems encountered in any radiograph work related to image sharpness and resolution (i.e., blurring, under/over exposure), the most common difficulty for imaging pulp chambers is positional errors. The angle at which the radiograph is taken can affect how you observe the shape of the pulp horns. Incorrect positioning can cause elongation, foreshortening, or superimposition (overlapping) of the pulp chambers. We recommend that the positioning of the maxilla and/or mandible be as perpendicular to the X-ray beam as possible in order to get the intersection of the beam in a buccolingual direction. Ideally, the X-ray beam should be as close to a right angle relative to the molar as possible. Faulty observations based on radiographs that are not set up at right angles to the X-ray beam can be seen in the example below (Fig. 4 and Fig. 5a, b, c). The pulp chambers appear chair shaped on all three mandibular molars radiographically; however, only the pulp horns in the 1st molar were constricted when observed histologically.

Where only one molar is available for radiographic analysis, we recommend that the tooth be buttressed on the X-ray plate in a buccolingual direction to obtain the correct right or perpendicular angle. If teeth cannot be removed take three radiographs, one for each tooth.

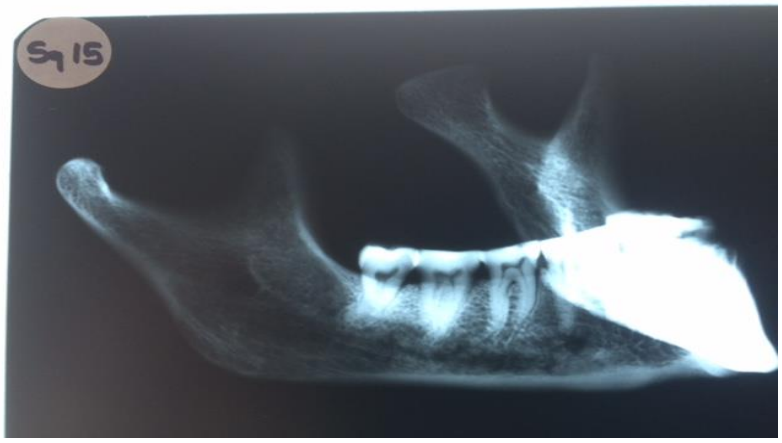


Fig. 4. Radiograph of St. Etienne SQ 15 (with past deficiency; IIGD observed in the 1st molar histologically), showing mandibular right 1st, 2nd, and 3rd molars. The pulp horns appear constricted and/or chair shaped in all three molars.

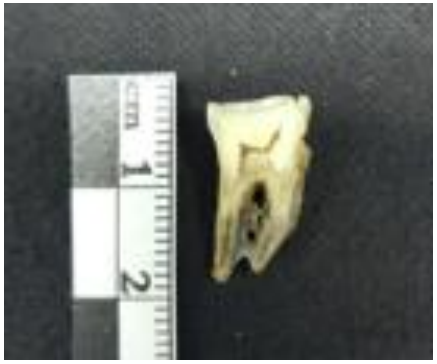
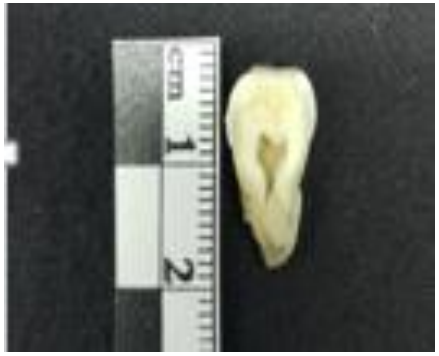
a  b  c 

Fig. 5. Sectioned teeth (seen in above radiograph, Fig. 4) from individual St. Etienne SQ 15; **a)** right mandibular 1st molar; **b)** right mandibular 2nd molar; **c)** right mandibular 3rd molar. The pulp horns are constricted and chair shaped in the right mandibular 1st molar, which is where Grade 1 IIGD was observed histologically. Note: The 1st molar has significant occlusal wear. Grade 1 IIGD appears to be insufficient to affect the shape of the pulp chamber during development, however dental wear may cause the development of reparative dentin and the significant wear seen on the 1st molar likely resulted in morphological changes to the pulp chamber.

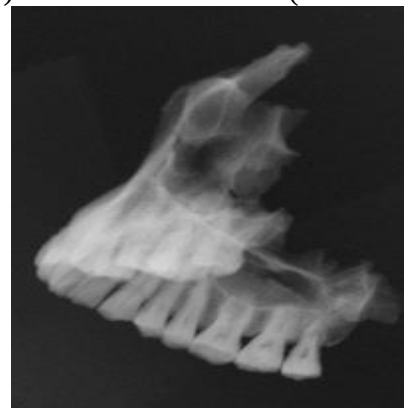
Table 3: Results of Blind Radiograph Test

Case	Blind test answers	Individual 1	2	3	4	5	6	% Correct
1	No	Possible yes	No	No	No	Possible yes	No	4/6=66%
2	Yes	Yes	Yes	Yes	Yes	Yes	Yes	6/6=100%
3	Yes	Undecided	Undecided	Undecided	Yes	No	Undecided	1/6=17%
4	No	No	No	No	No	No	No	6/6=100%
5	No	Undecided	No	No	No	No	No	5/6=83%
6	Yes	Yes	Yes	Yes	Yes	Yes	Yes	6/6=100%
7	No	No	No	No	Yes	Possible yes	No	4/6=66%
8	Yes	Yes	Yes	Yes	No	No	No	3/6=50%
9	Yes	Yes	Undecided	No	Yes	No	Yes	3/6=50%
10	Yes	Yes	Yes	Yes	No	No	Yes	4/6=66%
11	No	No	No	No	No	No	No	6/6=100%
12	Yes	Yes	Yes	Yes	Yes	Yes	Yes	6/6=100%
	Total Correct:	9/12=75%	10/12=83%	10/12=83%	9/12=75%	6/12=50%	10/12=83%	

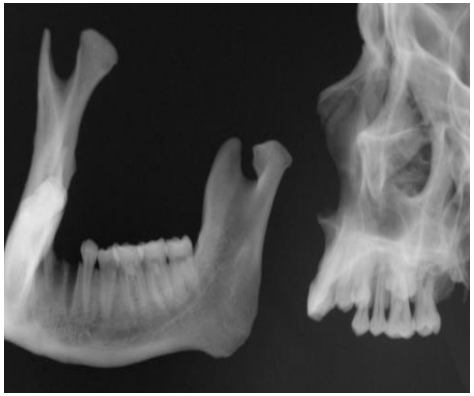
Total Correct Answers: 54/72=75%

Examples from radiograph blind test results

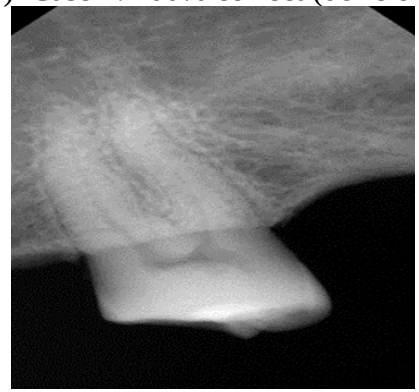
1) Case 3: 17% correct (deficient).



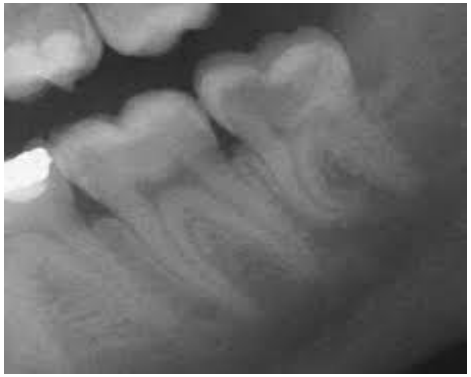
2) Case 9: 50% correct (deficient).



3) Case 2: 100% correct (deficient).



4) Case 11: 100% correct (no deficiency).



Supplementary Data C

Table 1: Pulp horn and pulp chamber measurements on living individuals.

Identifier	Past deficiency	Tooth Type	PH1-h (mm)	PH2-h (mm)	PC-h (mm)	PH1-w (mm)	PH2-w (mm)	Pulp horn Ratio x:1
KT1	✓*	RM_1	3.3	4.6	1.5	0.9	0.6	1.4
		RM_2	4.5	4.4	2.3	0.5	0.3	1.0
		LM_1	3.2	1.4	1.2	0.7	0.9	2.2
		LM_2	2.8	2.0	1.7	0.6	0.9	2.7
		RM^1	3.1	1.1	1.8	0.6	0.8	2.9
		LM^1	2.5	1.9	1.5	0.8	0.5	1.3
TT1	✗*	RM_1	3.6	3.5	1.5	1.3	1.1	1.0
		RM_2	3.8	3.8	1.8	1.1	1.2	1.0
		LM_1	3.8	3.6	1.5	1.2	1.2	1.0
		LM_2	3.0	3.2	1.8	1.1	1.2	1.1
		RM^1	3.0	3.0	2.4	1.0	1.0	1.0
		LM^1	3.5	3.4	2.0	1.2	1.3	1.0
KT2	✓	RM_1	4.2	6.5	2.5	0.9	0.8	1.6
		RM_2	5.0	8.6	6.1	0.9	0.8	1.8
		LM_1	8.1	4.7	4.5	0.9	0.8	1.7
		RM^1	6.3	4.3	4.0	0.5	0.8	1.4
		LM^1	5.7	3.0	1.9	1.1	0.8	1.9
KT3	✓	RM_1	2.0	2.8	0.8	0.5	0.3	1.6
		RM_2	2.5	2.9	1.5	0.9	0.5	1.2
		LM_1	3.2	1.9	0.7	0.5	0.5	1.6

Identifier	Past deficiency	Tooth Type	PH1-h (mm)	PH2-h (mm)	PC-h (mm)	PH1-w (mm)	PH2-w (mm)	Pulp horn Ratio x:1
		LM_2	3.2	2.0	1.3	0.8	0.6	1.6
		RM^1	2.9	3.5	1.3	0.5	0.3	1.4
		LM^1	2.8	0.9	0.8	0.4	0.3	3.0
M11	✓	LM_1	3.5	2.2	1.9	0.9	1.0	1.7
M12	×	LM^1	1.9	1.8	1.1	1.1	1.2	1.1
M13	×	LM^1	1.2	1.2	0.9	1.1	1.1	1.0
M16	×	RM_1	2.5	1.4	1.1	1.3	1.4	1.8
M17	×	LM^1	2.6	2.6	2.3	1.2	1.3	1.0
M18	×	RM^1	2.6	2.7	2.5	1.1	1.0	1.1
M19	×	LM^1	2.3	2.0	1.3	1.5	1.2	1.2
M111	×	RM^1	2.1	2.1	1.5	1.1	1.2	1.0
M22	×	LM_2	2.1	1.6	0.8	1.1	1.0	1.3
M24	×	RM_2	2.5	2.6	1.6	1.3	1.5	1.1
M25	×	LM_2	2.3	2.4	1.6	1.0	1.0	1.1
M27	×	LM^2	1.6	1.5	0.7	1.1	1.1	1.1
M28	×	RM_2	1.6	1.5	1.3	1.1	1.4	1.1
M29	×	RM_2	1.7	2.1	1.6	1.2	1.2	1.3
M210	✓	LM_2	3.0	2.1	1.4	0.8	0.7	1.5
M31	✓	LM^3	3.8	2.2	2.5	0.7	0.5	1.7
M32	×	RM_3	3.2	3.2	1.4	1.3	1.5	1.0
M33	×	LM_3	3.4	3.3	2.3	1.2	1.2	1.0
M34	×	RM_3	3.7	3.7	2.0	1.6	1.7	1.0

Identifier	Past deficiency	Tooth Type	PH1-h (mm)	PH2-h (mm)	PC-h (mm)	PH1-w (mm)	PH2-w (mm)	Pulp horn Ratio x:1
M36	✖	LM^3	3.7	3.4	3.0	1.4	1.9	1.1
M37	✖	LM^3	3.2	3.0	1.7	1.3	1.2	1.0
M38	✖	RM^3	3.3	3.0	2.5	1.5	1.5	1.1
M39	✖	RM^3	2.3	2.4	1.3	1.1	2.2	1.0
M310	✖	LM_3	4.8	4.9	3.8	1.5	1.4	1.0

*✖ = no deficiency; ✓= deficiency

Table 2: Pulp horn and pulp chamber measurements on archaeological individuals.

Identifier	Past deficiency	Tooth Type	PH1-h (mm)	PH2-h (mm)	PC-h (mm)	PH1-w (mm)	PH2-w (mm)	Pulp horn Ratio x:1
19G37-M02	✖*	RM_1	2.9	3.0	2.6	1.1	1.1	1.0
		RM_3	4.7	5.0	4.0	1.2	1.3	1.0
		RM^1	3.7	3.7	2.8	1.0	0.9	1.0
		RM^2	3.5	3.5	2.7	0.8	0.8	1.0
		LM^1	3.7	3.6	2.9	1.2	1.0	1.0
		LM^2	2.9	2.9	2.8	0.9	0.9	1.0
19G37-E03	✓*	RM_1	0.6	1.0	0.7	0.3	0.4	1.9
		RM_2	3.1	1.9	1.2	0.3	0.3	1.6
		RM_3	2.1	1.7	1.3	0.6	0.5	1.2
		RM^1	3.7	2.4	1.6	0.3	0.7	1.5
		RM^2	2.3	1.2	0.7	0.5	0.6	1.9

Identifier	Past deficiency	Tooth Type	PH1-h (mm)	PH2-h (mm)	PC-h (mm)	PH1-w (mm)	PH2-w (mm)	Pulp horn Ratio x:1
		LM_1	2.9	1.6	0.6	0.7	0.7	1.8
		LM_2	3.2	1.6	1.1	0.5	0.5	2.0
		LM_3	1.7	1.1	0.8	0.7	0.6	1.6
19G37- M01	✓	LM_1	2.1	2.4	0.6	0.4	0.5	1.2
		LM_2	2.3	2.7	1.0	0.6	0.4	1.2
		LM_3	2.0	2.3	1.1	0.6	0.5	1.1
		RM^1	3.8	4.3	1.8	0.6	0.5	1.1
		RM^2	4.9	2.5	1.6	0.9	0.9	1.9
		RM^3	3.3	3.7	2.9	1.0	1.9	1.1
19G37-M03	✓	LM_1	4.5	1.9	1.6	0.4	0.2	2.4
		LM_2	2.0	1.1	0.8	0.7	0.6	1.8
		LM_3	2.2	1.9	1.2	1.1	0.8	1.1
		RM^1	3.6	2.3	1.9	0.8	0.5	1.5
		LM^2	2.2	1.4	1.6	0.6	0.0	1.6
39G6-B2	✓	RM_1	3.4	1.6	1.3	0.6	0.4	2.1
		RM_2	4.3	2.9	2.1	0.5	0.0	1.5
		RM^2	2.7	3.3	1.9	0.9	0.5	2.0
		RM^3	2.5	1.8	1.1	0.5	0.3	1.5
		LM^1	1.0	1.3	0.5	0.2	0.2	1.3
		LM^2	2.1	3.1	1.9	0.6	0.5	1.5
		LM^3	3.3	4.2	2.4	0.6	0.9	1.8
		LM_1	2.0	1.1	0.8	0.6	0.8	1.7

Identifier	Past deficiency	Tooth Type	PH1-h (mm)	PH2-h (mm)	PC-h (mm)	PH1-w (mm)	PH2-w (mm)	Pulp horn Ratio x:1
19G37-F05	✖	RM^1	2.2	2.1	1.7	0.9	1.2	1.1
19G37-N01	✓	RM_1	2.6	3.6	2.4	0.8	0.4	1.4
		RM_2	3.9	4.7	2.1	0.9	0.9	1.2
		RM_3	4.3	3.5	2.7	0.5	0.5	1.2
19G41-D01	✓	RM_1	3.2	1.5	0.7	0.5	0.4	2.2
		RM_2	2.5	1.4	1.3	0.5	0.4	1.8
		RM^1	2.3	2.5	0.3	0.2	0.6	2.0
		RM^2	3.2	2.5	1.7	0.3	0.3	2.2
		LM^1	3.3	1.3	0.4	0.3	0.2	2.6
		LM_1	2.9	2.9	0.5	0.2	0.7	1.0
		LM_2	1.1	1.6	1.4	0.2	0.2	1.5
		LM_3	3.6	1.8	1.8	0.7	0.0	4.3
19G35-H01	✖	RM^1	2.5	2.5	1.2	1.2	1.2	1.0
19G37-F04	✖	RM_1	1.4	1.2	1.2	1.3	1.1	1.2
		RM_2	2.6	1.9	2.6	1.1	1.0	1.4
		RM_3	4.2	4.2	3.5	1.2	1.2	1.0
		RM^1	2.2	2.3	1.8	1.0	0.9	0.9
		RM^2	2.9	2.9	2.9	0.0	0.0	1.0
		RM^3	2.6	2.4	2.7	0.0	0.0	1.1
		LM^1	2.4	2.4	2.3	1.1	1.0	1.0
		LM^2	2.5	2.2	2.0	1.3	1.4	1.2
		LM^3	3.0	3.2	3.2	0.0	0.0	1.0

Identifier	Past deficiency	Tooth Type	PH1-h (mm)	PH2-h (mm)	PC-h (mm)	PH1-w (mm)	PH2-w (mm)	Pulp horn Ratio x:1
		LM_1	2.5	2.4	2.2	1.0	1.1	1.0
		LM_2	2.6	2.9	2.8	0.0	1.1	1.1
		LM_3	3.1	3.3	3.2	0.0	0.0	1.1
15A-S36	✓	RM^2	3.4	2.9	2.4	0.8	0.7	1.8
		LM^3	2.6	1.8	1.3	0.4	0.4	1.7
STA 18K 55	×	LM^1	2.3	2.2	1.3	0.9	1.2	1.1
STA 25C 55	×	RM_1	4.8	4.9	4.0	1.1	1.4	1.0
		RM_2	2.0	2.0	0.9	0.9	1.1	1.0
		RM_3	1.6	1.4	0.8	1.1	1.4	1.2
STA 25A 53	×	RM_1	1.2	1.2	0.4	0.7	0.8	1.0
		RM_2	3.9	3.8	2.5	1.0	1.0	1.0
		LM_1	2.0	1.6	0.4	1.1	1.1	1.3
		LM_2	2.6	2.3	1.4	1.3	1.0	1.2
STA 22A 511	×	LM_3	2.8	2.8	1.8	1.1	1.0	1.0
		RM_2	2.2	2.3	1.8	1.1	1.2	1.0
		RM_3	2.7	2.6	1.9	1.1	1.4	1.0
STA 25C 520	×	RM_1	1.8	1.1	0.8	1.1	0.9	1.6
		RM_2	2.7	1.9	1.3	0.9	1.2	1.4
		RM_3	2.1	1.8	1.4	1.1	1.2	1.2
STA 25C 518	✓	RM_1	3.1	2.0	1.8	0.5	0.6	1.6
		LM_1	2.7	1.6	1.4	0.6	0.6	1.6
PAT 7A9 513	×	RM_1	1.6	1.7	0.8	1.0	1.0	1.0

Identifier	Past deficiency	Tooth Type	PH1-h (mm)	PH2-h (mm)	PC-h (mm)	PH1-w (mm)	PH2-w (mm)	Pulp horn Ratio x:1
		RM_2	1.9	1.7	1.2	1.2	1.1	1.1
		RM^1	2.6	2.4	1.8	1.5	1.5	1.1
		LM^1	3.4	3.4	2.3	1.2	1.2	1.0
		LM^2	4.1	3.9	2.1	1.2	1.4	1.1
PAT 7A9 546	×	LM_1	2.2	2.2	1.9	1.0	1.1	1.0
PAT 7A11 560	×	RM_3	1.6	1.5	1.1	1.3	1.2	1.0
		LM_3	1.4	1.3	1.1	1.3	1.0	1.0
PAT 7A11 561	×	LM^2	2.1	2.2	1.8	1.1	1.2	1.0
		RM^2	1.6	1.6	1.4	1.2	1.1	1.0
SJ 384	✓	RM_1	2.5	0.9	0.6	0.3	0.4	2.6
		RM_2	2.3	0.6	0.3	0.4	0.4	4.0
		RM^2	2.1	1.0	0.4	0.4	0.3	2.0
		LM^1	1.4	0.7	0.4	0.5	0.6	2.0
		LM^2	2.5	1.2	0.6	0.6	0.6	2.1
		LM_2	1.5	0.5	0.4	0.5	0.2	2.7
SJ 562	✓	LM^1	1.1	1.5	0.9	0.1	0.1	1.4
		RM^1	2.1	2.4	1.4	0.8	0.7	2.2
SJ 892	✓	RM_1	2.1	0.9	0.6	0.9	0.4	2.4
		RM_2	1.8	1.3	0.6	0.4	0.5	1.4
		RM^2	2.8	1.4	1.0	0.7	0.8	2.0
		RM^3	2.9	0.2	1.5	1.1	0.8	3.8
		LM^2	2.6	1.7	1.0	0.6	0.7	1.5
		LM_2	1.3	1.1	0.5	0.3	0.4	1.2

Identifier	Past deficiency	Tooth Type	PH1-h (mm)	PH2-h (mm)	PC-h (mm)	PH1-w (mm)	PH2-w (mm)	Pulp horn Ratio x:1
SJ 970	✓	LM_3	1.8	1.8	1.0	1.0	1.0	1.0
		RM_1	1.2	1.0	0.3	0.5	0.1	1.3
		RM^1	1.5	1.0	0.5	0.6	0.5	1.5
		RM^3	1.5	1.1	0.8	0.5	0.4	1.4
		LM^1	2.0	1.0	0.7	0.4	0.4	1.9
		LM^2	1.7	0.7	0.6	0.4	0.4	2.6
		LM_1	1.4	0.7	0.4	0.3	0.4	1.9

*✖ = no deficiency; ✓ = deficiency

Supplementary Data D

Table 1: Radiograph measurements of KT3's 1st molar over 7-year time period.

Identifier	Tooth Type	PH1-h (mm)	PH2-h (mm)	PC-h (mm)	PH1-w (mm)	PH2-w (mm)
KT3, Year 1	RM_1	2.8	2.0	1.2	0.5	0.3
KT3, Year 2	RM_1	2.8	2.1	1.2	0.6	0.3
KT3, Year 3	RM_1	2.8	2.0	1.2	0.5	0.4
KT3, Year 4	RM_1	2.8	2.1	1.2	0.5	0.3
KT3, Year 5	RM_1	2.8	2.0	1.3	0.5	0.3
KT3, Year 6	RM_1	2.8	2.0	1.2	0.6	0.3
Averages:		2.8	2.0	1.2	0.5	0.3
MAE*		±0.01	±0.03	±0.01	0	±0.01

*MAE= mean absolute error

Brief Communication: Age estimation in older adults: Use of pulp/tooth ratios calculated from tooth sections

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Abstract:

Objectives: Accurate age estimates are foundational for bioarchaeological research, yet the ability to accurately age older adult skeletons remains elusive. This study uses a new version of pulp/tooth area calculations to investigate chronological age of older archaeological individuals.

Materials and Methods: Pulp/tooth area ratios were calculated on modern control teeth (n=10) that were first radiographed and then sectioned for comparative analysis. Pulp/tooth area ratios were determined on sectioned teeth using ImageJ software for: 1) modern individuals of known age (n=26); 2) individuals from Belleville, Ontario, Canada (1821-1874) with documented age (n=50); 3) Belleville individuals with skeletally estimated age (n=122).

Results: Calculations from tooth sections on modern teeth (n=10) resulted in a mean absolute error (MAE) of ±3.9 years, whereas the radiographic method for the same teeth had an MAE of ±14.45 years. Results indicate that sectioned pulp/tooth area ratios are a significant predictor of chronological age ($p < 0.005$), with MAEs of ±4.53 years for Belleville and ±3.77 years for modern individuals. There were no statistically significant differences in age estimations between modern and archaeological individuals, or with respect to tooth type, sex, or intra/inter-observer estimations.

Discussion: This study provides a new more accurate method for estimating age-at-death, particularly for individuals in the 50+ age category. Sectioning the teeth and directly measuring exposed pulp chambers results in age estimations that were within ±4.15 years for both modern and archaeological individuals, thus presenting a method that will enhance the ability to age older individuals.

Keywords: dental histology, bioarchaeology, older adult age estimation

Accurate estimation of age-at-death in older adults is both crucial and problematic in bioarchaeology as age is a fundamental characteristic needed to investigate past communities. There are a number of methodological issues that interfere with the ability to investigate older age groups in skeletal samples, hence the widespread use of the broad 50+ age category for older adults. The most commonly used age-at-death estimates for the adult skeleton are based on macroscopic analysis of different skeletal elements, such as; the pubic symphysis (e.g., Suchey and Katz, 1998); the auricular surface of the ilium (e.g., Lovejoy et al., 1985; Buckberry and Chamberlain, 2002); sternal rib ends (e.g., İşcan and Loth, 1989); and cranial sutures (e.g., Meindl and Lovejoy, 1985). These techniques rely on visual assessment of skeletal development and degeneration, and generally produce large, imprecise age ranges for individuals beyond 50 years of age (Chamberlain, 2006; Martrille et al., 2007). Transition analysis, a technique that uses multiple age indicators by combining cranial suture, auricular surface, and pubic symphyseal measurements, produce age estimation that become uncertain in the 40-70 age range (Milner and Boldsen, 2012). Currently, skeletal methods result in age-at-death estimations that are often too narrow (e.g., ages 18-19, Todd, 1920), too broad (e.g., ages 25-83, phase 5 in Brooks and Suchey, 1990), or simply too vague (e.g., ages 50+).

The human skeleton ceases systematic growth after the age of 35, therefore changes in the human skeleton become increasingly random (Angel, 1984; Lucy and Pollard, 1995a; Lucy et al., 1995b). These factors have the potential to skew the age of older adults when using the standard skeletal methods of age-at-death estimation (Milnar and Boldson, 2012). Researchers using skeletal aging techniques to estimate age have

reported bias in age estimates, referred to as 'attraction towards the middle' (e.g., Bocquet-Appel and Masset, 1996; Boldsen et al., 2002; Hoppa and Vaupel, 2002b; Prince and Ubelaker, 2002). This results in a tendency to consistently overestimate age in younger individuals while underestimating age in older individuals, as estimated ages are closer to the mean age of the sample population rather than the actual chronological age (Aykroyd et al., 1997). In bioarchaeological research, underestimating age-at-death for older adults may lead to the conclusions that: 1) older adults were excluded from the community being examined, or 2) that survivorship did not occur beyond age 50 years (Buikstra and Konigsberg, 1985). The difficulty in accurately aging the older individual affects demographic profiles by excluding mortality data on individuals over age 50 (Walker et al., 1988). Including the elderly in the age distribution of a community is essential as many pathological conditions are age-cumulative (e.g., degenerative joint disease, osteoporosis) (Glencross and Sawchuck, 2003). Current techniques in age estimation use open-ended terminal intervals (typically 46+ or 50+), therefore little can be said about pathological conditions in older age groups, other than certain conditions were present.

Continuing problems with estimating age-at-death in the older skeleton is why a closer examination of techniques that use teeth may provide a way forward. Teeth, particularly the dentin found in teeth, retain characteristics valuable for bioarchaeological studies. Tooth development and morphology is controlled by a strict genetic program specific to humans (Scott, 1997), and while there is inter-population variation in tooth dimensions, they are less than those found in body shape and size (Ruff, 2002), making

teeth an ideal tissue to investigate adult age estimation. For example, Bishara et al. (1989) analysed crown dimensions in three populations from Egypt, Mexico, and the United States, and found that differences were of very small magnitude, and were not considered clinically significant. Bodecker (1925) first determined that the apposition of secondary dentin was inversely correlated with chronological age. Gustafson (1950) later pioneered a study on secondary dentin deposition in which dentin transparency and secondary dentin calculations showed the highest correlation with age. Secondary dentin begins to develop once the tooth crown is fully formed and the root complete. The apposition of secondary dentin reduces the size of the pulp chamber due to the continuous secretion of dentin by odontoblasts. Dentin is a living tissue containing odontoblasts that form the bulk of the tooth and as one ages, the odontoblasts deposit concentric layers of secondary dentin that gradually obliterate the pulp chamber (Bodecker, 1925; Vasiliadis et al., 1983). Physiologically, secondary dentin formation is similar in both permanent multi-rooted and single-rooted teeth. Secondary dentin is slowly deposited and metabolism of the dentin is maintained by pulp tissues (Benzer, 1948). The average rate of increasing dentinal thickness is approximately 6.5 μm per year for the crown and 10 μm per year for the root (DeLuca et al., 2010). As secondary dentine is laid down at the pulpal boundary of primary dentine, the pulp cavity decreases in size with age (Gustafson, 1950; Morse, 1991; Solheim, 1992; Hillson, 2002). Based on this physiological process researchers have measured the length, width, and area of the pulp chamber to correlate a reduction in size with increasing age (e.g., Solheim, 1992; Cameriere et al., 2007a, b). Solheim (1992) reported a positive correlation between the growth of secondary dentin and increasing age

by measuring pulp width relative to tooth width in sectioned teeth. This demonstrates the importance of analysing tooth sections, as secondary dentin is a continually accruing tissue that is not remodelled; once layers are deposited, they remain throughout life to serve as a permanent indicator of age.

The potential to evaluate secondary dentin growth using dental radiographs has been investigated by researchers such as Cameriere et al. (2007a, b) who studied the relationship between age-at-death and the ratio of the pulp/tooth area in periapical radiographs of upper and lower canines in Italian skeletons. Cameriere and colleagues (2007a, 2007b) have clearly demonstrated that secondary dentin deposition is correlated with age using the pulp/tooth ratio radiograph technique in individuals from Italian cemeteries. Joseph et al.'s (2013) study applied Cameriere et al.'s (2007a, b) method successfully on radiographs of premolars for 120 modern Indian individuals, where prior to this, other studies on Indian populations (e.g., Babshet et al., 2010) had a mean error of >10 years. Studies using cone-beam CT provide a three-dimensional virtual section of the tooth and have been used to age living individuals (e.g., Yang et al., 2006; Star et al., 2011). However, similar to some radiographic studies (e.g., DeLuca et al., 2010), there were few individuals over the age of 60 years included in these studies and while this technique is non-destructive, it is not always available to researchers.

Older adults form an important component of archaeological skeletal collections that at present remain an un-tapped source of information. This study compares age-at-death estimates from dental radiographs with those derived from tooth sections of modern teeth to test the hypothesis that features of secondary dentin deposition observed will

provide a more accurate means of estimating age-at-death for adults over the age of 50 years. We then apply this method to individuals from the 19th century Belleville skeletal sample to determine if this method can be successfully used on archaeological skeletal material.

MATERIALS AND METHODS

2.1 | Comparison of age calculations from radiographs and tooth section images for known aged modern individuals

Single-rooted teeth were chosen for this study as they are often present in old age and are less likely than molars to undergo attrition or abrasive wear. Single-rooted teeth have pulp chambers that are similar in size and shape, therefore are the easiest to conduct pulp/tooth area ratio measurements (Azevedo et al., 2014). Note that maxillary 1st premolars were also excluded as they have two roots, but all other premolars (2nd maxillary premolars, 1st and 2nd mandibular premolars), maxillary and mandibular canines, and central and lateral incisors were used as they have one root (see Additional Supporting Information A and B). Teeth with carious lesions, fillings or crowns were excluded from the study. To negate the effects that heavy dental wear has on pulp/tooth area ratio measurements and avoid potentially under-aging some of the individuals in this study, teeth that scored more than a grade 2 using the dental wear table developed by Molnar (1971:178) were also excluded. Kvaal et al.'s (1995) study did not find significant differences in measurements between teeth from the left or right side of the maxilla or mandible; therefore, teeth from both sides were used in this study.

To test the utility of calculations from tooth sections versus the radiographic aging method, a sample (n=10) of randomly chosen modern teeth from individuals of known

age (HIREB ethics approval 14-670-T) were radiographed. These teeth were used as a control for direct comparison of age calculations between the histological and radiographic methods. Permanent canines, incisors and premolars were radiographed with a Vidisco FlashX Pro radiograph machine. The teeth were placed individually on the X-ray plate at a distance of 120cm from the source. To ensure consistency and facilitate comparison with the histological images, the teeth were positioned perpendicular to the X-ray beam in order to obtain the intersection of the X-ray beam in a mesial-distal orientation. The number of pulses was selected (~20 pulses), which equates to 1.4 seconds of exposure time, with photon energy of 150 kVp. The images were then converted to JPEG files and saved to a database.

2.2 | Age calculations for modern and archaeological teeth

The second phase of this study calculated age for three other groups of individuals using sections of teeth. The first group were modern individuals of known age (n=36) from Ancaster, Ontario, that acted as controls and included the original 10 teeth used for comparative analysis. Modern individuals volunteered their age at time of tooth extraction. The second and third groups were archaeological tooth samples from Belleville, Ontario, Canada (n=172; 50 with documented age from parish records; 122 with no documented age). Age-at-death for the undocumented Belleville individuals was estimated by Rogers (1991) using skeletal morphological methods (i.e., dental development and long bone length for subadults; pubic symphyseal and auricular surface features for adults). These individuals were interred in St. Thomas' Anglican Church cemetery located in Belleville, Ontario (1821-1874), (Fig. 1). In 1989, 579 graves were

excavated and the skeletal remains were investigated, followed by reburial in 1990 at St. Thomas' after the project was completed. Permission was obtained by McMaster University to retain samples from the Belleville collection and the teeth were used for this research.

Fig. 1. Belleville located in southern Ontario, Canada.

The Belleville tooth samples were previously prepared by Saunders et al. (2007) for analysis. All samples were ultrasonicated for 15 minutes in distilled water. The samples were embedded in a chemical-setting resin (Epothin), then temporarily bonded with melted dental sticky wax to a chuck cutting using a Buehler IsoMet 1000 saw. The sawn face of the tooth was adhered to a microscope slide using a UV-activated adhesive. The thin-sections 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 3µm

diamond polish, followed by 1 μm diamond polish on a microcloth pad. The polished samples were ultrasonicated for 15 minutes in distilled water. Modern tooth samples were not embedded in resin, but were adhered to a chuck with dental sticky wax and sectioned longitudinally in a buccolingual direction into two equal half sections with the Buehler IsoMet 1000 saw. Both the Belleville thin-sections and the sectioned modern teeth were imaged using a Nikon DsR:1 camera attached to an Olympus BX51 digital microscope (no magnification) and saved as high-resolution JPEG files.

2.2.1 | Pulp to tooth area ratio measurements from images to calculate age

The radiographs from the first set of control teeth (n=10) and microscopic images for all of the sectioned teeth (n=208) were imported into the image editing software program ImageJ to perform the area measurements. The scale on the microscope images of tooth sections and a one-inch ball on the radiographs were used to calibrate the images imported into ImageJ. Using the Straight Line Tool in ImageJ, each image was calibrated either to the microscopic scale, where 1 mm equaled ~70 pixels or the one-inch ball where1 mm equaled ~ 6.5 pixels. The outline of the tooth and the pulp chamber were marked separately using the Polygon Selection Tool and the area was automatically derived for the tooth and the pulp chamber. Approximately fifty points from each tooth outline and 40 for each pulp outline were identified and connected with the Line tool to determine the area of both tooth and pulp chamber (Fig. 2). The pulp/tooth area ratio was calculated (mm^2) by dividing pulp area by the tooth area. Pulp/tooth area ratios were used to predict individual age using the appropriate regression formula.

Fig. 2a. Outline of modern tooth (M170) using ImageJ; maxillary incisor; b. Outline of pulp chamber for M170 in ImageJ. Dots indicate points taken using ImageJ software.

Regression formulae developed by Cameriere et al. (2007a, b) and Joseph et al. (2013) were used to estimate age on both the radiographs and images of sectioned teeth. These formulae were employed in this study as they were successfully used to estimate age of living individuals (Joseph et al., 2013), and to estimate age-at-death for forensic and archaeological individuals of unknown age (Cameriere et al., 2007a, b, 2013; DeLuca et al., 2010). As we directly compared calculated age estimates from both radiograph

images and tooth sections from the same individuals (controls), the same regression formulae were used to prevent introducing any bias into the test. Secondly, these equations could age people above age 60 years, as other equations cut off at age 60 years or less (e.g., Zaher et al., 2011). These equations were also formulated on the same tooth types that we analysed. Cameriere et al.'s (2007a, b) linear regression equations were used for canines and Joseph et al.'s (2013) equation for premolars and incisors:

Upper canine: Age = 99.937 – 532.775 x (pulp/tooth ratio) (Cameriere et al. 2007a, b)

Lower canine: Age = 89.456 – 461.873 x (pulp/tooth ratio) (Joseph et al. 2013)

Premolar/Incisor: Age = 89.778 – 379.020 x (pulp/tooth ratio) (Joseph et al. 2013)

2.3 | Statistical analysis:

Statistical analysis was conducted using statistiXL, a Microsoft excel program. Descriptive statistics and the mean absolute error (MAE) were calculated for all three groups of individuals. The mean absolute errors (MAE) were determined to measure how far calculated age differed from known age or skeletally-estimated age. Paired t-tests on the MAEs determined any significant differences between calculations from radiographic versus images of tooth sections. To test intra-observer error reproducibility, a random sample of 30 images (10 modern and 20 Belleville) were re-examined after an interval of 6 months. Inter-observer error was conducted on the same 30 images by two individuals with no prior experience using ImageJ. Paired t-tests were performed to reveal any statistically significant differences between inter- and intra-observer measurements, tooth types, and males and females. Regression equations were used to measure the association

between calculated age from the pulp/tooth area ratio method and known or documented/estimated age.

RESULTS

3.1 | Phase 1: Comparison of age calculations from radiographic versus microscopic images of tooth sections

Age calculations were performed on radiographic and images of tooth sections for the same teeth in a subsample of modern individuals (n=10) for comparative analysis (see Supporting Information A). The mean absolute error (MAE) from the radiograph images ranged from ±5.9 to ±20.5 years (average 14.45 years), whereas the MAEs for the images of sectioned teeth ranged from ±0.3 to ±8.8 years (average ±3.9 years). When comparing calculations from radiographic versus images of tooth sections, paired t-tests determined this difference was significant (p= 0.00002). Known and calculated ages are compared graphically in Figure 3 for all 10 modern individuals.

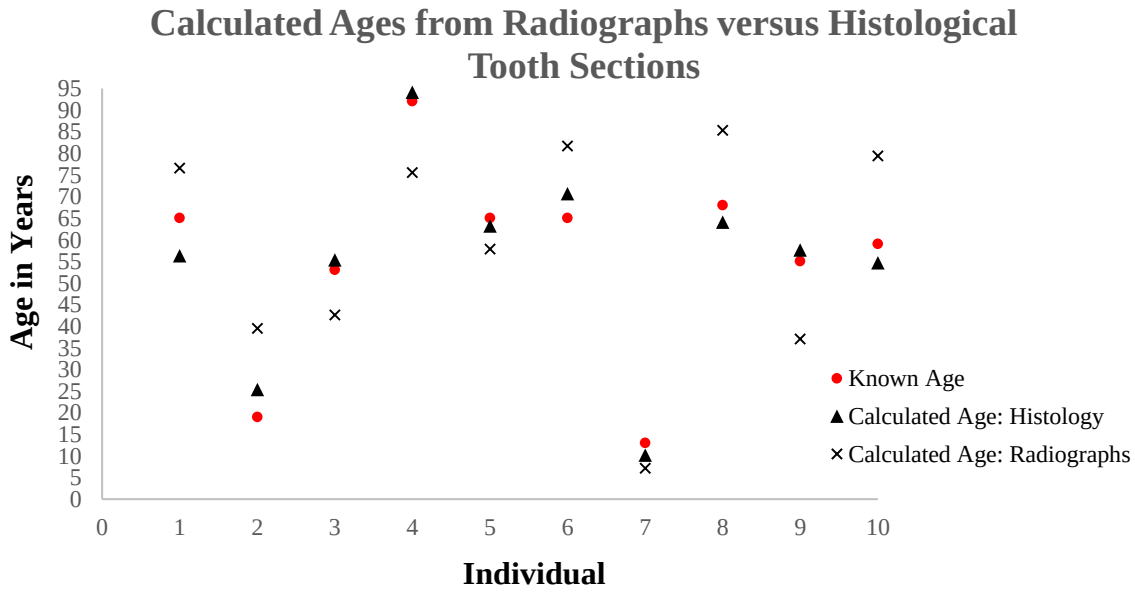

Fig. 3. Calculated ages from images taken from radiographs and histologic tooth sections for modern individuals of known age (n=10).

3.2 | Phase 2: Age calculations from images of tooth sections

Table 1 presents descriptive statistics for modern and Belleville individuals and the mean absolute error (MAE) between known or skeletally-estimated age (in years) for each group is shown in Table 2. The pulp/tooth area ratio calculations for all modern and Belleville individuals are presented in full in Additional Supporting Information B.

Table 1: Descriptive statistics for modern and Belleville individuals.

Identifier	N	Mean of Ages	Min (years)	Max (years)
Modern known age	36	62.2	13	98
Belleville documented age	50	49.3	1	89
Belleville skeletally estimated age	122	41.7	0.8	80+

Table 2: Mean absolute errors (MAE) for all individuals.

Identifier	N	Mean absolute error in years (MAE)
Average MAE for modern known age individuals	36	±3.77 years
Average MAE for Belleville documented age individuals	50	±3.93 years
Average MAE for Belleville skeletally age estimated individuals	122	±5.12 years

Paired t-tests revealed no significant intra- or inter-observer variation, and no significant differences based on sex in the modern individuals and the Belleville documented/estimated ages. There were also no significant differences in calculations from different tooth types (incisors vs canines vs premolars) (p values ranged from 0.419-0.989). Pulp/tooth area ratios for modern and Belleville individuals were significantly inversely correlated with documented/estimated and known ages (all p-values <0.05, Table 3); that is, the pulp/tooth area ratios progressively decreased as age increased.

Table 3 shows the R^2 values, which provide an estimate of the strength of the relationship between pulp/tooth area ratios and age. Regression formulae on tooth sections resulted in accurate age estimates to within ±3.77 to ±5.12 years for both modern and documented Belleville individuals (Table 3), even though many individuals tested were in the 50+ range (Belleville: 69/172 over age 50; modern: 32/36 over age 50). Graphs of the inverse relationship between pulp tooth area ratios versus documented and skeletally-estimated ages for Belleville individuals and known aged modern individuals are displayed in the Additional Supporting Information B (Figs. 1-3). Figures 4-6 present documented/estimated and known age versus calculated age for the Belleville and modern individuals.

Table 3: Summary of R^2, standard errors, and p-values for pulp-tooth area ratios.

Identifier	R^2	Standard Error	P-value
Modern known age	0.928	5.930	0.005
Belleville documented age	0.942	5.198	0.003
Belleville skeletally estimated age	0.744	8.876	0.002

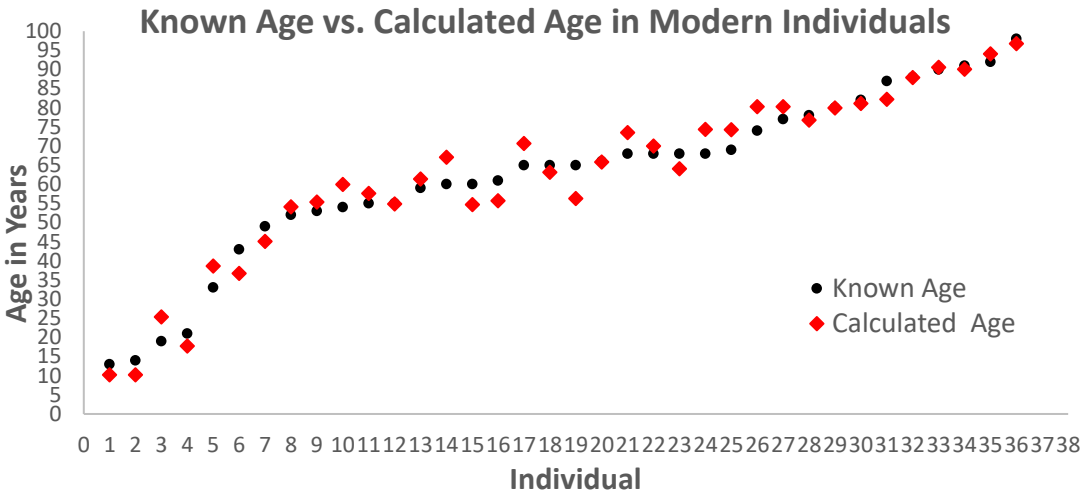

Fig. 4. Known age versus calculated age in modern individuals.

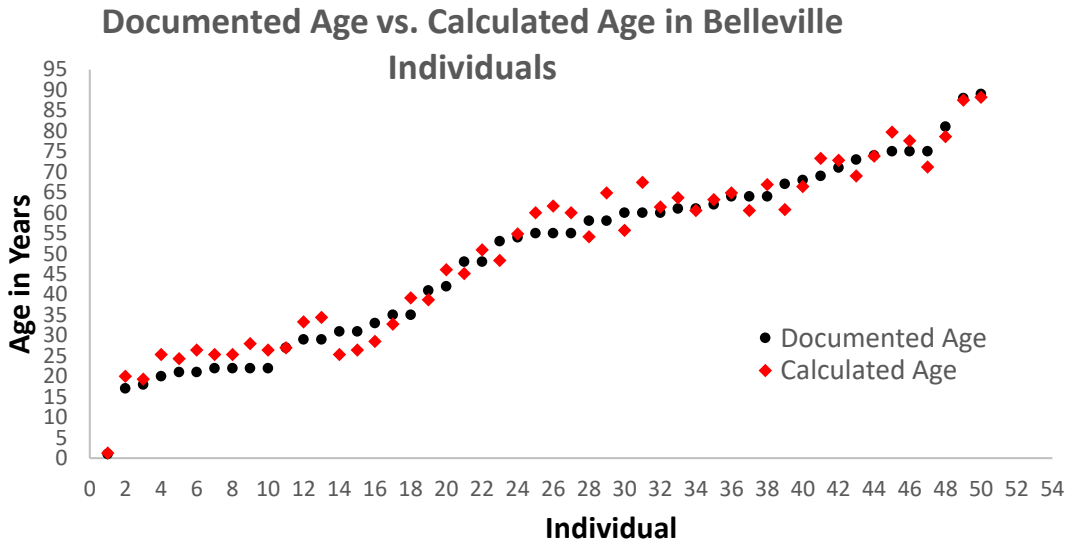

Fig. 5. Documented age versus calculated age in Belleville individuals. Age-at-death was documented in St. Thomas' Anglican Church's parish records.

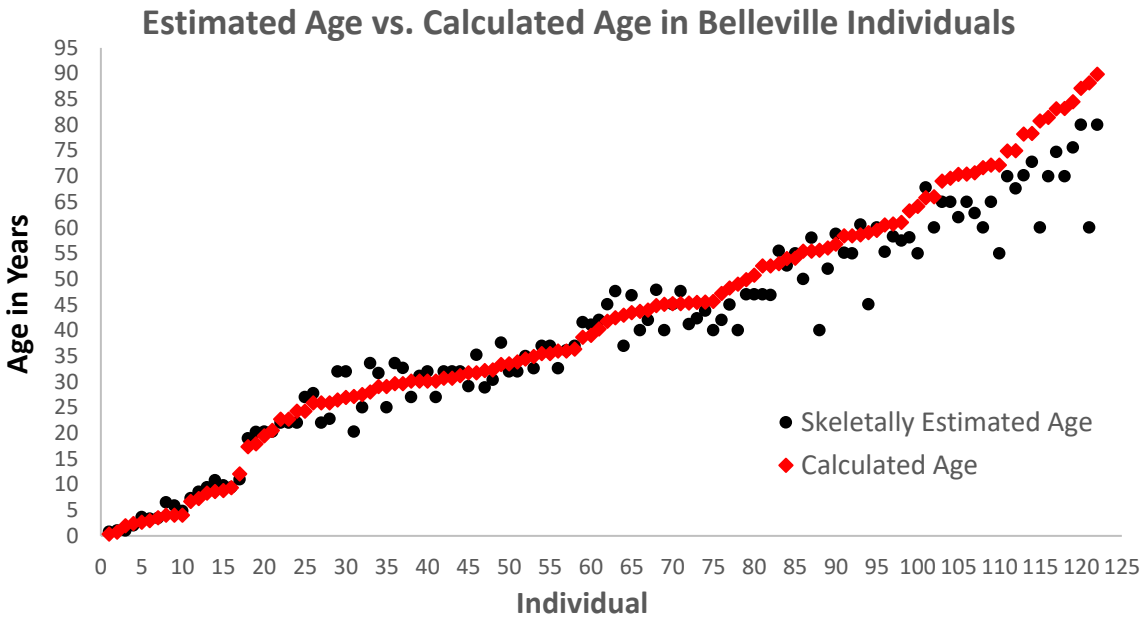

Fig. 6. Estimated age versus calculated age for Belleville individuals. Age-at-death was estimated using skeletal morphological methods (e.g., pubic symphysis and auricular surface).

DISCUSSION

Estimation of age-at-death, particularly in older adults, is one of the most challenging aspects of bioarchaeological research. This study investigated the estimation of age-at-death using direct measurements of tooth sections to calculate the pulp/tooth area ratios developed by Cameriere et al. (2007a, b). To assess the applicability of a pulp/tooth area ratio technique, we first conducted pulp/tooth area ratio age calculations on modern teeth that had been both radiographed and sectioned (n=10) to directly compare the two methods. The difference between the MAEs for the radiographic versus the technique conducted on tooth sections was significant (p= 0.00002), with MAEs for

the radiographed teeth averaging ±14.45 years, and MAEs averaging ±3.9 years for the method using tooth sections (Fig. 3). The more precise results of the MAEs for this method led to the second phase of the study that determined pulp/tooth area ratio age calculations on another group of known aged modern individuals (n=26) and Belleville individuals (n=172) with documented or skeletally-estimated age. There were no significant differences in precision of age estimates between males and females, suggesting that it is not necessary to know the sex of the individual when applying this method (modern known age group ($p = 0.461$); Belleville documented age group ($p = 0.272$); Belleville skeletally-estimated age group ($p = 0.536$). This is useful in bioarchaeological studies when sex is unknown, particularly for older individuals.

The paired t-tests revealed low intra-and inter-observer error (p-values = 0.964 and 0.999 respectively), indicating that the method is easily replicable. There were also no significant differences in calculations for different tooth types, indicating that the method works well for all of the teeth tested ($p= 0.419$-0.989), suggesting that any single-rooted tooth can be used. This is useful for older skeletons as they often have teeth missing antemortem and this increases the range of teeth that can be used for age estimation. As anticipated, the pulp/tooth area ratios consistently decreased as age increased along the whole age continuum (Figs. 4-6). Table 4 presents the MAEs for different age ranges and indicates that they remained low even as age increased in individuals of known age. The Belleville documented age group and modern individuals had MAEs between ±3.77 to ±5.12 (Table 2), signifying that each individual was correctly aged to within approximately ±4 to 5 years.

Table 4: MAEs for different age ranges in modern and both groups of Belleville individuals.

Age range (years)	MAEs for modern individuals: known-age	MAEs for Belleville individuals: documented age	MAEs for Belleville individuals: skeletally estimated age
0-19	±4.3	±3.7	±1.1
20- 29	±3.2	±4.4	±3.4
30-39	±5.7	±4.8	±3.3
40-49	±5.1	±3.6	±5.2
50-59	±2.6	±4.7	±6.3
60-69	±4.8	±3.6	±8.8
70-79	±3.6	±3.2	±6.2
80-89	±1.5	±1.4	±7.2
90+	±2.6	N/A	N/A

The regression equation used in Cameriere et al.'s (2004) study found that age explained 84.9% of the total variance observed ($R^2 = 0.849$). The present study obtained more accurate results with both Belleville and modern individuals where ages were documented or known (Table 3). This study found that age explained between 92.8 - 94.2% total variance using the regression equations for Belleville individuals (Additional Supporting Information B, Figs. 1-3). Our results show that more precise data can be obtained by direct measurement of the exposed pulp chamber versus the measurements calculated from pulp chambers observed on radiograph images. Reasons for less accurate results obtained using radiographs are related to image sharpness and resolution (i.e., blurring, under/over exposure), and the most common difficulty for imaging pulp chambers is positional errors. Incorrect positioning can cause elongation, foreshortening, or superimposition (overlapping) of the pulp chambers. Positioning of the tooth should be as perpendicular to the X-ray beam as possible in order to get the intersection of the beam in a buccolingual direction. (D'Ortenzio et al., in press).

The lower R^2 values (0.744) found in the skeletally-aged Belleville individuals was likely due to the original age estimations being conducted skeletally versus those conducted on individuals with known ages. This was anticipated, as results from adult skeletal aging techniques are often imprecise, particularly in older age groups (Milnar and Boldsen, 2012). This tendency was noted in the skeletally-aged Belleville individuals (Fig. 5), where older individuals appeared to be consistently under-aged skeletally starting at approximately 40 years of age.

When comparing this technique with other dental age estimation methods, results appear to be consistent with the others and, in most cases, surpass the results achieved by other dental age estimations methods. Stott et al. (1982) counted cementum annuli and achieved an accuracy of ±4 years deviation from true age. Critical issues with this technique is a lack of consistency in counting the rings (Jackes, 2011), and that accuracy diminishes with increasing age (particularly over 50+ years) due to a reduction in the alternating light and dark bands needed to discern the cementum rings (Lipsinic et al., 1986; Condon et al., 1986), making this technique unsuitable for aging older individuals. Tooth root translucency is a method that also showed initial promise (e.g., Bang 1972, 1989) with good correlations between estimated and actual age (R^2= ~0.7), but later studies found lower correlations (e.g., R^2= 0.35, Lopez-Nicolas, 1990) and large standard errors (±12.8 years) (Drusini et al., 1991). Cameriere et al.'s (2004) study investigated pulp/tooth area ratios using radiographs as an indicator of age and using maxillary canines, they found that pulp/tooth area ratios were better correlated with chronological age than Solheim's (1992) tooth, root, pulp length, and width linear measurements

(Cameriere et al., 2004). While the authors found a high level of accuracy in age prediction, their study sample only included 12 (out of 100) individuals above the age of 60 years and no individuals over the estimated age of 73 years. Similarly, Joseph et al. (2013) reported an average MAE of ±5.38 using premolars, however age estimations were far more accurate in the 36-50-year age range.

Current methodological limitations in bioarchaeology render the elderly invisible, as precise ages are not known, which further confounds our understanding of older individuals in past communities. Age is an important aspect in the creation of a biological profile, yet the nature of aging and old age remains challenging to explore in detail (Gowland, 2007; Appleby, 2010, 2011). All bodies do not age in a uniform manner nor according to the same timetable, therefore it is crucial to age older individuals accurately (Gowland, 2007; Sofaer, 2011). Teeth, however, are durable and evolutionarily conservative making them less susceptible to the variability found in the skeleton (Smith et al., 1997). There are ways to address these inherent problems related to age-at-death estimates using dentally-based methods. The use of pulp/tooth area ratios on sectioned teeth is directly quantifiable (Rösing and Kvaal, 1998) and eliminates the necessity of placing a skeletal element into an age phase. An advantage of this technique is that it covers the whole adult age range, whereas, with the exception of the auricular surface method (Lovejoy et al., 1985b), most skeletal methods have an upper age limit of 50 years. As this is a preliminary study, future research will test this method on different populations to determine its wide-scale applicability. An approach to enhance age prediction may be to use additional teeth and develop multiple regression models for

several types of teeth. An informed knowledge of aging is a critical component to understanding the evolution of the human life span and using the technique outlined above can assist in constructing a more nuanced picture of old age.

CONCLUSIONS

Inherent challenges related to adult age-at-death estimates can be addressed by calculating the pulp/tooth ratio directly from tooth sections. This study found a significant correlation between chronological age and the size of pulp chambers due to apposition of secondary dentin. Based on this variable, chronological age could be determined to 92.8% and 94.2% accuracy in Belleville and modern individuals (documented and known ages), with MAEs between ±3.77 to ±3.93 years, respectively. This age estimation method showed a clear relationship between decreasing pulp chamber size and increasing age permitting more accurate age estimations for individuals in the 50+ age range. The ability to age older archaeological individuals can be addressed through the investigations of techniques using pulp/tooth ratios in teeth enabling enhanced engagement with old age in a bioarchaeological context.

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LITERATURE CITED

Angel, J. L. (1984). Variation in estimating age at death of skeletons. *Collegium Antropologicum*, *8*(2), 163-168.

Appleby, J. E. (2010). Why we need an archaeology of old age, and a suggested approach. *Norwegian Archaeological Review*, *43*(2), 145-168.

Appleby, J. E. P. (2011). Bodies, burials and aging: accessing the temporality of old age in prehistoric societies. *Oxford Journal of Archaeology*, *30*(3), 231-246.

Aykroyd, R. G., Lucy, D., Pollard, A. M., & Solheim, T. (1997). Regression analysis in adult age estimation. *American Journal of Physical Anthropology*, *104*, 259-265.

Azevedo, A. C., Michel-Crosato, E., Biazevic, M. G. H., Galić, I., Merelli, V., De Luca, S., & Cameriere, R. (2014). Accuracy and reliability of pulp/tooth area ratio in upper canines by peri-apical X-rays. *Legal Medicine*, *16*(6), 337-343.

Babshet, M., Acharya, A., Venkatesh, N.G. (2010). Age estimation in Indians from pulp/tooth area ratio of mandibular canines. *Forensic Science International*, 197, 125.e1-125.e4.

Bang, G. (1972). Factors of importance in dental identification: five case reports. *Forensic Science*, *1*(1), 91-102.

Bang, G. (1989). Age changes in teeth: Developmental and regressive. In: Işcan M.Y., (Ed.), *Age Markers in the Human Skeleton*. Thomas, Springfield, pp. 211-235.

Bishara, S.E., Jakobsen, J.R., Abdallah, E.M. and Garcia, A.F.1989. Comparisons of mesiodistal and buccolingnal crown dimensions of the permanent teeth in three populations from Egypt, Mexico, and the United States. *American Journal of Orthodontics and Dentofacial Orthopedics*, *96*(5), 416-422.

Benzer, S. (1948). The development and morphology of physiological secondary dentin. *Journal of Dental Research*, *27*(5), 640-646.

Bocquet-Appel, J. P., & Masset, C. (1996). Paleodemography: expectancy and false hope. *American Journal of Physical Anthropology*, *99*(4), 571-583.

Bodecker, C. F. (1925). A consideration of some of the changes in the teeth from young to old age. *Dental Cosmos*, *67*(6), 543-549.

Boldsen, J. L., Milner, G. R., Konigsberg, L. W., Wood, J. W. (2002). Transition analysis: a new method for estimating age from skeletons. In: Hoppa R. D., Vaupel J. W., (Eds.), *Paleodemography: Age Distributions from Skeletal Samples*. Cambridge University Press, pp. 73-106.

Brooks, S. T., Suchey, J. M. (1990). Skeletal age determination based on the os pubis: A comparison of the Acsádi-Nemeskéri and Suchey-Brooks methods. *Journal of Human Evolution* 5, 227-238.

Buckberry, J. L., & Chamberlain, A. T. (2002). Age estimation from the auricular surface of the ilium: a revised method. *American Journal of Physical Anthropology*, *119*(3), 231-239.

Buikstra, J. E., & Konigsberg, L. W. (1985). Paleodemography: critiques and controversies. *American Anthropologist*, *87*(2), 316-333.

Cameriere, R., Ferrante, L., & Cingolani, M. (2004). Variations in pulp/tooth area ratio as an indicator of age: a preliminary study. *Journal of Forensic Science,* 49(2), 1-3.

Cameriere, R., Ferrante, L., Belcastro, M. G., Bonfiglioli, B., Rastelli, E., & Cingolani, M. (2007a). Age estimation by pulp/tooth ratio in canines by peri-apical x-rays. *Journal of Forensic Sciences*, 52(1), 166-170.

Cameriere, R., Ferrante, L., Belcastro, M. G., Bonfiglioli, B., Rastelli, E., & Cingolani, M. (2007b). Age estimation by pulp/tooth ratio in canines by mesial and vestibular peri-apical x-rays. *Journal of Forensic Sciences*, 52(5), 1151-1155.

Cameriere, R. E., Cunha, S. N., Wasterlain, S., De Luca, E., Sassaroli, F., Pagliara, E., Nuzzolese, M., Cingolani, L. Ferrante. (2013). Age estimation by pulp/tooth ratio in lateral and central incisors by peri-apical X-ray. *Journal of Forensic and Legal Medicine*, 20(5), 530-536.

Chamberlain, A. T. (2006). *Demography in archaeology*. Cambridge: Cambridge University Press.

Condon, K., Charles, D. K., Cheverud, J. M., & Buikstra, J. E. (1986). Cementum annulation and age determination in Homo sapiens. II. Estimates and accuracy. *American Journal of Physical Anthropology*, 71(3), 321-330.

De Luca, S., Alemán, I., Bertoldi, F., Ferrante, L., Mastrangelo, P., Cingolani, M., & Cameriere, R. (2010). Age estimation by tooth/pulp ratio in canines by peri-apical X-rays: reliability in age determination of Spanish and Italian medieval skeletal remains. *Journal of Archaeological Science*, 37 (12), 3048-3058.

D'Ortenzio, L., Ribot, I., Kahlon, B., Bertrand, B., Bocaege, E., Raguin, E., Schattmann, A., Brickley, M. (in press). The rachitic tooth: the use of radiographs as a screening technique. *International Journal of Paleopathology.*

Drusini, A., Calliari, I., Volpe, A. (1991). Root dentine transparency: age determination of human teeth using computerized densitometric analysis. *American Journal of Physical Anthropology*, 85(1), 25-30.

Glencross, B., & Sawchuk, L. (2003). The person-years construct: aging and the prevalence of health related phenomena from skeletal samples. *International Journal of Osteoarchaeology*, 13(6), 369-374.

Gowland, R. (2007). Age, ageism and osteological bias: the evidence from late Roman Britain. *Journal of Roman Archaeology; Supplementary series*, 65, 153-169.

Gustafson, G. (1950). Age determinations on teeth. *The Journal of the American Dental Association*, 41(1), 45-54.

Hillson, S. (2002). *Dental Anthropology*. UK: Cambridge University Press.

Hoppa, R. D., Vaupel, J. W. (2002b). The Rostock Manifesto for paleodemography: the way from stage to age. In: Hoppa R. D., Vaupel J. W., (Eds.), *Paleodemography: Age Distributions from Skeletal Samples*. Cambridge: Cambridge University Press, pp. 1-8.

İşcan, M. Y., Loth, S. (1989). Osteological manifestation of age in the adult. In: İşcan, M.Y., Kennedy K. A. R., (Eds.), *Reconstruction of Life from the Skeleton.* Wiley-Liss, New York, NY, pp. 23-40.

Jackes, M. (2011). Representativeness and bias in archaeological skeletal samples. *Social Bioarchaeology*, 107-146.

Joseph, C. C., Reddy, B. H. S., Cherian, N. M., Kannan, S. K., George, G., Jose, S. (2013). Intraoral digital radiography for adult age estimation: a reliable technique. *Journal of Indian Academy Oral Medicine Radiology*, 25 (4), 287-290.

Kvaal, S. I., Kolltveit, K. M., Thomsen, I. O., & Solheim, T. (1995). Age estimation of adults from dental radiographs. *Forensic Science International*, 74(3), 175-185.

Lipsinic, F. E., Paunovich, E., Houston, G. D., & Robison, S. F. (1986). Correlation of age and incremental lines in the cementum of human teeth. *Journal of Forensic Science*, 31(3), 982-989.

Lopez-Nicolas, M., Canteras, M., & Luna, A. (1990). Age estimation by IBAS image analysis of teeth. *Forensic Science International*, 45(1-2), 143-150.

Lovejoy, C. O., Meindl, R. S., Pryzbeck, T. R., & Mensforth, R. P. (1985). Chronological metamorphosis of the auricular surface of the ilium: a new method for the determination of adult skeletal age at death. *American Journal of Physical Anthropology*, 68(1), 15-28.

Lucy, D., & Pollard, A. M. (1995). Further comments on the estimation of error associated with the Gustafson dental age estimation method. *Journal of Forensic Science*, 40 (2), 222-227.

Lucy, D., Pollard, A. M., & Roberts, C. A. (1995). A comparison of three dental techniques for estimating age at death in humans. *Journal of Archaeological Science*, 22(3), 417-428.

Martrille, L., Ubelaker, D. H., Cattaneo, C., Seguret, F., Tremblay, M., & Baccino, E. (2007). Comparison of four skeletal methods for the estimation of age at death on white and black adults. *Journal of Forensic Sciences*, 52 (2), 302-307.

Meindl, R. S., & Lovejoy, C. O. (1985). Ectocranial suture closure: A revised method for the determination of skeletal age at death based on the lateral-anterior sutures. *American Journal of Physical Anthropology*, 68(1), 57-66.

Milner, G. R., Boldsen, J. L. (2012). Transition analysis: A validation study with known-age modern American skeletons. *American Journal of Physical Anthropology*, 148 (1), 98-110.

Molnar, S. (1971). Human tooth wear, tooth function and cultural variability. *American Journal of Physical Anthropology*, 34(2), 175-189.

Morse, D.R. (1991). Age-related changes of the dental pulp complex and their relationship to systemic aging. *Oral Surgery, Oral Medicine, Oral Pathology*, 72(6), 721-745.

Prince, D. A., & Ubelaker, D. H. (2002). Application of Lamendin's adult dental aging technique to a diverse skeletal sample. *Journal of Forensic Science*, 47(1), 107-116.

Rogers, T. (1991). Sex determination and age estimation: Skeletal evidence from St. Thomas' Cemetery Belleville, Ontario. Master's Thesis. Department of Anthropology, McMaster University.

Rösing, F. W., Kvaal, S. I. (1998). Dental age in adults. A review of estimation methods.

In: Alt K. W., Rösing F. W., Teschler-Nicola M, (Eds.), *Dental Anthropology*. Fundamentals, Limits and Prospects. Wien: Springer, pp. 443-468.

Ruff, C. (2002). Variation in human body size and shape. *Annual Review of Anthropology*, *31*(1), 211-232.

Saunders, S. R., Chan, A. H., Kahlon, B., Kluge, H. F., & FitzGerald, C. M. (2007). Sexual dimorphism of the dental tissues in human permanent mandibular canines and third premolars. *American Journal of Physical Anthropology*, 133(1), 735-740.

Smith, P., Gomorri, J. M., Spitz, S., & Becker, J. (1997). Model for the examination of evolutionary trends in tooth development. *American Journal of Physical Anthropology*,102(2), 283-294.

Sofaer J. 2011. Towards a social bioarchaeology of age. In Agarwal S. C., Glencross B. A., (Eds.), *Social Bioarchaeology*. West Sussex (UK): Wiley-Blackwell Publishers, pp. 285-311.

Solheim, T. (1992). Amount of secondary dentin as an indicator of age. *European Journal of Oral Sciences*, 100(4), 193-199.

Suchey, J. M., Katz, D. (1998). Application of pubic age determination in a forensic setting. In: Reichs K. J., (Ed.), *Forensic Osteology. Advances in Identification of Human Remains*. Springfield, Illinois: C.C. Thomas Publisher, pp. 204-236.

Star, H., Thevissen, P., Jacobs, R., Fieuws, S., Solheim, T. and Willems, G. (2011). Human Dental Age Estimation by Calculation of Pulp–Tooth Volume Ratios Yielded on Clinically Acquired Cone Beam Computed Tomography Images of Monoradicular Teeth*. *Journal of Forensic Sciences*, 56, S77–S82.

Stott, G. G., Sis, R. F., & Levy, B. M. (1982). Cemental annulation as an age criterion in forensic dentistry. *Journal of Dental Research*, 61(6), 814-817.

Todd, T. W. (1920). Age changes in the pubic bone. I. The male white pubis. *American Journal of Physical Anthropology*, 3(3), 285-334.

Vasiliadis, L., Darling, A. I., & Levers, B. G. H. (1983). The amount and distribution of sclerotic human root dentine. *Archives of Oral Biology*, 28(7), 645-649.

Walker, P. L., Johnson, J. R., & Lambert, P. M. (1988). Age and sex biases in the preservation of human skeletal remains. *American Journal of Physical Anthropology*, 76(2), 183-188.

Yang, F., Jacobs, R., Willems, G. (2006). Dental age estimation through volume matching of teeth imaged by cone-beam CT. *Forensic Science International*, Volume 159, Supplement, 2006.

Zaher, J. F., Fawzy, I. A., Habib, S. R., Ali, M. M. (2011). Age estimation from pulp/tooth area ratio in maxillary incisors among Egyptians using dental radiographic images. *Journal of Forensic and Legal Medicine*, 18(2), 62-65.

Additional Supporting Information A: Age calculations for modern individuals of known age comparing calculations taken from radiographic images versus those taken from images of histological teeth.

Table 1: Calculated age data set for modern individuals taken from histological tooth sections.

Modern	Tooth Type	Known Age	Sex	Tooth area mm^2	Pulp area mm^2	Pulp/tooth ratio mm^2	Cameriere's et al. (2007a, b)/Joseph's et al. (2013) regression	Calculated age (years)
M170	maxillary central incisor	60	F	46.64	3.83	0.082	43.69	56.2
M90	maxillary canine	19	M	55.78	7.82	0.140	74.59	25.3
M141	maxillary canine	53	F	64.85	5.43	0.084	44.61	55.3
M113	maxillary lateral incisor	92	M	47.22	0.55	0.011	5.86	94.1
M179	mandibular lateral incisor	65	M	39.99	2.79	0.069	36.76	63.2
M166	mandibular central incisor	65	F	40.86	2.28	0.055	29.30	70.6
M127	maxillary 2nd premolar	13	F	79.5	16.73	0.210	79.59	10.18
M161	mandibular canine	68	F	60.13	3.32	0.055	25.40	64.1
M57	mandibular 1^{st} premolar	55	F	64.5	5.47	0.085	32.14	57.6
M20	mandibular central incisor	59	M	49.69	4.24	0.085	45.29	54.7

*Note: Maxillary first premolars were excluded as they have two roots.

Table 2: Calculated age data set for modern individuals taken from radiographs.

Modern	Tooth Type	Known Age	Sex	Tooth area mm^2	Pulp area mm^2	Pulp/tooth ratio mm^2	Cameriere's et al. (2007a, b)/Joseph's et al. (2013) regression	Calculated age (years)
M1	maxillary central incisor	60	F	91.19	4	0.044	23.36	76.6
M2	maxillary canine	19	M	104.74	11.88	0.113	60.42	39.5
M3	maxillary canine	53	F	142.27	15.31	0.108	57.33	42.6
M4	maxillary lateral incisor	92	M	139.19	6.39	0.046	24.46	75.5
M5	mandibular lateral incisor	65	M	81.38	6.46	0.079	42.09	57.8
M6	mandibular central incisor	65	F	82.87	2.83	0.034	18.19	81.7
M7	maxillary 2nd premolar	13	F	141.6	24.67	0.174	92.8	7.13
M8	mandibular canine	68	F	117.96	3.25	0.028	14.68	85.3
M9	mandibular 1^{st} premolar	55	F	120.31	14.2	0.118	62.88	37
M10	mandibular central incisor	59	M	95.54	3.68	0.039	20.52	79.4

*Note: Maxillary first premolars were excluded as they have two roots.

Additional Supporting Information B: Age calculations for modern and Belleville documented/estimated aged individuals

Table 1: Calculated age data set for Belleville archaeological individuals with documented age.

Identifier	Type of Tooth	Documented Age (years)	Sex	Tooth area mm^2	Pulp area mm^2	Pulp/tooth ratio mm^2	Cameriere's et al. (2007a, b)/Joseph's et al. (2013) regression	Calculated age (years)
B19	maxillary canine	17	M	73.44	11.05	0.15	79.92	20
B71	maxillary canine	20	M	79.75	11.18	0.14	74.59	25.3
B2	maxillary canine	21	M	54.71	7.8	0.142	75.65	24.3
B269A	maxillary canine	21	F	50.46	6.99	0.138	73.52	26.4
B002	maxillary canine	21	M	54.96	8.58	0.156	83.11	16.8
B423	maxillary canine	22	F	47.91	6.72	0.14	74.59	25.3
B351	maxillary canine	22	F	79.12	11.1	0.14	74.59	25.3
B465	maxillary canine	22	F	50.78	6.88	0.135	71.93	28
B514	maxillary canine	22	F	47.52	6.57	0.138	73.52	26.4
B115	maxillary canine	27	M	57.31	7.8	0.141	75.12	27
B400	maxillary canine	29	F	66.86	8.38	0.125	66.6	33.3
B437	maxillary canine	29	F	56.34	6.94	0.123	65.53	34.4
B472	maxillary canine	31	M	71.82	10.13	0.14	74.59	25.3
B527A	maxillary canine	31	M	89.15	12.34	0.138	73.52	26.4
B111	maxillary canine	33	F	54.27	7.28	0.134	71.39	28.5
B385	maxillary canine	35	M	71.7	9.05	0.126	67.13	32.8
B317	maxillary canine	35	F	49.3	5.65	0.114	60.74	39.2
B133	maxillary canine	41	M	49.67	5.72	0.115	61.27	38.7
B92A	maxillary canine	42	F	60.77	6.18	0.101	53.81	46.1
B464	maxillary canine	46	F	51.75	5.03	0.097	51.68	48.3
B191	maxillary canine	48	M	63.25	6.18	0.103	54.87	45.1

Identifier	Type of Tooth	Documented Age (years)	Sex	Tooth area mm^2	Pulp area mm^2	Pulp/tooth ratio mm^2	Cameriere's et al. (2007a, b)/Joseph's et al. (2013) regression	Calculated age (years)
B287	maxillary canine	48	M	41.9	3.88	0.092	49.02	50.9
B429A	maxillary canine	53	M	67.49	6.57	0.097	51.68	48.3
B43	maxillary canine	54	F	48.56	4.11	0.085	45.09	54.8
B443	maxillary canine	54	M	63.69	5.14	0.08	42.62	57.3
B156	maxillary canine	55	M	47.99	3.62	0.075	39.96	60
B338	maxillary canine	55	M	66.75	4.81	0.072	38.36	61.6
B502	maxillary canine	55	M	47.83	3.6	0.075	39.96	60
B303	maxillary canine	58	M	76.86	6.78	0.086	45.82	54.1
B32	maxillary canine	58	F	50.93	3.38	0.066	35.16	64.8
B485	maxillary canine	60	M	54.23	4.52	0.083	44.22	55.7
B312	maxillary canine	60	F	54.69	3.36	0.061	32.6	67.4
B47	maxillary canine	61	M	69.86	4.79	0.068	36.23	63.7
B518	maxillary canine	61	M	66.63	4.95	0.074	39.43	60.5
B245	maxillary canine	62	F	60.84	4.23	0.069	36.76	63.2
B467	maxillary canine	64	M	66.43	4.43	0.066	35.16	64.8
B374	maxillary canine	64	M	55.57	4.12	0.074	39.43	60.5
B39	maxillary canine	64	M	69.49	4.32	0.062	33.03	66.9
B407	maxillary canine	68	M	59.34	3.77	0.063	33.57	66.4
B544	maxillary canine	71	F	54.7	2.82	0.051	27.17	72.8
B470	maxillary canine	73	F	45.66	2.66	0.058	30.9	69
B542	maxillary canine	74	M	59.37	2.91	0.049	26.106	73.8
B375	maxillary canine	75	M	69.97	2.68	0.038	20.25	79.7
B334	maxillary canine	75	F	64.76	2.77	0.042	22.38	77.6
B297	maxillary canine	75	M	51.29	2.8	0.054	28.77	71.2

Identifier	Type of Tooth	Documented Age (years)	Sex	Tooth area mm^2	Pulp area mm^2	Pulp/tooth ratio mm^2	Cameriere's et al. (2007a, b)/Joseph's et al. (2013) regression	Calculated age (years)
B512	maxillary canine	76	F	66.29	2.81	0.042	22.38	77.6
B335	maxillary canine	81	F	55.57	2.23	0.04	21.31	78.6
B491	maxillary canine	88	M	68.52	1.59	0.025	13.32	86.6
B395	maxillary canine	88	F	62.32	1.84	0.029	15.45	84.5
B516	maxillary canine	89	F	58.99	1.3	0.022	11.74	88.2
B326	mandibular canine	1	U	47.36	9.06	0.191	88.22	1.2
B19	mandibular canine	17	M	52.83	7.69	0.145	66.97	22.5
B433	mandibular canine	18	M	51.95	7.9	0.152	70.20	19.3
B269A	mandibular canine	21	F	42.78	5.75	0.134	61.89	27.6
B423	mandibular canine	22	F	33.08	5.11	0.154	71.13	18.3
B465	mandibular canine	22	F	45.4	6.28	0.138	63.74	25.7
B514	mandibular canine	22	F	44.37	6.9	0.155	71.59	17.9
B115	mandibular canine	27	M	48.81	6.76	0.138	63.74	25.7
B400	mandibular canine	29	F	44.85	6.28	0.140	64.66	24.8
B472	mandibular canine	31	M	55.68	7.83	0.140	64.66	24.8
B527A	mandibular canine	31	M	56.71	7.83	0.138	63.74	25.7
B317	mandibular canine	35	F	39.28	4.31	0.109	50.34	39.1
B133	mandibular canine	41	M	36.92	4.41	0.119	54.96	34.5
B92A	mandibular canine	42	F	45.52	4.21	0.092	42.49	47.0
B484	mandibular canine	42	F	41.25	3.98	0.096	44.34	45.1
B464	mandibular canine	46	F	35.44	3.16	0.089	41.11	48.3
B191	mandibular canine	48	M	43.17	3.38	0.078	36.03	53.4
B429	mandibular canine	53	M	40.58	3.45	0.085	39.27	50.2
B429A	mandibular canine	53	M	51.06	4.81	0.094	43.42	46.0

Identifier	Type of Tooth	Documented Age (years)	Sex	Tooth area mm^2	Pulp area mm^2	Pulp/tooth ratio mm^2	Cameriere's et al. (2007a, b)/Joseph's et al. (2013) regression	Calculated age (years)
B443	mandibular canine	54	M	46.86	4.4	0.093	42.95	46.5
B156	mandibular canine	55	M	40.92	2.71	0.066	30.48	59.0
B32	mandibular canine	58	F	45.58	2.67	0.058	26.79	62.7
B312	mandibular canine	60	F	44.55	2.48	0.055	25.40	64.1
B245	mandibular canine	62	F	47.42	2.19	0.046	21.25	68.2
B374	mandibular canine	64	M	43.54	2.73	0.062	28.64	60.8
B39	mandibular canine	64	M	53.31	2.17	0.040	18.47	71.0
B100	mandibular canine	67	M	50.21	3.13	0.062	28.64	60.8
B447	mandibular canine	69	M	39.83	1.41	0.035	16.17	73.3
B447	mandibular canine	69	M	39.31	1.34	0.034	15.70	73.8
B544	mandibular canine	71	F	47.43	1.86	0.039	18.11	71.3
B512	mandibular canine	76	F	45.83	1.39	0.030	13.86	75.6
B395	mandibular canine	88	F	50.38	0	0.000	0.00	89.5
B516	mandibular canine	89	F	54.19	0.09	0.001	0.46	89.0
B433	maxillary 2nd premolar	18	M	50.63	8.48	0.167	63.30	26.5
B437	maxillary 2nd premolar	29	F	62.06	10.57	0.170	64.43	25.3
B111	maxillary 2nd premolar	33	F	42.22	5.71	0.135	51.17	38.6
B385	maxillary 2nd premolar	35	M	55.23	7.21	0.130	49.27	40.5
B484	maxillary 2nd premolar	42	F	49.65	5.98	0.120	45.48	44.3
B338	maxillary 2nd premolar	55	M	40.27	3.33	0.083	31.34	58.4
B26	maxillary 2nd premolar	60	M	54.14	4.07	0.075	28.43	61.4
B470	maxillary 2nd premolar	73	F	44.63	1.2	0.026	9.85	79.9
B542	maxillary 2nd premolar	74	M	38.08	1.35	0.035	13.27	76.5
B375	maxillary 2nd premolar	75	M	52.31	2.66	0.050	18.95	70.8
B334	maxillary 2nd premolar	75	F	61.44	1.61	0.025	9.48	80.3
B491	maxillary 2nd premolar	88	M	45.68	0.3	0.006	2.27	87.5

Table 2: Calculated age data set for Belleville archaeological individuals with skeletally estimated age.

Identifier	Type of Tooth	Skeletally Estimated Age	Sex	Tooth area mm^2	Pulp area mm^2	Pulp/tooth ratio mm^2	Cameriere's et al. (2007a, b)/Joseph's et al. (2013) regression	Calculated age (years)
B307	maxillary canine	33.6	M	66.51	8.82	0.132	70.33	29.6
B196	maxillary canine	60.6	M	67.02	5.2	0.078	41.34	58.6
B193	maxillary canine	42	F	51.57	5.81	0.112	59.67	40.3
B179	maxillary canine	46.8	M	69.45	7.4	0.106	56.47	43.5
B182	maxillary canine	22	U	53.37	8.05	0.145	77.25	22.7
B192	maxillary canine	25	F	50.72	6.74	0.133	70.86	29.1
B424	maxillary canine	70	F	48.36	2.31	0.047	25.04	74.9
B490	maxillary canine	5.86	U	28.07	5.07	0.180	95.90	4.0
B489	maxillary canine	11.01	U	49.24	8.14	0.165	87.91	12.0
B388	maxillary canine	32	M	67.76	9.37	0.138	73.52	26.4
B356	maxillary canine	35	M	74.29	9.13	0.123	65.53	34.4
B117	maxillary canine	37	F	45.68	5.55	0.121	64.47	35.5
B176	maxillary canine	57.5	F	52.36	3.84	0.073	38.89	61.0
B172	maxillary canine	65	U	59.53	3.48	0.058	30.90	69.0
B171	maxillary canine	35.2	M	78.29	10.04	0.128	68.20	31.7
B226	maxillary canine	55	M	68.25	5.88	0.086	45.90	54.0
B263	maxillary canine	43.8	M	73.22	7.48	0.102	54.43	45.5
B277	maxillary canine	47.85	M	68.92	7.13	0.103	55.12	44.8
B501	maxillary canine	9.42	U	50.06	8.52	0.170	90.57	9.4
B94	maxillary canine	9.84	U	71.1	12.2	0.171	91.11	8.8
B411	maxillary canine	20.25	F	43.99	6.56	0.149	79.38	20.6
B405	maxillary canine	45.1	M	63.05	4.84	0.077	40.90	59.0
B410	maxillary canine	3.44	U	23.94	4.32	0.181	96.43	3.5

Identifier	Type of Tooth	Skeletally Estimated Age	Sex	Tooth area mm^2	Pulp area mm^2	Pulp/tooth ratio mm^2	Cameriere's et al. (2007a, b)/Joseph's et al. (2013) regression	Calculated age (years)
B483	maxillary canine	36.1	M	81.25	9.8	0.120	63.93	36.0
B138	maxillary canine	65	M	69.56	3.85	0.055	29.49	70.4
B137	maxillary canine	27.75	M	48.12	6.65	0.139	74.06	25.9
B102A	maxillary canine	45	U	61.05	5.92	0.097	51.66	48.3
B399	maxillary canine	65	F	50.62	2.64	0.052	27.79	72.2
B477	maxillary canine	67.8	M	55.76	3.59	0.064	34.10	65.8
B480	maxillary canine	32.7	F	59.02	7.82	0.132	70.33	29.6
B310	maxillary canine	32	F	71.42	8.88	0.124	66.06	33.9
B11	maxillary canine	47	M	69.58	6.22	0.089	47.42	52.5
B461	maxillary canine	30.35	M	54.71	6.96	0.127	67.66	32.3
B243	maxillary canine	32	M	63.58	8.29	0.130	69.26	30.7
B260	maxillary canine	42	M	60.84	6.03	0.099	52.75	47.2
B272	maxillary canine	60	M	58.99	3.76	0.064	33.96	66.0
B481	maxillary canine	60	M(?)	74.04	1.637	0.022	11.78	88.2
B471	maxillary canine	20.25	M(?)	66.11	10.03	0.151	80.45	19.5
B132A	maxillary canine	1.81	U	30.5	5.63	0.185	98.56	1.4
B364	maxillary canine	7.34	U	51.5	9.04	0.175	93.24	6.7
B355	maxillary canine	45.1	M	56.59	6.18	0.109	58.18	41.8
B157	maxillary canine	60	M	74.4	5.68	0.076	40.49	59.4
B442	maxillary canine	32.6	F	62.07	7.58	0.122	65.00	34.9
B438	maxillary canine	74.75	F	61.41	1.934	0.031	16.78	83.2
B313	maxillary canine	55	M	45.18	3.56	0.078	41.56	58.4
B101	maxillary canine	29.1	M	62.5	8.01	0.128	68.20	31.7

Identifier	Type of Tooth	Skeletally Estimated Age	Sex	Tooth area mm^2	Pulp area mm^2	Pulp/tooth ratio mm^2	Cameriere's et al. (2007a, b)/Joseph's et al. (2013) regression	Calculated age (years)
B130	maxillary canine	47	U	63.49	5.86	0.092	49.17	50.8
B102	maxillary canine	8.54	U	47.92	8.35	0.174	92.70	7.2
B98	maxillary canine	20.2	M	69.45	10.73	0.154	82.05	17.9
B131	maxillary canine	55	M	63.57	4.27	0.067	35.79	64.2
B416	maxillary canine	62	F	41.07	2.28	0.056	29.58	70.4
B345	maxillary canine	70.2	M	68.45	2.79	0.041	21.72	78.2
B499	maxillary canine	9.42	U	76.93	13.25	0.172	91.64	8.3
B363	maxillary canine	80	M	73.29	1.39	0.019	10.10	89.8
B341	maxillary canine	37	F	58.17	6.23	0.107	57.01	42.9
B342	maxillary canine	47.6	F	50.15	5.15	0.103	54.71	45.2
B343	maxillary canine	52.6	M	70.83	6.11	0.086	45.96	54.0
B40	maxillary canine	40	M	61.46	5.88	0.096	50.97	49.0
B45	maxillary canine	32	M	82.45	10.77	0.130	69.26	30.7
B48	maxillary canine	32	M	43.24	5.95	0.137	72.99	26.9
B182	maxillary canine	27	F	61.34	8.26	0.131	69.79	30.1
B12	maxillary canine	37	M	62.69	7.61	0.121	64.47	35.5
B58	maxillary canine	60	M	59.33	3.15	0.053	28.29	71.7
B281	maxillary canine	55.1	M	57.2	4.48	0.078	41.56	58.4
B475	maxillary canine	75.6	M	74.8	2.17	0.029	15.46	84.5
B76	maxillary canine	27	M	55.03	7.82	0.142	75.65	24.3
B368	maxillary canine	22	F	46.78	6.79	0.145	77.25	22.7
B372	maxillary canine	32.6	F	46.15	5.56	0.120	63.93	36.0
B242	maxillary canine	25	M	65.34	8.89	0.136	72.46	27.5

Identifier	Type of Tooth	Skeletally Estimated Age	Sex	Tooth area mm^2	Pulp area mm^2	Pulp/tooth ratio mm^2	Cameriere's et al. (2007a, b)/Joseph's et al. (2013) regression	Calculated age (years)
B369	maxillary canine	80	F	45.82	1.1	0.024	12.79	87.1
B49	maxillary canine	70	M	45.28	1.57	0.035	18.47	81.5
B228	maxillary canine	50	M	66.65	5.57	0.084	44.52	55.4
B298	maxillary canine	46.85	M	72.44	6.48	0.089	47.42	52.5
B300	maxillary canine	37.6	F	60.38	7.56	0.125	66.60	33.3
B301	maxillary canine	58.8	M	71.86	5.85	0.081	43.16	56.8
B541	maxillary canine	4.85	U	26.42	4.77	0.180	95.90	4.0
B539	maxillary canine	52	F	53.16	4.38	0.082	43.90	56.0
B89	maxillary canine	55	M	84.87	4.42	0.052	27.75	72.2
B169	maxillary canine	40	F	57.02	6.02	0.106	56.25	43.7
B165	maxillary canine	62.8	M	65.68	3.61	0.055	29.28	70.7
B161	maxillary canine	65	M	75.62	4.3	0.057	30.30	69.6
B159	maxillary canine	70	F	51.51	1.62	0.031	16.76	83.2
B210A	maxillary canine	58	F	64.75	5.41	0.084	44.51	55.4
B210	maxillary canine	55.5	M	48.48	4.27	0.088	46.93	53.0
B204	maxillary canine	45.1	M	64.95	6.67	0.103	54.71	45.2
B412	maxillary canine	47.6	M	72.96	7.87	0.108	57.47	42.5
B262	maxillary canine	42.35	F	64.62	6.61	0.102	54.50	45.4
B250	maxillary canine	31.1	M	74.23	9.75	0.131	69.79	30.1
B197	maxillary canine	22	U	53.11	7.57	0.142	75.65	24.3
B333A	maxillary canine	22	M	87.62	12.22	0.139	74.06	25.9
B170	maxillary canine	72.8	M	57.94	2.35	0.041	21.61	78.3
B87	maxillary canine	27	F	61.17	8.04	0.131	69.79	30.1
B519	maxillary canine	41.6	F	60.65	6.99	0.115	61.27	38.7

Identifier	Type of Tooth	Skeletally Estimated Age	Sex	Tooth area mm^2	Pulp area mm^2	Pulp/tooth ratio mm^2	Cameriere's et al. (2007a, b)/Joseph's et al. (2013) regression	Calculated age (years)
B131A	maxillary canine	31.7	F	61.27	8.1	0.133	70.86	29.1
B132	maxillary canine	40	M	58.89	4.9	0.083	44.33	55.6
B21	maxillary canine	40	F	53.93	5.5	0.102	54.34	45.6
B30	maxillary canine	42	F	54.9	5.77	0.105	56.00	43.9
B37	maxillary canine	19	M	58.02	15.39	0.155	82.58	17.4
B428	maxillary canine	55.3		56.79	4.29	0.074	39.43	60.5
B268	maxillary canine	40	F	49.14	5.08	0.103	54.88	45.1
B488	maxillary canine	41.1	M	62.71	7.17	0.114	60.92	39.0
B505	maxillary canine	3.36	U	31.08	5.67	0.182	96.97	3.0
B503	maxillary canine	32	F	62.04	8.17	0.131	69.79	30.1
B148	maxillary canine	32.85	M	69.86	9.4	0.134	71.39	28.5
B383	maxillary canine	47	F	42.35	3.98	0.094	50.07	49.9
B370	maxillary canine	33.6	M	49.05	6.63	0.135	71.93	28.0
B537	maxillary canine	22.75	M	64.82	9.04	0.139	74.06	25.9
B307	mandibular canine	33.6	M	53.62	7.15	0.133	61.43	28.0
B196	mandibular canine	60.6	M	49.27	2.57	0.052	24.02	65.4
B193	mandibular canine	42	F	43.24	4.7	0.109	50.34	39.1
B179	mandibular canine	46.8	M	44.53	4.52	0.101	46.65	42.8
B182	mandibular canine	22	U	47.08	7.23	0.153	70.67	18.8
B192	mandibular canine	25	F	48.88	6.46	0.132	60.97	28.5
B490	mandibular canine	5.86	U	34	6.31	0.185	85.45	4.0
B388	mandibular canine	32	M	47.35	6.56	0.139	64.20	25.3
B117	mandibular canine	37	F	35.75	4.3	0.120	55.42	34.0
B222A	mandibular canine	0.8	U	37.26	7.21	0.193	89.14	0.3

Identifier	Type of Tooth	Skeletally Estimated Age	Sex	Tooth area mm^2	Pulp area mm^2	Pulp/tooth ratio mm^2	Cameriere's et al. (2007a, b)/Joseph's et al. (2013) regression	Calculated age (years)
B226	mandibular canine	55	M	44.98	3.66	0.081	37.41	52.0
B263	mandibular canine	43.8	M	47.17	4.407	0.093	43.15	46.3
B277	mandibular canine	47.85	M	60.62	5.47	0.090	41.68	47.8
B323	mandibular canine	6.51	U	46.34	8.59	0.185	85.45	4.0
B452	mandibular canine	20.25	M	41.78	5.65	0.135	62.35	27.1
B94	mandibular canine	9.84	U	56.65	9.32	0.164	75.75	13.7
B411	mandibular canine	20.25	F	43.07	5.82	0.135	62.35	27.1
B138	mandibular canine	65	M	41.63	2.09	0.050	23.19	66.3
B137	mandibular canine	27.75	M	40.12	5.14	0.128	59.12	30.3
B102A	mandibular canine	45	U	38.28	3.49	0.091	42.11	47.3
B399	mandibular canine	65	F	43.1	1.91	0.044	20.32	69.1
B477	mandibular canine	67.8	M	47.47	1.67	0.035	16.17	73.3
B480	mandibular canine	32.7	F	49.85	6.38	0.128	59.12	30.3
B310	mandibular canine	32	F	50	6.92	0.138	63.74	25.7
B11	mandibular canine	47	M	51.5	4.15	0.080	36.95	52.5
B461	mandibular canine	30.35	M	47.83	5.3	0.110	50.81	38.6
B243	mandibular canine	32	M	47.12	5.21	0.110	50.81	38.6
B260	mandibular canine	42	M	42.5	3.8	0.089	41.11	48.3
B272	mandibular canine	60	M	46.36	1.61	0.034	15.70	73.8
B471	mandibular canine	20.25	M(?)	42.8	5.59	0.130	60.04	29.4
B132A	mandibular canine	1.81	U	21.75	4.01	0.184	84.98	4.5
B364	mandibular canine	7.34	U	26.34	4.75	0.180	83.14	6.3
B355	mandibular canine	45.1	M	38.86	3.97	0.102	47.11	42.3
B157	mandibular canine	60	M	57.37	2.42	0.042	19.40	70.1

Identifier	Type of Tooth	Skeletally Estimated Age	Sex	Tooth area mm^2	Pulp area mm^2	Pulp/tooth ratio mm^2	Cameriere's et al. (2007a, b)/Joseph's et al. (2013) regression	Calculated age (years)
B442	mandibular canine	32.6	F	47.56	6.22	0.130	60.04	29.4
B438	mandibular canine	74.75	F	56.58	2.19	0.039	17.88	71.6
B313	mandibular canine	55	M	37.29	1.12	0.030	13.87	75.6
B130	mandibular canine	47	U	52.18	4.68	0.090	41.43	48.0
B98	mandibular canine	20.2	M	52.72	8.08	0.153	70.67	18.8
B345	mandibular canine	70.2	M	53.97	2.45	0.045	20.97	68.5
B499	mandibular canine	9.42	U	54.46	9.75	0.179	82.68	6.8
B341	mandibular canine	37	F	45.92	5.21	0.113	52.40	37.1
B342	mandibular canine	47.6	F	39.92	3.8	0.095	43.88	45.6
B343	mandibular canine	52.6	M	52.3	3.68	0.070	32.33	57.1
B13	mandibular canine	10.79	U	52.32	9.16	0.175	80.83	8.6
B182	mandibular canine	27	F	43.34	5.01	0.115	53.12	36.3
B12	mandibular canine	37	M	53.15	5.22	0.097	44.80	44.7
B475	mandibular canine	75.6	M	42.57	1.36	0.032	14.76	74.7
B76	mandibular canine	27	M	54.14	7.2	0.132	60.97	28.5
B368	mandibular canine	22	F	37.71	5.76	0.152	70.20	19.3
B242	mandibular canine	25	M	45.66	5.44	0.119	54.96	34.5
B228	mandibular canine	50	M	51.52	1.9	0.036	16.63	72.8
B298	mandibular canine	46.85	M	60.09	4.12	0.068	31.41	58.0
B541	mandibular canine	4.85	U	19.04	3.55	0.186	85.91	3.5
B539	mandibular canine	52	F	40.62	4.01	0.098	45.26	44.2
B89	mandibular canine	55	M	60.62	3.35	0.055	25.40	64.1
B169	mandibular canine	40	F	50.71	5.14	0.101	46.82	42.6
B161	mandibular canine	65	M	49.78	2.51	0.000	0.00	89.5

Identifier	Type of Tooth	Skeletally Estimated Age	Sex	Tooth area mm^2	Pulp area mm^2	Pulp/tooth ratio mm^2	Cameriere's et al. (2007a, b)/Joseph's et al. (2013) regression	Calculated age (years)
B204	mandibular canine	45.1	M	51.69	2.33	0.045	20.78	68.7
B413	mandibular canine	28.85	F	40.11	5	0.124	57.27	32.2
B262	mandibular canine	42.35	F	48.23	3.92	0.081	37.41	52.0
B261	mandibular canine	3.66	U	14.61	2.75	0.188	86.83	2.6
B250	mandibular canine	31.1	M	47.82	5.81	0.121	55.89	33.6
B167	mandibular canine	58.3	F	44.71	2.78	0.062	28.72	60.7
B333A	mandibular canine	22	M	43.92	6.88	0.156	72.05	17.4
B170	mandibular canine	72.8	M	43.57	1.11	0.025	11.55	77.9
B87	mandibular canine	27	F	57.44	6.93	0.120	55.42	34.0
B378	mandibular canine	37	F	46.9	5.43	0.115	53.12	36.3
B428	mandibular canine	55.3	M	50.98	4.52	0.088	40.64	48.8
B268	mandibular canine	40	F	46.49	5.14	0.110	50.81	38.6
B488	mandibular canine	41.1	M	48.34	5.08	0.105	48.54	40.9
B503	mandibular canine	32	F	46.55	6.31	0.135	62.35	27.1
B148	mandibular canine	32.85	M	52.08	5.75	0.110	50.81	38.6
B383	mandibular canine	47	F	34.9	2.76	0.079	36.49	53.0
B422	mandibular 2nd premolar	67.6	M	53.71	2.11	0.039	14.78	75.0
B481	mandibular 1st premolar	60	M(?)	69.05	1.97	0.029	10.81	79.0
B131	mandibular 2nd premolar	55	M	52.82	3.52	0.067	25.26	64.5
B363	mandibular 2nd premolar	80	M	54.29	0.742	0.014	5.18	84.6
B75	mandibular 1st premolar	1.05	U	48.58	11.42	0.235	89.07	0.7
B484	mandibular 1st premolar	58.1	F	44.12	3.12	0.070	26.53	63.2
B165	mandibular 2nd premolar	62.8	M	55.21	3.49	0.063	23.88	65.9
B159	mandibular 1st premolar	70	F	40.43	1.74	0.043	16.30	73.5

Identifier	Type of Tooth	Skeletally Estimated Age	Sex	Tooth area mm^2	Pulp area mm^2	Pulp/tooth ratio mm^2	Cameriere's et al. (2007a, b)/Joseph's et al. (2013) regression	Calculated age (years)
B210A	mandibular 2nd premolar	58	F	48.21	4.16	0.086	32.71	57.1
B210	mandibular 1st premolar	55.5	M	36.34	3.08	0.085	32.12	57.7
B412	mandibular 1st premolar	47.6	M	54.1	5.54	0.102	38.81	51.0
B132	mandibular 2nd premolar	40	M	53.24	4.43	0.089	33.73	56.0
B370	mandibular 1st premolar	33.6	M	53.48	7.63	0.142	53.82	36.0

*Note: Maxillary first premolars were excluded as they have two roots.

Table 3: Calculated age data set for modern individuals

Identifier	Tooth Type	Known Age (years)	Sex	Tooth area mm^2	Pulp area mm^2	Pulp/tooth ratio mm^2	Cameriere's et al. (2007a, b)/Joseph's et al. (2013) regression	Calculated Age (years)
M39	maxillary 2nd premolar	14	F	56.97	12.01	0.210	79.59	10.18
M119	maxillary 2nd premolar	74	F	67.9	1.75	0.025	9.48	80.30
M168	maxillary 2nd premolar	68	M	62.69	2.75	0.043	16.30	73.48
M189	maxillary 2nd premolar	78	F	66.55	2.29	0.034	13.04	76.74
M106	maxillary 2nd premolar	87	M	61.06	1.25	0.020	7.58	82.20
M162	maxillary 2nd premolar	68	F	65.53	3.42	0.052	19.78	70.00
M127	maxillary 2nd premolar	13	F	79.5	16.73	0.210	79.59	10.18
M112	maxillary 2nd premolar	43	F	68.7	9.63	0.140	53.06	36.72
M52	maxillary 2nd premolar	52	F	56.99	5.36	0.094	35.65	54.13
M92	maxillary 2nd premolar	77	F	69.87	1.76	0.025	9.48	80.30

Identifier	Tooth Type	Known Age (years)	Sex	Tooth area mm^2	Pulp area mm^2	Pulp/tooth ratio mm^2	Cameriere's et al. (2007a, b)/Joseph's et al. (2013) regression	Calculated Age (years)
M9	mandibular 1st premolar	60	F	48.91	2.96	0.060	22.74	67.0
M57	mandibular 2nd premolar	55	F	64.5	5.47	0.085	32.14	57.6
M11	mandibular 1st premolar	21	F	47.71	9.08	0.190	72.01	17.8
M15	mandibular 2nd premolar	59	M	69.69	5.24	0.075	28.43	61.4
M48	mandibular 1st premolar	80	F	53.58	1.4	0.026	9.85	79.9
M165	mandibular 1st premolar	69	F	61.54	2.56	0.041	15.54	74.2
M82	mandibular 2nd premolar	88	F	42.93	0.22	0.005	1.90	87.9
M90	maxillary canine	19	M	55.78	7.82	0.140	74.59	25.3
M35	maxillary canine	54	F	34.75	2.61	0.075	39.96	60.0
M103	maxillary canine	33	M	62.96	7.29	0.115	61.27	38.7
M141	maxillary canine	53	F	64.85	5.43	0.084	44.61	55.3
M161	mandibular canine	68	F	60.13	3.32	0.055	25.40	64.1
M41	mandibular canine	82	M	47.47	0.86	0.018	8.31	81.1
M51	mandibular lateral incisor	90	M	42.68	0.75	0.018	9.36	90.6
M181	mandibular central incisor	68	F	49.3	2.4	0.048	25.57	74.4
M20	mandibular central incisor	60	M	49.69	4.24	0.085	45.29	54.7
M93	mandibular lateral incisor	91	M	44.19	0.823	0.019	9.92	90.0
M166	mandibular central incisor	65	F	40.86	2.28	0.055	29.30	70.6

Identifier	Tooth Type	Known Age (years)	Sex	Tooth area mm^2	Pulp area mm^2	Pulp/tooth ratio mm^2	Cameriere's et al. (2007a, b)/Joseph's et al. (2013) regression	Calculated Age (years)
M107	mandibular central incisor	61	M	42.88	3.58	0.083	44.22	55.7
M57	mandibular 1st premolar	55	F	46.18	3.91	0.085	45.11	54.8
M179	mandibular lateral incisor	65	M	39.99	2.79	0.069	36.76	63.2
M170	maxillary central incisor	60	F	46.64	3.38	0.072	38.36	61.6
M113	maxillary lateral incisor	92	M	47.22	0.55	0.011	5.86	94.1
M178	maxillary lateral incisor	66	F	37.9	2.47	0.064	34.10	65.8
M100	maxillary central incisor	98	F	40.82	0.26	0.006	3.20	96.7

*Note: Maxillary first premolars were excluded as they have two roots.

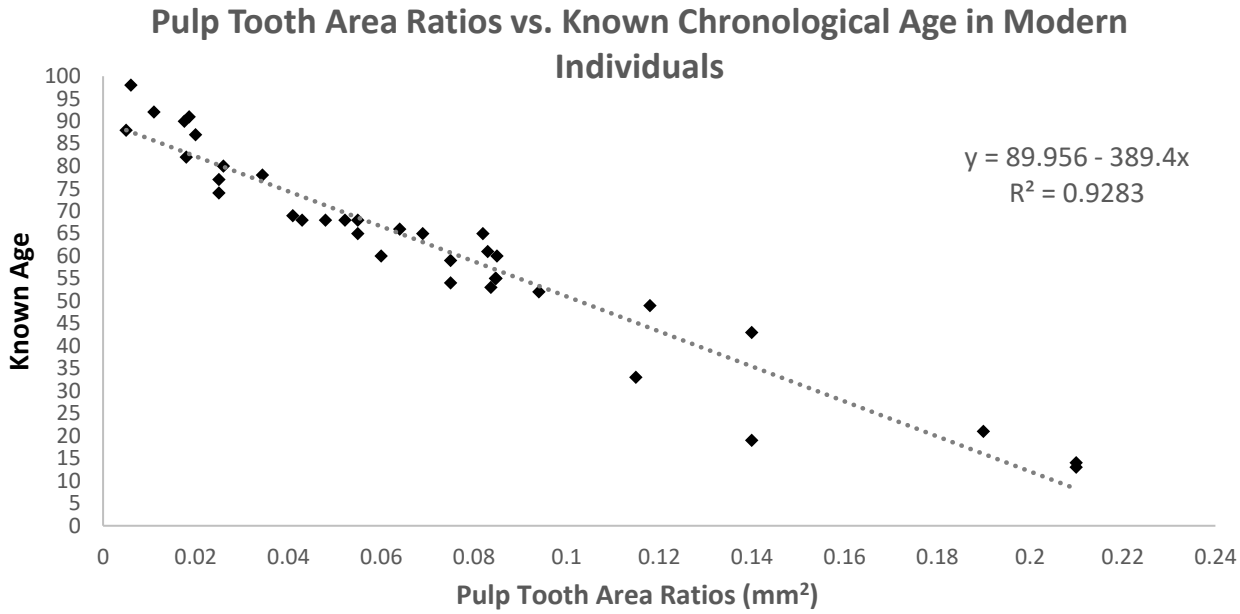

Fig. 1. Regression of pulp/tooth area ratios versus known age in modern individuals. Note R^2 explains 92.8% of variation as age.

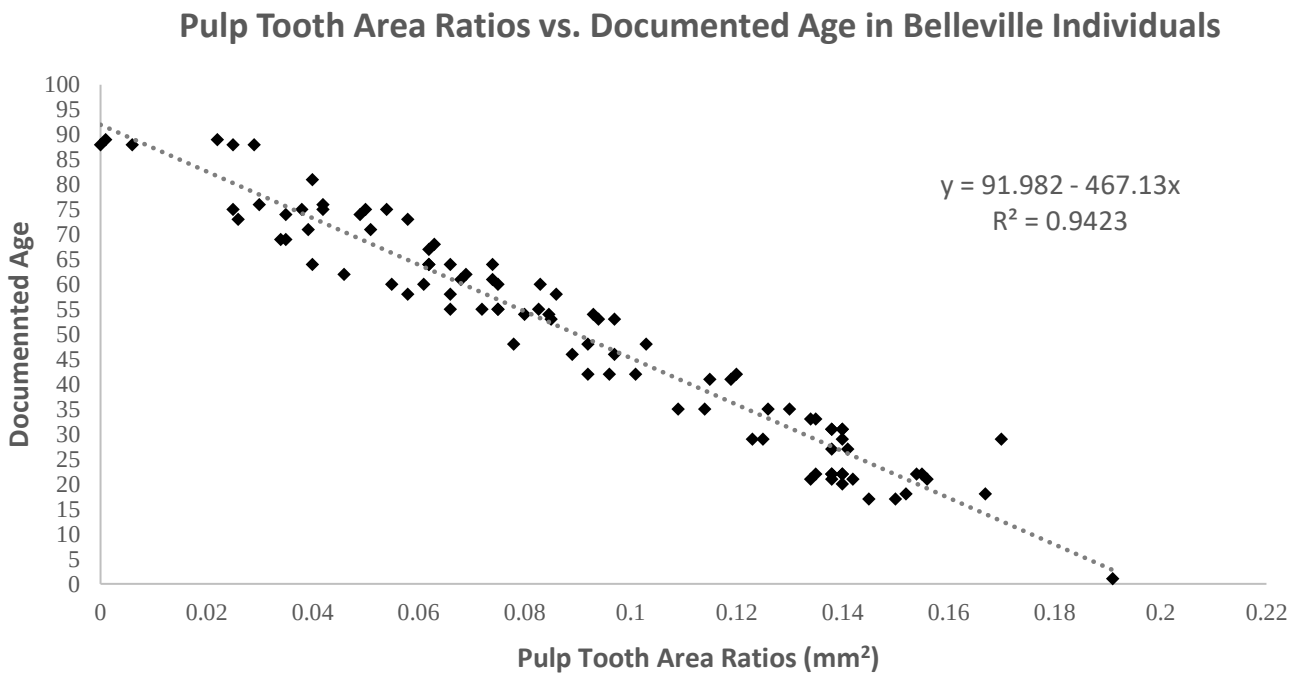

Fig. 2. Regression of pulp/tooth area ratios versus documented age in Belleville individuals. Note R^2 explains 94.2% of variation as age.

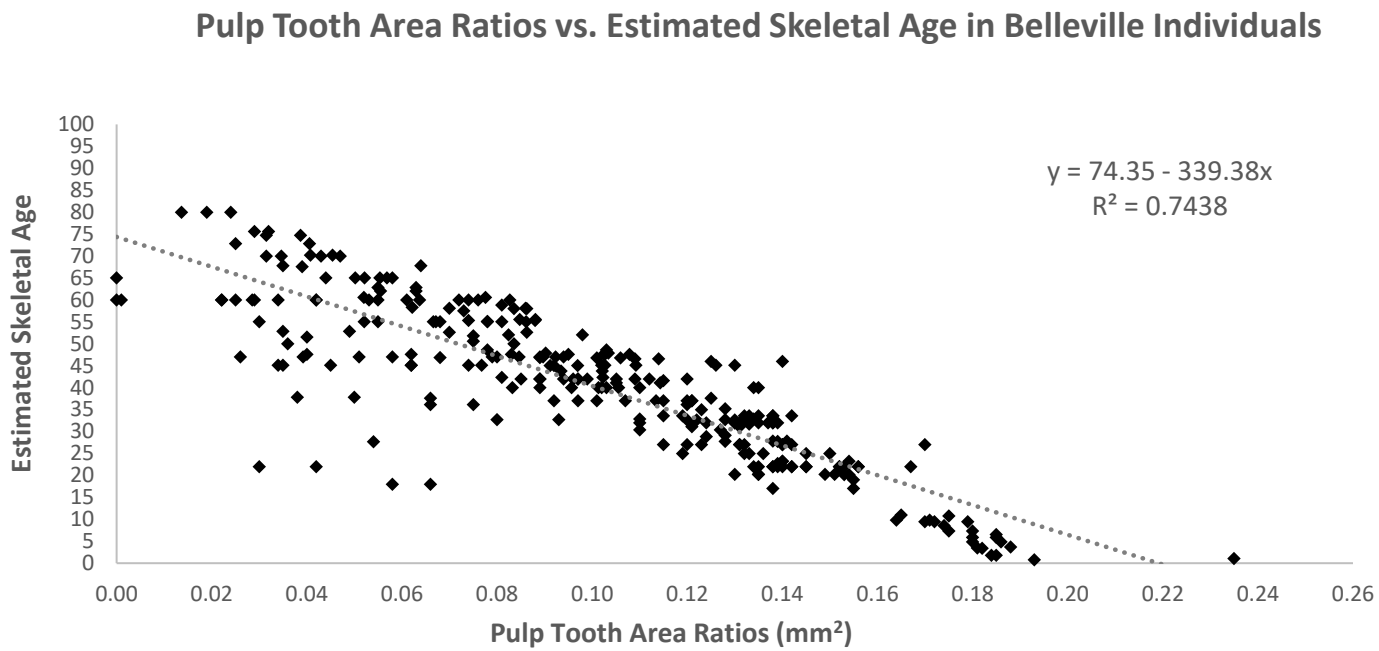

Fig. 3. Regression of pulp/tooth area ratios vs estimated skeletal age in Belleville individuals. Note R^2 is found to explain 74.3% of variation is age.

CHAPTER 5: DISCUSSION and CONCLUSIONS

The purpose of this thesis was to explore the use of internal dental structures as a biomarker for vitamin D deficiency and for improved age-at-death estimation over the lifecourse for both modern and archaeological individuals. The adverse outcomes of vitamin D deficiency in children and in adults are well documented (e.g., Holick, 2007, 2008), however this body of research linked vitamin D deficiency to mineralisation defects found in teeth, suggesting additional benefits of vitamin D for dental health. This thesis also used tooth dentin to investigate a method to improve accuracy of age-at-death for individuals over the age of 50 years, as age is a critical component to the study of human remains. Using a lifecourse perspective requires that age estimates be more accurate than current methods produce to provide precise data that are foundational to interpretations in bioarchaeology (Sofaer, 2011).

These concepts were explored through three specific sets of questions:

1) Is there a way to detect vitamin D deficiency in a tissue other than bone? Is IGD in tooth dentin a reliable indicator to aid in the diagnosis of this deficiency in archaeological samples? Is it possible to use teeth to determine the severity and age at which a deficient episode occurred?

2) What is the potential of radiographs in diagnosing vitamin D deficiency in skeletal samples? Is this a technique that can lend itself to identification of individuals with deficiency, as it is readily available to most researchers? This research also addressed important questions such as how severe do deficient conditions have to be before a

change in pulp shape occurs, at what ages do deficiencies occur, and what other factors might cause pulp shape changes?

3) Are age-at-death estimates derived from tooth section measurements of pulp/tooth ratios more accurate than those derived from radiographs and other available methodologies? Can this method be used to more precisely age older adult individuals in skeletal samples?

I address these questions through a theoretical framework that encompasses the lifecourse perspective. Exploring these questions has produced a body of work that opens up new avenues for the use of radiographic and histological analysis of tooth structures to investigate pathological conditions, specifically vitamin D deficiency, and these same internal dental structures can be effectively used to accurately age older in individuals in archeological samples.

The 21st century is now witnessing a dramatic return of vitamin D deficiency, particularly amongst individuals living at higher latitudes (Harris, 2004). In the late 1970s a wave of rickets was reported in industrialized countries, particularly in children of immigrants and in children who experienced prolonged breastfeeding (Goel et al., 1976; Welch et al., 2000). During the last few decades, several researchers have reported vitamin D deficiency in both developing and industrialized countries (e.g., Belachew et al., 2005; Dawodu et al., 2005; Al-Mustafa et al., 2007). It is thought that this resurgence has been the result of a combination of factors of modern lifestyles, such as the use of covering clothing, overuse of sunscreen, and an indoor lifestyle (Huh and Gordon, 2008; Brickley et al., 2014). As vitamin D is not naturally present in most food, with the

exception of oily fish and egg yolks, most vitamin D is synthesized in the body via ultraviolent rays in sunlight. Currently, Health Canada's daily recommended intakes for vitamin D are 400 international units for infants, 600 IU for children aged one through adulthood, and 800 IU for people over 70 (Hanley et al., 2010). The WHO and other health agencies are considering bone health and have identified clear evidence that maternal vitamin D levels are linked to bone development (Cooper et al., 2006; Javaid et al., 2006; Harvey et al., 2010). Adequate intakes are particularly important for pregnant women as they need to ensure they get enough vitamin D for proper fetal bone and tooth development, as permanent teeth are already forming in utero. Breast milk does not have enough vitamin D so infants who are not regularly exposed to sunlight need to be given drops of the vitamin to prevent nutritional rickets (Atkinson, 2011). It is noteworthy, that the Food and Drug Administration in the United States recently announced it has changed its regulations in 2016 to allow manufacturers to increase the level of vitamin D in milk and add fortification to more plant-based dairy beverages and yogurt alternatives. Health Canada is currently undergoing similar considerations to improve the vitamin D content of available foods in the marketplace (Osteoporosis Canada, 2017).

Vitamin D deficiency has also been a problem for past human societies, and has been the focus of extensive research through the analysis of visible indicators of deficiency on the skeleton (e.g., Brickley and Ives, 2008; Beck-Nielsen, 2012). Vitamin D deficiency can cause severe skeletal deformities with classic indicators including bowing of the weight-bearing bones, rachitic rosary of the costochondral rib junctions, and swelling of the distal metaphyses such as the wrists and ankles (Holick, 2007), which can

be identified in archaeological juveniles. However, many of these skeletal changes are subtle and are not present in an adult skeleton due to remodelling in bone growth and development. For example, alterations such as porosity found on the growth plate will be eradicated once vitamin D is subsequently obtained (Brickley et al., 2010). Similarly, a number of conditions exist that can mimic rickets by causing bowing deformities of the long bones during growth and development. Blount's disease, congenital defects, and Paget's disease are examples of conditions that can cause pathological deformity to long bones. As only approximately 10-25% of cases of rickets that result in observable leg bowing are retained in the adult skeleton, subtle changes associated with vitamin D deficiency will not be recognizable in the adult skeleton (Hess, 1930; Brickley et al., 2010). In some cases, it is not possible to diagnose vitamin D deficiency by assessing the skeleton.

To address these methodological issues, the first paper in this thesis demonstrated the effective use of tooth dentin, specifically interglobular dentin (IGD), to detect vitamin D deficiency in individuals who experienced deficiency during infancy and childhood but survived to adulthood. I amalgamated information from the clinical dental literature that showed that vitamin D deficiency and related conditions cause a failure in dentin mineralisation, and though it is currently reported in rare genetic cases, most of these conditions would not have been survivable prior to modern health care. The use of IGD permits increased recognition and identification of adult individuals who experienced deficiency in childhood and provides information on deficiency at other stages of the lifecourse (D'Ortenzio et al., 2016).

Chapter 2 of my thesis demonstrated that conditions disrupting vitamin D, calcium, and phosphate pathways cause systemic mineralisation defects in teeth, which are observed as clear bands of bubble-like spaces that follow incremental lines within the dentin matrix (i.e., interglobular dentin). This research found a clear association of IGD with conditions that cause vitamin D deficiency through scanning electron analysis (SEM) and histological examination. My results indicated that all of the archaeological individuals (n=6) who showed skeletal evidence of past deficiency also displayed the formation of IGD, whereas IGD was absent in the modern healthy controls (n=3). As dentin was considerably more disturbed in individuals with marked skeletal evidence of deficiency, I proposed that a temporary inhibition of dentin growth leads to modification of calcospherite shape and size, resulting in the characteristic interglobular spaces in individuals with deficiency.

One important contribution of this research was the creation of a scoring system for IGD in order to develop a link between IGD severity and the severity of deficiency experienced by the individual. 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). IGD was compared relative to normal dentin observed in the field of view in the microscope eyepiece. 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 2 had interglobular spaces that covered between 25 to 50% of the region of interest and were moderately large and more numerous than Grade 1. 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. The scoring system allows future researchers to quantify the severity of an episode of deficiency, which up until now, has only been conducted in the clinical literature (see Seow et al., 1989). I was able to correlate the most severe or marked cases of deficiency observed skeletally (e.g., marked bowing deformities in the long bones) with the presence of the most severe Grade 3 level of IGD. Conversely, individuals with less severe skeletal indicators of deficiency tended to have a less severe IGD score (Grade 1 or Grade 2). Further investigation is required to obtain evidence from histological and radiographic analysis to determine if duration of deficiency plays a role in the grade and type of dentin abnormality observed.

The ability to determine the approximate age when an episode of vitamin D deficiency may have occurred, using Moorrees et al.'s (1963) technique of assessing the dental age according to the degree of calcification observed in permanent teeth was a significant contribution of this research. The standards of Moorrees et al. (1963) were chosen as this method shows the formation of the tooth where initial cusp formation, root formation, and apex closure are all taken into account. The age at which an episode of past vitamin deficiency occurred was approximated by assessing the location of IGD within the tooth. Five out of six individuals had two to three teeth available that form at different ages and the location of IGD was noted. Use of this method enables paleopathologists to narrow down the age range at which a deficiency occurred during

childhood; however, the age is necessarily approximate, as dentin grows in concentric cones not horizontal layers.

Multiple episodes of vitamin D deficiency are impossible to accurately assess from macroscopic examination of the skeleton, even when clear skeletal changes are present, as individuals who have experienced one episode are likely to be vulnerable to experiencing further episodes (Brickley et al. 2014). This research found that multiple episodes of deficiency can be determined through the identification of fluctuating regions of IGD. While the tooth is in a growth stage, those with deficiency likely had periods of deficiency followed by periods of recovery, thus forming distinct bands of IGD that run parallel to the incremental lines within the dentin.

As vitamin D deficiency is systemic, IGD can be detected in several teeth the are forming at the same time. For example, if deficiency occurs in newborns, primary teeth and the 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 IGD in the roots of the first molar and under the crown of the second molars for two archaeological individuals. By correlating the age at which a deficiency occurred, it was possible to determine that three individuals had more than one episode of vitamin D deficiency. Although further research is needed with additional numbers of individuals examined, this paper was foundational in setting up research for the next paper where IGD enabled past episodes of vitamin D deficiency to be recognized in cases where skeletal indicators are not clearly distinguishable.

I recognize that this method is destructive and there will be some collections for which it will be impossible to apply this technique to, and this is why in the second paper in Chapter 3, I developed a non-destructive technique for identification of vitamin D deficiency. It is a technique that has direct comparability to the widely available information in the current population, hence it addresses the call by Wright and Yoder (2003) for stronger links between modern health research and paleopathology.

Chapter 3 explored whether radiographic images of internal tooth structures could be used to identify morphological changes in pulp chambers in the teeth of individuals with past vitamin D deficiency. Clinical studies have demonstrated that vitamin D deficiency from nutritional and genetic causes produces morphological changes in dental anatomy that can be detected radiographically (e.g., Seow and Latham, 1986; McDonnell et al., 1997). Based on this concept, the hypothesis tested in this paper was whether abnormal mineralisation related to vitamin D deficiency in childhood could be detected in both modern and archaeological samples through pulp chamber changes in permanent molars. This research showed that radiographs of pulp chamber shape can be successfully used to identify individuals with past deficiency.

Morphological examination of the pulp chamber, particularly the pulp horns, determined that those in healthy individuals were evenly matched, whereas pulp horns from individuals exhibiting deficiency were uneven (referred to as 'chair shaped'); or constricted (narrow). These observations were quantified by measurements taken of the pulp chambers. It was demonstrated that significant differences were found in: 1) pulp horn widths, and 2) for the ratios between pulp horn heights in both archaeological and

living individuals with deficiency versus those without. Measurements of pulp horn widths showed that pulp horns labelled morphologically as 'constricted' were less than 1mm and were associated with those individuals with vitamin D deficiency. In contrast, the pulp horns labelled 'normal' were greater than 1mm and were present in those individuals without deficiency. Those without deficiency had an approximate 1:1 ratio for pulp horn height, whereas those with deficiency had close to a 2:1 ratio. This signifies that the 'chair shape' observed on radiographs in those with deficiency was validated by the measurements, where one pulp horn was shorter relative to the other. As anticipated, measurements of pulp horn widths and heights taken on living individuals with deficiency were similar to archaeological individuals with deficiency and vise versa, further validating the results.

I used modern controls (with available medical and dental records) and three different groups of archaeological individuals who were radiographed and evaluated histologically for the presence of IGD. Results showed that all individuals with clear evidence of past deficiency (both skeletal and blood serum levels) and most (5/6) having slight skeletal indicators of deficiency, displayed constricted or chair shaped pulp horns. For individuals lacking both skeletal and radiological evidence of deficiency, two out of four had Grade 1 IGD, suggesting there is a threshold of severity of vitamin D deficiency that plays a role in pulp changes.

The discovery that severity of deficiency experienced by an individual influenced the use of radiographs of pulp chambers as a screening technique was an important aspect of research for this thesis. Grade 1 IGD appears to be insufficient to affect the shape of

the pulp chamber during tooth development. This result supported the findings from the first paper where it was found that less severe IGD (less than Grade 2) was not strongly associated with skeletal changes found in marked cases of severe deficiency. In cases where histological analysis revealed Grade 1 IGD (mild), that is the individuals who experienced deficiency leading to very slight mineralisation defects, it was determined that such individuals did not develop morphological changes in the pulp chamber and could be missed in radiological screening. Hence, by using the presence and severity of IGD in combination with pulp chamber changes, this can provide a baseline for future researchers to use when determining the severity of deficiency that an individual may have experienced.

A blind test was conducted to establish if other researchers could also use the instructions provided in this paper and consistently identify individuals with a previous episode of vitamin D deficiency. Using radiographs of known clinical cases and archaeological individuals with slight to marked skeletal indicators of vitamin D deficiency, the blind test revealed an accuracy of 75% among participants for selecting the correct answer. It was found that the results were clearly linked to the quality and proper angulation of the radiograph observed as all cases that received 100% correct scores involved just one or two teeth with good magnification and positioning.

Limitations of this study consisted of radiographing a tooth with the natural variation found in the 3rd molar (Scheid and Woelfel, 2007:409). Third molars, particularly the maxillary 3rd molar, have the most variable crown shape of all of the permanent molars. Therefore, caution should be used when interpreting pulp chamber

shape in 3rd molars as to date, no research has been conducted on whether this variation directly affects pulp chamber shape. Radiograph images that include all permanent molars provide a more comprehensive analysis of pulp shape. Incorrect positioning of a tooth on the X-ray plate can cause elongation, foreshortening, or superimposition (overlapping) of the pulp chambers. I recommend that the positioning of the maxilla and/or mandible be as perpendicular to the X-ray beam as possible in order to get the intersection of the beam in a buccolingual direction. A third consideration when using this technique is the presence of severe attrition on the occlusal surface of the dentition. Significant dental wear and large carious lesions may cause the development of reparative tertiary dentin, which in turn may result in morphological changes to the pulp chamber. The caveat is to try, where possible, to use permanent molars with no carious lesions and dental wear.

Individuals with morphological changes to their pulp chamber observed radiographically will certainly have experienced a condition that causes mineralisation defects. A differential diagnosis conducted in this paper indicates that only conditions linked to vitamin D, calcium, or phosphate imbalance cause these types of pulp chamber changes linked to abnormal mineralisation (see D'Ortenzio et al., 2016). This research found that it is possible to use non-destructive radiograph assessment of teeth as a screening technique for deficiency in past communities and that measurements taken of the pulp chamber directly corroborated the different pulp chamber shapes observed.

Although paleopathological research has made great advances in diagnosing deficiency using skeletal bone, it remains difficult to obtain a definitive diagnosis. While there is debate about individual variation in the ability to use the active form of vitamin D

(see Brickley and Ives, 2008), the medical clinician studying vitamin D deficiency in living populations has the advantage of analysing blood serum levels, which are more sensitive to nutritional changes. Even with the advantage of blood serum levels, it can be difficult to select appropriate indicators of vitamin D status, particularly when deficiencies are subclinical (Huss-Ashmore et al., 1982). The paleopathologist working with archaeological populations does not have the advantage of using most of the indicators available for living populations. There are, however, advantages to using tooth dentin from archaeological skeletons in vitamin D deficiency diagnosis. Archaeological dentition can provide a series of teeth for examination. Consequently, the paleopathologist can examine the whole dentition, in conjunction with the skeleton, to determine the effects of deficiency. In particular, the use of IGD can narrow down the age at which an individual experienced deficiency and can discern multiple episodes and severity of deficiency. Tooth dentin reflects the systemic effects of deficiency in cases where deficiency leave no skeletal changes.

The final paper in this thesis was undertaken using internal dental structures to identify older adult individuals who survived past the age of 50+. This technique found that pulp/tooth ratios can predict adult age-at-death with more accuracy and narrower age ranges than methods that use degenerative skeletal morphological analyses. By directly comparing pulp/tooth area ratios taken from radiograph images to those taken from histological images it was determined that age calculations from histological images were more accurate. This research created a further application of the ImageJ measuring technique, developed in the second paper, making available another method in a

bioarchaeologist's toolbox for age-at-death estimations, particularly for older individuals (50+). There are a number of methodological issues that interfere with the ability of paleopathologists to investigate old age. The current scarcity of bioarchaeological studies on ageing can be attributable to problems with age-at-death methodological issues. While there has been engagement by paleopathologists with the idea of age as an aspect of identity, this has usually concentrated on the earlier part of the lifespan, such as childhood (e.g., Halcrow and Tayles, 2008). Old age is mentioned tangentially most often in osteological studies, and is typically related to the search for more accurate ageing techniques (Hoppa and Vaupel, 2002b). Although important, these techniques are destined to fail at producing an age estimate that is both precise and accurate because of an absence of a one-to-one relationship between skeletal changes (due to variable degenerative issues) and chronological age (Appleby, 2010). A lack of direct correlation between chronological age and biological age means that bioarchaeological investigations are limited in analyzing constructed social categories of old age (Gowland, 2007). The results of this study are important methodologically as the histological pulp/tooth area ratios were a significant (P-values <0.005) predictor of chronological age with a mean absolute error (MAE) of ±3.93 to ±5.77 years for archaeological samples (Belleville) and ±3.77 years for modern individuals. When comparing this technique with cementum annulation, another technique that requires histological analysis of tooth sections, the results surpass the results achieved by counting annuli. While reasonably accurate age estimates have been produced (e.g., Stott et al., 1982), one critical issue with this technique is that accuracy diminishes with increasing age (particularly over 50+ years)

due to a reduction in the alternating light and dark bands needed to discern the cementum rings (Lipsinic et al., 1986; Condon et al., 1986), making this technique unsuitable for ageing older individuals. It is also a complex method requiring specialist equipment and training and the biological processes on which it is based is not completely understood (Wittwer-Backofen and Buba, 2002). With advancing age, it becomes increasingly difficult to clearly discern the cementum rings.

As with all adult age estimation methods, this technique I have outlined was not without limitations. For example, where single-rooted teeth were missing or severely worn, the method cannot be employed. One must ensure that the tooth section is cut precisely through the middle of the pulp chamber in a labiolingual plane for incisors or buccolingual direction for canines and premolars to expose the whole pulp shape for analysis. As with any histological technique, this requires precision tooth cutting instruments and the implementation of precise protocols.

The ages calculated from the histological images of sectioned teeth signified that this technique can be used for reliable age estimations, particularly in older age groups (50+). It is not only useful in assessing the chronological age-at-death in skeletal remains, but could be a reliable tool in forensic cases, where determining age is an important factor in identification. Currently, paleodemographic studies are based mainly on expected longevity and mortality rates (Hoppa and Vaupel, 2002b). This technique could contribute to such studies by opening up the potential to determine age, particularly in the 50+ age range, with greater precision and accuracy.

The results of the research presented in this thesis underscore the importance of internal dental structures to vitamin D deficiency and to adult age-at-death estimation. Secondary dentin is a vital tissue that continues to grow throughout the life of an individual and is influenced by systemic conditions of the body (Burke and Samarawickrama, 1995). Similarly, pulp chambers respond to pathological stimuli during an episode of vitamin D deficiency. This body of work offers new approaches in the identification of older individuals and those who may have suffered from vitamin D deficiency during childhood. These findings have a number of important implications for anthropological studies. Attempting to extend the study of the lifecourse by calculating more precise age-at-death estimations for older individuals permits future research to now include the elderly in the age distribution of a community. The inclusion of the elderly in the age structure of a community contributes to a more comprehensive understanding of health and disease. This is an essential component of any analysis as many pathological conditions are age-cumulative (e.g., degenerative joint disease, osteoporosis); the longer an individual is alive, the greater the exposure to factors leading to these conditions (Larson, 1997). The age-at-death technique presented in this thesis encourages active engagement with old age, making it possible for bioarchaeologists to contribute significantly to investigations of what ageing is and how the ageing process may have changed over time. In turn, this should assist in constructing a more nuanced picture of old age in the bioarchaeological literature.

By considering the relationship between IGD and vitamin D deficiency from early infancy to later adult life, through exploring a condition that affects health status, this

opens up lines of inquiry that emphasize the potential of vitamin D to impact skeletal, cardiovascular, and metabolic health. As childhood vitamin D deficiency is known to affect skeletal morphology, one may be able to identify hidden lifestyle factors that contribute to age-specific deficiency by focusing on the dentition of individuals who survived to adulthood. As an example, one may find that age-specific rates or severity of IGD, for example, differ among individuals who were weaned at a young age, versus those who were breastfed for a longer period of time.

Improved adult age-at-death estimations in combination with better identification of vitamin D deficiency in archaeological individuals can begin to tackle complex questions raised by the osteological paradox which suggests that individuals with clear evidence of dental defects are those who managed to survive stressful events, while those lacking dental defects succumbed without having time to develop a response to the disruptive impact of deficiency (Wood et al., 1992). One can now evaluate the individuals who survived the most critical early phase of life (i.e., from birth to approximately age five) and determine if there is a relationship between age-at-death and number and severity of mineralisation defects. By directly comparing those with deficiency who died early in childhood, during the presumed risky periods (i.e., weaning ages), to those who survived to an older age, one could determine whether IGD is more frequent and severe in the vulnerable younger individuals, which would conform to the expectations proposed in Wood et al.'s (1992) theory. Conversely, if numerous episodes of severe IGD are more common in older age groups, this suggests a higher robusticity or lower frailty that lends support to Wood et al.'s (1992) predictions. Recognition of diagnostic features in internal

dental structures provides information on the earliest part of the lifecourse, particularly the childhood of individuals who survived episodes of deficiency during their growth and development. This could also contribute information to such lifestyle factors as socioeconomic status and cultural factors that affect infants and children in different communities (Holick, 2006; Brickley et al., 2010). The overall contribution of this thesis is not only limited to archaeological individuals as modern individuals with low levels of vitamin D can experience an inhibition in dentin mineralisation that has adverse effects on dental health. This research encourages potential studies to be carried out on the teeth of modern 'at risk' groups that may add to the evidence considered by public health authorities when making decisions on vitamin D fortification of foods.

Future Research Directions

Areas of future research will involve investigation of adults with osteomalacia to determine if IGD is present in secondary dentin 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, interglobular dentin may be observed. For example, the modern individual in Chapter 4 with a seven-year radiological history had almost no secondary dentin develop over this time period. This indicates that vitamin D deficiency would need to be very long-standing to produce severe IGD, and illustrates that using the methods in this thesis can better connect current health data to paleopathological research (Wright and Yoder, 2003).

Relationships between enamel hypoplasia (EH) and IDG could also be explored. If vitamin D deficiency only affects dentin formation, consequently, the EHs are not caused by vitamin D deficiency, it would be interesting to know if there is a correlation between severity of IDG and the formation of EHs.

While the papers in this thesis analysed permanent teeth, deciduous teeth could provide valuable information related to the intrauterine environment of mothers with vitamin D deficiency. Deciduous teeth could be investigated for prenatal vitamin D deficiency by using the neonatal line as a guide for location of IGD in subadults and adults who have their 1st molars.

Additional work using clinical dental radiographs and the associated medical data of modern individuals for the pulp chamber shape data will be helpful in fully developing this radiograph technique. This will further our understanding of the pathogenesis of pulp tissue to obtain detailed insight into the formation of dentin and pulp chambers in individuals with vitamin D deficiency. Further studies are needed to explore questions such as whether there is a differential sensitivity to deficiency in other tooth types as changes thus far appear most marked in 1st molars. It would be useful to see what results could be achieved by using tooth sections instead of radiographs to evaluate pulp chamber shape, as tooth sections produced more accurate measurements for age estimates.

Future research envisioned might also entail investigating the pulp/tooth area ratio ageing technique presented in this thesis on different populations. It would be advantageous to expand this technique for use in different communities to develop a data

set to enable a comparison of calculated ages found in the Belleville community. It may also be useful to experiment with the use of different tooth types using this technique, particularly molar teeth to test if this method could be adapted for use with the more complex pulp chambers found in molars. Future research will aim at acquiring larger sample sizes, in order to reduce the errors of age estimation, and could potentially study the effects of ancestry, culture, and life expectancy on the technique parameters. Another future direction would be to investigate the use of several teeth together to develop a composite picture of dental age estimations. One might also consider the use of microCt imaging equipment to explore 3-D volume measurements of the pulp chamber.

Literature on congenital rickets has found that maternal deficiency led to significant bone impairment in the fetus (Paterson and Ayoub, 2014). Wolfe (1935) 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 presence of IGD in teeth could contribute to research on the Developmental Origin of Health and Disease (DOHaD) theory, where studies on deciduous teeth could explore if stressors such as vitamin D deficiency, early in an individual's life have negative health consequences, later in life. As vitamin D appears to play a role in insulin resistance, high blood pressure, and immune function, IGD can contribute to research on how vitamin D relates to chronic conditions such as heart disease, diabetes, and cancer. The theory that certain adult-onset diseases may have their origin in insults acquired in utero or early childhood has been difficult to confirm, but the concept that early life stress affect adult health outcomes has become a widely accepted

phenomenon and has biological plausibility (e.g., Barker et al., 1989; Barker, 1994; Armelagos et al., 2009; Feltes et al., 2011). The exploration as to whether this theory can be applied to vitamin D deficiency could be conducted on the Belleville, Ontario, individuals who may have experienced deficiency during childhood. One could investigate if those with evidence of early childhood vitamin D deficiency experienced adverse effects that persisted throughout the lifecourse resulting in potentially reduced longevity.

The importance of internal dental structures in understanding past lifestyles cannot be underestimated as conditions such as vitamin D deficiency and its effects impact the human body. Dentin and pulp chambers are underutilized as tissues that offer improved age-at-death estimations for those in the older age category, and provide insights into the risk factors and health status experienced by those who lived in the past. A comprehensive analysis of teeth can provide a direct record of past lives, information that might otherwise not be retrievable from the archaeological record. This may help emphasize the importance of adequate vitamin D concentrations throughout the lifecourse. Teeth can play a key role in the development and maintenance of a healthy body and are an important component to a systemic approach to vitamin D fragility from before the cradle to the grave.

References

Al-Mustafa, Z.H., Al-Madan, M., Al-Majid. H.J., Al-Muslem, S., Al-Ateeq, S., Al-Ali, A.K. 2007. Vitamin D deficiency and rickets in the Eastern Province of Saudi Arabia. Ann Trop Paediatr, 27 (1), 63-67.

Appleby, J. 2010. Why we need an archaeology of old age, and a suggested approach. Nor Archaeol Rev, 43 (2), 145-168.

Armelagos, G.J., Goodman, A.H., Harper, K.N., Blakey, M.L. 2009. Enamel hypoplasia and early mortality: Bioarcheological support for the Barker hypothesis. Evol Anthropol Rev, 18 (6), 261-271.

Atkinson, S.A. 2011. Determinants of Vitamin D Status in Early Infancy. J Am Diet Assoc, 111 (12), 1820-1821.

Barker, D., Osmond, C., Winter, P., Margetts B. 1989. Weight in infancy and death from ischaemic heart disease. Lancet, 9, 577-580.

Barker, D. 1994. Mothers, babies, and disease later in life. London: BMJ Publishing Group.

Beck-Nielsen, S.S. 2012. Rickets in Denmark. Dan Med J, 59 (2), B4384-B4384.

Belachew, T., Nida, H., Getaneh, T., Woldemariam, D., Getinet, W. 2005. Calcium deficiency and causation of rickets in Ethiopian children. East Afr Med J, 82 (3), 153-159.

Brickley, M., Ives, R. 2008. The bioarchaeology of metabolic bone disease. San Diego; Academic Press.

Brickley, M., Mays, S., Ives, R. 2010. Evaluation and interpretations of residual rickets deformities in adults. Int J Osteoarchaeol 20, 54-66.

Brickley, M.B., Moffat, T., Watamaniuk, L. 2014. Biocultural perspectives of vitamin D deficiency in the past. J Anthropol Arch, 36, 48-59.

Burke, F.M. and Samarawickrama, D.Y.D., 1995. Progressive changes in the pulpo-dentinal complex and their clinical consequences. Gerodontology, 12 (2), pp.57-66.

Condon, K., Charles, D.K., Cheverud, J.M., Buikstra, J.E. 1986. Cementum annulation and age determination in Homo sapiens. II. Estimates and accuracy. Am J Phys Anthropol, 71 (3), 321-330.

Cooper, C., Westlake, S., Harvey, N., Javaid, K., Dennison, E., Hanson, M. 2006. Review: developmental origins of osteoporotic fracture. Osteoporos Int, 17, 337-347.

Dawodu, A., Agarwal, M., Sankarankutty, M., Hardy, D., Kochiyil, J., Badrinath, P. 2005. Higher prevalence of vitamin D deficiency in mothers of rachitic than nonrachitic children. J Pediatr, 147 (1), 109-111.

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.

Feltes, B.C., de Faria Poloni, J., Bonatto, D. 2011. The developmental aging and origins of health and disease hypotheses explained by different protein networks. Biogerontol, 12 (4), 293-308.

Gluckman, P., Hanson M. 2006. The developmental origins of health and disease: an overview in developmental origins of health and disease. Gluckman P., Hanson, M. (Eds.). Cambridge: Cambridge University Press, pp.1-5.

Goel, K.M, Sweet, E.M., Logan, R.W., Warren, J.M., Arneil, G.C, Shanks, R.A. 1976. Florid and subclinical rickets among immigrant children in Glasgow. Lancet, 1 (7970), 1141-1145.

Goldberg, M., Smith, A.J. 2004. Cells and extracellularmatrices of dentin and pulp: a biological basis for repair and tissue engineering. Crit Rev Oral Biol Med, 15:13–27.

Gowland, R. 2007. Age, ageism and osteological bias: The evidence from late Roman Britain. J Rom Archaeol Supplemental Series, 65,153-169.

Halcrow, S.E., Tayles, N. 2008. The bioarchaeological investigation of childhood and social age: problems and prospects. J Archaeol Method and Theory, 15, 190-215.

Hanley, D.A., Cranney A., Jones, G., Whiting S.J., Leslie W.D., Cole D.E.C, Atkinson S.A., Josse R.G., Feldman S., Kline G.A., Rosen C. 2010.Vitamin D in adult health and disease: a review and guideline statement from Osteoporosis Canada. Can Med Ass J, 182 (12), E610-E618.

Harris, R.J. 2004. Nutrition in the 21st century: what is going wrong? Arch Dis Child, 89, 154-158.

Harvey, N.C., Javaid, M.K., Arden, N.K., Poole, J.R., Crozier, S.R., Robinson, S.M., Inskip, H.M., Godfrey, K.M., Dennison, E.M., Cooper, C., Team, S.W.S.S. 2010. Maternal predictors of neonatal bone size and geometry: the Southampton Women's

Survey. J Dev Orig Health Dis, 1, 35-41.

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

Holick, M.F. 2006. Resurrection of vitamin D deficiency and rickets. J Clin Invest, 116 (8), 2062-2072.

Holick, M.F. 2007. Vitamin D deficiency. N Engl J Med, 357 (3), 266-281.

Holick, M.F. 2008. Vitamin 1 D: a D-lightful health perspective. Nutrition Rev, 66, S182-S194.

Hoppa, R.D., Vaupel, J.W. 2002b. The Rostock Manifesto for paleodemography: the way from stage to age. In Paleodemography: Age Distributions from Skeletal Samples, Hoppa, R.D., Vaupel, J.W., (Eds.) Cambridge: Cambridge University Press, pp. 1-8.

Huh, S.Y. Gordon, C.M. 2008. Vitamin D deficiency in children and adolescents: Epidemiology, impact and treatment. Rev Endoc Metabol Disorders, 9, 161-170.

Huss-Ashmore R., Alan, H., Goodman A.H., Armelagos J. 1982. Advances in Archaeol Method Theory, 5, 395-474.

Javaid, M.K., Crozier, S.R., Harvey, N.C., Gale, C.R., Dennison, E.M., Boucher, B.J., Arden, N.K., Godfrey, K.M., Cooper, C., Princess Anne Hospital Study G. 2006. Maternal vitamin D status during pregnancy and childhood bone mass at age 9 years: a longitudinal study. Lancet, 367, 36-43.

Katz, S., Foulks, E. 1969. Mineral metabolism and behavior abnormalities of calcium homeostasis. Am J Phys Anthropol, 32, 299-304.

Larson, C.S. 1997. Bioarchaeology: Interpreting Behavior from the Human Skeleton. Cambridge Studies in Biological Anthropology. Cambridge: Cambridge University Press.

Lipsinic, F.E., Paunovich, E., Houston, G.D., Robison, S.F. 1986. Correlation of age and incremental lines in the cementum of human teeth. J Forensic Sci, 31 (3), 982-989.

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. 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.

Osteoporosis Canada, 2017. Electronic document, retrieved April 27, 2017, http://www.osteoporosis.ca/news/press-releases/new-vitamin-d-guidelines/

Paterson, C.R., Ayoub, D. 2014. Congenital rickets due to vitamin D deficiency in the mothers. Clin Nutr, 34, 793-798.

Pinhasi, R., Mays, S. 2008. (Eds.). Advances in human palaeopathology. West Sussex, U.K: John Wiley & Sons.

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., 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.

Scheid, R.C., Woelfel, J.B. 2007. Woelfel's dental anatomy: Its relevance to dentistry. 7th edition, Maryland; Lippincott Williams and Wilkins.

Sofaer, J., 2011. Towards a social bioarchaeology of age. In: Agarwal S.C., Glencross, B.A., (Eds.), Social bioarchaeology. West Sussex, U.K: Wiley-Blackwell Publishing Ltd. Pp. 283-311.

Stewart, R.J.C. 1975. Bone pathology in experimental malnutrition. World Rev Nut Dietetics, 21, 1-74.

Stott, G.G., Sis, R F., Levy, B.M. 1982. Cemental annulation as an age criterion in forensic dentistry. J Dent Res, 61 (6), 814-817.

Uauy, R., Kain, J., Corvalan, C. 2011. How can the Developmental Origins of Health and Disease (DOHaD) hypothesis contribute to improving health in developing countries? Am J Clin Nutr, 94 (6 Suppl), 1759S-1764S.

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.

Welch, T.R., Bergstrom, W.H., Tsang, R.C. 2000. Vitamin D-deficient rickets: the reemergence of a once-conquered disease. J Pediatr, 137 (2), 143-145.

Wells, C. 1975. Prehistoric and historic changes in nutritional diseases and associated conditions. Prog Food Nut Sci, 1 (2), 729-779.

Wittwer-Backofen, U. and Buba, H., 2002. Age estimation by tooth cementum annulation: perspectives of a new validation study. In Paleodemography: Age Distributions from Skeletal Samples, Hoppa, R.D., Vaupel, J.W., (Eds.) Cambridge: Cambridge University Press, pp. 107-128.

Wolfe, J. J. Teeth in fetal rickets. 1935. Am J Dis Child, 49 (4), 905-911.

Wood, J.W., Milner, G.R., Harpending, H.C., Weiss, K.M., Cohen, M.N., Eisenberg, L.E., Hutchinson, D.L., Jankauskas, R., Cesnys, G., Česnys, G. and Katzenberg, M.A., 1992. The osteological paradox: problems of inferring prehistoric health from skeletal samples [and comments and reply]. Curr Anthropol, 33 (4), 343-370.

Wright, L.E. and Yoder, C.J., 2003. Recent progress in bioarchaeology: approaches to the osteological paradox. J Archaeol Res, 11 (1), 43-70.