THE WISDOM IN THE TOOTH

THE WISDOM IN THE TOOTH: THE MEASUREMENT AND ANALYSIS OF INTERGLOBULAR DENTINE IN A SAMPLE OF TEETH FROM 19TH CENTURY MADRID, SPAIN

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LAY ABSTRACT

This research measures a tooth mineralization defect known as interglobular dentine (IGD), often associated with vitamin D deficiency (VDD), in a sample of 26 individuals from pre-industrial Spain (19th century CE). The individuals ranged in age between 7 months in-utero to 6 years. The aims of this thesis are threefold: (1) to establish an objective methodology to quantify the total amount of IGD in each tooth; (2) to analyze and interpret the IGD data connecting it to the overall health of the individual; and (3) to make inferences on the vitamin D status of the individual's mother and cultural practices (i.e., breastfeeding and weaning periods) through the vitamin D status of the infant or child. Of the 26 individuals examined in this thesis, 21 had evidence of IGD in at least one tooth (33/44 teeth). Of these 33 teeth with IGD, their amounts relative to the unaffected surrounding dentine ranged between 0.05% - 26.22%, with 15 teeth having an episode of IGD ending <10 months of age (prior to weaning onset). By analyzing the teeth and measuring the timing and amount of IGD within them, this thesis presents the physical evidence of VDD in women and children in pre-industrial Spain, providing information on members of society often under-represented in the historical literature.

ABSTRACT

Interglobular dentine (IGD), a tooth mineralization defect, is associated with vitamin D deficiency (VDD) and its resultant conditions (e.g., rickets). Using a sample of 44 infant and child teeth (34 deciduous; 10 permanent) from 26 individuals (aged 7 months in-utero – 6 years) from pre-industrial (19th century CE) Madrid, Spain, this thesis provides insight into the health of both mothers and their children by answering the following research questions: (1) How can the amount of IGD in an entire tooth be quantified rather than in a "region of interest"? (2) How can this methodology contribute to the interpretation of the IGD data using the mother-infant nexus as a theoretical framework? (3) How can the IGD data be used to understand the risks of VDD in infants and their mothers in Spain during the 19th century? The results of this study indicated that 21 of 26 individuals, who had skeletal evidence of rickets, had at least one tooth with evidence of IGD (33/44 teeth). The amount of IGD present ranged between 0.05% - 26.22% relative to the surrounding unaffected dentine area. Of the 33 teeth with evidence of IGD, 15 had an episode of IGD ending before the onset of weaning (i.e., <10 months of age), with 13 teeth belonging to individuals who had skeletal evidence of rickets. These results indicate that the VDD experienced by the infants from this study were likely a result of the health and/or the cultural practices of the mother. The objective measurement of IGD amounts, timing, and length of the episodes recorded, in combination with historic literature provide insight into the health, socio-economic status and environmental conditions of women and children in 19th century Spain.

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ABBREVIATIONS AND DENTAL NOTATION

CE	Common Era
DEJ	Dental-Enamel Junction
DIGD	Developmental Interglobular Dentine
fl oz	Fluid Ounces
g	Grams
IGD	Interglobular Dentine
IU	International Units
mo	Month
TD	Trinitarias Descalzas
UVB	Ultraviolet B Radiation
VDBP	Vitamin D Binding Protein
VDD	Vitamin D Deficiency
yrs	Years

DENTAL NOTATION

ri^1	Deciduous Maxillary Right First Incisor
li^1	Deciduous Maxillary Left First Incisor
li_1	Deciduous Mandibular Left First Incisor
rm^1	Deciduous Maxillary Right First Molar
lm^1	Deciduous Maxillary Left First Molar
rm_1	Deciduous Mandibular Right First Molar
lm_1	Deciduous Mandibular Left First Molar
lm_2	Deciduous Mandibular Left Second Molar
RM^1	Permanent Maxillary Right First Molar
LM^1	Permanent Maxillary Left First Molar
RM_1	Permanent Mandibular Right First Molar
LM_1	Permanent Mandibular Left First Molar

DECLARATION OF ACADEMIC ACHIEVEMENT

I declare that I am the sole author of this thesis, working under the supervision of Dr. Tracy Prowse. I developed a new methodology for quantifying interglobular dentine using a sample of teeth provided by Dr. Megan Brickley. I was trained by the laboratory co-ordinator, Bonnie Kahlon, of the Anthropology department at McMaster University to prepare tooth sections for microscopic analysis. I interpreted this data with the assistance and guidance of Dr. Prowse. Feedback on my thesis was provided by my second committee member, Dr. Amanda Wissler and my third reader Dr. Andrew Roddick.

CHAPTER 1: INTRODUCTION

1.1 Research Objectives and Overview

The recognition, interpretation, and understanding of children and childhood in the past have been a growing focus of research in anthropology and archaeology over the past 25 years (e.g., Perry 2005, Sofaer 2007, Baxter 2008, Crawford et al. 2018): however, infants (i.e., under 1 year of age) have not yet received the same degree of attention. The connection between the health of a foetus or infant in relation to their mother's health, particularly during pregnancy and/or times of breastfeeding and weaning is critical for long term health, but has not been widely explored in anthropological and archaeological literature. This relationship is acutely evident when we look at the outcomes of adverse health conditions experienced by the mother while pregnant and post-partum. In particular, vitamin D deficiency (VDD) in the mother has consequences for the health of the infant. Clinically, VDD can be easily detected through a blood sample; however bioarchaeologically, such a deficiency must be interpreted through other means to understand this mother-infant connection.

The often-ephemeral nature of skeletal evidence makes it difficult for bioarchaeologists to analyze past populations. Teeth, however, are more enduring. The process of tooth development captures information that may otherwise be lost or remodeled during skeletal development or taphonomic processes once deceased. Teeth can provide information on the health of the individual and the individual's mother (if the tooth was forming during pregnancy and/or breastfeeding). This thesis identifies and analyzes the timing, length, age of onset and amount of a tooth mineralization defect

(interglobular dentine - IGD) associated with VDD. The results are interpreted using the mother-infant nexus framework to understand diet and/or environmental conditions experienced by the individual during tooth development. Specifically, this thesis aims to answer the following research questions:

1) How can the amount of IGD in an entire tooth be quantified rather than only in a 'region of interest'?
2) How can the methodology from question 1 (under the framework of the mother-infant nexus) contribute to the interpretation of the IGD data?
3) How can the IGD data be used to understand the risks of VDD in infants and their mothers in Spain during the 19th century?

With regards to research question (1), this thesis will assess existing methodologies for quantifying IGD and present a novel method for measuring the amount of the mineralization defect (as a percentage). Previous studies of IGD (e.g., D'Ortenzio et al. 2016) have used a 'region of interest' method, which targets a specific area of the tooth to quantify the amount of IGD present by visually estimating and assigning a percentage range to said region. By using one region of interest on a tooth to visually estimate a percentage of IGD, information from the rest of the tooth can be overlooked or misinterpreted. This thesis is the first study to analyse and quantify the entire dentine space in a tooth with regards to IGD, instead of focusing on a region of interest.

The second research question (2) will be addressed using a sample of deciduous and permanent teeth from infants and children discovered in a 19th century crypt under the church of Las Trinitarias Descalzas in Madrid, Spain. The individuals in this sample had a high prevalence of rickets in their skeletal remains, so their teeth were intentionally chosen by Dr. Megan Brickley specifically to analyse this deficiency. The relationship between the mineralization defect and biological or social factors that contributed to VDD in these infants and children will be explored.

The final research question (3) will use historic data in combination with the IGD data to discuss and understand the potential health outcomes for infants and children in 19th century Spain. This thesis will interpret the IGD data collected from the Trinitarias Descalzas sample and explore the relationship(s) between the data and the health of mothers, infants, and children from the sample. The data are interpreted using the framework of the mother-infant nexus, a concept that examines the relationship between a mother and her infant as a synergistic unit rather than two separate entities.

1.2 Theoretical Orientation – Mother-Infant Nexus

The important relationship between a mother and their infant has been explored in multiple health-related disciplines (e.g., pediatrics, obstetrics, epidemiology, psychology, nutrition) since the 1970s. In these health-related fields, the well-known concept of the mother-infant dyad emphasizes the importance of the biological, psychological, and social relationship between an infant and their mother in all aspects of life, which have important consequences for infant and childhood development (Mäntymaa et al. 2003,

Dieterich et al. 2013, White-Traut et al. 2013, Sansavini et al. 2015, Géa-Horta et al. 2016, Provenzi et al. 2018, Zietlow et al. 2019, Neugebauer et al. 2021).

When specifically discussing the mother-infant dyad in relation to vitamin D, current clinical literature presents evidence that maternal vitamin D status during pregnancy and breastfeeding can impact the vitamin D status of the infant (e.g., Dawodu and Tsang 2012, Thiele et al. 2017), although there is disagreement about the protocols necessary to provide accurate vitamin D regulations for the general population and, in particular, pregnant and breastfeeding women and their infants. Fiscaletti et al. (2017), Moon et al. (2020), Mustapa Kamal Basha et al. (2020), Wang et al. (2024), and Yang et al. (2024), are a few of the many researchers to note that there is an abundance of observational evidence that links VDD to many different types of diseases or health outcomes, but randomized controlled trials have yet to confirm this information. The observational evidence notes that a lack of vitamin D in a pregnant woman can lead to significant negative health outcomes for both the mother and infant. Gestational hypertension, pre-eclampsia, and gestational diabetes (Moon et al. 2024, Wang et al. 2024) are a few examples of complications the mother could face, while the infant could be pre-term, have a low birth weight, be small for their gestational age and be even more susceptible to develop immunological complications if vitamin D remains low (Thiele et al. 2017, Wang et al. 2024).

The focus on the important relationship between mothers and infants received comparatively less attention in anthropology and archaeology until recent work by Han and colleagues (2018) and Gowland (2018) advocated for a greater focus on foetus and

infants as critical areas of research in these fields. In the anthropological literature, physical evidence from the past, such as skeletal and dental remains, historic documentation, and other archaeological evidence are used to understand and interpret the relationship between mother and infant in different contexts throughout time.

The recent development of the 'mother-infant nexus' framework in anthropology is the result of the collective efforts of multiple individuals from different disciplines at a workshop organized by Dr. Rebecca Gowland and Dr. Siân Halcrow. Gowland and Halcrow (2020) led this workshop to focus on the sharing, collaboration and discussion of the challenges researchers faced when trying to comprehend the mother-infant nexus. Their resulting data was used to develop a volume incorporating the new theoretical and methodological developments discussed and expand on four research themes surrounding health, nourishment, social interactions and infant death in the archaeological record. The focus of this volume was to highlight an area often overlooked in multiple disciplines (i.e., the relationship between a mother and their infant). Subsequent research has adopted the mother-infant nexus as a framework for investigating the health of both infants and their mothers (e.g. Riccomi et al. 2021, Nava 2023, Chiappa and Schrader 2025, Gowland 2025).

Using the mother-infant nexus framework in this thesis is vital to understand the data collected from the Trinitarias Descalzas sample and interpret what the data tells us about the life and health of mothers and their offspring in 19th century Spain. The data from the infant teeth can be used to understand the environment they experienced during significant periods of development, both biologically and culturally, as a result of their

mother's health and care. Biologically, the mother-infant nexus can be employed to make inferences concerning the health of the mother through the health of the infant during the infant's development. Culturally, it considers infant care customs, such as breastfeeding and weaning, as critical periods of this relationship. By using this framework, this thesis can contribute to discussions of both infant and maternal health, specifically their synergistic health effects in 19th century Spain.

1.3 Thesis Structure

Chapter 2 provides an overview of the metabolism of vitamin D in the body and discusses how this process changes during pregnancy, breastfeeding, and weaning. The effects of vitamin D on the skeleton and, notably, the teeth are then explained. Before the specifics of IGD are discussed, a brief overview of tooth structure and development are included as it is necessary information to understand the process, development, and causes of IGD.

Chapter 3 begins with a brief introduction on the health, diets, and cultural practices of mothers and their infants in 19th century Spain. An introduction of the site from which the sample was collected, providing geographic, social, historic and archaeological context is then presented. Following this information, the methodologies used to prepare the slides for data collection are described, as well as the examination and analysis processes.

Chapter 4 presents the results obtained and begins with information on the demography of the sample, including age-at-death, tooth type, and evidence of rickets. This chapter reports the data collected on the prevalence of IGD and organizes the

presence of the data by individual and then by tooth. The teeth identified with IGD are then analyzed to determine the amount of IGD present per tooth, as well as by tooth type (i.e., deciduous incisor, deciduous molar, permanent molar). The timing and number of IGD episodes are then presented. Statistical analyses of the data are presented in this chapter, concluding with a summary of the information provided in the entire chapter.

Chapter 5 interprets the results presented in the previous chapter and how they relate to prior research on IGD. Four interpretations are presented for the atypical patterns seen within the data. The utility of the method presented in this thesis for quantifying IGD is discussed at the end of this chapter. The final chapter (Chapter 6) summarizes the findings from this thesis and presents recommendations for future research.

CHAPTER 2: BACKGROUND

2.1 Introduction

The purpose of this chapter is to provide the information necessary to understand the importance of vitamin D, its role in the human body, and its influence on the health of mothers, infants, and children viewed through the lens of the mother-infant nexus. Beginning with the importance of vitamin D and its metabolism, this chapter then focuses on how vitamin D deficiency (VDD) impacts mothers during pregnancy and lactation. The skeletal evidence of VDD and the conditions associated with it are briefly summarized prior to the explanation for how VDD can be seen in dental remains with the presence of a tooth mineralization defect known as interglobular dentine (IGD).

2.2 Vitamin D

2.2.1 Vitamin D and its Importance

Vitamin D is more than just a vitamin, it is also considered a *pleiotropic hormone* (i.e., its main biological function is performed in multiple organ systems) (Verstuyf et al. 2010, De Martinis et al. 2021, Durá-Travé and Gallinas-Victoriano 2023). There are two forms of vitamin D in human biology. Vitamin D_2 (ergocalciferol) originates from the photosynthesis of plants, fungi or yeast, while vitamin D_3 (cholecalciferol) originates from animals (Jones 2018). Both forms can be ingested and absorbed though dietary sources; however, D_3 is mainly synthesized within the skin of humans after exposure to ultraviolet B (UVB) radiation (i.e., sunlight) and accounts for most of our vitamin D intake, at 80-90% (Sahota 2014). Vitamin D_2 and D_3 are both considered biologically inactive, meaning they cannot carry out their proper functions within the body until they

are activated. The activation (i.e., metabolism) of vitamin D is discussed in the next section (2.2.2).

The physiological function of vitamin D was initially thought to only aid in bone homeostasis and mineralization through the regulation of calcium and phosphate. However, recent clinical research also considers vitamin D to be an essential regulator of gene expression and cell differentiation (Sutton and MacDonald 2003, DeLuca 2004, Snoddy et al. 2016). With this knowledge, connections have been made between VDD and the risk of developing and/or exacerbating multiple types of diseases and illnesses. An increase in risk of colon cancer (Garland and Garland 2006) and breast cancer (Voutsadakis 2021) are linked to VDD. Vitamin D and its metabolizing components have recently been linked with the regulation of immune function (Hewison 2012, Charoenngam and Holick 2020, Bishop et al. 2021); therefore individuals with extremely low levels of vitamin D are more susceptible to infections like tuberculosis (Nnoaham and Clarke 2008, Aibana et al. 2019), auto-immune disorders (such as such as rheumatoid arthritis) (Adorini and Penna 2008), diabetes (Charoenngam and Holick 2020) and multiple sclerosis (Spiezia et al. 2023). An increased risk of cardiovascular disease is also linked to a deficiency in vitamin D (Zittermann et al. 2021).

As it relates to this thesis and the framework of the mother-infant nexus, connections have also been made between VDD during pregnancy and adverse repercussions for both the mother and foetus (Agarwal et al. 2018). Some of the maternal effects associated with VDD during pregnancy in modern populations include

preeclampsia (high blood pressure), gestational diabetes, preterm delivery, the necessity for cesarean section and post-partum depression (Fiscaletti et al. 2017, Agarwal et al. 2018, Elsori and Hammoud 2018). The foetal outcomes for mothers with VDD include low birth weight, small size for gestational age, respiratory tract infections, and general lowered immunity (Agarwal et al. 2018, Elsori and Hammoud 2018). To further understand the role of vitamin D and its importance throughout an individual's life, it is necessary to learn about its discovery.

The road to the discovery of vitamin D (deficiency) began in the 16th century, though O'Riordan and Bijvoet (2014) argued that it was earlier with the emergence of a bone-deforming disorder affecting children. Physicians in the 17th century described this 'new' disorder as having multiple skeletal abnormalities, including spine and femur curvature, joint enlargement of long bones and ribs, and shortened stature (O'Riordan and Bijvoet 2014, Holick 2023). This disease was referred to as rickets or the 'English Disease' in the 17th century (O'Riordan and Bijvoet 2014, Jones 2018). Although its emergence and 'discovery' was noted in England, there is evidence of rickets in Italy in the 16th century among children of high socio-economic status – the Medicis (Guiffra et al. 2015). The increase in the occurrence of rickets (or an increased awareness), as well as the debate concerning its cause, continued throughout Europe into the 18th century (Holick 2023). With the increased awareness came various types of treatments or remedies including venesection of the ear (bloodletting), ingestion of crow (rook) and/or frog liver, and use of shark liver ointment and cod liver oil (Parapia 2008, O'Riordan and Bijvoet 2014, Jones 2018). Most people argued for the sole use of cod liver oil in rickets

treatment; however, it was not until early in the 19th century that sunlight was suggested as a cure for rickets by the Polish doctor, J. Sniadecki (Jones 2018, Aguilar-Shea 2021). Through the observation of the occurrence of rickets between rural and urban children during the Industrial Revolution, Sniadecki noted that an environmental factor was likely involved (Jones 2018, Holick 2023).

In the early 1900's a connection was made between rickets and (newly discovered) vitamins. The British physician Sir Edward Mellanby conducted a study on beagles (*Canis lupus familiaris*), inducing rickets via an oatmeal diet, followed by curing them with cod liver oil, and noted a nutritional deficiency was likely involved in the development of rickets (O'Riordan and Bijvoet 2014, Jones 2018, Holick 2023). According to Holick (2023), the American biochemist Elmer McCollum did not agree with others that vitamin A (found in cod liver oil) was the antirachitic element. To prove his hypothesis, McCollum heated cod liver oil to destroy the vitamin A (as it was very sensitive to heat) and found that the oil still helped with rickets; thus, he named the new micronutrient vitamin D (Holick 2023).

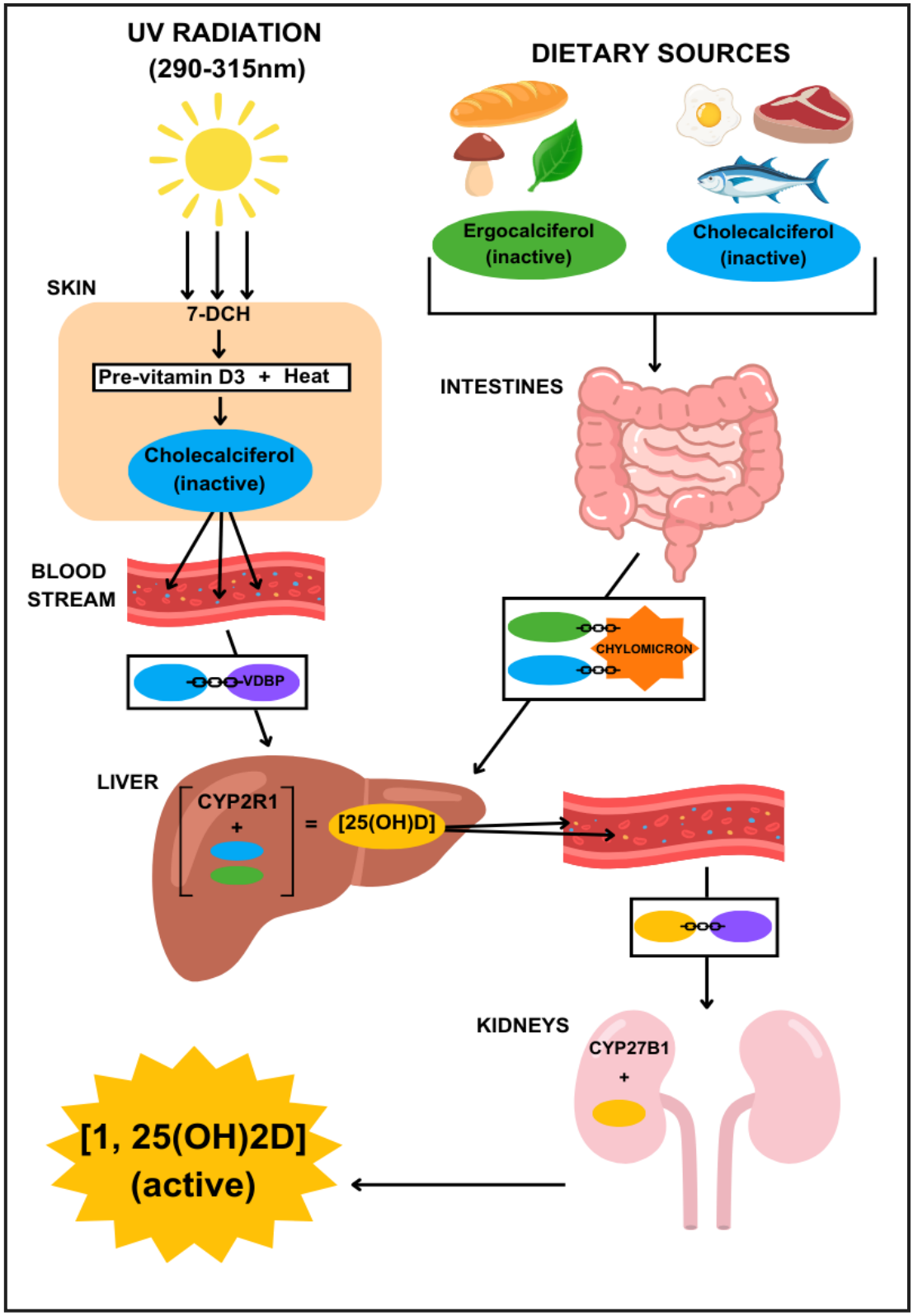
2.2.2 Metabolism and Sources of Vitamin D

The metabolism of vitamin D requires multiple biologic elements and various organ systems to ensure its proper function (Figure 2.1). Once exposed to UVB radiation, ideally between the 290-315 nm wavelengths, epidermal 7-dehydrocholesterol (pro-vitamin D_3) is synthesized into pre-cholecalciferol (pre-vitamin D_3). Pre-vitamin D_3 is thermodynamically unstable and rapidly transforms into cholecalciferol (the inactive form

vitamin D_3) under the influence of body temperature, through a process known as phytolysis (Wagner and Greer 2008, Charoenngam et al. 2019, Durá-Travé and Gallinas-Victoriano 2023). As it diffuses into the bloodstream, cholecalciferol is first transported to the liver via the vitamin D binding protein (VDBP) (Bikle 2014, Karras et al. 2018). A reaction between the enzyme cholecalciferol-25-hydroxylase (CYP2R1) and cholecalciferol within the hepatocytes (liver cells) results in 25-hydroxycholecalciferol [25(OH)D] or *calcidiol* (Ross et al. 2011). This is known as the first hydroxylation stage as it adds a hydroxyl group (OH) to the chemical. Calcidiol is then released back into the bloodstream to be transported by the VDBP to the kidneys. The enzyme 1-α-hydroxylase (CYP27B1) then converts calcidiol [25(OH)D] into 1,25-dihydroxycholecalciferol [$1,25(OH)_2D$] or *calcitriol*, the biologically active form of vitamin D (Ross et al. 2011). Once activated, calcitriol binds to the vitamin D receptor (VDR), which helps initiate its many biologic functions (Karras et al. 2018, Durá-Travé and Gallinas-Victoriano 2023).

When food containing vitamin D is ingested, it is broken down in the gut and transported from the intestines to the liver via lipid proteins, known as chylomicrons (Ross et al. 2011). From this stage, the ingested vitamin D continues through the same process as mentioned above beginning with the first hydroxylation stage (Figure 2.1).

Figure 2.1 *Diagram of Vitamin D Metabolism. Image by L. Godawa*



Current recommendations state that adults and children between the ages of 1-70 years old should consume 400-600 International Units (IU) of vitamin D daily to maintain adequate levels for skeletal health, while assuming UVB exposure is minimal (Ross et al. 2011).

Foods that are considered high in vitamin D include fatty fish, such as salmon (570 IU per 3 oz), mackerel (80 IU per 2.6 oz) and tuna (36 IU per 2.6 oz), egg yolks (44 IU per 1 large egg), white mushrooms (366 IU per ½ cup), sardines (46 IU per 2 sardines) and trout (645 IU per 3 oz) (Hossein-nezhad and Holick 2012, Haytowitz et al. 2019, NIH 2024). Table 2.1 provides examples of the food consumed in 19th century Spain, along with their vitamin D content. The information was derived from food distribution plans from hospital records between 1852-1931 in Spain. Though the information was gathered through hospital records, Medina-Albaladejo and Calatayud (2021) note that the institutional diet was very similar to the diet of the general population of Europe at this time. Foundlings (orphans housed at the hospital) were, however, treated differently than the other patients with a restriction on pork fat, poultry, and eggs (Medina-Albaladejo and Calatayud 2021) (see Section 3.2.3). Abeledo (2012) noted that with the increase in salting plants toward the latter half of the 19th century, sardines became a dietary staple in the working class for both men and women. Advertisements and Spanish medical journals link the consumption of cod liver oil to the late 1800's and early 1900's, however its distribution and acceptance is unclear (Vallejo and González 2014).

Table 2.1 *Common Dietary Items Consumed in 19th Century Spain and Their Vitamin D Levels (Based on Modern Studies)*

Food Sources	Serving Size	Vitamin D Content per Serving
Brown bread (a)	1 slice (28g)	0 IU (NIH 2024)
White bread (a)	1 slice (28g)	0 IU (Haytowitz et al. 2019)
Rice (a)	1 cup	0 IU (NIH 2024)
Chickpeas(a)	100g	0 IU (Haytowitz et al. 2019)
Potatoes (a)	100g	0 IU (Haytowitz et al. 2019)
Meat (veal) (a)	100g	0 IU (Jakobsen and Christensen 2021)
Olive Oil (a)	1 tablespoon	0 (Haytowitz et al. 2019)
Wine (a)	29.5g (1 fl oz)	0 IU (Haytowitz et al. 2019)
Milk (a) (unfortified, whole)	1 cup	2 IU (Haytowitz et al. 2019)
Chocolate (a) (dark)	100g	219 IU (Kühn et al. 2018)
Sardines (b)	2 sardines	46 IU (NIH 2024)
Cod liver oil (c)	1 tablespoon	1360 IU (NIH 2024)

Sources: (a) Medina-Albaladejo and Calatayud 2021; (b) Abeledo 2012; (c) Vallejo and González 2014

Using abattoir bookkeeping data from Barcelona, Guardia and colleagues (2018) examined the changes in consumption of meat between 1709-1935. In their research, they noted that both bread and meat were considered staple food items within the urban population and the municipalities regulated their prices due to the subsistence crises prior to the 20th century (Guardia et al. 2018). Toward the latter half of the 19th century, however, as meat (i.e., beef, mutton, goat, pork) consumption increased, the consumption of fish, which is high in vitamin D content, decreased (Guardia et al. 2018).

As mentioned above, egg yolks have a high vitamin D content, however, Nos and Andreu (2005) noted that eggs only accounted for 12% of the total protein intake in Barcelona during the latter part of the 19th century. Though this information was from Barcelona and not Madrid, it was still a large urban center and comparable to Madrid. Another high vitamin D food, mushrooms, were part of the regular diet, however to what

extent is undocumented (Chauvin-Payan 2024). Sources like cookbooks and newspapers mention the cultivation and use in recipes of mushrooms, though actual consumption details are lacking in the historical record. A specific example can be seen in a section of the Madrid newspaper *La Correspondencia de España* on April 3, 1892, when it describes that a horticulturalist named Juan Gras has discovered a way to obtain fertile mushroom beds that can produce 15 kilos of mushrooms (Ultima Edicion 1892). It is clear that mushrooms were being grown and sold, however their prevalence in diet of the Spanish population (for any age, sex or socio-economic status) is ambiguous.

Considering the information above, it is clear that the people of Spain during this time were generally lacking in dietary vitamin D. Although it was beyond the scope of this thesis, it would be interesting to note if the dietary insufficiency of vitamin D was universal across other populations during this time period. Toward the latter half of the 19th century, leading into the 20th century, European countries saw a significant change in diet from predominately vegetable and cereal based to higher caloric and nutrient rich food (such as animal proteins and fats), known as the 'nutritional transition' (Medina-Albaladejo and Calatayud 2021). The nutritional transition positively affected the health and life expectancy of European populations.

2.2.3 Vitamin D Metabolism During Pregnancy and Lactation

The physical manifestations of the connection between a mother and her infant are undeniable. From conception, a mother's body adapts to ensure the maturing embryo and foetus develops in a stable intrauterine environment for a successful delivery (Karras et

al. 2018). During pregnancy and lactation, the metabolism of vitamin D is crucial to the health and development of the foetus. One of the major adaptations to vitamin D metabolism during pregnancy is the significant increase in circulating calcitriol from both the mother's kidney and placenta, almost tripling by the third trimester (Wagner and Hollis 2020).

As early as 4 weeks of gestation (i.e., the formation of the placenta), 25(OH)D (calcidiol) can diffuse from mother to foetus via the placenta, however it still must undergo activation by the enzyme 1-α-hydroxylase (CYP27B1) before it is useful (Kaludjerovic and Vieth 2010, Kiely et al. 2020). The active form of vitamin D (calcitriol) cannot cross the placenta from mother to foetus, necessitating an increase in CYP27B1 on the foetal side of the placenta (Kaludjerovic and Vieth 2010, Kiely et al. 2020).

The increase in vitamin D metabolism experienced by the mother during pregnancy returns to normal levels after birth. The amount of vitamin D (in IU) per litre in breastmilk (at less than 25IU/L – 78IU/L) is insufficient to provide the required vitamin D necessary for the infant (Wagner and Greer 2008). Recent studies showed that exclusively breastfed infants obtain less than the 20% of the required daily amount of vitamin D (Møller et al. 2012, við Streym et al. 2016), and with no vitamin D supplementation or UVB exposure can develop VDD (Kovacs 2008, 2015, Heo et al. 2022).

As breastmilk does not provide enough vitamin D and no supplementation was prescribed or available in the 19th century, the sooner an infant could be weaned, the less

likely they would develop VDD. During the 19th century, infants were typically weaned between 6 - 11 months (Reher et al. 1997). However, using stable isotope analysis on a portion of the sample of teeth from Trinitarias Descalzas, Smith (2018) concluded that the onset of weaning began around 10-14 months after birth. For the purpose of this thesis, Smith's (2018) findings will be standard. Further discussion on Smith's (2018) work is mentioned in Section 5.2.

2.2.4 The Importance of Vitamin D in Bone Health

Vitamin D helps to maintain sufficient levels of calcium and phosphate in both the bloodstream and the skeleton (Charoenngam et al. 2019). These micronutrients are necessary for bone mineralization. Bone mineralization is particularly important to a growing skeleton between birth and ~17 years of age (Halcrow and Tayles 2008, Fiscaletti et al. 2017). Bone forms through two different processes: (1) intramembranous ossification, where bone develops directly from undifferentiated connective tissue (e.g., bones in the cranium); and (2) endochondral ossification, where a cartilage (connective tissue) precursor develops and is gradually replaced with mineralized bone (e.g., long bones) (White and Folkens 2005). When these processes are affected by nutritional or environmental deficiencies the growing skeleton is impacted. As a child grows and begins to crawl, stand and walk, their bones need to be sufficiently mineralized to withstand the body's weight without bending. If there is a disruption in the mineralization process of the bones at this stage, the bones will start to bend or deform under the pressure of weight bearing (Ortner and Mays 1998). The bending or deformation of weight bearing bones (e.g., long bones) is often seen in severe cases of rickets (Brickley et al. 2014, 2020b).

2.2.5 Vitamin D Deficiency

In clinical terms, vitamin D status is defined by the amount of calcidiol within the blood (Hossein-nezhad and Holick 2013). Current research categorizes an individual's level of vitamin D (calcidiol) into three separate categories: sufficiency, insufficiency and deficiency. Vitamin D sufficiency means an individual's calcidiol levels are equal to or higher than 30ng/mL (≥75nmol/L); insufficient levels of calcidiol range between 29-20 ng/mL (74 – 51nmol/L), while deficient levels are lower than 20ng/mL (<50nmol/L) (Hossein-nezhad and Holick 2013, Heo et al. 2022, Durá-Travé and Gallinas-Victoriano 2023). Simply, a deficiency in vitamin D is the result of a lack of exposure to sunlight (via UVB radiation) and/or a diet lacking vitamin D rich foods. Apart from diet or sunlight exposure, there are many other factors than can influence an individual's ability to absorb and/or metabolize vitamin D (see Table 2.2).

Table 2.2 *Factors Influencing Absorption or Metabolism of Vitamin D*

Factor	Explanation	Source
Biological		
Skin pigmentation	Higher melanin levels (i.e. darker skin) slows the absorption of UV radiation	Datta et al. 2019
Aging	Reduced vitamin D synthesis ability in skin	Nair and Maseeh 2012
Gastrointestinal diseases (Crohn's disease, irritable bowel syndrome)	Inflammation of the gut makes absorption of vitamin D difficult	Charoenngam and Holick 2020
Geographic		
High latitude	Diminished levels of UVB radiation further away from the equator	Mays et al. 2018
Socio-cultural		
Clothing preferences/ religion	Women who completely cover their bodies for cultural/religious reasons do not get proper sun exposure on skin	Buyukuslu et al. 2014, Al-Yatama et al. 2019
Medications	Certain medications can interfere with vitamin D metabolism and absorption	Gröber and Kisters 2012, Wakeman 2021
Sunscreen	High UVA and UVB sunscreens can reduce absorption of vitamin D	Passeron et al. 2019
Swaddling	The process of wrapping an infant in a tight blanket or special binding to limit mobility or secure the infant to their caregiver can limit the infant's skin exposure to UV radiation	Veselka et al. 2015

The earliest written record of a vitamin D deficiency (VDD) is mentioned in historical documentation from the early 17th century in England (O'Riorden and Bijvoet 2014, Jones 2018). The term 'rickets' was mentioned in a domestic receipt book in 1632, while it was noted as a cause of death in the 1634 London Bill of Mortality (O'Riorden and Bijvoet 2014, Jones 2018). It was not until the early 20th century that rickets was connected to a deficiency of vitamin D, through the work of Mellanby, McCullum, and Hess (cited in Holick 2023).

There are many causes that can lead to a deficiency of vitamin D, whether hereditary, environmental, or nutritional, however, only a few are identifiable in the bioarchaeological record. As its main physiological role is the maintenance of bone homeostasis, prolonged periods of VDD can lead to various problems involving the skeleton (Lockau and Atkinson 2018).

2.2.6 Vitamin D Deficiency During Pregnancy, Lactation

An important aspect of VDD is the relationship between a mother and their foetus/infant. A foetus can become deficient through their mother during pregnancy (if she is deficient in vitamin D) or as an infant within the first few years of life (Elsori and Hammoud 2018, Lockau and Atkinson 2018). If a pregnant woman is deficient in vitamin D, her foetus will also be deficient (Pilz et al. 2018). Holick (2006) reported that vitamin D helps with the development of growth plates and the growing foetus could develop severe consequences or complications from VDD.

After birth, if a mother relies solely on their breast milk (with minimal sunlight exposure) to provide an adequate amount of vitamin D necessary for their infant, that infant will develop VDD and likely rickets if it continues (Heo et al. 2022). Current studies of exclusively breastfed infants in areas with sufficient sunlight/UV radiation (i.e., those with a latitude ≤ 35° North or South) such as Qatar (between 24-26° N) (Salameh et al. 2016) and India (between 8-36° N) (Trivedi et al. 2020) had a high incidence of VDD with 83.0% and 91.1% in the participants, respectively. Salameh et al. (2016) noted that most mothers in their study had minimal skin exposure (i.e., they were fully clothed from head to foot) and spent less than an hour outside every week. Their infants wore less clothing; however, they also spent less than an hour outside a week. Despite vitamin D supplementation in the mothers, both the mothers and infants had a high incidence of VDD. Similarly, Trivedi et al. (2020) found that both mothers and infants in their study received an inadequate amount of UV exposure per week. Sunlight exposure and vitamin D supplements are necessary to ensure an adequate amount of vitamin D is received by the infant, especially in the first year of life (Holick 2006, Fink et al. 2019). The supplementation of vitamin D was not introduced until the 20th century, so it is possible that unless the infants in 19th century Spain were receiving daily UVB exposure, they were likely deficient (Jones 2018, Pilz et al. 2018). It is unclear if women in 19th century Spain actively avoided sunlight, however, their conservative garments would have severely limited their UV absorption regardless of their sunlight exposure (Casal-Valls 2016).

2.2.7 Evidence of Vitamin D Deficiency in Human Skeletal Remains

Bioarchaeological and paleopathological literature primarily considers VDD a metabolic bone disease (Snoddy et al. 2016). Though true, this statement does not encompass the magnitude of effects an insufficiency or deficiency of vitamin D can have on the body when reading clinical works. As mentioned earlier, vitamin D plays a larger role in the body than calcium and phosphorus homeostasis and the bioarchaeological literature is lacking in a discussion of these broader effects.

VDD can be grouped into three categories based on the origins of the condition. Most commonly seen in the archaeological record in children (typically <4 years of age) is (1) 'nutritional rickets'. It manifests as a result of insufficient vitamin D in a child's diet and/or inadequate exposure to UVB rays via sunlight (for recent studies see: Brickley and Mays 2019, Lockau et al. 2019, Brickley et al. 2020a, Bowers et al. 2024, Snoddy et al. 2024). Adult skeletons can show lesions indicative of a childhood case of rickets, known as (2) 'residual rickets' (Brickley et al. 2017, Brickley et al. 2020b). This is not necessarily considered an active disease but is more an indication of a condition that the individual had previously suffered from. While the term *rickets* is used to describe the juvenile skeletal manifestation of VDD, the third category: (3) 'osteomalacia*'* is reserved for adults. These terms, however, describe the effects of VDD on two different processes – endochondral bone growth in the case of rickets and cell turnover for maintenance of osseous tissue in osteomalacia (Snoddy et al. 2016). Rickets mainly affects areas of rapid endochondral bone growth which occurs during skeletal development and sometimes in early adolescents, during puberty, thus is associated with juveniles. The maintenance of

bone tissue, however, is also a process involved in skeletal development and continues throughout life. Prior to the fusion of the growth plates (regions of cartilage involved in bone growth), the cells involved in maintenance can be affected by VDD, thus resulting in areas of the bone that are poorly mineralized. Therefore, technically, both processes occur in juveniles and as a result they would concurrently experience BOTH rickets and osteomalacia, while adults would only suffer from the latter (Brickley et al. 2014, Snoddy et al. 2016).

Snoddy and colleagues (2016) noted, it is rare, if not impossible, to see the visual impact of vitamin D insufficiency in archaeological assemblages. Vitamin D insufficiency is determined by the blood serum levels of calcidiol (74-51nmol/L) (see Section 2.3.5) and is often subclinical (i.e., the conditions symptoms are unclear or not yet observable), therefore it is highly unlikely skeletal lesions would have developed enough to distinguish a problem or anomaly (Hossein-nezhad and Holick 2013, Snoddy et al. 2016). The identification of multiple types of macroscopic skeletal lesions is necessary to associate with a rickets diagnosis, and most develop from a severe deficiency over an extended period of time. It must be noted that skeletal evidence of vitamin D insufficiency is unlikely to be identified in archaeological assemblages.

According to Brickley and colleagues (2010), it is difficult to diagnose residual rickets in archaeological samples as the bones would have likely remodeled over time and possibly concealed the evidence of the previous condition. The more severe the case of rickets experienced as a child, the less likely the bone will have remodelled away the

evidence (Brickley et al. 2010). The literature is unclear on how severity was measured within the study by Brickley and colleagues (2010), apart from a visual assessment of the bones when compared to others within the sample which were noted as 'marked' when a skeletal deformity was instantly observable in multiple aspects.

1) (Nutritional) Rickets

Nutritional rickets, often referred to simply as *rickets*, is a metabolic disorder that can appear macroscopically on the skeleton between the ages of four months to four years (Brickley and Mays 2019). Foetal, infant (<1 year), and child skeletal remains provide information on the health (or lack thereof) of a population, but also insights into the health of their mothers as well (Blake 2018). The timing of the appearance of the most obvious and often pathognomonic symptoms of rickets (i.e., bowing of the long bones) often coincides with specific developmental phases in the life of an infant or child (Table 2.3). An infant's ability to learn to bear its own weight through crawling, standing and/or walking can result in the bowing of long bone(s) if they have VDD, as their bone mineralization is inadequate (Figure 2.2). Although there is considerable variability, infants typically start crawling around 6-9 months and standing/walking around 12-15 months (Zubler et al. 2022).

Table 2.3 *Macroscopic Lesions of Rickets in Infants and Children*

Cranial Lesions*
Porosity of cranial vault
Porosity of orbital roof
Deformation of mandibular ramus
Thickening of cranial vault
Post-Cranial Lesions*
Porosity and flaring of costo-chondral (sternal) rib ends
Deformation of costo-chondral (sternal) rib ends
Porosity underlying long bone growth plate(s)
Porosity and flaring of metaphyseal ends of long bone(s)
Deformity or bowing of lower limb(s) (femur, tibia, fibula)
Deformity or bowing up upper limb(s) (humerus, radius, ulna)
Deformity of ilium
Thickening of long bone(s)

*Sources: Ortner and Mays (1998), Brickley and Mays (2019), Brickley et al. (2020b), Veselka et al. (2021)

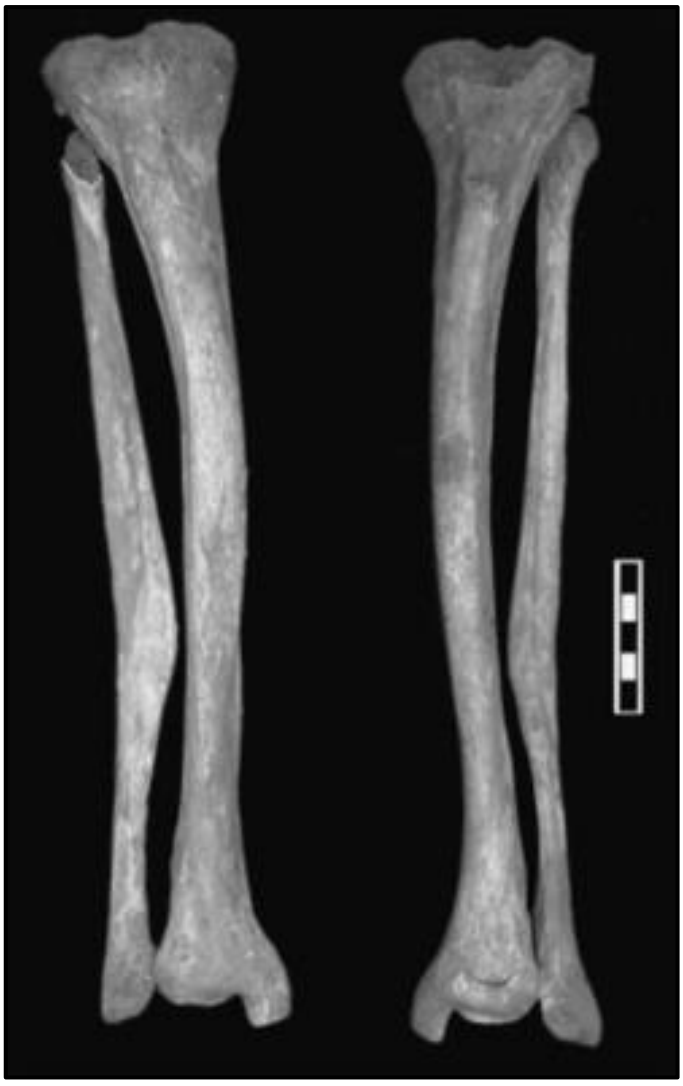
Figure 2.2 *Image of the Femora (left) and Tibiae (right) from Individual N4-4 (age-at-death: 6 years) from the Trinitarias Descalzas Site, Showing Bowing of Long Bones Attributed to Rickets (Source: Aranzadi, n.d. p. 39)*

2) Residual Rickets

Residual rickets is a term used to indicate that an individual had vitamin D deficiency in infancy, childhood, or early adolescence. Depending on the timing and severity of rickets experienced during childhood, adults may retain visible skeletal deformities (Brickley and Mays 2019). Macroscopic lesions of residual rickets include rib, sternal, sacral and vertebral column curvature; as well as the bending of lower (femur, tibia, fibula) and/or upper (humerus, ulna, radius) limb long bones (Brickley et al. 2020b,

Veselka et al. 2021) (Figure 2.3). If an adult has residual rickets, it does not reduce, or promote the individual's risk to develop osteomalacia, unless they are consistently without sufficient vitamin D (Brickley et al. 2017).

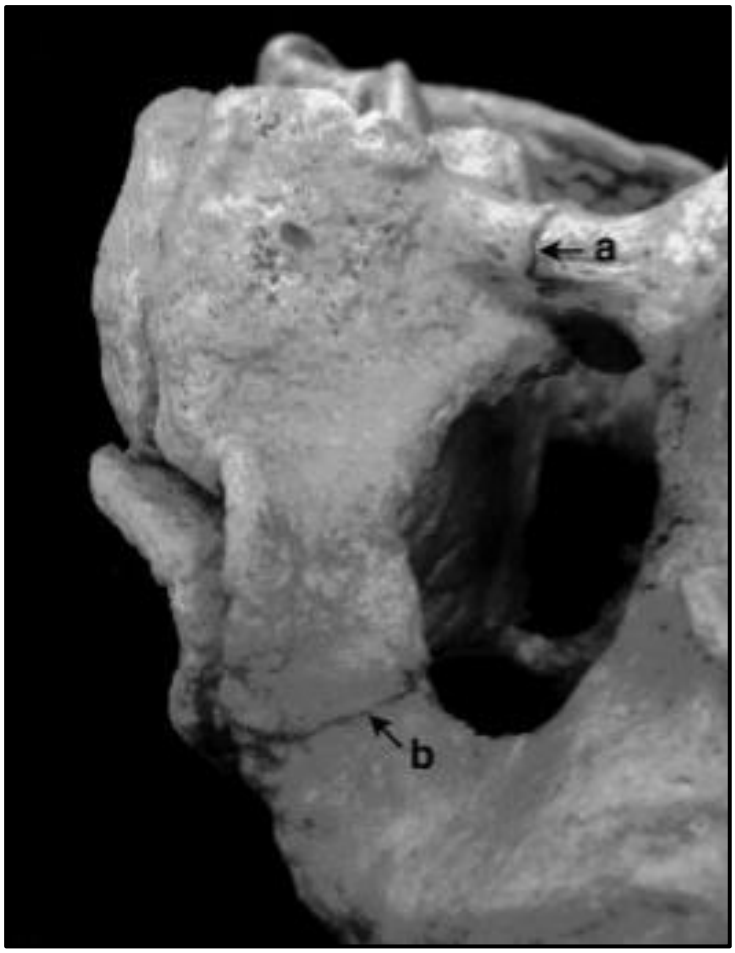
Figure 2.3 *Lower Leg Bones (Tibiae and Fibulae) of an Individual (HB320) from St. Martin's Churchyard in Birmingham, England (18th-19th Century) with Bowing Deformities Related to Residual Rickets (Source: Fig. 6 from Brickley et al. 2010: p. 61)*



3) Osteomalacia

Osteomalacia is often referred to as ‘adult rickets’ and is identifiable by defects present in remodelled bone (Haduch et al. 2009). These defects are usually pseudofractures or biomechanical deformities (Brickley and Mays 2019, Brickley et al. 2020b) (Figure 2.4).

Figure 2.4 *Example of Pseudofracture turned true fracture on pelvis at (a) superior pubic ramus and (b) inferior pubic ramus on individual from the historic skeletal collection housed at the Federal Museum for Pathological Anatomy in Vienna, Austria (Source: Figure 9e from Brickley et al. 2005: p. 398)*



Once bone growth is complete, bone turnover continues throughout adulthood. The rate of bone turnover in adults is slower than the rate of bone growth and turnover in growing children and adolescents, around 3-4% per year, depending on the sex (Quinn 2024). Therefore, the skeletal manifestations of osteomalacia are also often more subtle than those of rickets as they usually take longer to manifest (Table 2.4).

Table 2.4 *Macroscopic Manifestations of Osteomalacia*

Bone(s)	Deformation	Pseudofracture	Compression Fracture	Porosity
Ribs	✔	✔		
Scapulae	✔	✔		
Iliac crest	✔	✔		
Pubic bone	✔	✔		
Vertebrae – vertebral body			✔	
Vertebrae – transverse process		✔		
Upper and lower long bones	✔	✔		
Sternum	✔			
Sacrum	✔			
Cranium	✔			✔

Sources: Brickley and Mays (2019), Brickley et al. (2020b)

2.3 Evidence of Vitamin D Deficiency in Teeth

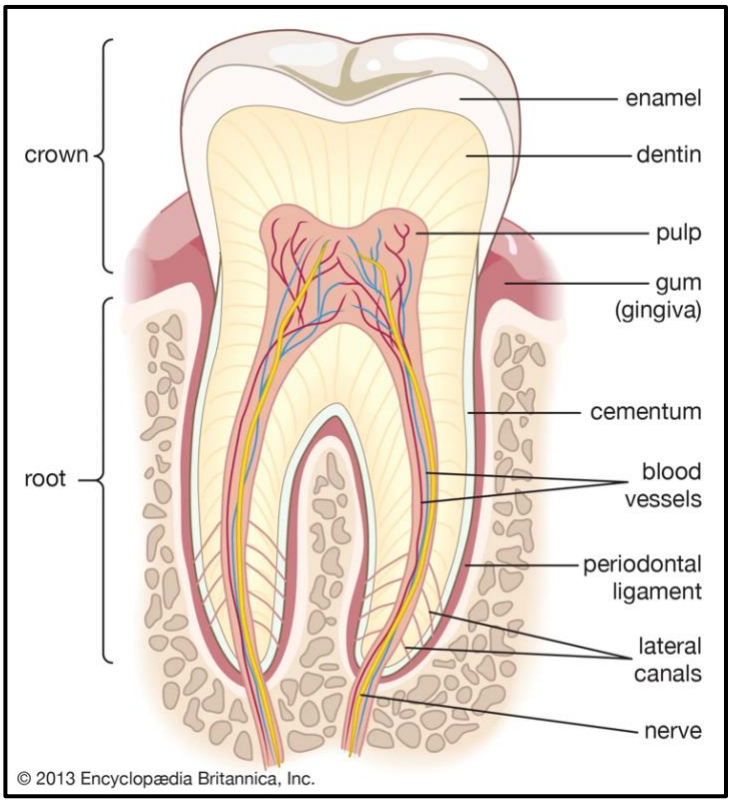
2.3.1 Introduction

Skeletal remains are not always discovered perfectly preserved in archaeological settings. In some cases, it is due to the physical environment or the burial practices, but when a disease or condition causes bone deformation and porosity (such as rickets or osteomalacia), it is the pathological condition itself that can inhibit the preservation (Assis et al. 2015). The weakened bone structure at the time of burial can make it more susceptible to diagenesis (Assis et al. 2015, Grupe and Harbeck 2015). Diagenesis is a term used to describe the biological, chemical and/or physical interactions between remains and the burial environment (Grupe and Harbeck 2015). Diagenetic conditions include, but are not limited to temperature, moisture content (humidity), soil chemistry, and/or bacterial presence (Assis et al. 2015). Grupe and Harbeck (2015) observed that accelerated decomposition can occur as a result of ante-mortem injury, infection, or pathology that caused tissue breakdown during life. VDD in skeletal remains causes a weakening of the tissue and as a result is likely to be affected by diagenesis post-burial. This is why teeth are invaluable when it comes to studying VDD, because teeth are more resistant to post-mortem diagenesis (Hollund et al. 2015). They do not remodel, and they can preserve microscopic evidence of conditions or diseases experienced during tooth formation (Hollund et al. 2015). To understand how VDD can be reflected in tooth samples, it is important to first understand a tooth's structure as well as its formation and development.

2.3.2 Tooth Structure

The structure of a tooth is made of 3 tissues – enamel, cementum and dentine, which surround the pulp chamber (Figure 2.5).

Figure 2.5 *Diagram of Tooth Cross Section*
(Source: https://www.britannica.com/science/tooth-anatomy)



Enamel forms the outer layer of the tooth crown and (once matured) is made up of a mostly (96%) inorganic matrix known as hydroxyapatite. The remaining 4% of enamel is organic, consisting mainly of water and a small mixture of proteins and peptides (Hillson 2024). Once tooth mineralization is complete, the structure of enamel remains largely unchanged. Enamel can be altered/worn purposefully though cultural practices (tooth filing, decoration, etc.) or unintentionally though daily activities (tooth grinding,

eating, pipe smoking, cavities, natural breakage/chipping) or occupations (such as needlework) (White and Folkens 2005).

Cementum is a tissue that surrounds the tooth roots and varies in thickness as well as in its organic and inorganic matrix. Important features of this tissue are the collagen fibres that aid in the attachment of the tooth to the alveolar bone via the periodontal ligament (Hillson 2024). Cementum is constantly remodelling and grows annually, however, it is often poorly preserved in archaeological specimens (Hillson 2024).

Dentine forms the inner portion of the tooth and is composed of 18% collagen, 72% hydroxyapatite and 8% water, while the remaining 2% are non-collagenous organic compounds (Hillson 2024). Hillson (2024) summarized three different types of dentine matrix that are secreted during mineralization: mantle, intertubular and peritubular, with each type containing varying combinations of collagen fibers and ground substance – a non-fibrous component.

2.3.3 Tooth Development

The initiation and development of deciduous tooth germs (a combination of cells that form the precursor to the tooth) begin as early as 6 weeks after fertilization and can be separated into four main stages (Hillson 2024). The following descriptions of tooth development are described by Hillson (2024): at the first stage, initiation, a tissue lining the surfaces of an embryo's developing mouth (epithelium), divides into the vestibular and dental lamina; the bud stage follows with the development of a round epithelial structure (enamel organ) at the distal end of the dental lamina. Next, at the cap stage, the

once round structure becomes indented, forming a cap-like pattern around a hollow space. During the final bell stage, the enamel organ further hollows to form a bell shape that will eventually define the crown shape of the tooth. The tooth germ can now begin forming the enamel and dentine layers to solidify the tooth shape and structure (Hillson 2024).

Following the development of the tooth germ, crown and root formation occurs in three main stages, generally by type of tissue. Starting with odontogenesis, odontoblasts (dentine forming cells) move inward from the dentine-enamel junction (DEJ) toward where the pulp cavity will develop (Hillson 2024). As the cells move, they secrete hydroxyapatite in the organic matrix, which mineralizes at a set rate. According to Dean and Scandrett (1995), in permanent teeth, the rate is approximately 4 – 6 μm per day in the crown portion of the tooth, while the cervical region mineralizes at a slower rate of 1.3 – 1.5 μm per day (Dean and Scandrett 1995). In deciduous teeth, this rate is likely faster due to the rapid period of growth, though a specific range of mineralization has yet to be confirmed (Dean and Scandrett 1995).

Shortly after the first stage, ameloblasts (enamel-forming cells) move outward from the DEJ toward the surface of the cusp then down the sides of the crown. The ameloblasts also secrete hydroxyapatite that is followed by waves of mineralization. In general, development happens inside the mandible and maxilla and starts around 14 weeks after conception (Hillson 2024). Once the dentine has completed the structure of the tooth root, cementoblasts secrete hydroxyapatite that mineralizes into cementum. Table 2.5 summarizes the timing of tooth development for the deciduous incisors

(maxillary and mandibular), deciduous molars (maxillary and mandibular), and permanent maxillary molars used in this thesis.

Table 2.5 *Tooth Development Timing (Adapted from information from Hillson 2024)*

Tooth Type	Start of Crown Mineralization	Crown Completion	Tooth Eruption	Root Completion
Deciduous first incisors (maxillary)	4-5 mo in-utero	2-4 mo	6-11 mo	18-24 mo
Deciduous first incisors (mandibular)	3.5-5 mo in-utero	birth-4 mo	4-10 mo	18-24 mo
Deciduous first molars (maxillary)	4.5-5.5 mo in-utero	5-7 mo	12-18 mo	24-30 mo
Deciduous first molars (mandibular)	5 mo in-utero	5-7 mo	12-18 mo	24-30 mo
Permanent first Molars (Maxillary)	Birth	2.5-4.5 yrs	5-8 yrs	9-11.5 yrs

Notes: mo = months; yrs = years

There are three types of dentine. Primary dentine does not remodel and therefore leaves a record of events during tooth formation. Dentine in deciduous teeth gets slowly resorbed, mainly within the roots as the permanent teeth develop (Hillson 2024). This allows the deciduous teeth to be exfoliated as the permanent teeth begin erupting into the mouth.

Once permanent teeth have completed the growth stage, secondary dentine slowly forms within the pulp cavity (Hillson 2024). In general, the older the individual, the less space within the pulp cavity. The final type of dentine, known as tertiary dentine, forms in

response to injury (i.e., trauma or wear). Assuming the injury does not continue or worsen, tertiary dentine can repair the damage (Hillson 2024).

2.4 Interglobular Dentine

2.4.1 Introduction

Improper mineralization of dentine, known as interglobular dentine (IGD), sometimes referred to as interglobular spaces in earlier research (e.g., Isokawa et al. 1963), results from a disruption in the metabolism of vitamin D (Mellanby 1928, D'Ortenzio et al. 2016, Hillson 2024). When there is a lack of vitamin D in the body dentine can become hypomineralized (deficient in calcium) (Botelho et al. 2020). Calcospherites, calcium salts involved in the mineralization wave, fail to coalesce (i.e., the rate of growth of the calcosphereites is slower than that of the matrix secretion) and leave spaces within the dentine (D'Ortenzio et al. 2016, Hillson 2024). These poorly mineralized sections are called interglobular spaces or IGD.

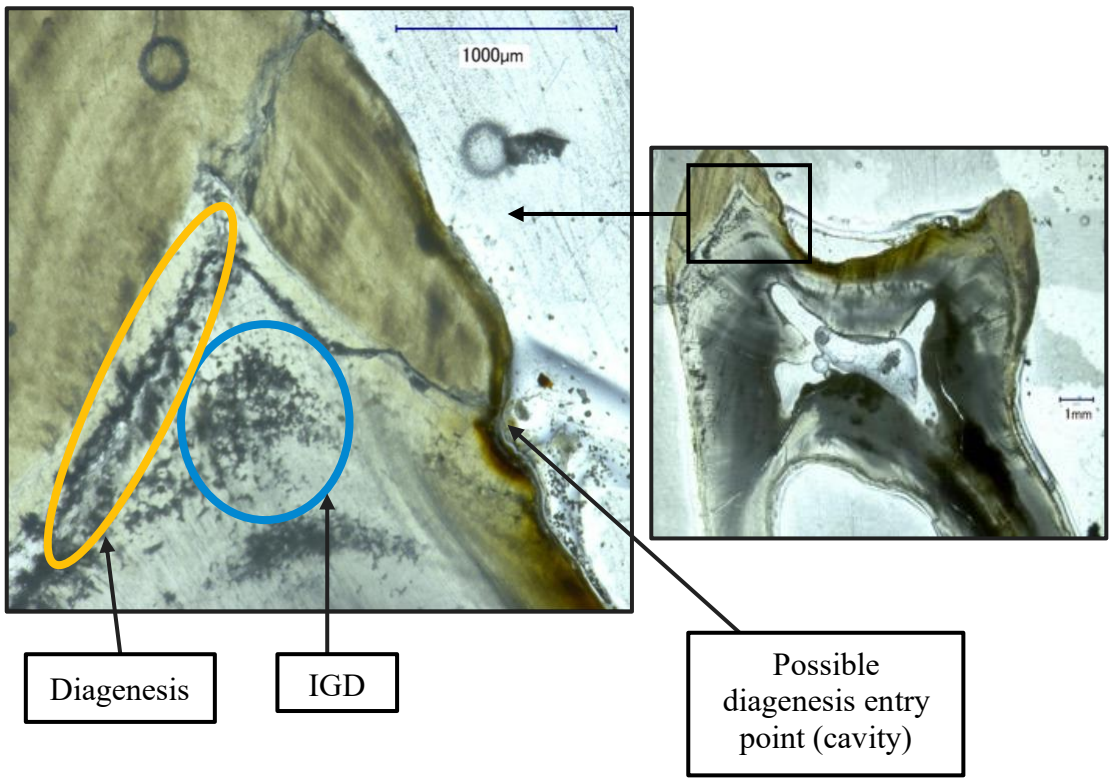
IGD was first described in 1850 by Johann Czermák in his paper *Beiträge zur mikroskopischen Anatomie der menschlichen Zähne* (Translation from German: *Contributions to the microscopic anatomy of human teeth*) (Cited in Brickley et al. 2017 and Hillson 2024). As mentioned earlier, Mellanby's controlled dietary experiments with VDD in dogs noted a correlation between VDD and the development of IGD (Holick 2023, Hillson 2024). Work continues to be published on the correlation between IGD and VDD, although it has not been confirmed whether it is pathognomonic for VDD.

Boukpessi et al. (2006), Vital et al. (2012), and Ribeiro et al. (2015) published papers on the relationship between dentine mineralization alterations (i.e., IGD) and hypophosphatemic rickets – a genetic form of rickets affecting the absorption of phosphate. D'Ortenzio et al. (2016), summarized all the knowledge on IGD and VDD up to that point in both the clinical and anthropological literature, and developed a method to grade the severity of IGD, which had not been done up to that point. Using a scanning electron microscope (SEM) and histological analyses, D'Ortenzio et al. (2016) scored the presence and severity of IGD in 11/12 teeth of 6 individuals from an archaeological assemblage with skeletal evidence of vitamin D deficiency. See Section 2.4.2 below for further discussion of the methods used to identify and quantify IGD in teeth.

According to Hillson (2024), IGD appears as dark parabolic, circular, or cloud-like patches within the dentine that can vary in size, shape, and distribution. IGD can appear parallel to the dental enamel junction (DEJ) in a zigzag pattern, when viewed cross-sectionally (Hillson 2024). Other patches can form a band parallel to the incremental lines of dentine, however, severe cases of IGD can occupy most of the dentine space with no clear pattern. IGD is also more commonly seen in the crown portion of the tooth rather than the root, though root dentine can still be affected (Sato et al. 2000, Jayawardena et al. 2009). The literature is unclear as to why IGD can be found mainly in the crown; however, Jayawardena et al. (2009) suggested it is likely due to the dentine formation processes.

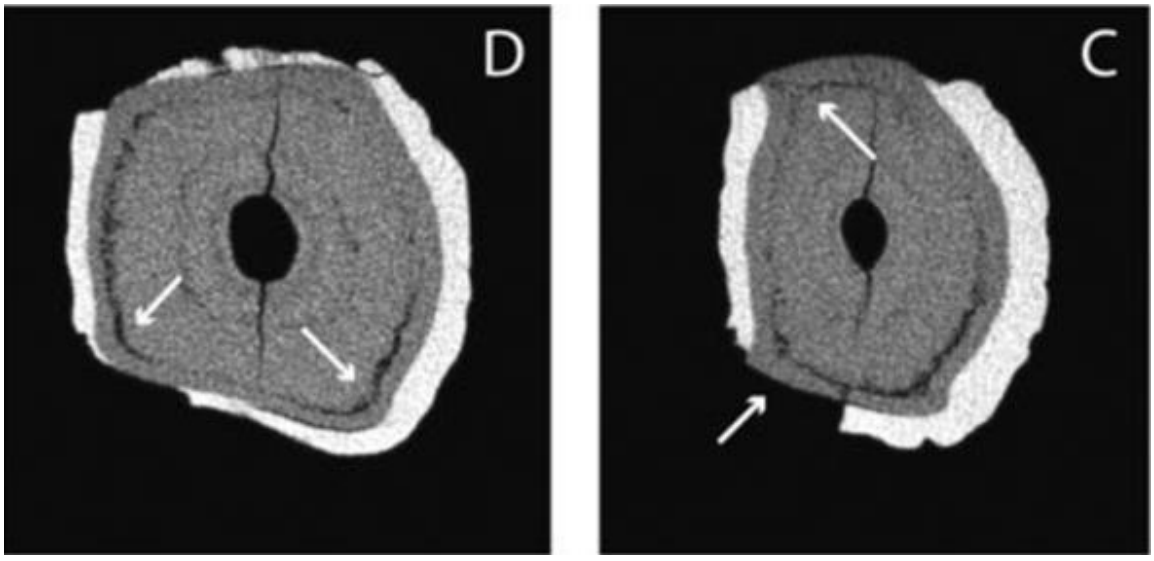
To the untrained eye, it is possible to confuse IGD with diagenesis. IGD can be differentiated from diagenesis by its bilateral symmetry. Diagenetic changes in the dentine will differ in patterning from IGD and sometimes have a distinct entry point (usually a cavity or crack from the enamel to dentine) and follow a path not in line with the dentinal tubules (Hollund et al. 2015) (see Figure 2.6).

Figure 2.6 *Enhanced Image of Diagenesis and IGD in 15A-S36 (RM[1]) From an Archaeological Site in Quebec City, Canada (c. 1771-1860 CE). Images by L. Godawa*



As discussed above, the formation of a tooth follows a typical pattern of growth and mineralization, therefore the presence of IGD during formation will reflect this. In a transverse cross section of a tooth using micro-CT, IGD will appear as a ring within the dentine sections (Figure 2.7) (Veselka et al. 2019, Huberthür et al. 2021).

Figure 2.7 *Transverse Section of a Tooth (RC^1) from Individual HT15S130 from Hattem, Gelderland, The Netherlands (17^{th} – 19^{th} Centuries) Using Micro-CT; White Arrows Point to IGD Rings (Source: Veselka et al. 2019, Figure 3, p. 127)*



D'Ortenzio et al. (2018) later noted that caution is advised when diagnosing vitamin D deficiency solely through the presence of IGD. Developmental IGD (DIGD) is a "less severe" form of IGD distinguished by its location, size, and amount within the tooth, and may not be associated with vitamin D deficiency. DIGD is a defect similar to IGD in appearance, however, it does not follow the incremental growth lines, nor does it appear bilaterally within a tooth (D'Ortenzio et al. 2018). This is discussed further in Section 3.2.4.

D'Ortenzio et al. (2018) also noted that other dental abnormalities can be misidentified as IGD. The analysis of the results from this thesis considers D'Ortenzio et al.'s (2018) findings and recognizes that caution is necessary when interpreting the IGD data (see Chapter 5 for further discussion).

Colombo et al. (2019) used both micro-CT and histological analyses to detect IGD in two archaeological samples from France (Saint-Jacques: 1225-1798 CE, Saint-Étienne de Toulouse: 11th – 13th century CE) with known macroscopic and radiologic evidence of vitamin D deficiency. Their results showed that both DIGD and IGD were present in 3 tooth samples, despite the one individual having no skeletal or radiological features of rickets (Colombo et al. 2019).

Research throughout the 20th and 21st centuries confirmed the link between VDD and IGD; however, much of the early research (e.g., Rosebury and Karshan 1931, Blackberg and Berke 1932, Bernick and Bavetta 1957, Rees et al. 1966) was conducted on animals. It was not until the study by Molnar and Ward (1975), that a link was made between IGD and a low quality of health in primates and humans. As discussed earlier in Chapter 2, the concept of a vitamin D deficiency was not yet clearly understood, so the link between vitamin D deficiency and IGD was limited by the knowledge of the time. In the early 1980's work by Ivanhoe (1982) on the relationship between presence and severity of IGD in individuals with sunshine deficiency rickets in archaeological collections, a shift was made from animals to a focus on IGD in humans. As an understanding of the process of vitamin D metabolism and IGD development increased in the 21st century the once synonymous rickets and vitamin D deficiency were differentiated.

2.4.2 Identification and Quantification Procedures

Histological examination and identification through microscopic analysis is the most reliable way to identify IGD (D'Ortenzio et al. 2016, 2018). With a lack of a standardization for the 'severity' of IGD, histological examination is prone to inter- and intra-observer error. Other methods for identification and scoring of severity of IGD have been established as a means to reduce these errors. In 1934, Mellanby developed a system of letters and symbols to indicate severity of IGD, where "No S" refers to no IGD and "S++" is a severe case of IGD (Mellanby 1934, D'Ortenzio et al. 2016). A three-tiered grading system was later developed by Seow and colleagues (1989), to distinguish the amount of IGD by size (thickness) occupied in dentine space (Grade I – Minimal; Grade II – Moderate; Grade III – Large).

Using aspects from both Mellanby (1934) and Seow et al. (1989), D'Ortenzio and colleagues (2016) developed an IGD scoring system based on the amount of IGD seen within a 'region of interest' to its surrounding normal dentine. The selection process or parameters of the region of interest is not described. D'Ortenzio et al. (2016) viewed a section of tooth (at 100X magnification) and visually estimated what percentage of IGD encompassed the field of view. The estimated percentages of IGD observed within the specified region of interest were translated into grades (Grade 0 – Grade 3) and used to describe severity (D'Ortenzio et al. 2016). Grade 0 was assigned to teeth with no interglobular spaces; Grade 1 was described as a mild form of IGD (<25% of the field of view had IGD); Grade 2 noted a moderate expression of IGD (25-50%); and Grade 3 had

a severe manifestation of IGD (>75%). Though only a small portion of the tooth was examined, the entire tooth was assigned that one grade.

Recognizing the potential bias and subjectivity of the above methods, Snoddy et al. (2020) developed a method using photo imaging software as a means to quantify IGD. Sections of tooth dentine containing IGD were isolated from the rest of the visual noise and converted into a percentage based on the distribution of grayscale elements. This eliminated the subjectivity of visual estimations; however, this method still had limitations. The lack of a defined region of interest, as well as how to choose such an area is not described thus leading to inconsistencies in replicability. Natural (diagenetic changes) and artificial (air bubbles or artifacts added during the embedding and mounting procedures) present challenges for the software and can be mistaken for IGD.

Knowing the limitations of the previous methods allowed for the development of a more robust methodology by L. Godawa for this thesis that included more than just one area of interest, as will be discussed in more detail in Chapter 3.

2.4.3 Causes of Interglobular Dentine (IGD)

Research in both anthropology and dental biology have shown that the most common causes of IGD are VDD and fluorosis (i.e., an excess of fluoride in the body). However, there are a variety of hereditary and acquired conditions that can lead to VDD and, as a result, potentially lead to IGD. Brickley et al. (2020a) noted that any conditions affecting tooth mineralization have the potential to cause IGD. In addition to VDD and

fluorosis, these conditions include renal disorders, celiac disease, and gastrointestinal disease.

Dental fluorosis is caused by the ingestion of fluoride in high levels during tooth formation and maturation (Ramesh et al. 2017). Despite its beneficial effects in preventing caries and its use in modern dentistry and dental health practices, an excess of fluoride in a developing tooth can cause mineralization defects, including IGD (Ramesh et al. 2017). Once the teeth have erupted, these defects primarily affect the enamel, but prolonged exposure and a weakened/porous enamel can affect the underlying dentine. Ramesh and colleagues (2017) reported an increase in the presence of IGD in teeth exposed to high quantities of fluoride in contaminated drinking water. Other studies by Fejerskov et al. (1974), Priyadharsini et al. (2015), and Shahroom et al. (2019) also observed a correlation between an increase in fluorosis and increased IGD in teeth. It is not clear whether the individuals involved in these studies were also suffering from some level of VDD in combination with the fluorosis. The diagnosis of fluorosis is more common in modern tooth samples and although it is difficult to prove in archaeological samples, it is still important to note (Hillson 2024). Lukacs and colleagues (1985) studied the levels of fluoride in permanent teeth from a Neolithic/Chalcolithic site in Mehrgarh, Pakistan, and noted the concentrations varied significantly throughout the enamel.

Water quality guidelines are currently in place in multiple countries for monitoring the levels of fluoride in freshwater. The World Health Organization and National Institute of Health state a maximum concentration of 1.5 mg/L should be the standard for most

countries (WHO 2004, NIH 2024). The current concentration of fluoride in water in Spain is 1.7 mg/L, six times higher than the natural fluoride levels in its freshwater sources (Camargo 2024). It is likely that the individuals in 19th century Spain did not suffer from fluorosis because they would have obtained most of their water from freshwater sources and/or rainwater.

2.5 Conclusion

For a bioarchaeologist, bones and teeth may be all that is available to understand the health and/or lifestyle of a population. Vitamin D plays a vital role in the formation of teeth and bones. Recent research has alerted us to the importance of vitamin D in a number of biological processes that can provide insight into an individual's or population's quality of health. Bones of an individual afflicted with mineralization defects (such as VDD) may not survive the burial conditions as well as teeth might. This makes teeth an invaluable source for the analysis of mineralization defects (e.g., IGD) associated with VDD. Analyzing IGD present in the teeth of infants and children from a 19th century Spanish cemetery provides further insights into the mother-infant nexus and the health of members of a population who are under-represented in the historical records.

CHAPTER 3: MATERIALS AND METHODS

3.1 Introduction

The purpose of this chapter is threefold; (1) to provide historic context on women and children in 19th century Spain; (2) to discuss, in detail, the sample of teeth used for this thesis; and (3) to describe the methodologies applied within this study. As the sample of teeth used for this thesis are from infants and children from a 19th century Spanish site, information on the health, cultural practices, and socio-economic status of women and children at that time is provided. Following the contextual information, the sample of teeth and its origin are presented. The final section of this chapter outlines the methodologies used to prepare, document and analyze the sample and resulting data.

3.2 Mothers and Infants in 19th Century Spain

The economic, political and socio-cultural status of Spain during the 19th century was turbulent, marked by wars, widespread inequality, and frequent regime changes (see Phillips and Phillips 2015). The constant wars and shifts in power continually drained both the economy and resources of cities in 19th century Spain and it was mainly the civilian population that suffered the most casualties (Prados de la Escosura and Santiago-Caballero 2022). Civilian hardships were felt particularly for women (and their children) because of their status and overall role(s) in Spanish society. Ongoing conflicts led to a significant production decrease in the agricultural sector, further leading to a steady decline in the consumption of meat and dairy products (Prados de la Escosura and Santiago-Caballero 2022). Disease was also common with the constant movement of peoples, both within Spain and into Spain. Migration into urban centers often resulted in a

high concentration of people living in close proximity with poor sanitary practices (Barona 2020). Adversities experienced by the Spanish population in the 19th century were even more keenly felt in women and their infants resulting in adverse health conditions.

3.2.1 Roles, Status and Health of Women

The analysis and interpretation of historic documents (such as censuses, advertisements and event documentation) from 19th century Spain must be viewed with a critical eye to account for socio-cultural and/or socio-economic biases of the creators of such documents, particularly in relation to the understanding of women's roles. Abeledo (2012) noted that documents (such as national censuses) often overlook or conceal the contribution that women make to their families and, in particular, to their local economy.

Most women worked in the primary sector (agriculture) during the 19th century on family farms as unpaid labourers (Shubert 2005). When women had the luxury of payment during peak harvesting seasons, their wages were between half to two thirds that of men's wages (Shubert 2005). The secondary sector (industry) had women working in manufacturing operations in textiles, tobacco, and other labour-intensive trades. The manufacturing of cigarettes was a mostly female-dominated occupation with women being paid on a piecework basis (Abeledo 2012). Despite having to pay for work materials and a midday meal, women in tobacco factories were often paid better than women working in other types of factories or sectors (Shubert 2005). The tertiary sector (service) was also heavily dominated by women who worked as domestic servants and

wet-nurses, the latter a common practice among both the Spanish elites and the working class (see Section 3.2.2).

The expectation of a woman, similar to other countries during this time, was to get married, and be a good wife and mother. Though women could not vote, nor be involved in any legal or political positions, a woman was allowed to conduct business in her own right once age of majority was reached between 23-25 years (Shubert 2005). Married women were further disadvantaged with regards to their legal rights. Once married, a woman lost what little legal rights she had and was barred from business unless approved by her husband (Shubert 2005, Socolow 2015). It was up to the husband to determine what activities he would allow of his wife to do, with severe punishment on the woman's part for any sort of disobedience. Outside of domestic responsibilities, a married woman was allowed to participate in charity work, however, this was considered part of her role as a woman, rather than an occupation (Shubert 2005). The social rules were a little less restrictive for noble women, who were permitted almost near autonomy in their charitable endeavours (Serrano 2013).

Historic documentation on the health of women in 19th century Spain is limited; however, assumptions can be made on a broader scale when analysing women's health in pre-industrial Europe. Prior to modern medical practices (specifically antisepsis techniques), it was common for women in Europe to die during pregnancy or birth (Loudon 1992, Scalone 2014). The maternal mortality rate in the latter half of the 19th century was around 40 deaths per 1000 live births, compared to less than 4 per 1000 live

births in the early 20th century (Loudon 1992, Manfredini 2020). There is conflicting evidence in the literature about the potential causes of maternal mortality in the past, but the main sources relate to nutritional stress during pregnancy, socio-economic conditions, and improper care during the birthing process (i.e., immediately before, during, and after labour). Scalone (2014) examined historical data of short-term economic crises from six villages in Germany between 1766-1863 to determine if socio-economic status impacted maternal mortality. The results of this study noted that, following a rise in the price of grain, maternal mortality increased (likely as a result of malnutrition), particularly among the wives of individuals working outside of the agricultural sector. Scalone (2014) also noted that infections developed between 2-6 weeks post-childbirth were significantly impacted by these nutritional crises.

In a study on maternal mortality in 19th-20th century Italy, Manfredini (2020) stated that poor nutritional status from a caloric deficient diet in combination with high energy expenditures before, during, and post-childbirth weakened the overall health of the mother. This weakened state resulted in a lowered immunity, leading to the development of infectious diseases during and after pregnancy. Though this study is focused on the women from Italy in the 19th-20th centuries, Manfredini's (2020) conclusions likely apply to maternal health throughout Europe.

In addition to the health issues noted above, biomechanical abnormalities complicated childbirth and often resulted in the death of both mother and foetus. Physical deformities related to prolonged vitamin D deficiency (VDD) could affect a woman's

ability to give birth (Biehler-Gomez et al. 2024) (see Section 5.2 for further explanation). The mother-infant nexus in these cases is not limited to pregnancy and infant care. The mother's heath, even as a child could impact her future children.

Though the literature on the causes for high maternal mortality in the past varies, it is likely that there was not necessarily one specific cause, but a combination of socio-economic, nutritional, and sanitary conditions that negatively impacted mothers in 19th century Europe. Further, the conditions not only effect mothers but also their children. Childcare practices, including nursing and feeding, must be considered when discussing the health status of both women and children.

3.2.2 Wet nurses

Wet-nurses (or *amas de leche*), were common throughout 19th century Spain (Siles-Gonzáles et al. 2020). The history and regulation of these women are important to this thesis, as they could influence the health of the nursing infant(s). Regardless of the socio-economic status of the individuals examined for this thesis, historical evidence suggests that most infants/children had a wet-nurse for at least a portion of their breastfeeding/weaning years. Abeledo (2012) noted the necessity to account for biases in historic writing, and Crifasi (2023) emphasizes that most of what is known about the history of wet-nurses and breastfeeding comes from documents written from the perspective of male elites. These men believed that breastmilk from women other than the infant's mother threatened their social, cultural, and religious beliefs (Crifasi 2023). Doctors even went as far as publishing medical treatises shaming women who did not

breastfeed their own children and stated that they were violating the laws of religion. This, however, did not deter women from advertising their wet-nursing availability in papers such as the *Diario de Avisos*, nor did it prevent the regulation of wet-nurses (Crifasi 2023, Siles-Gonzáles et al. 2020). The practical knowledge of wet-nurses was often prioritized over the anti-wet-nurse propaganda.

Fildes (1986) states that during the 19th century the quality of breastmilk was advertised and often determined by "age" – meaning the length of time from production. The best time period for nursing was between 2-8 months post-birth, as it was believed that colostrum (a fluid produced during initial lactation) was unpurified and mother's body post-delivery would corrupt the breastmilk (Fildes 1986, Musumeci and Musumeci 2013). Colostrum is now known to be crucial for the development of a newborn, with proteins that assist in infant development and immunity (Musumeci and Musumeci 2013).

A wet-nurse in 19th century Spain could be classified by three different tiers of socio-economic status (Siles-Gonzáles et al. 2020). Wet-nurses hired by the monarchy or "elites" of society were provided a better quality of life for herself and her family. They would receive better resources including housing, food and clothing to ensure they remained healthy enough to feed the infants they nursed (Siles-Gonzáles et al. 2020). The majority of women advertising as a wet-nurse would be hired from a middle-class family. They would not receive the luxuries of the monarchy who employed wet-nurses, but earnings were often sufficient for daily living expenses. The final tier of women offering

to wet-nurse did not receive fair payment and often worked in the hospitals with foundlings (Siles-Gonzáles et al. 2020).

In some cases, the wet-nurse would act as a surrogate mother to care for the child, particularly during the mother's recovery period after birth (Siles-Gonzáles et al. 2020, Crifasi 2023). Siles-Gonzáles and co-authors (2020) noted that although a wet-nurse was typically hired by the wealthy or the monarchy, laws were established to protect infants of very low socio-economic status. These infants were often orphans or foundlings who were placed in charity establishments to ensure they received adequate care.

Wet-nurses were regulated to attempt to alleviate the rates of infanticide (an act punishable by death) (Siles-González et al. 2020). Prior to the industrialization of many European countries, both the active and passive acts of infanticide (and in some cases neonaticide), were common (Hanlon 2023). Active infanticides were purposeful acts used to control the regulation of family numbers or sexes within household (often as a means to ensure survivability and maturation of children >2years old), or to avoid social or cultural stigmas (e.g., infants born from unwed mothers or through infidelity) (Hanlon 2023). Passive infanticide resulted from accidental death due to improper care (e.g., inadequate feeding practices, premature weaning, general neglect) or unintentional suffocation (i.e., sleeping adults rolling on top of infants when sharing the same bed) (Hanlon 2023). The regulation of wet-nurses through foundling hospitals or institutions, enabled mothers to avoid active or passive infanticide through abandonment (a permitted practice) (Sarasúa et al. 2023). Therefore, rather than committing infanticide, the mothers

or family members could give up their child to an institution that employed wet nurses where the infant could receive adequate care.

Within the literature on the mother-infant relationship, the use of a wet nurse has rarely been incorporated into bioarchaeological studies. Knowing the importance of the wet-nurse in Spanish society and incorporating the data from this thesis can aid in the development of the understanding of connection between an infant and their feeding source(s) (i.e., their mother and/or wet-nurse(s)).

3.2.3 Infant and Children's Health

The infant mortality rate in Spain during the late 19th and early 20th centuries was one of the highest of all the European countries, fluctuating between 171-365 deaths per thousand live births (Reher et al. 1997, Siles-González et al. 2020). Other countries during this time had lower infant mortality rates, such as Greece (<100) (Hionidou 1997), Italy (75-240) (Pinnelli and Mancini 1997), and France (175-225) (Cole 1996) per thousand births. There is conflicting evidence in the historical and archaeological record that the employment of a wet-nurse had a positive impact on the chance of survival of the infant. Siles-González and colleagues (2020) argued that a wet-nurse increased the survival of children, however, this does not account for the high rates of infant mortality in Spain compared to other European countries from this time.

In their study on the discrepancy in infant and childhood sex ratios in 19th century Spain, Beltrán Tapia and Gallego-Martínez (2020) hypothesized that sex discrimination against females at a very young age could account for the higher female morbidity and

mortality rates. Although there is no written evidence of female infanticide or abuse from the period, Beltrán Tapia and Gallego-Martínez (2020) suggested this discrepancy could be attributed to inadequate care or treatment within the household when compared to their male counterparts. It is also possible that there was a lack of information provided in censuses on the females of the households, unless they were actively working (Shubert 2005).

As mentioned in Chapter 2, Medina-Albaladejo and Calatayud (2021) studied the diet of foundlings (20 months to 7 years of age) in a hospital of Valencia in eastern Spain between 1852-1931 through the hospital's archives. The amount of food delivered to the hospital, number of patients receiving food and what specifically was served, as well as the number of days of the stay of the patient was recorded by the nun running the kitchen at the time (Medina-Albaladejo and Calatayud 2021). Medina-Albaladejo and Calatayud (2021) noted that though the individuals who received treatment at the hospital were likely those of low socio-economic status, the food provided by the hospital was similar to the domestic diet of the working class.

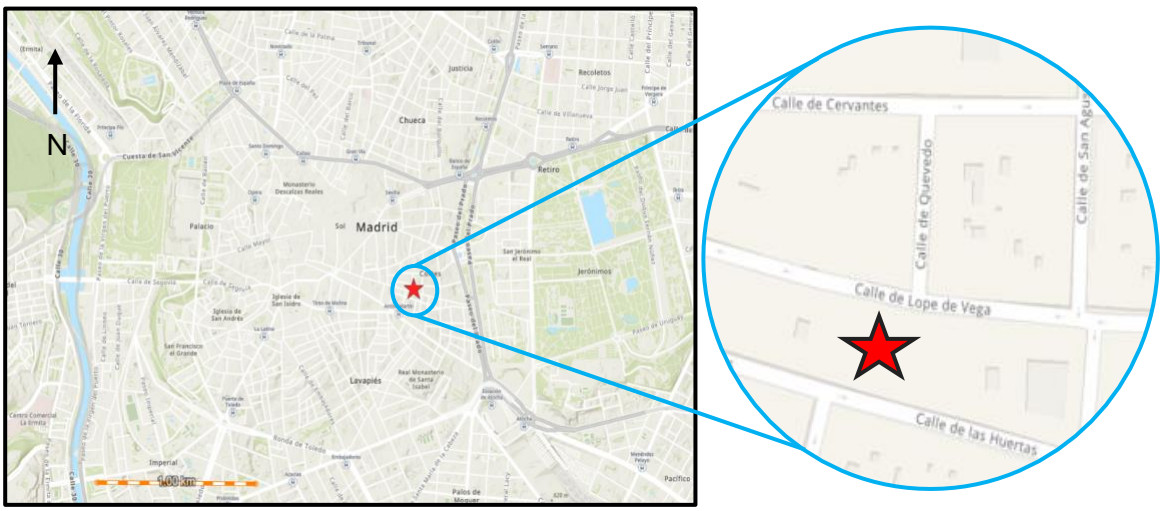
When breaking down the products and nutrients in the average daily diet of the foundlings, Medina-Albaladejo and Calatayud (2021) reported that the nutrient least represented was vitamin D. With a diet of bread, rice, chickpeas and veal, the foundlings had sufficient protein and carbohydrates, but were severely lacking in several vitamins and minerals necessary for proper growth (Medina-Albaladejo and Calatayud 2021).

3.3 Materials

3.3.1 Crypt at the Church of San Ildefonso de Las Trinitarias Descalzas de Madrid

While searching for the remains of Miguel de Cervantes, the 16-17th century Spanish author of *Don Quixote*, archaeologists discovered an undocumented burial ground beneath the convent of *San Ildefonso de Las Trinitarias Descalzas de Madrid* (Ríos et al. 2016, Labajo et al. 2017). Located in the neighborhood of Las Letras in the central district of Madrid, Spain (Figure 3.1), the cemetery was unearthed within a crypt that yielded approximately 500 individuals under the age of six years (García-Rubio et al. 2015, Ríos et al. 2016, Aranzadi n.d.).

Figure 3.1 *Geographic Location of the Church of the Trinitarias Descalzas (Sources: ARCGIS, Eris; MapQuest)*



The convent was established in 1612 (Figure 3.2), however the current church, where the cemetery was discovered, was built between 1673 and 1736 CE (Figure 3.3) (Paz de Miguel Ibáñez et al. 2017). During the construction of the current church, the

location of the first church and its associated burials were lost. Some of the historic literature on the site suggested that the current church was built over the old one (Roca de Togores, 1870; Astrana Marín, 1948-1958, cited in Paz de Miguel Ibáñez et al. 2017), however this was proven false by a document from the convent archive. This document recorded a payment in 1697 to the sacristan *Miguel de Ortigosa* for the transfer of deceased bodies from the old church to the new one (García-Rubio et al. 2015).

Figure 3.2 *Historical Map From 1622 Illustrating the Convent of San Ildefonso de Las Trinitarias Descalzas Prior to its Reconstruction (Source: ARCGIS, Eris)*

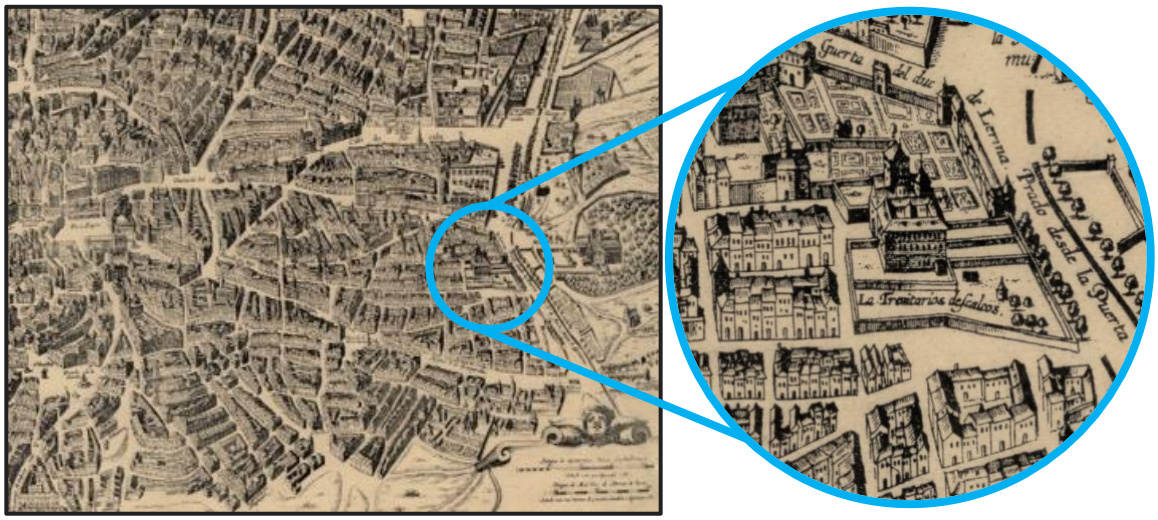
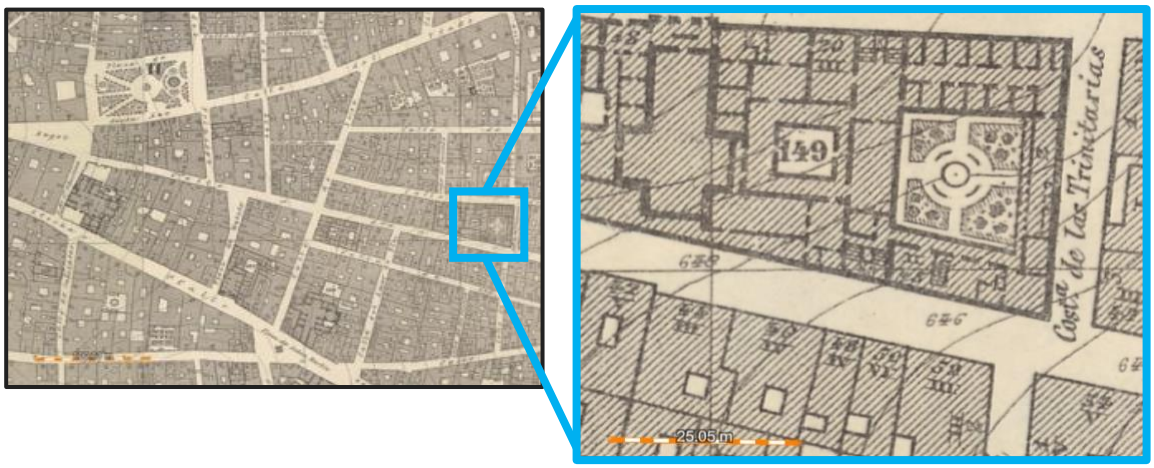


Figure 3.3 *Historical Map From 1875 Illustrating the Location of the Current Church of the Trinitarias Descalzas (Source: ARCGIS, Eris)*



The primary focus of the excavation of the crypt beneath the church was its floor and the niches within the North wall. Rectangular tiles next to the North wall indicated seven possible graves beneath the crypt floor (García-Rubio et al. 2015). Upon excavation of supposed burials, archaeologists concluded that there was no connection between the subsoil burials and the covering tiles. This was due to the combination of both children and adult burials, many of which were disarticulated, indicating a previous disturbance. As a result, the crypt was separated into six sections, with three distinct burial levels (García-Rubio et al. 2015).

Most of the adults and children discovered in the crypt were interred in a manner indicating a primary burial – they were discovered in-situ and had not been disturbed (Duday et al. 2014, Osterholtz et al. 2014). Others were found disarticulated, suggesting that they were reburied from their original burial site in a secondary burial (Osterholtz et al. 2014, Haddow and Knüsel 2017, Aranzadi n.d.). Evidence of primary and secondary

burials were found in both the floor within the crypt and the niches within its North wall (Aranzadi n.d.).

The types of burials, as well as their preservation levels, varied within the crypt. The garments worn by the deceased also varied from specific funerary garments adorned with lace or silk to other clothing that would have been considered simple everyday wear for the majority of the civilians (García-Rubio et al. 2015, Aranzadi n.d.) (Figure 3.4).

Figure 3.4 *Examples of Clothing Found on Individuals Buried in Wooden Coffins Within the Trinitarias Descalzas Crypt (Source: García-Rubio et al. 2015, p. 6)*

It was more common for the infants and children to be found buried in a shroud than in a coffin. Where coffins were discovered, they were either made of cork or wood. In a unique case, an individual in Niche 18 was discovered in a coffin made of zinc and

covered in wood (Aranzadi n.d.) (Figure 3.5). Due to the different levels of preservation at the Trinitarias Descalzas site, individuals exhumed without fabric remains were often found with fastening pins and/or buttons to indicate there was likely a shroud or clothing on the individual prior to the decomposition of the clothing (Aranzadi n.d.). The clothing and grave goods suggested the burials ranged between the 18th and 19th centuries (García-Rubio et al. 2015, Paz de Miguel Ibáñez et al. 2017, Aranzadi n.d.).

Figure 3.5 *Two Different Styles of Coffins from the Trinitarias Descalzas Crypt. Left: Niche 18 Made of Zinc, Enclosed in Wood. Right: Niche 23 Made of Cork. (Source: Aranzadi, n.d., p. 4)*

Two of the infant burials discovered contained preserved documentation related to the deceased (Aranzadi n.d.). These grave goods allowed archaeologists to search church records and associate a date with that burial level (Figure 3.6).

Figure 3.6 *Document Shown in a Hermetically Sealed Glass Jar Enclosed in a Lead Case Found in the Trinitarias Descalzas Crypt (Source: Aranzadi n.d., p. 11)*

The paper inside the jar reads: "*Sepultura de D. Trinidad Ribelles y Goya, hijo del que firma Julián de Ribelles. Muerto de 1º año*" (Aranzadi n.d, p. 11). The English translation is: "Burial of Mr. Trinidad Ribelles and Goya, son of whom Julián de Ribelles signs. Dead in 1st year". Based on the names provided in combination with archival information, researchers were able to determine the date of birth, date of baptism, address, immediate genealogy and social status of this individual and their family.

During a brief analysis of the remains during exhumation, the researchers noted that over 60% of the child and infant skeletons showed numerous indicators of rickets (vitamin D deficiency) (Labajo et al. 2017, Paz de Miguel Ibáñez et al. 2017). Using literature from Ortner and Mays (1998), Mays et al. (2006), Lewis (2007), Brickley et al.

(2010, 2014), as well as their own findings, Aranzadi and colleagues (n.d.) prepared recording sheets to document the frequency of disease (mainly rickets) within the sample. To diagnose an individual as having rickets, they followed the criterion described by Mays et al. (2006), which states that at least three of the 16 skeletal features associated with rickets must be evident (Table 3.1).

The high incidence of rickets (approx. 60% of children and infants excavated) led the researchers to want to understand the sample more, as well as the possible causes of VDD in this sample (Aranzadi n.d.). As a result of this find, an in-depth analysis was completed on both the sample and history of the individuals and their life at this time in Madrid, Spain.

Table 3.1 *Skeletal Features Associated with Rickets*

Skeletal Features
Cranial vault porosity
Orbital roof porosity
Deformed mandibular ramus
Rib deformity
Costochondral rib flaring
Costochondral rib porosity
Ilium concavity
Deformed leg bones
Deformed arm bones
Long-bone metaphysical flaring
Long-bone general thickening
Long-bone metaphysical porosity
Superior flattening of femoral metaphysis
Coxa vara (reduced angle of the neck of the femur)
Porosis/roughening of bone underlying long-bone growth plates
Long-bone concave curvature porosity

Sources: Ortner and Mays (1998), Brickley et al. (2010, 2014), Lewis (2007), Mays et al. (2006)

3.3.2 Sample Information

Both permanent and deciduous teeth were collected from the Trinitarias Descalzas sample in 2017 by Dr. Megan Brickley. She collected 86 teeth from 42 individuals, ranging in age from 7 months in utero to 6 years, most of which showed skeletal evidence of rickets, but some teeth were selected from individuals with no evidence of rickets. Individual age estimates were determined by Dr. Brickley using dental development and eruption charts when she examined the skeletons in Madrid. The teeth are currently housed at McMaster University. Of the 86 teeth identified, 44 teeth were selected for this study. The selection process was based on Dr. Brickley's notes on the presence of skeletal evidence of rickets for each individual. Five (of the 44) teeth were chosen from the group labeled 'older children with no skeletal evidence of rickets'. It is possible that these individuals had healed or obscured evidence of rickets, but the notes were inconclusive.

Of the 44 teeth selected for this thesis, 14 had been previously sectioned and analyzed for a stable isotope study in an M.A. thesis published in September of 2018 (Smith 2018). All of the teeth involved in this thesis are either molars (n=33) or incisors (n=11) (Table 3.2). Each individual was assigned a code by the researchers in Madrid. A unique 'MAD' code was assigned to each tooth here at McMaster, so they could be properly identified with the individuals included who had more than one tooth represented in this thesis (e.g., Individual N12-1 had two teeth – MAD01 and MAD02).

Table 3.2 *Identification and Tooth Types Used in This Study*

Original Individual Code	MAD ID	Age at Death Estimate*	Deciduous/ Permanent	Class
N12-1	MAD01	2.5 years	Deciduous	Incisor
N12-1	MAD02	2.5 years	Deciduous	Molar
N5-1	MAD03	6 years	Deciduous	Molar
SCIV-16	MAD04	3 years	Deciduous	Molar
SC4-15	MAD05	1 year	Deciduous	Incisor
SC4-12	MAD06	2 years	Deciduous	Molar
SC1-F2-18	MAD07	1 year	Deciduous	Incisor
SC1-F2-5	MAD08	2 years	Deciduous	Incisor
SC1-F2-5	MAD09	2 years	Deciduous	Molar
SC1-F1-2	MAD10	1 year	Deciduous	Molar
N4-4	MAD11	6 years	Deciduous	Molar
N4-4	MAD12	6 years	Permanent	Molar
N23-5	MAD13	2.7 years	Deciduous	Molar
SC4-79	MAD14	3 years	Deciduous	Molar
SC4-42	MAD15	2 years	Deciduous	Molar
SC4-12	MAD16	2 years	Deciduous	Molar
N23-2	MAD17	1.2 years	Deciduous	Molar
N5-1	MAD18	6 years	Permanent	Molar
N23-2	MAD19	1.2 years	Permanent	Molar
N7-2	MAD20	6 months	Deciduous	Molar
N4-3	MAD21	6 months	Deciduous	Incisor
N4-3	MAD22	6 months	Permanent	Molar
N18-1	MAD23	9 months	Deciduous	Incisor
N18-1	MAD24	9 months	Deciduous	Molar
N36-1	MAD25	7th month in-utero	Deciduous	Incisor
N36-1	MAD26	7th month in-utero	Deciduous	Molar
N14-5	MAD27	Just before birth	Deciduous	Incisor
N14-5	MAD28	Just before birth	Deciduous	Molar
N7-2	MAD29	6 months	Deciduous	Incisor
SC1-F2-9	MAD30	9 - 11 months	Permanent	Molar
SC1-F2-9	MAD31	9 - 11 months	Deciduous	Incisor
SC4-8	MAD32	11 months	Deciduous	Molar
SC4-8	MAD33	11 months	Permanent	Molar
SC4-51	MAD34	1 year	Permanent	Molar
SC4-51	MAD35	1 year	Deciduous	Molar
SC4-55	MAD36	9 months	Deciduous	Molar
SEP3-10	MAD39	~1 year	Deciduous	Incisor

Original Individual Code	MAD ID	Age at Death Estimate*	Deciduous/ Permanent	Class
SEP3-10	MAD40	~1 year	Deciduous	Molar
N14-1	MAD41	8 months	Deciduous	Molar
N14-1	MAD42	8 months	Permanent	Molar
N24-1	MAD43	9 months	Deciduous	Molar
N24-1	MAD44	9 months	Permanent	Molar
N16-5	MAD45	1 year	Permanent	Molar
N16-5	MAD46	1 year	Deciduous	Molar

Note: *Age-at-death estimate provided by notes from Dr. M. Brickley

3.4 Methods

3.4.1 Tooth Identification and Initial Imaging

While this is the second thesis at McMaster to focus on the analysis of the teeth from the TD site in Madrid, Spain, re-examination, and identification was completed for all of the teeth used in this thesis (n=44) to ensure accuracy and consistency. Using information from Brown (1985), White and Folkens (2005), Cunningham et al. (2016), and Hillson (2024), the types of teeth used in this thesis were identified and confirmed by McMaster's laboratory coordinator and supervisor B. Khalon. The process used to identify each tooth focused on the analysis and interpretation of three main tooth features: (1) crown size and shape, (2) root structure and (3) wear (Table 3.3). There are five key elements necessary to catalogue a tooth – the dentition layout (deciduous or permanent), side (right or left), location (maxillary or mandibular), class (incisor, canine, premolar or molar) and position within the dental arcade sequence (central/lateral for incisors or first/second for premolars and first/second/third for molars). To avoid confusion and simplify tooth identification across disciplines, tooth notation systems were developed.

Table 3.3 *General Tooth Identifying Features*

Crown	Root	Wear
Occlusal surface	Number	Occlusal surface
Lingual surface	Shape	Mesial/distal facets
Buccal/labial surface	Fossae	
Number/shape of cusps	Apical skew	
Mesial/distal ridges	Lack of roots	
Occlusal shape	Shape of pulp cavity	
Presence or absence of tubercles		
Location and shape of cervical margin		

Sources: Brown (1985), White and Folkens (2005), Cunningham et al. (2016), Hillson (2024)

In this thesis, each tooth was given a unique label (MAD##) to differentiate between the samples. The original identification used for the sample only noted the individual the tooth came from. As most (18 of the total of 26) of the individuals within this sample have more than one tooth included, it was necessary to establish a unique label for each individual tooth (Table 3.2).

Once the teeth from the Trinitarias Descalzas site were identified and labeled, they were imaged using the Keyence® (VHX-2000) digital microscope to document their features, prior to any altering or destructive procedures. The images included the occlusal, apical, medial, distal, buccal/labial, and lingual/palatal sides at 20x magnification.

3.4.2 Developmental Tooth Age Estimation

Due to their resistance to chemical and physical destruction when compared to other skeletal elements, as well as their consistent formation and eruption times, teeth are often used to estimate the age-at-death of individuals suspected to be under the age of 18 years (White and Folkens 2005). Although there is minor variation between the sexes and populations, tooth development is more accurately associated with chronological age than

age estimates assessed from skeletal elements (White and Folkens 2005). Chronological age refers to the time since birth, often described in years whereas physiological or biological age refers to the biological changes experienced by the body (Ginn and Arbor 1995, Sofaer 2006, Halcrow and Tayles 2008). Dental development charts, such as those created by Moorrees et al. (1963a, b), Demirjian et al. (1973), Gustafson and Koch (1974), Buikstra and Ubelaker 1994, and AlQahtani et al. (2010) use data from individuals with known chronological age to describe their physiological or biological age (Sofaer 2006, Halcrow and Tayles 2008).

Prior to this thesis, Dr. Megan Brickley had estimated the age-at-death of the individuals in the Trinitarias Descalzas sample, using their dental and skeletal remains. As the skeletal remains reside in Spain, a reassessment of the age-at-death could not be completed. However, the developmental age of each tooth was estimated based on the type of tooth, and its crown and root completeness. The developmental age, determined from the tooth analysis, in combination with the age-at-death estimated from the skeletal remains, provide a more accurate assessment.

Using the methods developed by Gustafson and Koch (1974), in combination with White and Folkens' (2005) and the Moorrees et al.'s (1963a, b) tooth development stage descriptions, all of the teeth were assigned a developmental age range based on four stages of tooth development – mineralization, crown completion, tooth eruption and root completion (Gustafson and Koch 1974). This method was selected over other available methods used in biological anthropology (such as those outlined in Demirjian et al. 1973, Buikstra and Ubelaker 1994 or AlQahtani et al. 2010) because the data gathered by

Gustafson and Koch (1974) was based on a sample of individuals of European descent and would be closely matched with individuals from the Trinitarias Descalzas site in Madrid, Spain. The Gustafson and Koch (1974) method also analyzed each individual tooth from in utero to 16 years of age. This data is fairly complete when compared to Demirjian et al. (1973) or Moorrees et al. (1963a, b) as it covers both types of dentitions (deciduous and permanent) from both the mandible and maxilla. It is important to know the approximate developmental ages associated with the teeth as it can be used to help identify the timing of the periods of VDD and correlate that information with data on breastfeeding and weaning periods. This will be further discussed further in Chapter 5.

3.4.3 Sample Preparation

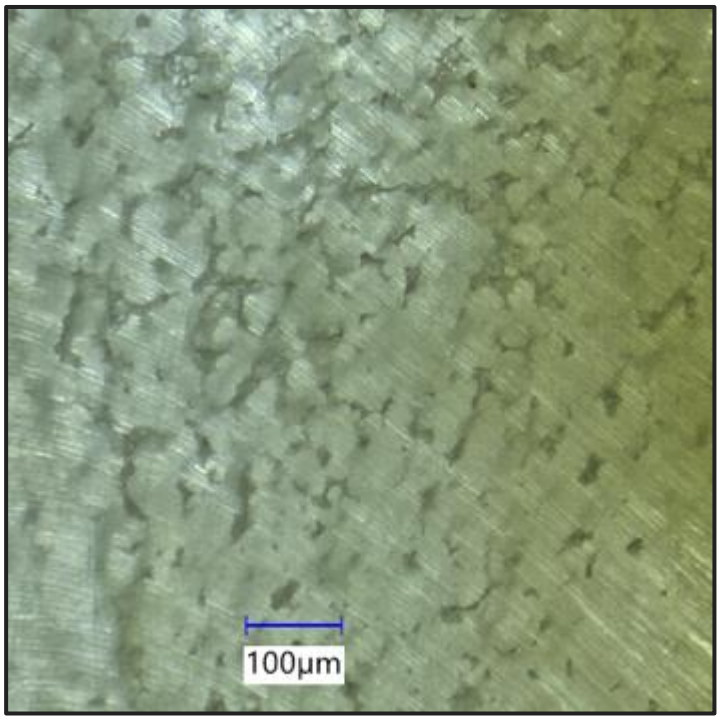
Following the procedures outlined in Saunders et al. (2007), the 30 unprocessed teeth from the Trinitarias Descalzas sample were first embedded in epoxy (Buehler EpoThin) to ensure stability during sectioning (the other 14 had been prepared by Smith for his MA thesis in 2018). After allowing 24 to 48 hours for the epoxy to set, each tooth was sectioned into two halves following a buccolingual direction using a Buehler IsoMet 1000 diamond wafering saw. One half of the tooth was set aside for possible future use, while the other half was ground and polished using 400, 600, 800 and 1200 grit paper. A drop of UV activated glue was then added to the center of a slide, which was placed onto an idle UV light source. The polished side of the sectioned tooth was carefully but firmly pressed into the glue and the UV light was activated. The slide remained on the light source for 2-3 minutes to ensure the sample adhered to the slide. With the tooth now secured to the slide, a second cut was made leaving a 3 mm section of tooth remaining

attached to the slide. Following this step, the newly sliced section was ground and polished using the four grades of grit paper noted above until the section measured 0.4 mm using a Mastercraft digital caliper.

3.4.4 Macroscopic Identification of Interglobular Dentine (IGD)

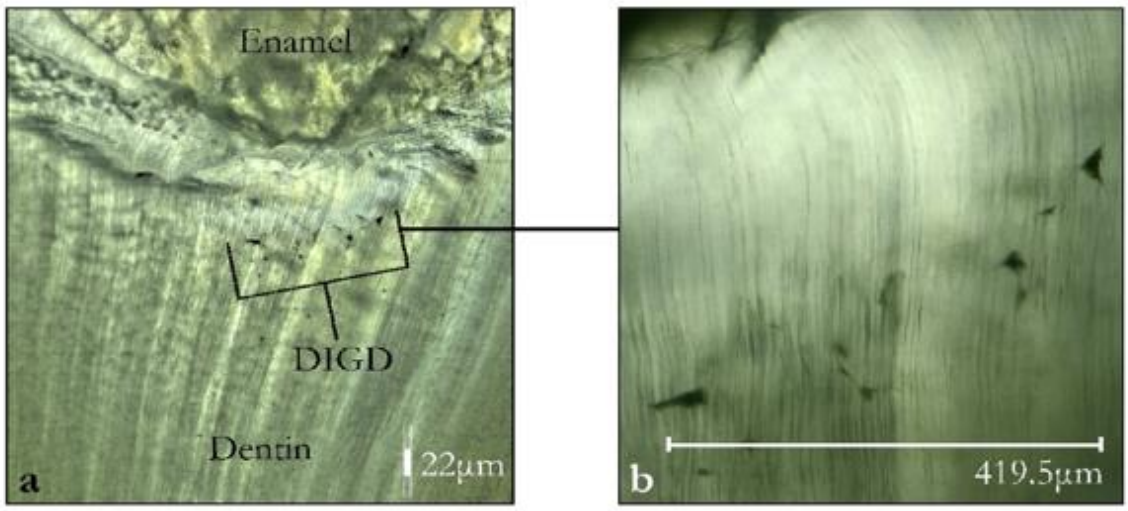
To identify IGD within this sample, each tooth section was examined using the Keyence® (VHX-2000) digital microscope at 20X, 50X, 100X and 200X magnification. The appearance of IGD, when viewed under a microscope on a tooth thin section, appears as semi-circular darkened patches of varying sizes (Figure 3.7) (Mellanby 1928, Sato et al. 2000, D'Ortenzio et al. 2016, Hillson 2024). The amount and location of IGD can vary both within a tooth and between multiple teeth (Sato et al. 2000, D'Ortenzio et al. 2016).

Figure 3.7 *Interglobular Dentine (dark patches) at 200X Magnification on MAD14 (lm^1). Image by L. Godawa*



At 20X and 50X magnification, it was easier to see the whole tooth (both the crown and root, where applicable) and identify areas of dentine that needed further examination. At 100X and 200X magnification, it was often easier to distinguish the parabolic-like shapes unique to IGD, regardless of the amount present. It is important to acknowledge the presence and/or absence of developmental interglobular dentine (DIGD) in this thesis. DIGD is a mineralization defect similar to IGD in appearance, however, it does not follow the incremental growth lines, nor does it appear bilaterally within a tooth, as seen in Figure 3.8 (from D'Ortenzio et al. 2018).

Figure 3.8 *Example of DIGD from Lisieux-Michelet, France (LM_2) (Source: D'Ortenzio et al. 2018, Fig. 4, p. 106)*



D'Ortenzio et al. (2018) noted that DIGD can often be mistaken as a low amount/grade of IGD, however, its origin and characteristics differ notably. The location, size, and amount of interglobular spaces can help determine whether the individual had IGD or DIGD (see Table 3.4). At this stage teeth were noted as a 'yes' or 'no' for presence

of IGD. DIGD was also noted. Those with the presence of IGD underwent further examination described in Section 3.4.5.

Table 3.4 *Comparing Characteristics of IGD vs DIGD*

Characteristics	IGD	DIGD
Probable Cause	Vitamin D deficiency or Fluorosis	Anomalies during tooth development in dentine formation
Appearance of interglobular spaces	Semi-circular shapes in varying sizes	Elongated, wave-like
Amount	Varies (low to high)	Minimal
Pattern	Follows along incremental growth lines	Scattered
Affected side of tooth	Bilateral	Localized on one side
Localization	Anywhere within the dentine, typically seen in the crown portion	Near the cemento-enamel junction

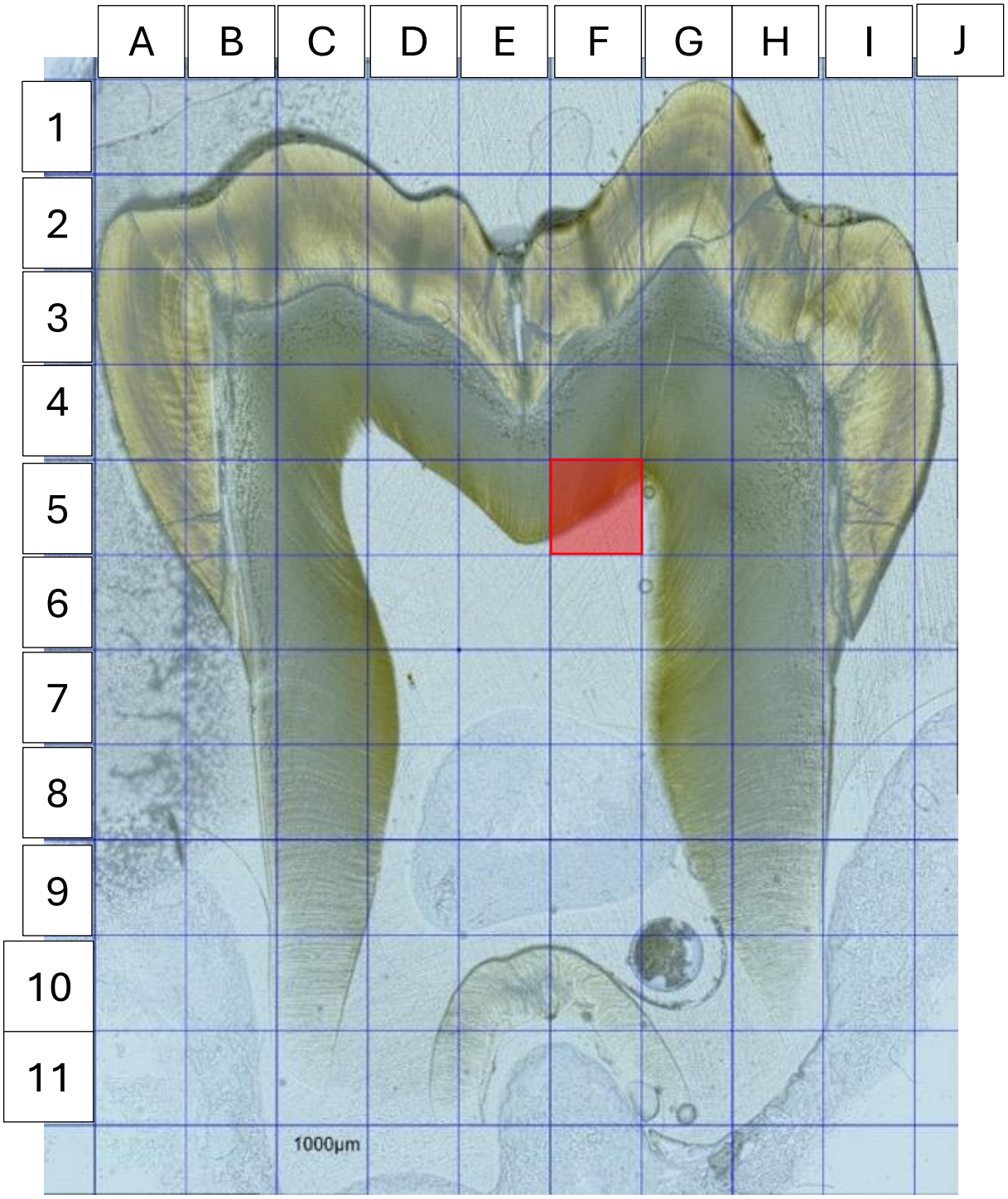
Sources: Sato et al. (2000), D'Ortenzio et al. (2018)

3.4.5 Quantifying Areas of IGD

Once the areas of IGD were identified in a tooth section, the following steps were used to measure the total area of IGD within the available dentine. For a better resolution of the images of the tooth sections, each tooth was set to 200x magnification and 'stitched' together using the software on the Keyence® (VHX-2000) digital microscope. The 'stitch' function takes multiple images of the subject in a specific area, determined by the user, and overlays them together to create a complete image of tooth at 200x magnification. This creates a high-resolution photograph of the entire tooth section. Once the images were stitched, a grid measuring 1000 µm X 1000 µm per cell was overlayed onto the image (Figure 3.9). Each cell was then coded using an alphanumeric numbering

system to differentiate between the different cells. The columns were represented by letters, while the rows were represented by numbers. Each cell was then measured using the area measurement feature on the Keyence® (VHX-2000) digital microscope.

Figure 3.9 *Cross-section of MAD12 (LM[1]), With Overlayed Grid and Alphanumeric Coding System. The image below has 62 cells with dentine and 24 of those have IGD. The red highlighted cell is F5. Image by L. Godawa*



As with any other biological component, teeth are not perfect, especially when they are from an archaeological setting. Diagenetic changes often alter the teeth and as a result, may disrupt the integrity of the sample (Bell et al. 1991, Kendall et al. 2018). To account for these changes, the amount of observable dentine present was measured (in μm^2) using a 'lasso' tool to outline the area of dentine within the cell to get an accurate measurement of the total dentine within that specific cell (Figures 3.10, 3.11). If IGD was present within that cell the 'lasso' tool was used again within the same dentine area in a separate measurement to outline each patch of IGD to be measured; however, during this step the contrast between the IGD and dentine was increased to differentiate between the unaffected dentine and the darkened patches of IGD. This number was then recorded (in μm^2) (Figure 3.12). The 'lasso' tool only outlined the area of IGD to be examined for each cell. The individual pieces of IGD within the cells did not have to each be lassoed as the software was able to highlight the contrasting areas of IGD (darkened patches) versus unaffected dentine (lighter areas) both around and within the patches of IGD.

Figure 3.10 *Cropped Image of MAD 11 (rm[1]), Cell C4 Outlined in Red. Area of Dentine in C4 is Circled on the Measurement Table (132,782 µm²). Image by L. Godawa*

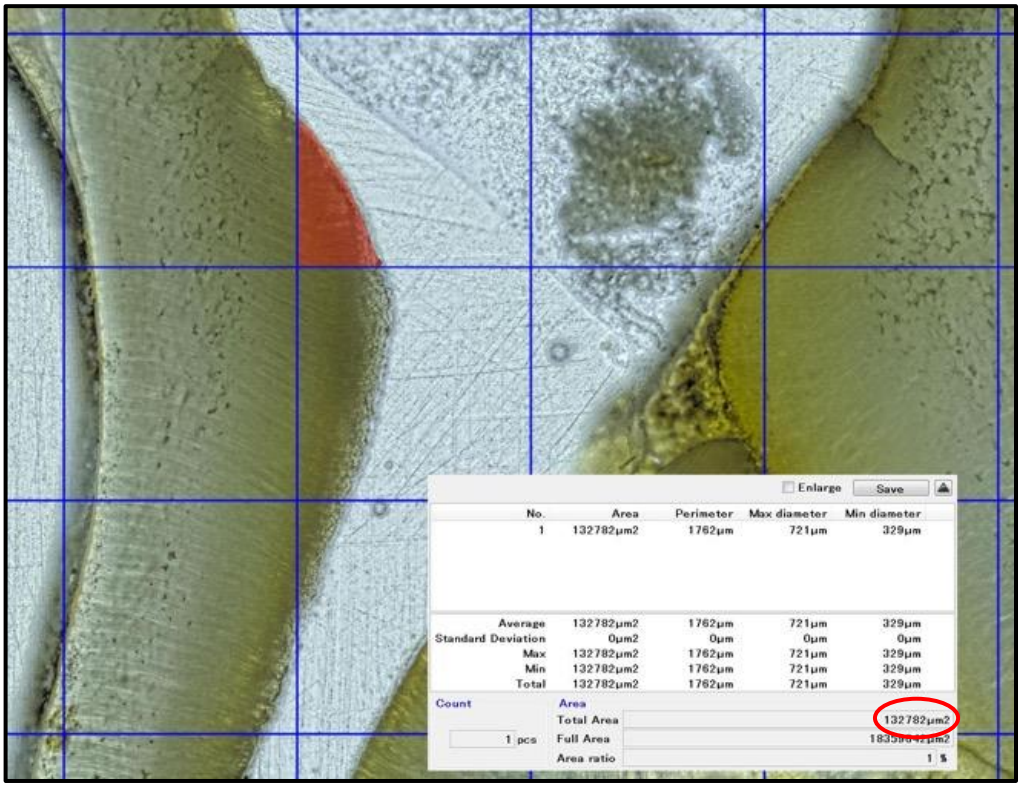


Figure 3.11 *Cropped Image of MAD11 (rm[1]) Showing a Cell Only Partially Filled with Dentine (Blue Highlighted Cell). Note that the red highlighted cell (E5) has dentine within the entirety of the 1000µm x 1000 µm square, while the blue highlighted cell (C4) does not. Image by L. Godawa*

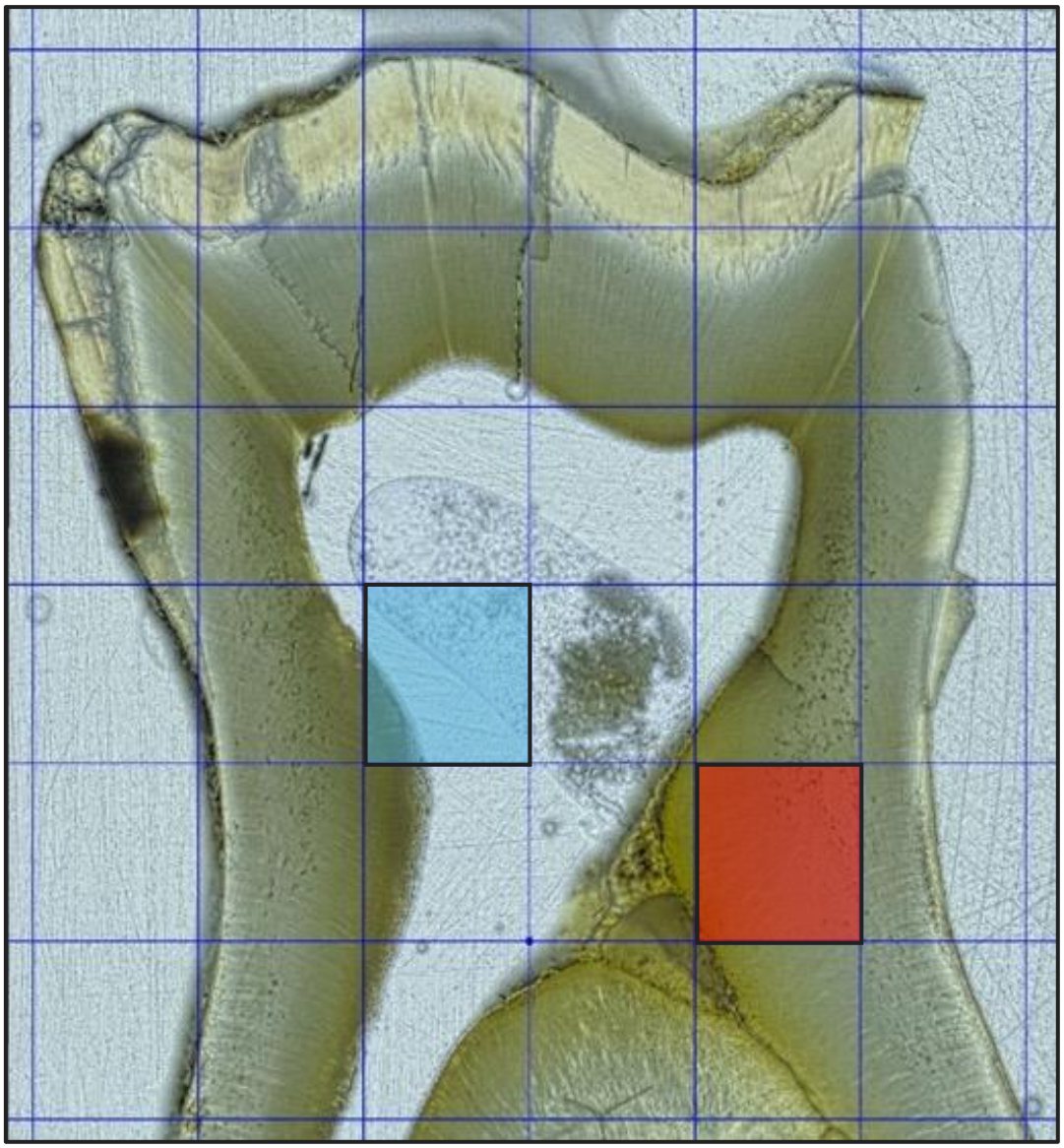
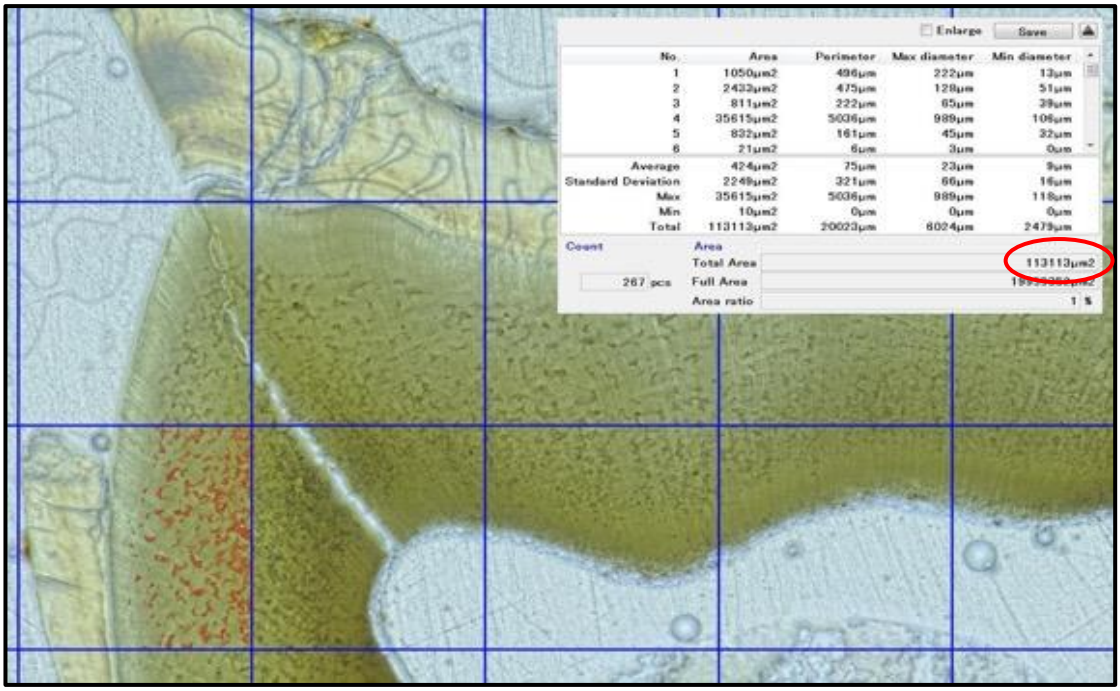


Figure 3.12 *Cropped Image of MAD15 (rm[1]), IGD in Cell A3 Measured. Area of IGD in A3 is Circled on the Measurement Table (113,113 µm²). Image by L. Godawa*



Once the dentine and IGD (where evident) in each cell was measured and recorded, the total amount of observable dentine was calculated. This was completed by adding the recorded areas of dentine from each individual cell in one tooth to have a sum of the dentine area per tooth. The total amount of IGD for each tooth was also calculated by adding the IGD areas from each individual cell. To calculate the percentage of IGD within each tooth, the total area of IGD within a tooth was divided by the total area of observable dentine within the same tooth. This method systematically accounts for all the dentine and IGD present in a tooth, whereas previous methods focused on an 'area of interest' (usually an area with significant IGD present). For further explanation see Section 5.4.

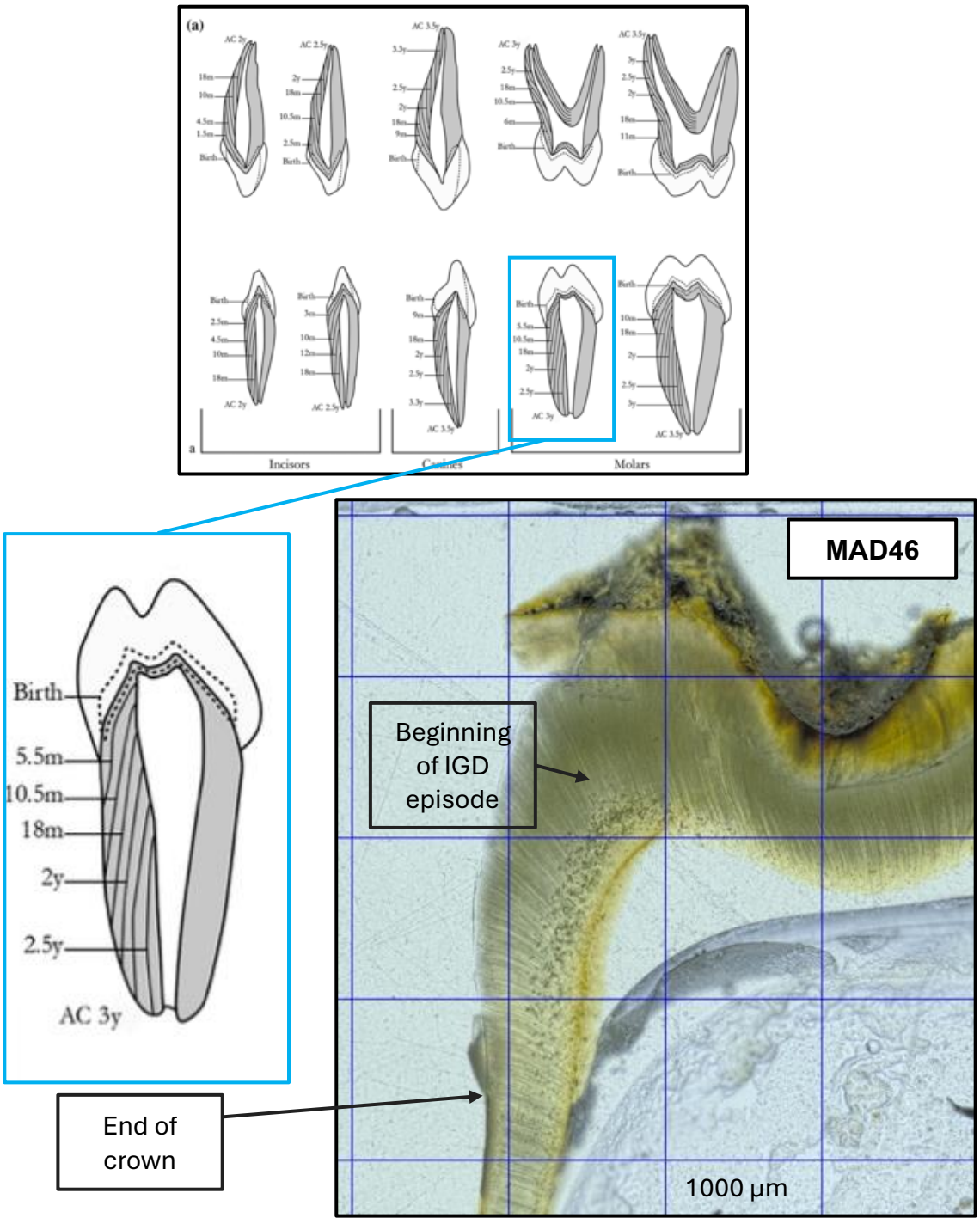
3.4.6 Timing of Episodes of IGD

The timing and duration of the IGD episodes were estimated to get a better understanding of when the occurrences of IGD took place during the individual's development (i.e., approximate age at start and end of episode), as well as the length of time they experienced this mineralization deficiency (i.e., how long the deficiency lasted). As mentioned earlier in this chapter, the timing data of IGD is crucial to understanding at what point(s) in the individual's development they experienced a period of VDD as it can help to infer possible cultural practices during pregnancy, or between the mother and infant during breastfeeding and weaning. Using Figure 3a (for deciduous teeth) and 3b (for permanent teeth) from Brickley et al. (2020a) in combination with the developmental age data compiled earlier in this thesis (Section 3.4.2) the number of episodes of IGD, their approximate timing of the start and end of the IGD episodes as well as the length of these episodes were established (Figure 3.13).

The figure developed by Brickley and colleagues (2020a) compiled data on the approximate ages of mineralization for both permanent and deciduous teeth to illustrate the incremental growth patterns within each tooth. The photos of the samples used for this thesis, with IGD present, were compared to Figure 3a and 3b (Brickley et al. 2020a) and the timing of the episodes was approximated based on the visual location of the IGD. This information was then compared to the percentages of IGD for each tooth. The teeth were only compared in groups based on the tooth type (i.e., deciduous molar to deciduous molar, deciduous incisor to deciduous incisor, and permanent molar to permanent molar). This was done because of the different growth rates between types of teeth. Figure 3.13

includes an image of 3a (deciduous teeth timing) from Brickley et al. (2020a), and highlights the tooth being examined (deciduous mandibular first molar) and a cropped image of the left crown portion of MAD46 (rm_1).

Figure 3.13 *Comparing Image of Figure 3a (Brickley et al. 2020a, p. 347) and MAD46 (rm_1) (Image by L. Godawa) to Determine Timing of IGD Episode. Enamel is White and Dentine is Grey in the Drawings. Coloured image of tooth section by L. Godawa*



3.4.7 Conclusion

One of the main research questions proposed in Chapter 1 of this thesis concerns the development of a means to quantify the amount of IGD present in an entire tooth instead of a region of interest within the tooth. Using the procedures developed here and described above, a replicable method of quantification was established. By measuring the total amount of dentine and IGD, and calculating a percentage of IGD within the dentine, the methodology provides more comprehensive data than previous documented methods. The amount of IGD, along with the timing and duration of periods of VDD (seen through the IGD episodes) provides insight into the connection between mother and infant at the time the dentine was forming. The dietary and environmental conditions experienced by the mother during pregnancy and by both the mother and infant, post childbirth are evidenced in the IGD.

CHAPTER 4: RESULTS

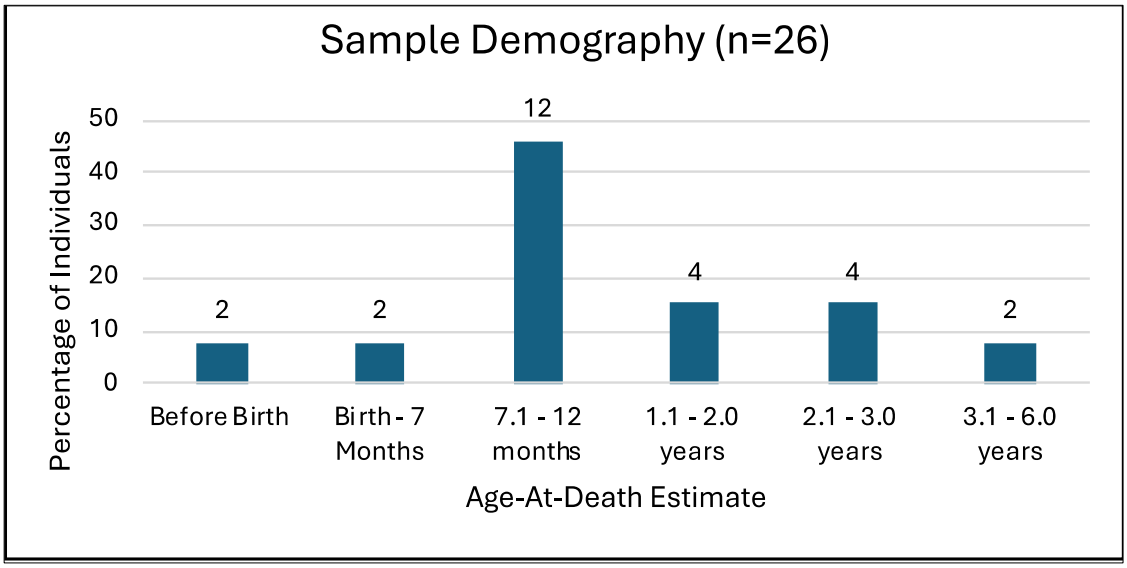
4.1 Introduction

The results presented in this chapter are based on the multiple types of analyses conducted on the teeth recovered from the Trinitarias Descalzas (TD) site. This chapter begins with information on the demography of the sample (previously calculated age-at-death estimates), the presence or absence of skeletal evidence of vitamin D deficiency (VDD), and the location of the IGD episodes in the tooth (e.g., crown vs root). The amount of IGD (in μm^2) in each tooth is then reported as a percentage based on the area in the dentine with IGD divided by the total observable dentine. Following this information, the number and timing of IGD episodes is described. Finally, a statistical analysis of the data presented in this chapter is discussed and analysed. It must be noted that of the 46 teeth originally selected for this study, 2 teeth were removed from analysis (MAD 37 and MAD 38) because of poor visibility in their thin sections. Therefore, the data presented below discusses the remaining 44 teeth from 26 individuals.

4.2 Demography of the Sample

There are 26 individuals in this sample ranging in age-at-death from 7 months in utero (MAD25 and MAD26) to 6 years of age (MAD11 and MAD12). The individuals most represented had an age-at-death estimate between 7.1 and 12 months. The age distribution of the sample is shown in Figure 4.1.

Figure 4.1 *Age Distribution of Individuals from the Sample (n=26)*



4.3 Tooth Samples Used in This Thesis

When the teeth were collected in 2017 by Dr. Brickley, each individual was assigned to one of four categories based on their age estimation (young individuals or older children) and the presence or absence of skeletal evidence of rickets (Table 4.1). In this thesis, 'young individuals' are those 1 year of age and under, while 'older children' are those older than 1 year. Specifically, young individuals ranged between 7 months in utero to 1 year old, while older children ranged in age between 1.2 years to 6 years. No teeth were analysed from Category 2 as there was minimal chance of evidence of healed rickets. Individuals included in the current study were primarily from Categories 1 and 3 (Table 4.1) as most displayed skeletal evidence of rickets (N= 23/26 = 88.5%). This was an intentional sampling choice because Dr. Brickley wanted to study the teeth from individuals who had known or possible skeletal evidence of rickets. Individuals from

Category 4 were also included as there was a chance that they could have had healed rickets or anomalies obstructing the observation of rickets on the skeletal materials.

Table 4.1 *Tooth Categories and Rickets Diagnosis by M. Brickley and Number of Teeth from the* Trinitarias Descalzas *Sample*

Category	Label	Number of Teeth used in this Thesis (N=44)
1	Young individuals (<1.0 year) with skeletal evidence of rickets	28 teeth (from 16 individuals)
2	Young individuals without skeletal evidence of rickets	No teeth
3	Older children (>1.2-6.0 years) with skeletal evidence of rickets	11 teeth (from 7 individuals)
4	Older children without skeletal evidence of rickets	5 teeth (from 3 individuals)

Note: Information from notes provided by Dr. M. Brickley and B. Kahlon.

Table 4.2 provides a detailed description of each tooth used in this study and their associated rickets category, as described in Table 4.1. If there are two teeth from the same individual, they are given their own code number (MAD## – *referring to Madrid*) to easily distinguish between the types of teeth present (e.g., individual N4-4 has two teeth, MAD11 – rm^1 and MAD12 – LM^1).

Table 4.2 *Tooth Identification of Samples Used in This Thesis*

Original Individual Code	MAD ID	Age-at-death Estimate*	Rickets Category†	Tooth				
				Deciduous/ Permanent	Maxillary/ Mandibular	Right/ Left	Class	Abbr.
N12-1	MAD01	2.5 years	4	Deciduous	Maxillary	Right	Incisor	ri^1
N12-1	MAD02	2.5 years	4	Deciduous	Maxillary	Right	Molar	rm^1
N5-1	MAD03	6 years	4	Deciduous	Maxillary	Right	Molar	rm^1
SCIV-16	MAD04	3 years	4	Deciduous	Mandibular	Right	Molar	rm_1
SC4-15	MAD05	1 year	1	Deciduous	Maxillary	Right	Incisor	ri^1
SC4-12	MAD06	2 years	3	Deciduous	Maxillary	Right	Molar	rm^1
SC1-F2-18	MAD07	1 year	1	Deciduous	Maxillary	Left	Incisor	li^1
SC1-F2-5	MAD08	2 years	3	Deciduous	Maxillary	Right	Incisor	ri^1
SC1-F2-5	MAD09	2 years	3	Deciduous	Maxillary	Left	Molar	lm^1
SC1-F1-2	MAD10	1 year	1	Deciduous	Mandibular	Left	Molar	lm_1
N4-4	MAD11	6 years	3	Deciduous	Maxillary	Right	Molar	rm^1
N4-4	MAD12	6 years	3	Permanent	Maxillary	Left	Molar	LM^1
N23-5	MAD13	2.7 years	3	Deciduous	Mandibular	Left	Molar	lm_1
SC4-79	MAD14	3 years	3	Deciduous	Maxillary	Left	Molar	lm^1
SC4-42	MAD15	2 years	3	Deciduous	Maxillary	Right	Molar	rm^1
SC4-12	MAD16	2 years	3	Deciduous	Mandibular	Left	Molar	lm_1
N23-2	MAD17	1.2 years	3	Deciduous	Maxillary	Right	Molar	rm^1
N5-1	MAD18	6 years	4	Permanent	Maxillary	Right	Molar	RM^1
N23-2	MAD19	1.2 years	3	Permanent	Maxillary	Left	Molar	LM^1
N7-2	MAD20	6 months	1	Deciduous	Maxillary	Right	Molar	rm^1
N4-3	MAD21	6 months	1	Deciduous	Maxillary	Right	Incisor	ri^1
N4-3	MAD22	6 months	1	Permanent	Mandibular	Left	Molar	LM_1
N18-1	MAD23	9 months	1	Deciduous	Maxillary	Right	Incisor	ri^1
N18-1	MAD24	9 months	1	Deciduous	Mandibular	Right	Molar	rm_1
N36-1	MAD25	7th month in-utero	1	Deciduous	Maxillary	Left	Incisor	li^1
N36-1	MAD26	7th month in-utero	1	Deciduous	Mandibular	Right	Molar	rm_1
N14-5	MAD27	Just before birth	1	Deciduous	Maxillary	Left	Incisor	li^1
N14-5	MAD28	Just before birth	1	Deciduous	Maxillary	Right	Molar	rm^1
N7-2	MAD29	6 months	1	Deciduous	Maxillary	Right	Incisor	ri^1
SC1-F2-9	MAD30	9 - 11 months	1	Permanent	Maxillary	Right	Molar	RM^1
SC1-F2-9	MAD31	9 - 11 months	1	Deciduous	Mandibular	Left	Incisor	li_1
SC4-8	MAD32	11 months	1	Deciduous	Maxillary	Right	Molar	rm^1
SC4-8	MAD33	11 months	1	Permanent	Maxillary	Right	Molar	RM^1

Original Individual Code	MAD ID	Age-at-death Estimate*	Rickets Cate-gory†	Tooth				
				Deciduous/ Permanent	Maxillary/ Mandibular	Right/ Left	Class	Abbr.
SC4-51	MAD34	1 year	1	Permanent	Mandibular	Right	Molar	RM_1
SC4-51	MAD35	1 year	1	Deciduous	Maxillary	Right	Molar	rm^1
SC4-55	MAD36	9 months	1	Deciduous	Mandibular	Right	Molar	rm_1
SEP3-10	MAD39	~1 year	1	Deciduous	Maxillary	Right	Incisor	ri^1
SEP3-10	MAD40	~1 year	1	Deciduous	Mandibular	Left	Molar	lm_2
N14-1	MAD41	8 months	1	Deciduous	Maxillary	Left	Molar	lm^1
N14-1	MAD42	8 months	1	Permanent	Maxillary	Right	Molar	RM^1
N24-1	MAD43	9 months	1	Deciduous	Mandibular	Left	Molar	lm_1
N24-1	MAD44	9 months	1	Permanent	Mandibular	Left	Molar	LM_1
N16-5	MAD45	1 year	1	Permanent	Mandibular	Right	Molar	RM_1
N16-5	MAD46	1 year	1	Deciduous	Mandibular	Right	Molar	rm_1

Notes: *Age-at-death estimate provided by notes from Dr. M. Brickley; †Category explanations found in Table 4.1

In addition to the age-at-death estimate by Dr. Brickley, the approximate dental (developmental) age range of each tooth (n=44) was estimated by L. Godawa for this thesis. The individual tooth development estimates may deviate from the age-at-death estimates because they are based on only one tooth, as this sample is not complete. A complete dental age analysis would involve all (where possible) of the teeth from an individual, as well as their tooth eruption levels and wear. However, both skeletal and dental age can yield different, but generally similar age ranges, the combination of the two helps provide a more thorough age analysis.

The dental development age of each tooth was estimated by comparing the stage of development observed on the tooth sample (i.e., the amount of crown and root present) to standards developed by Moorrees et al. (1963a, b) and Gustafson and Koch (1974). The developmental age of each tooth used in this study is presented in Table 4.3 along with Dr. Brickley's age-at-death estimate and presence/absence of skeletal evidence of rickets.

Table 4.3 *Teeth Chosen for This Study, Their Skeletal Evidence of Rickets, Associated Tooth Developmental Ages, and Estimated Age-At-Death*

MAD ID	Skeletal Evidence of Rickets	Individual Tooth (Developmental) Age Estimate	Age-At-Death Estimation*
MAD01	No	1.5 - 2 years	2.5 years
MAD02	No	2 - 2.5 years	2.5 years
MAD03	No	2.5+ years	6 years
MAD04	No	2.5 - 3 years	3 years
MAD05	Yes	10 months - 1 year	1 year
MAD06	Yes	2 - 2.5 years	2 years
MAD07	Yes	11 months - 1.5 years	1 year
MAD08	Yes	11 months - 1.5 years	2 years
MAD09	Yes	1.5 - 2 years	2 years
MAD10	Yes	1.5 - 2 years	1 year
MAD11	Yes	4.5 - 5.5 years	6 years
MAD12	Yes	4.5 - 5 years	6 years
MAD13	Yes	2.5 - 3.5 years	2.7 years
MAD14	Yes	2.5 - 3 years	3 years
MAD15	Yes	1.5 - 2.5 years	2 years
MAD16	Yes	1.5 - 2 years	2 years
MAD17	Yes	1 - 2.5 years	1.2 years
MAD18	No	5.5 - 6 years	6 years
MAD19	Yes	1.5 - 2 years	1.2 years
MAD20	Yes	4 - 10 months	6 months
MAD21	Yes	4 - 6 months	6 months
MAD22	Yes	4.5 - 10.5 months	6 months
MAD23	Yes	5 months - 1 year	9 months
MAD24	Yes	7 months - 1 year	9 months
MAD25	Yes	5 months in-utero - 2 months	7th month in-utero
MAD26	Yes	5 months in-utero - Birth	7th month in-utero
MAD27	Yes	Birth - 2 months	Just before birth
MAD28	Yes	5 months in-utero - 2 months	Just before birth
MAD29	Yes	2 - 6 months	6 months
MAD30	Yes	Birth - 10.5 months	9 - 11 months
MAD31	Yes	4 - 10 months	9 - 11 months

MAD ID	Skeletal Evidence of Rickets	Individual Tooth (Developmental) Age Estimate	Age-At-Death Estimation*
MAD32	Yes	7 - 12 months	11 months
MAD33	Yes	6 - 12 months	11 months
MAD34	Yes	7 months - 1 year	1 year
MAD35	Yes	10 months - 1.5 years	1 year
MAD36	Yes	10 months - 1.5 year	9 months
MAD39	Yes	7 months - 1 year	~1 year
MAD40	Yes	7 months - 1 year	~1 year
MAD41	Yes	7 months - 1 year	8 months
MAD42	Yes	6 - 9 months	8 months
MAD43	Yes	7 months - 1.5 years	9 months
MAD44	Yes	6 months - 1.5 years	9 months
MAD45	Yes	8 months - 1.5 years	1 year
MAD46	Yes	1.5 - 2 years	1 year

Note: *Age-at-death estimation completed by Dr. M. Brickley in 2017

4.4 Identification of Interglobular Dentine in the Sample

4.4.1 Prevalence of IGD by Individual

Twenty-one individuals showed clear evidence of IGD in their teeth (N=21/26 = 80.7%) (vs. 23/26 showing skeletal evidence of rickets) (see Table 4.4). In three cases (individuals N12-1, N18-1, SC1-F2-9), there was conflicting evidence of IGD in two teeth from the same individual, discussed below, and emphasized in bold in Table 4.4.

Table 4.4 *Presence of Interglobular Dentine and Evidence of Rickets Presented by Individual and Tooth*

Individual ID	MAD ID	Skeletal Evidence of Rickets	Evidence of IGD
N4-3	MAD21	Yes	Yes
N4-3	MAD22	Yes	Yes
N4-4	MAD11	Yes	Yes
N4-4	MAD12	Yes	Yes
N5-1	MAD03	No	Yes
N5-1	MAD18	No	Yes
N7-2	MAD20	Yes	No
N7-2	MAD29	Yes	No
N12-1	**MAD01**	**No**	**No**
N12-1	**MAD02**	**No**	**Yes**
N14-1	MAD41	Yes	Yes
N14-1	MAD42	Yes	Yes
N14-5	MAD27	Yes	No
N14-5	MAD28	Yes	No
N16-5	MAD45	Yes	Yes
N16-5	MAD46	Yes	Yes
N18-1	**MAD23**	**Yes**	**No**
N18-1	**MAD24**	**Yes**	**Yes**
N23-2	MAD17	Yes	Yes
N23-2	MAD19	Yes	Yes
N23-5	MAD13	Yes	Yes
N24-1	MAD43	Yes	Yes
N24-1	MAD44	Yes	Yes
N36-1	MAD25	Yes	No
N36-1	MAD26	Yes	No
SC1-F1-2	MAD10	Yes	Yes
SC1-F2-5	MAD08	Yes	Yes
SC1-F2-5	MAD09	Yes	Yes
SC1-F2-9	**MAD30**	**Yes**	**Yes**
SC1-F2-9	**MAD31**	**Yes**	**No**
SC1-F2-18	MAD07	Yes	Yes

Individual ID	MAD ID	Skeletal Evidence of Rickets	Evidence of IGD
SC4-8	MAD32	Yes	Yes
SC4-8	MAD33	Yes	Yes
SC4-12	MAD06	Yes	Yes
SC4-12	MAD16	Yes	Yes
SC4-15	MAD05	Yes	No
SCIV-16	MAD04	No	Yes
SC4-42	MAD15	Yes	Yes
SC4-51	MAD34	Yes	Yes
SC4-51	MAD35	Yes	Yes
SC4-55	MAD36	Yes	No
SC4-79	MAD14	Yes	Yes
SEP3-10	MAD39	Yes	Yes
SEP3-10	MAD40	Yes	Yes

Note: Bold indicates the individuals with conflicting IGD evidence in their teeth

As previously mentioned, this high prevalence of teeth with IGD is due to the sampling strategy employed by Dr. Brickley to intentionally select individuals who showed skeletal evidence of rickets. The individuals were sorted into 4 categories to distinguish patterns among the sample: individuals with or without rickets (assessed by Dr. Brickley) and teeth with or without IGD (assessed by L. Godawa). In a small number of cases (n=3), where one individual had multiple teeth analyzed, each tooth was assigned to a different IGD category. Individual N12-1 (2.5 years old) showed no skeletal evidence of rickets, and their ri^1, MAD01 (Figure 4.2), displayed no evidence of IGD; however, their rm^1, MAD02, displayed clear evidence of IGD (Figure 4.3). Similarly, individual N18-1 (9 months old) displayed skeletal evidence of rickets, but one of their teeth (MAD23, ri^1) did not show any evidence of IGD (Figure 4.4), while their other tooth

MAD 24 (rm_1), showed evidence of IGD (Figure 4.5). Finally, individual SC1-F2-9 (9-11 months old) also displayed skeletal evidence of rickets, and while one of their teeth (MAD31, li_1) did not have evidence of IGD, the other (MAD30, RM^1) had evidence of IGD (Figure 4.6 and 4.7, respectively).

In addition to these three individuals with conflicting evidence of IGD, 16 individuals with skeletal evidence of rickets also displayed IGD in their teeth, 5 individuals (SC4-15, N7-2, N36-1, N14-5, SC4-55) with skeletal evidence of rickets had no visible IGD in their teeth, and 2 individuals (N5-1 and SCIV-16) without skeletal evidence of rickets had IGD present in their teeth. The possible reasons for these discrepancies will be discussed in Chapter 5.

Figure 4.2 *MAD01 (ri[1]) With No Evidence of IGD (Individual N12-1). Image by L. Godawa*

Figure 4.3 *MAD02 (rm[1]) Showing Episodes of IGD in Blue Rectangles (Individual N12-1). Images by L. Godawa*

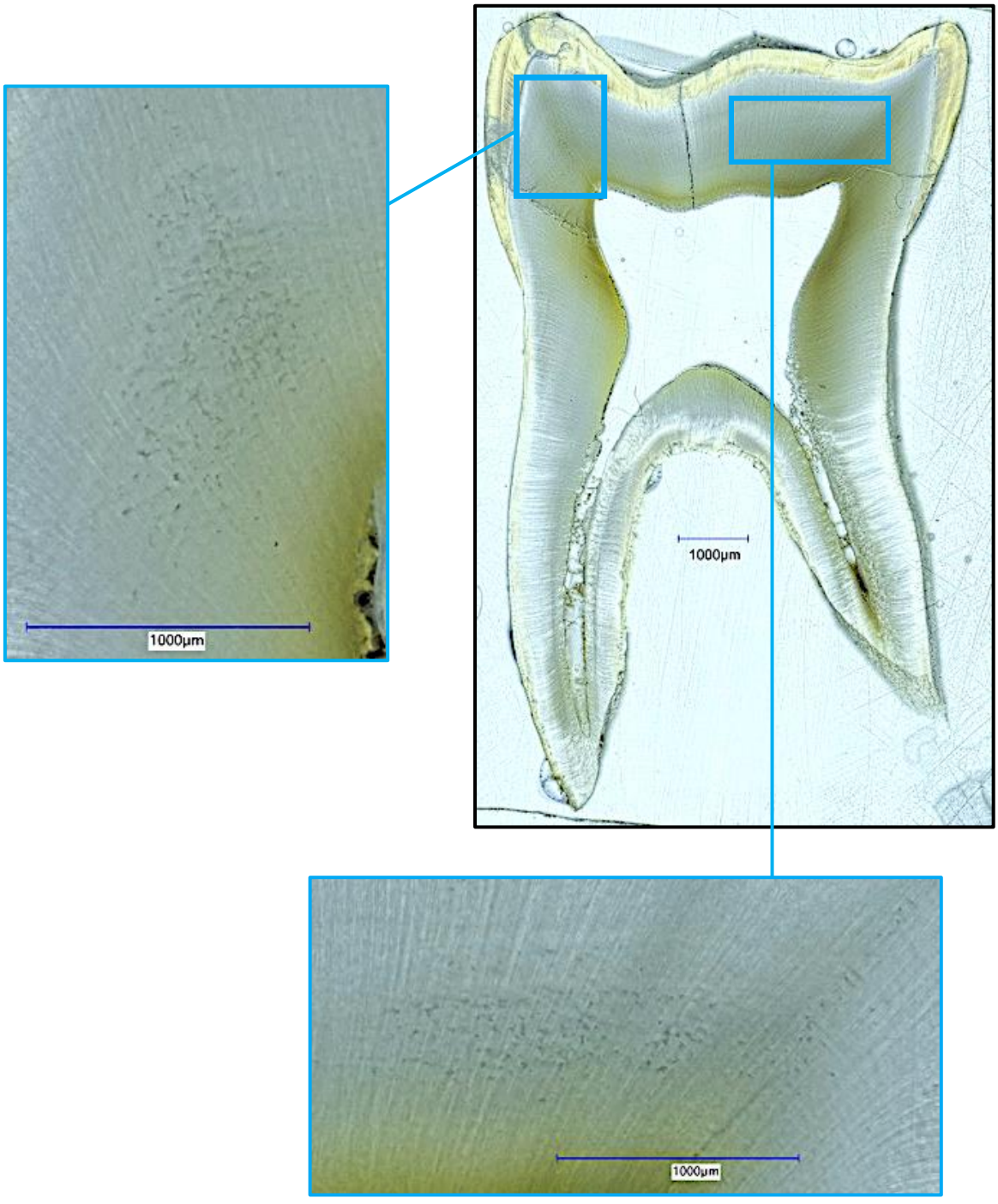


Figure 4.4 *MAD23 (ri[1]) With No Evidence of IGD (individual N18-1). Image by L. Godawa*

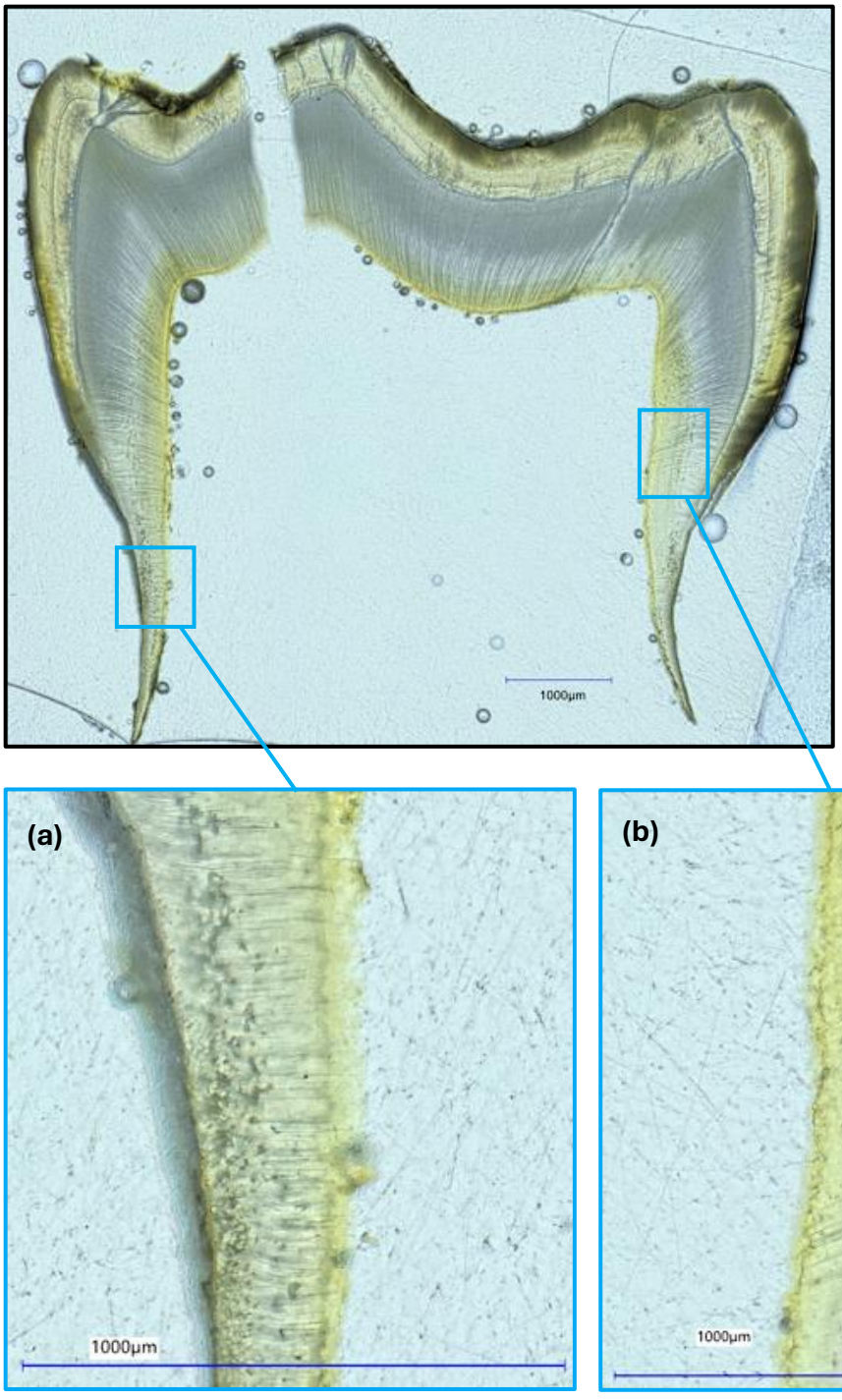


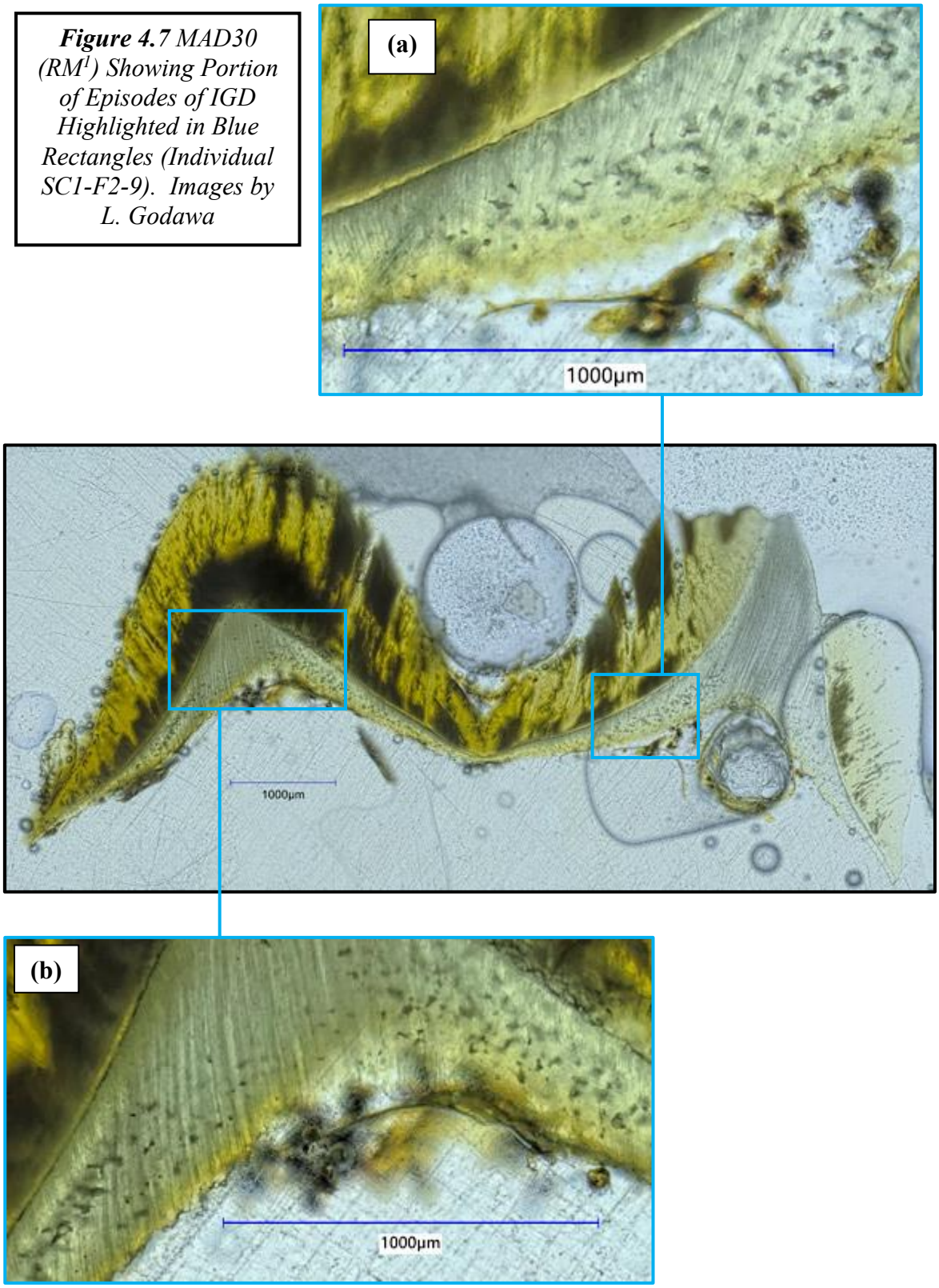
Figure 4.5
MAD24 (rm_1) Showing Continuous Episodes of IGD (individual N18-1).

Note: (a) and (b) only highlight a portion of the episode on each side of the tooth – highlighted by blue rectangles.

Images by L. Godawa

Figure 4.6 *MAD31 (li_1) With No Evidence of IGD (Individual SC1-F2-9). Image by L. Godawa*

Figure 4.7 *MAD30 (RM[1]) Showing Portion of Episodes of IGD Highlighted in Blue Rectangles (Individual SC1-F2-9). Images by L. Godawa*



4.4.2 Presence/Absence of IGD by Tooth

Out of the 44 teeth analyzed for this study, 33 displayed evidence of IGD (33/44 = 75%). All but one tooth (MAD04) (32/33= ~97%) had evidence of IGD within the crown, or in both the crown and roots. MAD04 was the only tooth that had IGD only in the roots, even though the crown was present. Of the 33 teeth with IGD, 9 had either no root growth or had just entered initial root formation as described by Moorrees et al. (1963a, b), so the prevalence of IGD in observable roots was 15/24 (62.5%). Only teeth with evidence of IGD in the crown or root are presented in Table 4.5, though all teeth were evaluated for the presence or absence of IGD (see Table 4.4).

Table 4.5 *Location of IGD Within the Dentine*

MAD ID	Tooth Type	IGD Location	Root Present?	Tooth Development Stage Description
MAD02	rm^1	Crown	Yes	Root length complete, apex open
MAD03	rm^1	Crown	Yes	Root length complete, apex closed
MAD04	rm_1	Root	Yes	Root length complete, apex closed
MAD06	rm^1	Crown and Root	Yes	Root length between 1/2 and 3/4 complete
MAD07	li^1	Crown and Root	Yes	Root length between 3/4 and complete
MAD08	ri^1	Crown and Root	Yes	Root length between 3/4 and complete
MAD09	lm^1	Crown and Root	Yes	Root length complete, apex closed
MAD10	lm_1	Crown	Yes	Root length between 3/4 and complete
MAD11	rm^1	Crown and Root	Yes	Root length between 3/4 and complete
MAD12	LM^1	Crown and Root	Yes	Root length between 1/4 and 1/2 complete
MAD13	lm_1	Crown and Root	Yes	Root length complete, apex closed
MAD14	lm^1	Crown and Root	Yes	Root length complete, apex closed

MAD ID	Tooth Type	IGD Location	Root Present?	Tooth Development Stage Description
MAD15	rm^1	Crown and Root	Yes	Root length between 3/4 and complete
MAD16	lm_1	Crown and Root	Yes	Root length between 1/2 and 3/4 complete
MAD17	rm^1	Crown and Root	Yes	Root length between 3/4 and complete
MAD18	RM^1	Crown	Yes	Root length between initial formation and 1/4 complete
MAD19	LM^1	Crown	No	Crown complete; initial root formation starting
MAD21	ri^1	Crown	Yes	Roots between 1/4 and 1/2 length
MAD22	LM_1	Crown	No	Crown between 1/2 and 3/4 complete
MAD24	rm_1	Crown and Root	Yes	Root length between initial formation and 1/4 complete
MAD30	RM^1	Crown	No	Crown between completion of cusp outline and crown 1/2 complete
MAD32	rm^1	Crown	Yes	Root length between 1/4 and 1/2 complete
MAD33	RM^1	Crown	No	Crown between completion of cusp outline and crown 1/2 complete
MAD34	RM_1	Crown	Yes	Root length between 1/4 and 1/2 complete
MAD35	rm^1	Crown	No	Crown between 1/2 and 3/4 complete
MAD39	ri^1	Crown and Root	Yes	Root length between 3/4 and complete
MAD40	lm_2	Crown	No	Crown complete; initial root formation starting
MAD41	lm^1	Crown	Yes	Root length between 1/4 and 1/2 complete
MAD42	RM^1	Crown	No	Crown between completion of cusp outline and crown 1/2 complete
MAD43	lm_1	Crown and Root	Yes	Root length between initial formation and 1/4 complete
MAD44	LM_1	Crown	No	Crown between completion of cusp outline and crown 1/2 complete
MAD45	RM_1	Crown	No	Crown between 1/2 and 3/4 complete
MAD46	rm_1	Crown and Root	Yes	Root length between 1/2 and 3/4 complete

4.5 Amount of Interglobular Dentine by Tooth

The total dentine area of each tooth was calculated by taking the sum of the measurements (in μm^2) of dentine from each cell. The total area of IGD in each tooth was also calculated through the sum of the measurements recorded from each cell with identified IGD (see Section 3.4.5). The amount of IGD relative to the surrounding unaffected dentine was then converted into a percentage (total area of IGD/total dentine area). The total areas of IGD ranged between 13,359 μm^2 (MAD42), which is a developing M^1 crown, so the overall area of dentine to observe was small (Figure 4.8) to 3,175,276 μm^2 (MAD15), an almost fully formed m^1, with a large area of observable dentine (Figure 4.9). The prevalence of IGD ranged between 0.05% (MAD04 – Figure 4.10) to 26.22% (MAD40 – Figure 4.11).

Figure 4.8 *MAD42 (RM^1 crown) with IGD Circled. Image by L. Godawa*

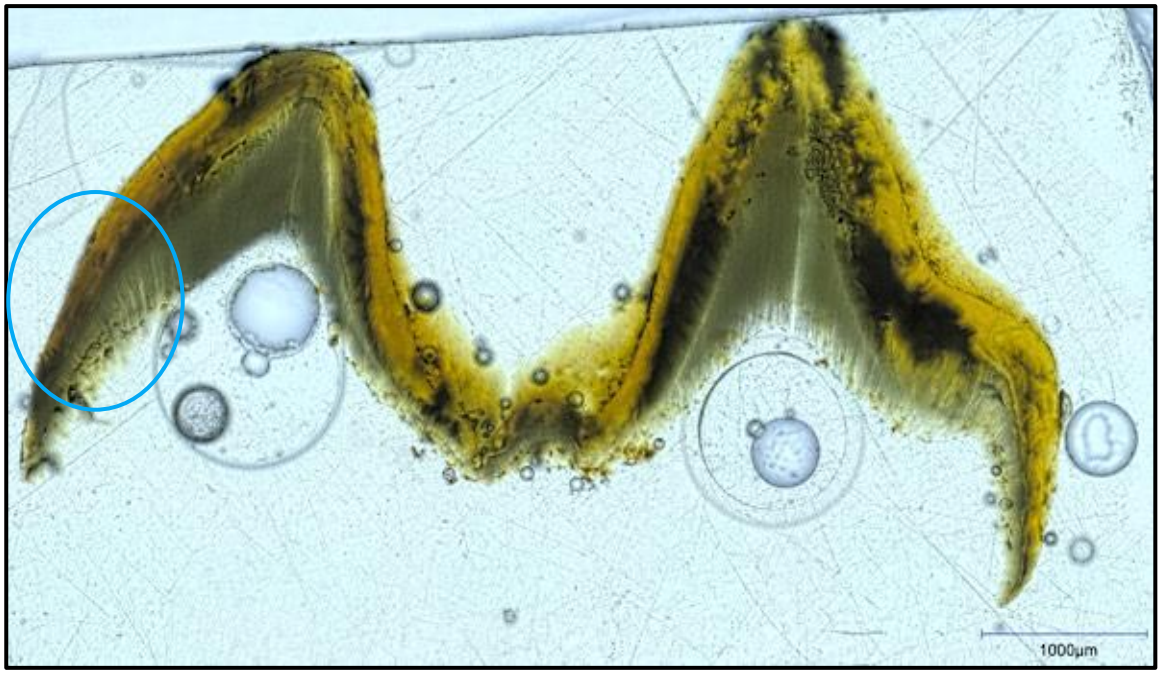


Figure 4.9 *MAD15 (rm[1]), IGD Present Throughout Most of Tooth Dentine. Image by L. Godawa*

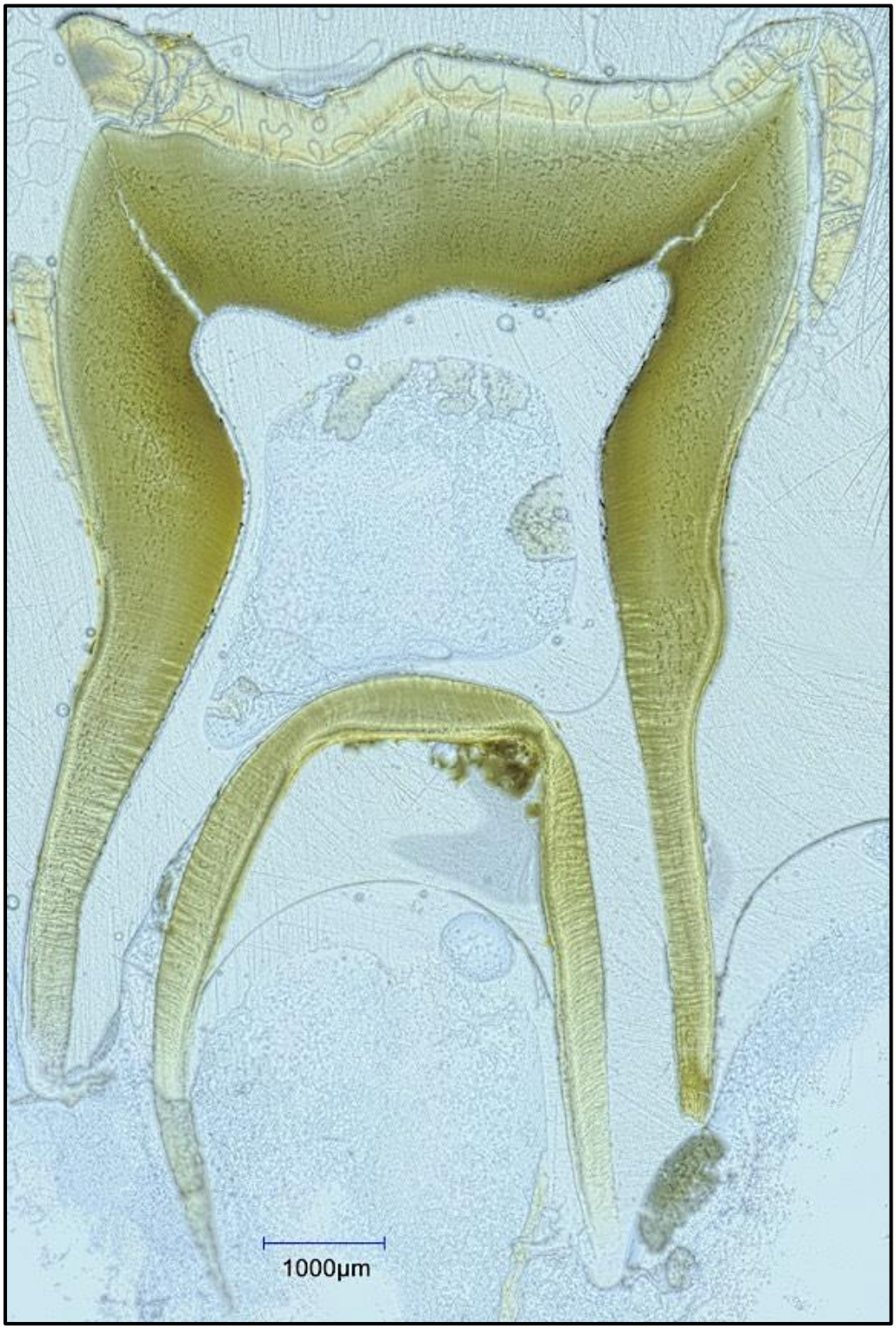


Figure 4.10 *MAD04 (rm_1) with IGD in Blue Oval. Image by L. Godawa*

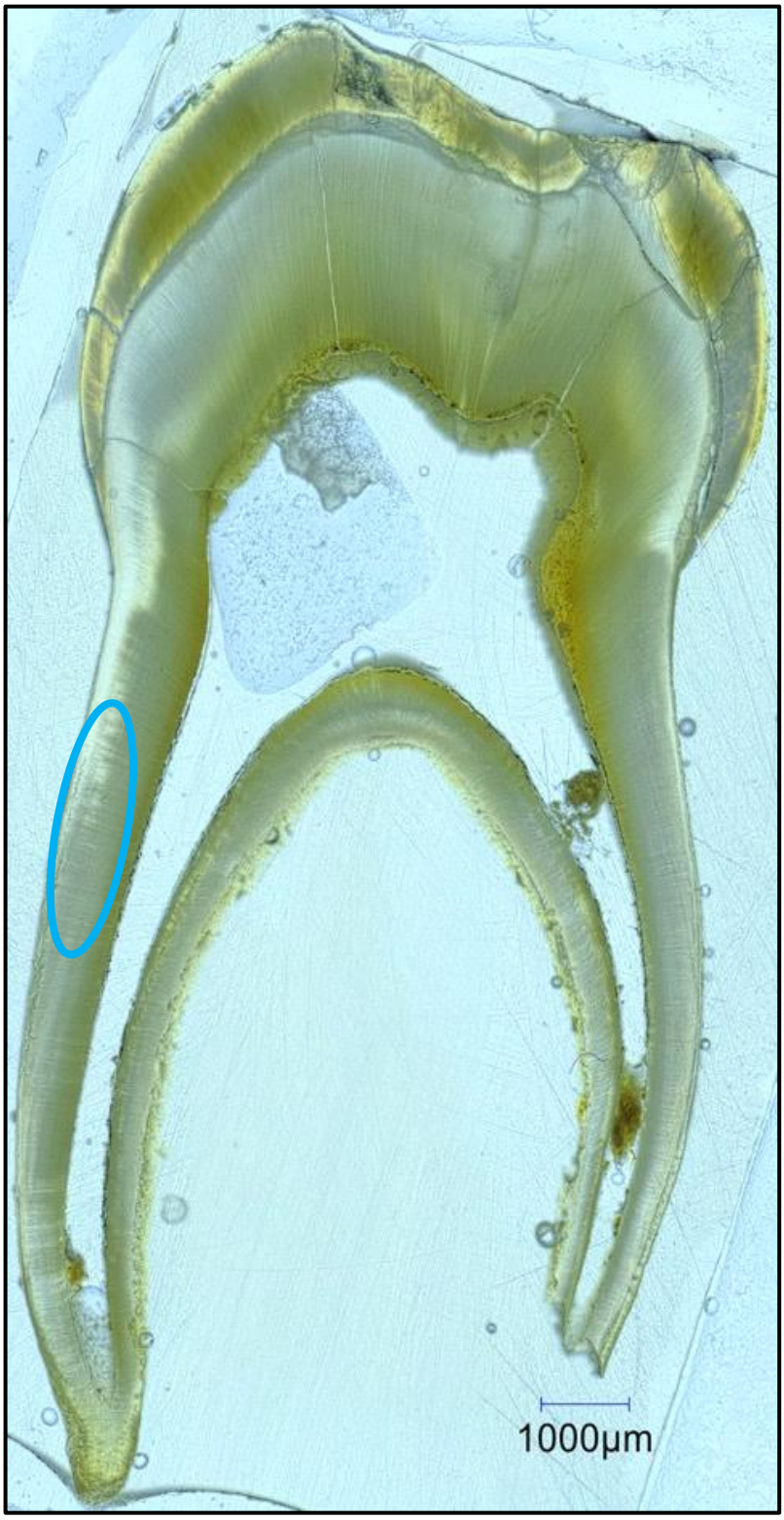


Figure 4.11 *MAD40 (lm_2 crown) Showing IGD Throughout Most of the Tooth Dentine. Image by L. Godawa*

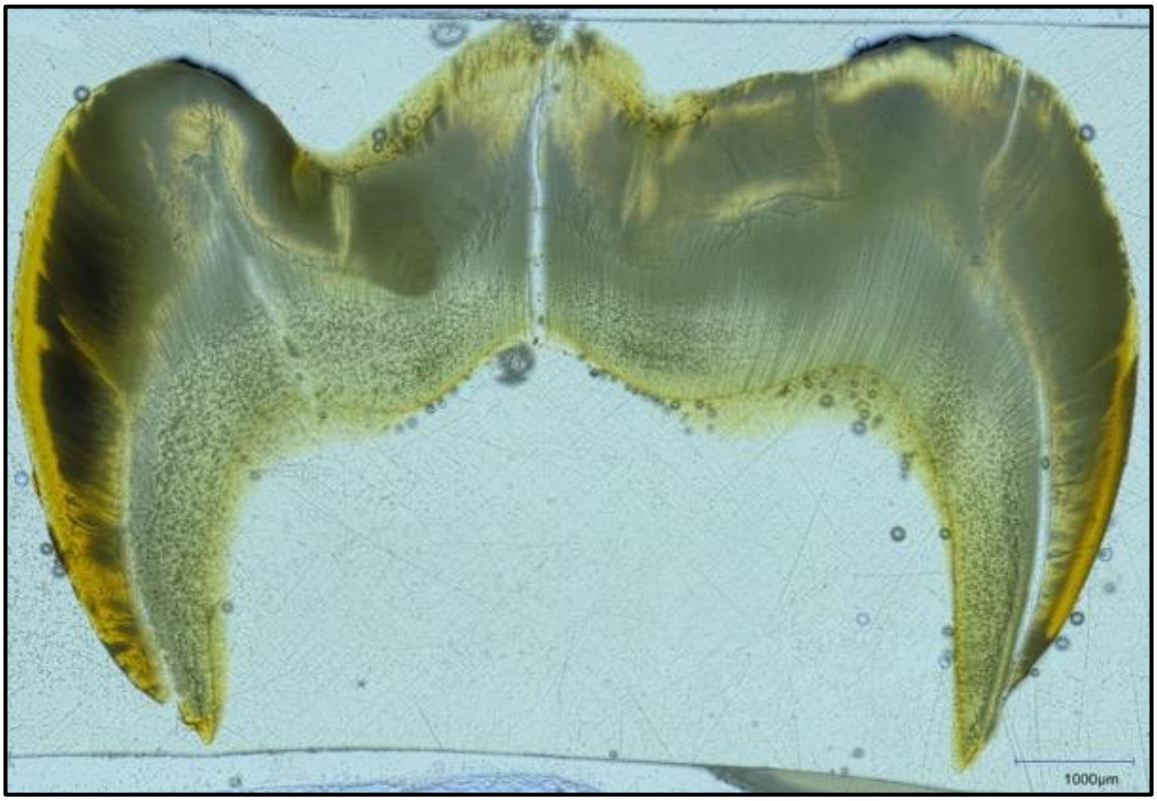


Table 4.6 presents the area of observable dentine, the area of IGD within the dentine, and the calculated percentages of IGD. The area of dentine and IGD were only calculated for teeth with evidence of IGD.

Table 4.6 *Amount of IGD in Observable Dentine*

MAD ID	Tooth Type	Area of Observable Dentine (µm²)	Area of IGD (µm²)	Percentage of IGD (%)	Notes
MAD01	ri[1]	-	-	-	No IGD
MAD02	rm[1]	28777679	228994	0.79	
MAD03	rm[1]	31486656	44304	0.14	
MAD04	rm$_1$	38710596	18889	0.05	
MAD05	ri[1]	-	-	-	No IGD
MAD06	rm[1]	16267603	174759	1.07	
MAD07	li[1]	14195990	916091	6.50	
MAD08	ri[1]	14334440	108712	0.76	
MAD09	lm[1]	17491881	500270	2.86	
MAD10	lm$_1$	21382987	230251	1.08	
MAD11	rm[1]	24666760	708903	2.87	
MAD12	LM[1]	30654448	2047480	6.68	
MAD13	lm$_1$	38167198	1922937	5.04	
MAD14	lm[1]	20970326	2970227	14.16	
MAD15	rm[1]	21667416	3175276	14.65	
MAD16	lm$_1$	16622878	385164	2.32	
MAD17	rm[1]	24168175	269738	1.12	
MAD18	RM[1]	36422218	175734	0.48	
MAD19	LM[1]	14628453	1139759	7.79	
MAD20	rm[1]	-	-	-	No IGD
MAD21	ri[1]	10777395	21641	0.20	
MAD22	LM$_1$	7836791	95957	1.22	
MAD23	ri[1]	-	-	-	No IGD
MAD24	rm$_1$	11743873	361082	3.07	
MAD25	li[1]	-	-	-	No IGD
MAD26	rm$_1$	-	-	-	No IGD
MAD27	li[1]	-	-	-	No IGD
MAD28	rm[1]	-	-	-	No IGD
MAD29	ri[1]	-	-	-	No IGD
MAD30	RM[1]	3768911	171135	4.54	
MAD31	li$_1$	-	-	-	No IGD
MAD32	rm[1]	14667987	354393	2.42	

MAD ID	Tooth Type	Area of Observable Dentine (µm²)	Area of IGD (µm²)	Percentage of IGD (%)	Notes
MAD33	RM^1	2551227	32265	1.26	
MAD34	RM_1	14667915	178432	1.22	
MAD35	rm^1	3901904	323007	8.28	
MAD36	rm_1	-	-	-	No IGD
MAD39	ri^1	14646787	301188	2.06	
MAD40	lm_2	11872742	3112667	26.22	
MAD41	lm^1	5538494	42113	0.76	
MAD42	RM^1	2585225	13359	0.52	
MAD43	lm_1	15686540	1445057	9.21	
MAD44	LM_1	5720975	770388	13.47	
MAD45	RM_1	6278446	707780	11.27	
MAD46	rm_1	11556263	523621	4.53	

To better understand and compare the percentages of IGD between the tooth samples, each tooth type (deciduous molar, deciduous incisor, and permanent molar) was compared to the others within that same category (Table 4.7 – 4.9). Essentially, Table 4.6 is broken into three sections to better evaluate the patterns within the same tooth type. An accurate comparison would be difficult between the different tooth types for this thesis because of the varying stages of development between the teeth. For example, the comparison between a deciduous molar to a permanent molar, though both have the same tooth class (molar), would not yield accurate conclusions since the developing dentine grows at a different rate between permanent and deciduous teeth, with deciduous dentine growing faster than permanent dentine (Dean and Scandrett 1995) (see Section 2.3.3).

Table 4.7 presents the data for deciduous molars only and shows that the deciduous molars have the greatest range in percentage of IGD (0.05% - 26.22%), with 4

of 23 having no IGD present. The deciduous incisors (Table 4.8) had more teeth without IGD (n=7) than with IGD (n=4). Their percentages of IGD were generally lower, with an average of 2.37% of IGD, and a range of 0.20% - 6.50%. Finally, all of the permanent molars had evidence of IGD, with an average of 4.85% of IGD, and a range of 0.48% - 13.47%.

Table 4.7 *Amount of IGD in Observable Dentine (Deciduous Molars Only)*

MAD ID	Tooth Type	Area of Observable Dentine (µm²)	Area of IGD (µm²)	Percentage of IGD (%)	Notes
MAD02	rm^1	28777679	228994	0.79	
MAD03	rm^1	31486656	44304	0.14	
MAD04	rm_1	38710596	18889	0.05	
MAD06	rm^1	16267603	174759	1.07	
MAD09	lm^1	17491881	500270	2.86	
MAD10	lm_1	21382987	230251	1.08	
MAD11	rm^1	24666760	708903	2.87	
MAD13	lm_1	38167198	1922937	5.04	
MAD14	lm^1	20970326	2970227	14.16	
MAD15	rm^1	21667416	3175276	14.65	
MAD16	lm_1	16622878	385164	2.32	
MAD17	rm^1	24168175	269738	1.12	
MAD20	rm^1	-	-	-	No IGD
MAD24	rm_1	11743873	361082	3.07	
MAD26	rm_1	-	-	-	No IGD
MAD28	rm^1	-	-	-	No IGD
MAD32	rm^1	14667987	354393	2.42	
MAD35	rm^1	3901904	323007	8.28	
MAD36	rm_1	-	-	-	No IGD
MAD40	lm_2	11872742	3112667	26.22	
MAD41	lm^1	5538494	42113	0.76	
MAD43	lm_1	15686540	1445057	9.21	
MAD46	rm_1	11556263	523621	4.53	
AVERAGE	**-**	**19755155.68**	**883771.2**	**5.30**	

Table 4.8 *Amount of IGD in Observable Dentine (Deciduous Incisors Only)*

MAD ID	Tooth Type	Area of Observable Dentine (µm²)	Area of IGD (µm²)	Percentage of IGD (%)	Notes
MAD01	ri^1	-	-	-	No IGD
MAD05	ri^1	-	-	-	No IGD
MAD07	li^1	14195990	916091	6.50	
MAD08	ri^1	14334440	108712	0.76	
MAD21	ri^1	10777395	21641	0.20	
MAD23	ri^1	-	-	-	No IGD
MAD25	li^1	-	-	-	No IGD
MAD27	li^1	-	-	-	No IGD
MAD29	ri^1	-	-	-	No IGD
MAD31	li_1	-	-	-	No IGD
MAD39	ri^1	14646787	301188	2.06	
AVERAGE	**-**	**13488653**	**336908**	**2.37**	

Table 4.9 *Amount of IGD in Observable Dentine (Permanent Molars Only)*

MAD ID	Tooth Type	Area of Observable Dentine (µm²)	Area of IGD (µm²)	Percentage of IGD (%)	Notes
MAD12	LM^1	30654448	2047480	6.68	
MAD18	RM^1	36422218	175734	0.48	
MAD19	LM^1	14628453	1139759	7.79	
MAD22	LM_1	7836791	95957	1.22	
MAD30	RM^1	3768911	171135	4.54	
MAD33	RM^1	2551227	32265	1.26	
MAD34	RM_1	14667915	178432	1.22	
MAD42	RM^1	2585225	13359	0.52	
MAD44	LM_1	5720975	770388	13.47	
MAD45	RM_1	6278446	707780	11.27	
AVERAGE	**-**	**12511460.9**	**533228.9**	**4.85**	

4.6 Timing of Interglobular Dentine Episodes

For the purpose of this thesis, an *episode* of IGD was considered to be any size/length of a mineralization defect that continued along the dentine growth lines within the tooth layers. Though not all the episodes within a tooth were connected to each other (such as those in MAD02, Figure 4.3), the location of the episodes of IGD within the same area on each side of the tooth suggests that they are likely from the same time of tooth formation. In these cases, the tooth was considered to only have one episode, as they would have happened at the same time. All teeth identified as having IGD, had 1 continuous mineralization defect episode. The length/timing of each episode was determined by using the age-at-death estimates initially estimated by Dr. Megan Brickley, the chart from Brickley et al. (2020a, Figure 3a, 3b) showing approximate ages of mineralization of teeth, as well as dental development charts from Gustafson and Koch (1974) (see Section 3.2.6). It is important to note that duration of episode does not directly correlate with amount of IGD observed.

Table 4.10 *Number and Timing of IGD Episodes*

MAD ID	Deciduous/ Permanent	Abbr.	Age at Death Estimate	Number of IGD Episodes	Length/Timing of Episode(s) in Age
MAD01	Deciduous	ri[1]	2.5 years	0	No IGD
MAD02	Deciduous	rm[1]	2.5 years	1	Birth – 6 months
MAD03	Deciduous	rm[1]	6 years	1	Birth – 6 months
MAD04	Deciduous	rm$_1$	3 years	1	10.5 – 18 months
MAD05	Deciduous	ri[1]	1 year	0	No IGD
MAD06	Deciduous	rm[1]	2 years	1	6 – 18 months
MAD07	Deciduous	li[1]	1 year	1	4.5 – 12 months
MAD08	Deciduous	ri[1]	2 years	1	1.5 – 10 months
MAD09	Deciduous	lm[1]	2 years	1	6 – 10.5 months
MAD10	Deciduous	lm$_1$	1 year	1	5.5 – 10.5 months
MAD11	Deciduous	rm[1]	6 years	1	Birth – 10.5 months
MAD12	Permanent	LM[1]	6 years	1	Birth – 2 years
MAD13	Deciduous	lm$_1$	2.7 years	1	5.5 – 10.5 months
MAD14	Deciduous	lm[1]	3 years	1	Birth – 18 months
MAD15	Deciduous	rm[1]	2 years	1	Pre-Birth – 10.5 months
MAD16	Deciduous	lm$_1$	2 years	1	5.5 – 10.5 months
MAD17	Deciduous	rm[1]	1.2 years	1	Birth – 10.5 months
MAD18	Permanent	RM[1]	6 years	1	1 – 2.5 years
MAD19	Permanent	LM[1]	1.2 years	1	Birth – 1 year
MAD20	Deciduous	rm[1]	6 months	0	No IGD
MAD21	Deciduous	ri[1]	6 months	1	Pre-birth – 2.5 months post-birth
MAD22	Permanent	LM$_1$	6 months	1	Pre-birth – 6 months
MAD23	Deciduous	ri[1]	9 months	0	No IGD
MAD24	Deciduous	rm$_1$	9 months	1	Just after birth – 5.5 months
MAD25	Deciduous	li[1]	7th month in-utero	0	No IGD
MAD26	Deciduous	rm$_1$	7th month in-utero	0	No IGD
MAD27	Deciduous	li[1]	Just before birth	0	No IGD

MAD ID	Deciduous/ Permanent	Abbr.	Age at Death Estimate	Number of IGD Episodes	Length/Timing of Episode(s) in Age
MAD28	Deciduous	rm^1	Just before birth	0	No IGD
MAD29	Deciduous	ri^1	6 months	0	No IGD
MAD30	Permanent	RM^1	9 - 11 months	1	Just after birth – 11 months
MAD31	Deciduous	li_1	9 – 11 months	0	No IGD
MAD32	Deciduous	rm^1	11 months	1	Birth – 6 months
MAD33	Permanent	RM^1	11 months	1	Just after birth – 11 months
MAD34	Permanent	RM_1	1 year	1	Birth – 1 year
MAD35	Deciduous	rm^1	1 year	1	Pre-birth – 6 months
MAD36	Deciduous	rm_1	9 months	0	No IGD
MAD39	Deciduous	ri^1	~1 year	1	1.5 – 4.5 months
MAD40	Deciduous	lm_2	~1 year	1	Birth – 10 months
MAD41	Deciduous	lm^1	8 months	1	Birth – 6 months
MAD42	Permanent	RM^1	8 months	1	Birth – 8 months
MAD43	Deciduous	lm_1	9 months	1	Birth – 9 months
MAD44	Permanent	LM_1	9 months	1	Birth – 9 months
MAD45	Permanent	RM_1	1 year	1	Birth – 1 year
MAD46	Deciduous	rm_1	1 year	1	5.5 – 10.5 months

The variation in amount (percentage) of IGD is due to the length of the time the individual had the mineralization deficiency as well as the tooth's development stage (i.e. amount of dentine that can be affected). It is important to note both of these factors when assessing the teeth as noting only the episode numbers or length does not provide a complete assessment of the individual's mineralization irregularities.

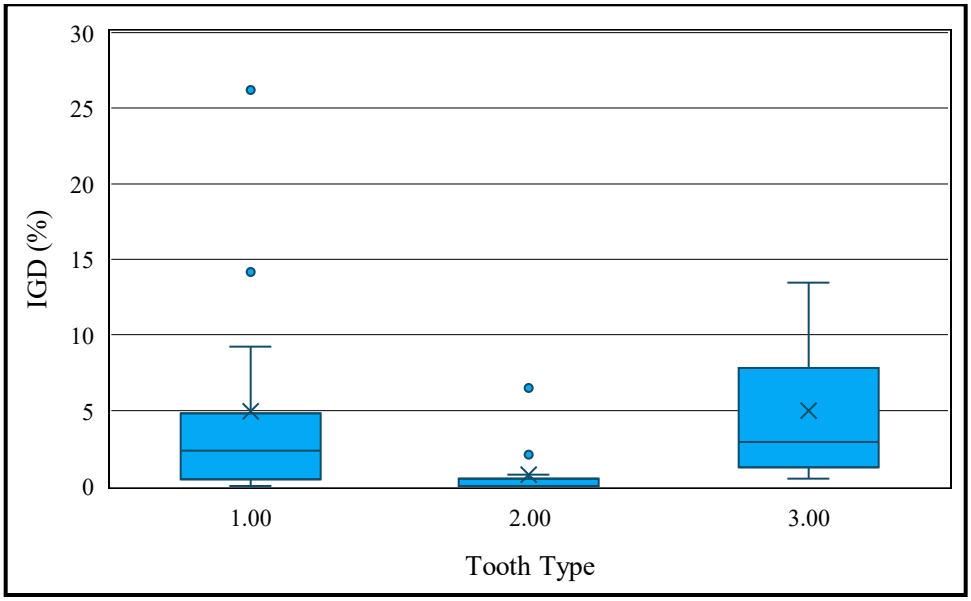
4.7 Statistical Analysis

Using Statistical analysis was used to answer three specific questions regarding the data from this thesis: (1) is there a significant correlation between the percentage of IGD and tooth type?; (2) is there a correlation between the age-at-death estimate and the percentage of IGD?; and (3) does the duration of the episode correlate with the amount of IGD? All statistical analyses were conducted using *IBM SPSS Statistics (Version 29)* (IBM Corp. 2022).

Prior to the analysis of these questions, two tests of normality (Kolmogorov-Smirnov and Shapiro-Wilk) were used to determine the type of statistical tests necessary for this dataset. Separating the data by tooth type and using IGD percentages as the dependent variable, the results showed that the data from this sample are not normal ($p = <0.001$) and therefore non-parametric tests must be used.

With regards to question (1), this information is important to determine if one of the tooth types examined tends to have more IGD that other teeth. Using the Kruskal-Wallis test, with a null hypothesis that the distribution of IGD is the same across the tooth type categories, the calculated result was $p = 0.006$ (i.e., there is a statistically significant difference between percentage of IGD and tooth type) and therefore the null hypothesis should be rejected. Thus, there is a significant correlation between the percentage of IGD and the tooth type (deciduous molars and/or permanent molars). According to this test molars (both deciduous and permanent) tend to have more IGD within this sample (Figure 4.12).

Figure 4.12 *Independent-Samples Kruskal-Wallis Test*



Note: 1.00 refers to deciduous molars, 2.00 refers to deciduous incisors and 3.00 refers to permanent molars.

The statistical analysis to determine a correlation between the percentage of IGD and the tooth type proved consistent with previous work on this subject. Isokawa and colleagues (1963) noted a higher prevalence of IGD (called *interglobular spaces* in their paper) in deciduous molars when compared to deciduous incisors and canines. Using histological methods, Isokawa et al. (1963) categorized 219 teeth into 3 groups correlating to the amount of IGD present. Teeth in Group I had no evidence of IGD, teeth in Group II had few interglobular spaces, while teeth from Group III had many. Of the 88 incisors examined, 83 were classified in Group I (no IGD), 4 in Group II (few IGD) and 1 in Group III (many IGD). The deciduous molars had almost the opposite result of the incisors with 8/89 in Group I, 11/89 in Group II and 70/89 in Group III. This research shows that of the different types of deciduous teeth to analyze for IGD, the molars consistently had more IGD than the incisors. This was expected as deciduous incisors and

molars begin mineralization around the same time (4-5 months in utero), but the incisor crowns (2-4 months of age) and roots (1.5-2 years of age) complete formation earlier, while molar crowns (5-7 months of age) and roots (1.75-3.25 years of age) take slightly longer to form, thus, having more time to develop IGD if rickets remains active during this time (Gustafson and Koch 1974).

It would be helpful to compare more information, but most studies use very few tooth samples when analyzing IGD (with the exception of Isokawa et al. 1963). There is also a lack of diversity of the type of tooth within these samples, necessitating more data on the comparison of patterns of occurrence, duration, amount and tooth type (i.e., between deciduous and permanent teeth).

To test question (2), the data was separated by tooth type (deciduous molars, deciduous incisors and permanent molars) and a Spearman correlation (Spearman's rho) was used. To determine if there is a significant correlation between the age-at-death estimate and the percentage of IGD, the p-value must be <0.05. For deciduous molars $p=0.153$ (Figure 4.13), for deciduous incisors $p=0.244$ (Figure 4.14) and for permanent molars $p=0.860$ (Figure 4.15), therefore there is no correlation between the age-at-death and the percentage of IGD. Thus, showing that individuals within this sample that lived longer did not have more evidence (amount) of IGD.

Figure 4.13 *Question (2) Results of Spearman's Rho Test on Deciduous Molars*

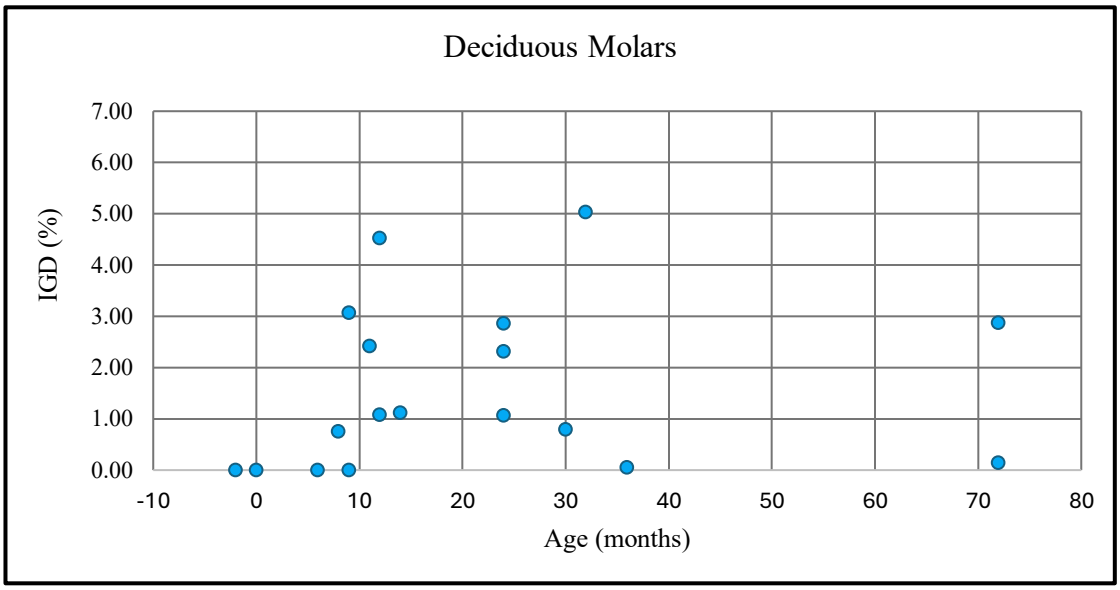


Figure 4.14 *Question (2) Results of Spearman's Rho Test on Deciduous Incisors*

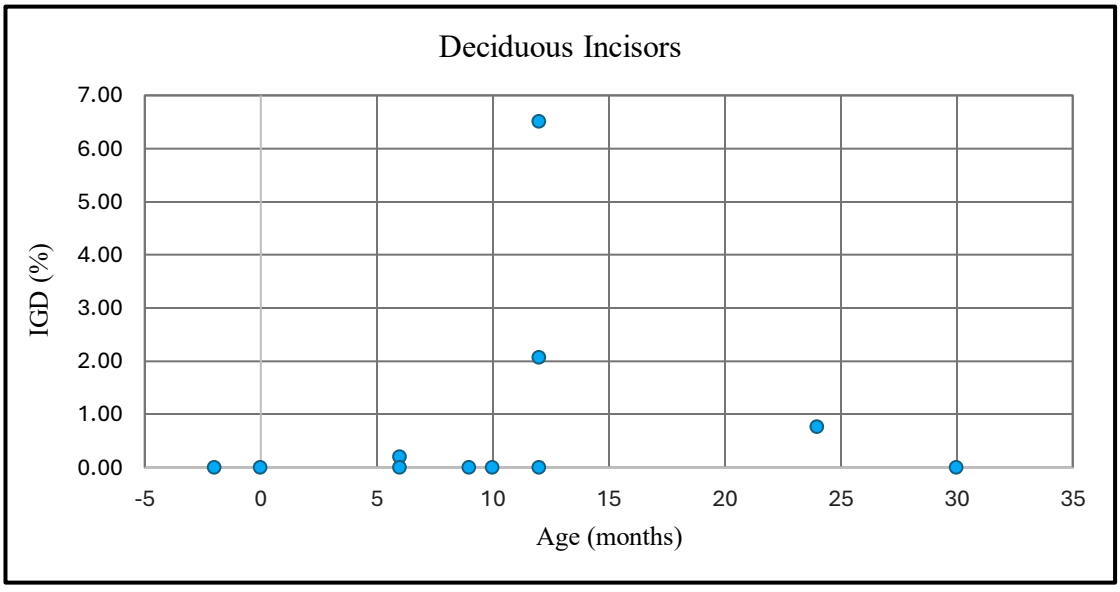
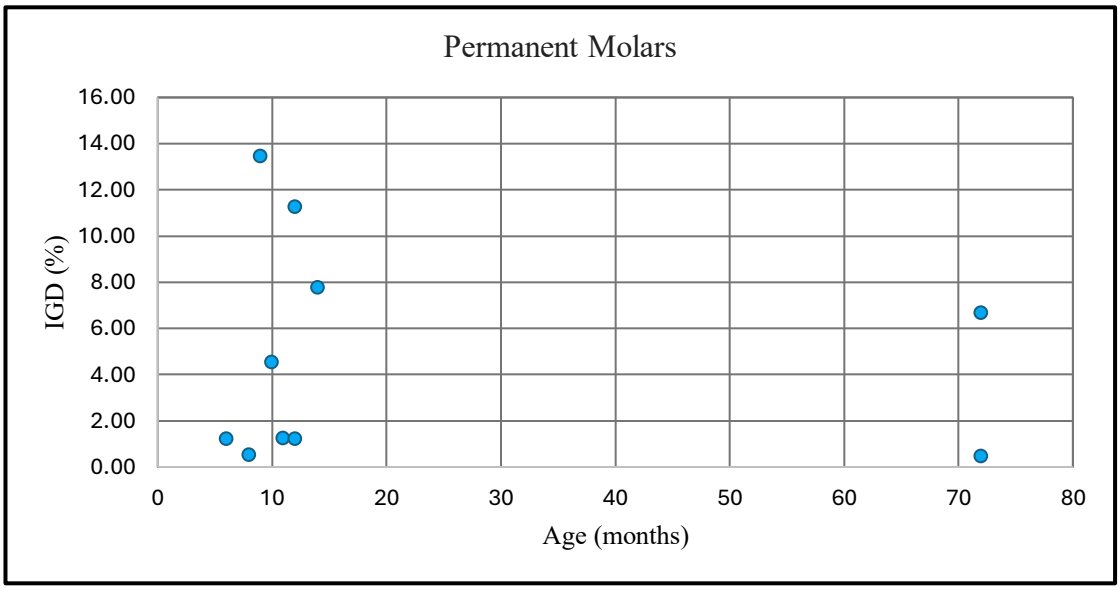


Figure 4.15 *Question (2) Results of Spearman's Rho Test on Permanent Molars*



For the purpose of question (3), the duration of IGD episodes needed to be converted to a percentage of life with IGD (rather than total months with IGD) for comparability with the percentage (amount) of IGD. The percentage of life with IGD was calculated by dividing the duration of IGD (in months) by the age-at-death estimate (in months) and multiplying by 100. Similarly to question (2) the teeth were separated by tooth type and Spearman's rho was used to answer question (3). The deciduous molars ($p=0.002$) and deciduous incisors ($p=<0.001$) were both statistically significant, while the permanent molars ($p=0.695$) were not. This means that there is a significant correlation between the percentage (amount) of IGD and the duration of the episode relative to the age-at-death estimate.

Figure 4.16 *Question (3) Results of Spearman's Rho Test on Deciduous Molars*

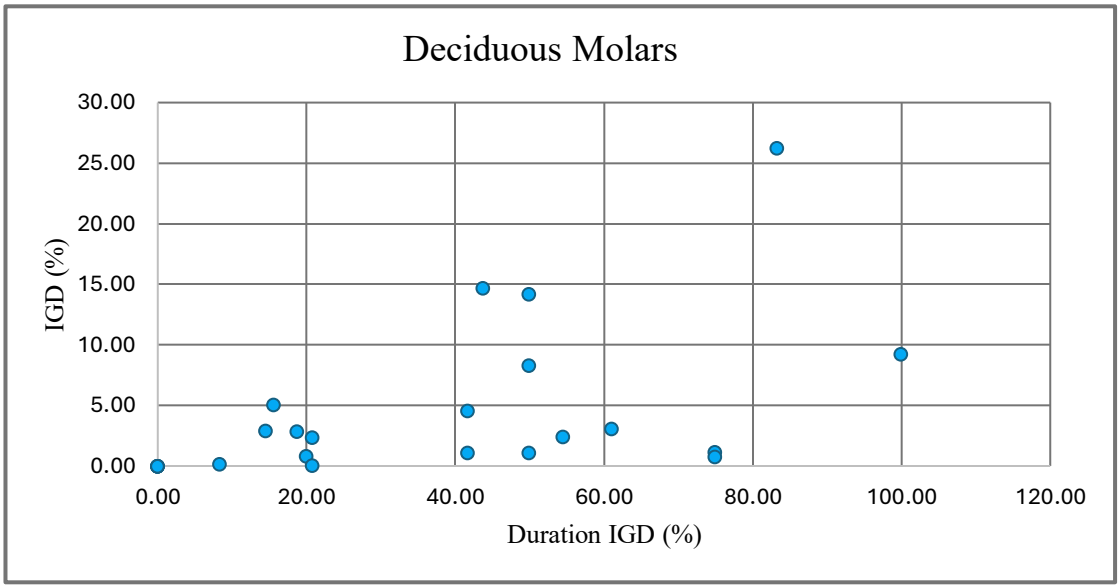


Figure 4.17 *Question (3) Results of Spearman's Rho Test on Deciduous Incisors*

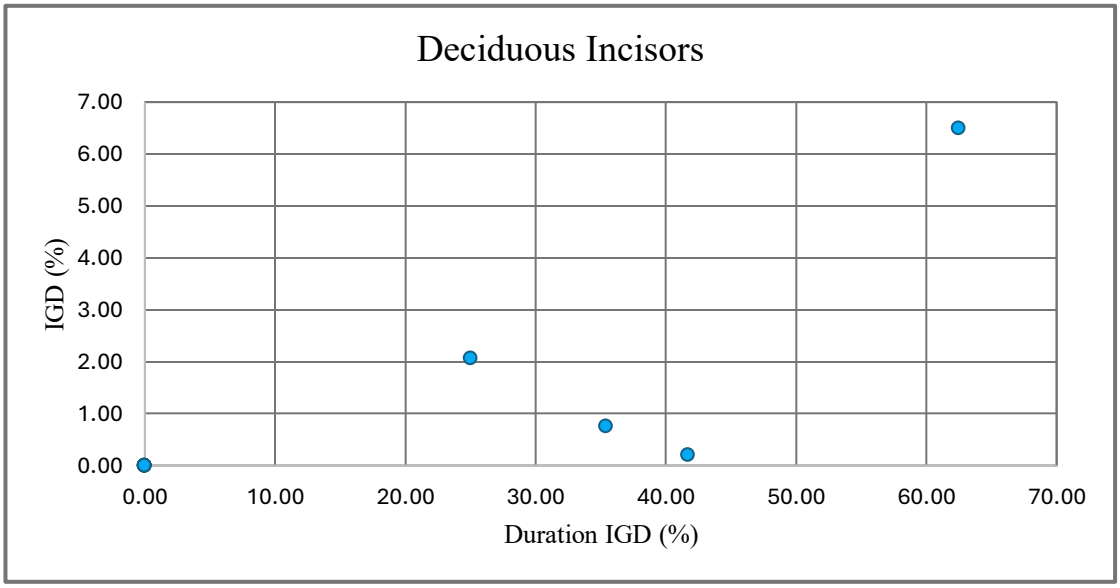
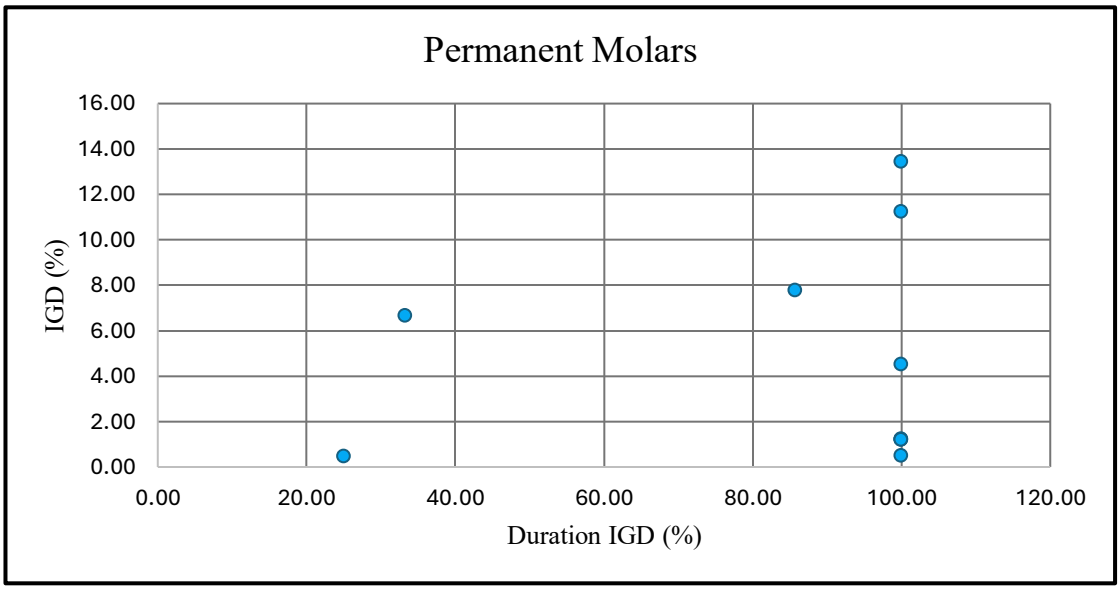


Figure 4.18 *Question (3) Results of Spearman's Rho Test on Permanent Molars*



4.8 Summary of Results

The data presented in this chapter described the different types of analyses conducted on the teeth from the Trinitarias Descalzas sample. By organizing the data by individual and then by tooth type, it is easier to understand the information provided at the level of an individual experiencing rickets, to the results of the mineralization defect (IGD) shown on an individual tooth. Though the teeth with IGD can provide a lot of information about the health of the individuals studied, the lack of IGD within this sample is just as important of a discussion topic. The information provided above begins to highlight patterns and inferences that the next chapter will discuss in context of maternal and childhood health, and infant rearing practices in 19th century Spain.

CHAPTER 5: DISCUSSION

5.1 Introduction

This chapter addresses the results observed from the analysis of teeth from the 19th century Trinitarias Descalzas cemetery. The results and their contribution to current academic research are discussed, along with the unique methodological developments utilized in this study and their potential for informing current and future methodological research on Vitamin D deficiency (VDD). This information can assist and advance our understanding of IGD and metabolic diseases in archaeological samples and help to better understand the influence of maternal vitamin D status on VDD in infants and children through the lens of the mother-infant nexus.

5.2 Existing Research and Observations

The analysis of the IGD in these 44 teeth supports existing research that IGD is an indicator of vitamin D deficiency and/or rickets (D'Ortenzio et al. 2016, Veselka et al. 2019). Of the 26 individuals included in this thesis, 23 were previously identified as having skeletal evidence of rickets by Dr. Brickley; however, the skeletons were unavailable for examination during this thesis. Further, there is no published osteobiography of all the individuals, and no internal report on the rickets diagnosis by the original researchers or other scholars since the excavation of the Trinitarias Descalzas site. As such, the methodology and morphological criteria used to diagnose rickets is likely based on morphological criteria published by Brickley et al. (2019). The accuracy of the diagnoses used for this thesis is not under question, but the interpretations discussed in this chapter are somewhat limited by knowing only presence/absence of

rickets and not, for example, the distribution of pathological lesions and the degree of expression on the bones of those individuals with rickets. Twenty-one of the 26 individuals had evidence of IGD in at least one of their teeth examined (33/44 teeth), regardless of their rickets diagnosis. The results of this study demonstrate that there is a relationship between the presence of IGD and rickets; however, in three cases (N12-1, N5-1, SCIV-16) individuals without skeletal evidence of rickets had IGD present. The presence of IGD in the teeth of individuals with no morphological evidence of rickets suggests that the relationship between VDD, the presence of IGD, and the skeletal expression of rickets is more complicated than a direct cause-effect relationship.

The findings of this thesis are consistent with the work of Boukpessi et al. (2006), Ribeiro et al. (2015) and D'Ortenzio et al. (2016) when discussing the relationship between the presence of IGD and skeletal evidence of rickets. Boukpessi et al. (2006) found evidence of IGD in all 15 deciduous teeth examined from modern children with hereditary (hypophosphatemic) rickets. Ribeiro et al. (2015) examined the mineralization patterns in 19 teeth (16 deciduous, 3 permanent) of 5 individuals from the same family, four of whom were affected by X-linked hypophosphatemic rickets, and one was not. This study concluded that the teeth from individuals with rickets had more evidence of mineralization deformities (i.e., IGD) than those from the individual without rickets. The study by D'Ortenzio et al. (2016) compared 12 permanent teeth from 6 archaeological individuals (with skeletal evidence of rickets) to 3 teeth (2 permanent, 1 deciduous) from 3 modern healthy individuals. All of the individuals from the archaeological sites that had

skeletal evidence of rickets also had evidence of IGD in their teeth, while none of the modern teeth had IGD.

However, little research into the timing of IGD – both the onset and episode duration – has been done. Within that limited research, even less work discusses and/or analyses the patterns in the timing of IGD. Generally, the existing research focuses on the presence or absence of IGD, number of episodes and their "severity". As there is not yet a threshold or scale for how much IGD within a tooth is considered a high or low amount, using the term "severity" when discussing amount of IGD in the episodes is subjective and should be avoided in future research. This chapter explores the timing of the episodes in relation to the mother-infant nexus and discusses conditions in 19th century Spain that may have contributed to rickets in these children.

Previous examination of 14 deciduous teeth from the Trinitarias sample by Smith (2018), using stable isotope analysis of carbon and nitrogen in dentine, determined that the age at onset of weaning was between 10-14 months, while weaning ended around 3 years of age (although the timing was variable). All of the 14 teeth (MAD01–MAD10, MAD14-MAD17) from the individuals studied by Smith (2018) were included in this thesis to supplement the data on these teeth. Only 2 (N5-1, N4-4) of the 26 individuals analyzed for this current thesis lived past 3 years of age (see Table 4.2). Of the 33 teeth analyzed in this thesis with evidence of IGD, 15 teeth had an IGD episode ending **before** the onset of the weaning process (i.e., <10 months), suggesting that VDD was occurring while the infant was still breastfeeding. In three cases (MAD21, MAD22, MAD35) the

IGD started prenatally and continued after birth (see Table 4.7). By using a combination of tooth development charts and Brickley et al.'s (2020a) Figure 3 of the approximate ages of mineralization for deciduous and permanent teeth, it is possible to identify the pre-natal evidence of IGD in MAD21, MAD22 and MAD35. For example, MAD21 is a deciduous maxillary incisor, therefore it starts forming dentine in-utero, and based on the location of the IGD within the tooth (i.e., close to the start of the mineralization process – the dentine-enamel junction), this indicates that the IGD episode began prior to birth and ended slightly afterward (2.5 months) meaning the mother was deficient during pregnancy and after birth. These results showing VDD during pregnancy and prior to weaning are not unexpected. As mentioned in Section 2.3.3, breastmilk alone is not an adequate source of vitamin D and pregnant mothers with inadequate dietary access to vitamin D or environmental exposure to sunlight (UVB radiation), will have a foetus with VDD if their maternal stores are not sufficient to provide the foetus with vitamin D. In their study of the skeletal evidence of stress indicators – factors negatively impacting the health and growth of an individual – from 136 foetal, perinatal and infant skeletons from post-medieval London, Gowland and Halcrow (2020) found "… many of the individuals, and subsequently their mothers, were chronically vitamin D deficient", (p.55). Where the living conditions of the mother impacted her access to adequate nutrition, the foetus and infant are negatively impacted. As mentioned in Chapter 2, the economy of 19th century Spain was frequently affected by political instability and recurrent wars. The consequences faced by civilians as a result of the economic and political unrest led to reduced access to food through decreased agricultural production and the raiding of food

reserves by soldiers (Prados de la Escosura and Santiago-Caballero 2022). Disease and demographic crises were also impacting health at this time, specifically, cholera epidemics and a high infant mortality rate compared to other countries in Europe during this time (Shubert 2005).

The societal expectations of female responsibilities, coupled with limited opportunities for financial independence led to restrictive conditions for women during 19th century Spain. As with other patriarchal societies, a woman was expected to marry, have children and perform their domestic duties. There is historical evidence to suggest that gender-based discrimination resulted in an unequal distribution of food and/or workloads for young girls and women, leading to nutritional deficiencies (Marco-Gracia and Beltran Tapia 2021). In a study on maternal mortality in 19th century Italy by Manfredini (2020), inadequate nutrition combined with the high energy demands of pregnancy and childbirth, contributed to the deterioration of the health of the mother.

Biehler-Gomez and colleagues (2024) examined the relationship between vitamin D deficiency, pregnancy and childbirth in early medieval Italy using skeletal evidence. Two tombs were analyzed from close, but separate necropoleis in Milan. In the first tomb, an incomplete female skeleton was uncovered with foetal remains (aged roughly 8 months in-utero) found *in situ* within the pelvic girdle. The second tomb had multiple comingled remains with a minimum number of seven individuals within, with the exception of a female and male in anatomical supine position. Another foetus (aged roughly 8 months in-utero) was also found within this tomb, though its position *in situ*

was lost during excavation. Following the examination of the female skeletons, Biehler-Gomez and colleagues (2024) concluded that both individuals suffered from extreme adult vitamin D deficiency (osteomalacia) that had resulted in biomechanical abnormalities, particularly in the pelvic girdle, leading to the impossibility of vaginal birth. They noted that it is likely both the woman and foetus died during childbirth due to these complications. Biehler-Gomez et al. (2024) considered the mother-infant nexus within their research and noted that the experiences from the mother throughout their life through ongoing or recurrent vitamin D deficiency, and physically demanding pregnancies negatively impacted both the mother and the foetus.

That the episodes in 15 teeth from the Trinitarias sample started and ended before the onset of weaning indicates that the mother (or wet-nurse) breastfeeding the infant, was also deficient in vitamin D as the infants only source of nutritional vitamin D at the time of the IGD episodes would have been breastmilk. When considering the episode timing of VDD in these 15 teeth, it is possible that some other indirect factor or factors were at play that caused an improvement in the infants' uptake of vitamin D prior to the onset of weaning. Here, cultural practices associated with the mother-infant nexus and environmental conditions must be considered along with potential biological factors. As noted previously (Section 2.3.1) our nutritional intake of vitamin D accounts for only 10-20% of our daily vitamin D intake, and it is necessary to get the rest via sunlight (Sahota 2014). Specific practices regarding exposing or restricting infants to sunlight in 19th century Spain are lacking from the literature, however we can hypothesize possible environmental and cultural factors that may have contributed to VDD. Jones (2022) noted

that the rise of the industrial revolution in Western Europe during the 19th century created smog and air pollution, limiting the amount of UV light available. The cultural practices of infant care in Spain are not well documented, however, if they were exposed to sunlight, it is possible that the air pollution limited their dermal absorption of vitamin D. Such was the case in post-medieval England (Gowland and Halcrow 2020). The development of industrialization in Spain occurred later than other European countries, such as England or France, due to the unstable economic and political climate, so industrialization alone may not be the main factor impacting VDD. Seasonality, time of day and latitude can also pose challenges for vitamin D absorption. According to Holick (2006, 2007) areas with a latitude ≥ 35° North or South are more likely to face challenges when absorbing vitamin D, especially during the winter months. Webb et al. (2011) noted that the amount of time needed for sufficient UV exposure increased during the winter and Serrano et al. (2017) argued that despite Spain having adequate UV exposure during the summer, there is insufficient sun exposure during the winter. With the latitude of Madrid (~40° N) above the threshold noted by Holick (2006, 2007), it is possible that the changes seen in the timing of IGD episodes on the teeth from this thesis could have been a result of insufficient UV exposure, particularly during the winter months. The samples of teeth in this thesis that had episodes of VDD ending prior to the start of the weaning period could have been born (and been breastfeeding) during the latter portion of the winter season and their uptake in vitamin D was a result of increased UV exposure in both the mother and infant.

Mothers may have also inadvertently contributed to their infants' VDD by the practice of swaddling – wrapping infants in bands or blankets tightly to restrict movement (Ellis 2010, van Sleuwen et al. 2007). Swaddling was used by mothers and midwives who believed the bindings would help with bone alignment (Lipton et al. 1965). Old Galenic teachings, often used prior to industrial Europe, on the treatment of newborns insisted the infants be salted (covered in soda ash), swaddled tightly and restricted in physical activity (Lipton et al. 1965). The age at which swaddling ceased varied across cultures and geographic location and it is unclear when this could have happened in 19th century Spain. The practice of swaddling gradually disappeared in Europe during the beginning of industrialization, as physicians deemed the practice to be detrimental to the health and development of the infant. It is possible that the dissolution of the practice of swaddling also lagged in Spain, thus leading to the development of VDD in the younger individuals from this sample.

The timing of IGD is important to the study of the relationship between a mother and their child – particularly during breastfeeding and weaning periods as it can shed light on the vitamin D health of the mother or surrogate in the cases of wet-nurses. Current research and medical practices advise vitamin D drops for infants during breastfeeding as it has been noted that even in mothers with sufficient vitamin D levels, their breastmilk only contains ≤ 25-50 IU/L, roughly 6.25-12.5% of the necessary intake of 400IU for infants (Kovacs 2008, 2015, Heo et al. 2022).

Fourteen teeth with episodes of IGD ending during the onset of weaning were also identified (10-14 months) and the remaining 4 teeth had IGD episodes during the process of weaning (14+ months). Since the episodes ended during this phase of the infant's life, it can be proposed that the introduction of new foods, supplementing breastmilk, may have contributed to the decline in the amount of IGD. Most of the information on the weaning diet, particularly in Spain, is unclear or lacking and primarily discusses the timing which, although important, does not report on the types of food introduced. Again, diet must be considered in combination with environmental factors and cultural practices in child rearing to account for the changes in the levels of vitamin D. The timing of the IGD episodes ending around weaning also coincides with another milestone in an infant's life – crawling and walking. Current research in child development notes that infants begin to crawl around 7 – 11 months of age and walk a few months afterward, though this can vary (Karasik et al. 2011, Hoch et al. 2020, Yamamoto et al. 2020). It is possible that as the infants learned to walk and crawl, they were swaddled less frequently and as a result had an increase in UV exposure. The potential increase in foods with vitamin D as well as increase in UV exposure could explain the timing of the episodes in the 18 individuals mentioned above.

The results of the timing of IGD in this thesis are expected since current research shows that breastmilk does not contain an adequate amount of vitamin D, even if the mother (or wet nurse) is not deficient (Heo et al. 2022). As mentioned in Chapter 2, utilizing wet nurses, as well as the profession of being a wet-nurse, were common practices in Spain during the 19th century regardless of socio-economic status (Siles-

Gonzáles et al. 2020). Wet nurses of lower socio-economic status tended to work in hospitals or charitable establishments for foundlings/orphans for meager wages (Siles-Gonzáles et al. 2020, Medina-Albaladejo and Calatayud 2021). The diet consumed by both the middle- and lower-class wet-nurses was similar to that of the foundlings (once weaned) and almost completely devoid of vitamin D (Medina-Albaladejo and Calatayud 2021). With healthy breastmilk already established to contain insufficient vitamin D levels, it follows that a mother/wet-nurse with insufficient dietary vitamin D would not be able to provide sufficient levels of that nutrient for the infant.

This research found that half of the individuals with IGD, which could be interpreted as VDD, exhibited episodes prior to weaning. With the information provided by the analysis of the teeth in this thesis, inferences can be made on the nutritional and cultural practices of women and their infants and children (or infants under their care) at this time in Spain. Considering that the teeth show episodes of IGD, it is likely that the women had both a dietary and environmental deficiency in vitamin D.

5.3 Atypical Patterns and Observations

From the analysis of the teeth from this sample there were a few unexpected results for which four interpretations are proposed. Three of the individuals (N12-1, N18-1, SC1-F2-9) examined in this thesis exhibited patterns atypical of the rest of the associated data.

Two teeth were examined from individual N12-1 (age-at-death – 2.5 years), MAD01 (ri^1), and MAD02 (rm^1). No skeletal evidence of rickets was reported by Dr.

Brickley and MAD01 showed no evidence of IGD. However, MAD02 displayed 1 episode of IGD that occurred between birth and 6 months of age. Individual N18-1 (age-at-death – 9 months) was reported to have skeletal evidence of rickets; however, one tooth (MAD23, ri^1) showed no evidence of IGD, while MAD24 (rm_1) had one episode of IGD. This episode occurred just after birth to around 5.5 months. Individual SC1-F2-9 (age-at-death: 9-11 months) contributed 2 teeth to this study as well – MAD30 (RM^1) and MAD31 (li_1). The presence of skeletal evidence of rickets was noted for this individual and, similar to N18-1, one of the teeth examined (MAD30) had evidence of IGD while the other (MAD31) did not. The episode of IGD for MAD30 occurred just after birth to 11 months of age.

5.3.1 Atypical Pattern Interpretation 1: Tooth Crown Formation

There are a few possibilities for the inconsistency in the presence of IGD between two teeth from the same individual, regardless of the pattern. The first possible explanation for this outcome is the timing of tooth crown formation (see Table 5.1). With regards to individuals N12-1 and N18-1, there is a period where the deciduous incisor crown forms (4-5 months in utero to 2-4 months), but the crown of the deciduous molar has not (it forms between 4.5-5.5 months in-utero and 5-7 months) (Hillson 2024). There is a possibility that the IGD began after the incisor crown had finished development but before the molar crown completed mineralization. The "period of IGD episode" does not necessarily indicate an exact range, but that the episode started and ended within that range (e.g., an episode of *birth-6 months* could mean 2-4 months or 1-3 months, etc.).

When making inferences about lifestyle, diet and/or health of an individual, knowing the timing of IGD episodes matters.

For individual SC1-F2-9 the differences in formation could also be explained by the space in developmental timing between the two teeth. The deciduous crown (MAD31) formed between 4-5 months in-utero to 2-4 months, while the permanent molar (MAD30, RM^1) forms between birth and 2.5-4.5 years (Table 5.1). It is therefore likely that the IGD episode happened post-crown completion for the incisor, and the timing of the IGD episode is estimated to be birth -11 months.

Table 5.1 *Tooth Crown Mineralization and Completion of Three Atypical Individuals from the Trinitarias Descalzas Sample*

Ind./ Tooth	Tooth	Skel. Evi. of R	IGD?	AAD Est.	Period of IGD Episode	Crown Mineralization*	Crown Complete*
N12-1							
MAD01	ri^1	No	No	2.5 yrs	No IGD	4-5 mo in-utero	2-4 mo
MAD02	rm^1	No	Yes	2.5 yrs	Birth – 6 mo	4.5-5.5 mo in-utero	5-7 mo
N18-1							
MAD23	ri^1	Yes	No	9 mo	No IGD	4-5 mo in-utero	2-4 mo
MAD24	rm_1	Yes	Yes	9 mo	Just after birth – 5.5 mo	5 mo in-utero	5-7 mo
SC1-F2-9							
MAD31	li_1	Yes	No	9-11 mo	No IGD	3.5-5 mo in-utero	2-4 mo
MAD30	RM^1	Yes	Yes	9-11 mo	Just after birth – 11 mo	Birth	2.5-4.5 yrs

Notes: Skel. Evi of R = Skeletal evidence of rickets; IGD = Interglobular Dentine; mo = months; yrs = years; AAD Est. = Age-at-death estimation; *information from Gustafson and Koch (1974)

5.3.2 Atypical Pattern Interpretation 2: Methodological Practices

Another possible explanation for the inconsistent evidence of IGD in the teeth of these individuals related to the methodology used during histological analysis of IGD. When examining IGD through transverse cross-sections on micro-CT, it appears as a dark, irregularly shaped band (Veselka et al. 2019). Depending on the location of the transverse section when cutting the tooth for histological analysis, and amount of IGD in the tooth, the "band" can appear incomplete (Veselka et al. 2019) (see Figure 2.6). It is possible that the sectioning of MAD01, MAD23 and MAD31 (the atypical teeth with no evidence of IGD) were cut between the bands, resulting in the conclusion that IGD was not present within that tooth. This methodological limitation necessitates extreme caution when definitively determining the presence or absence of IGD. Teeth with minimal amounts of IGD would be the most vulnerable to this sectioning inadequacy. To definitively say whether or not the sectioning process of the teeth resulted in the anomalies described above, a CT-scan of the tooth or the development of an additional cross-section could aid in the accuracy of this interpretation.

5.3.3 Atypical Pattern Interpretation 3: Differential Diagnoses

Two teeth were examined from individual N12-1 (age-at-death – 2.5 years): MAD01 (ri^1) and MAD02 (rm^1). No skeletal evidence of rickets was reported by Dr. Brickley and MAD01 showed no evidence of IGD. However, MAD02 had 1 episode of IGD that occurred between birth and 6 months of age. With the information from D'Ortenzio et al. (2016, 2018) and Brickley et al. (2020a, b) that IGD is not always pathognomonic for rickets, it is possible that individual N12-1 had a different condition

that led to the mineralization defect. As discussed in Chapter 2, the presence of IGD is primarily associated with VDD and/or fluorosis and has been well documented in the literature (D'Ortenzio et al. 2016, 2018, Ramesh et al. 2017, Brickley et al. 2020b). What is often overlooked or (not accounted for) are the rare conditions that can cause IGD and/or similar mineralization defects within the dentine (Table 5.2).

Table 5.2 *Conditions* Affecting Tooth Mineralization (adapted from D'Ortenzio et al. 2016 and Brickley et al. 2019)*

Condition	Type of Condition	Cause of Mineralization Deficiency	Sources
Vitamin-D-dependent rickets	Genetic disorders	Group of conditions affecting the activation of vitamin D during metabolism or maintenance of active vitamin D concentrations	Levine 2020
Vitamin-D-resistant rickets	Genetic disorder	Condition where the active form of vitamin D fails to bind to the vitamin D receptor and is unable to initiate biological functions	Kashyap et al. 2023
Autosomal dominant hypophosphatemic rickets; Autosomal recessive hypophosphatemic rickets; X-linked hypophosphatemic rickets; Tumour-induced osteomalacia	Genetic disorders	Mutation in a hormone (fibroblast growth factor 23) that decreases the absorption of phosphorus	Baroncelli and Mora 2021, Edouard and Linglart 2024, Mameli et al. 2021, Minisola et al. 2017
Hypophosphatasia	Genetic disorder	Loss-of-function mutation in a gene that aids in tissue mineralization	Mornet 2017

Condition	Type of Condition	Cause of Mineralization Deficiency	Sources
Celiac disease, Crohn's disease	Auto-immune disorder	Malabsorption of vitamin D	Infantino et al. 2022
Renal disorders	Varies	Reduction/absense in ability to convert vitamin D into active form	Kim and Kim 2014

Note: *Conditions other than those discussed in detail in this thesis (i.e., nutritional rickets, residual rickets, osteomalacia, and fluorosis)

Supplemental data from Snoddy et al. (2024) described the presence or absence of the macroscopic lesions associated with VDD (adapted from Brickley et al. 2020a) for each of their individuals examined. For two individuals with skeletal evidence of VDD but no episodes of IGD, only 1-2 lesions were present on the skeletons, all of which (on their own) were not pathognomonic for VDD. In these cases, the authors noted that these types of lesions could have been a result of "another condition or anatomical variation" (Snoddy et al. 2024, p. 15).

5.3.4 Atypical Pattern Interpretation 4: Timing/Duration of Vitamin D Deficiency

The last explanation considered for the discrepancy between MAD01 and MAD02 (N12-1) relates to the process of the development of lesions associated with rickets. Current literature notes that multiple factors can contribute to the manifestation and severity of skeletal lesions (e.g., Mays et al. 2018, Brickley et al. 2020b, Charoenngam and Holick 2020). Both environmental (timing/length of deficiency) and genetic (physiology, inherited conditions) factors can influence the severity of deficiency experienced (Brickley et al. 2020b). Pettifor (2012) and more recently Gentille and

Chicarelli (2021) noted that the development of skeletal evidence of rickets is usually first seen before 18 months of age. As the episode of IGD in MAD02 (rm^1) was between birth and 6 months and the amount of IGD present (0.79%) was low compared with others in the sample (see Table 4.6) it is possible that the deficiency was short in duration and/or "severity". This may help to explain how an individual without skeletal evidence of rickets, had an episode of IGD. Without the establishment of a standard or threshold for the amount of IGD relative to the vitamin D deficiency exhibited macroscopically, it is difficult to ascertain if this individual was developing rickets when they had the IGD episode, or if the IGD was caused by another condition as discussed in the previous section.

Individual N36-1 was noted as having skeletal evidence of rickets by Dr. Brickley and the age-at-death was estimated to be the 7th month in-utero. No IGD was observed on the teeth. Clinical literature states that visual (skeletal) evidence of rickets typically appears before 18 months of age with the most frequent bone deformities appearing between 4-12 months of age (Gentille and Chiarelli 2021). Possible explanations for the results of individual N36-1 could be due to a differential diagnosis, that is, there were skeletal indicators on the skeleton, but it was not due to rickets, or there was an error in methodological practices and/or age estimation of the individual. Future research should re-examine the skeletal evidence for rickets on this individual to confirm the age estimation.

5.4 Methodological Developments

The most important component of this thesis was the quantification of IGD. The process developed and used by this author to quantify the amount of IGD present in each tooth was adapted from the methods established by D'Ortenzio et al. (2016) and Snoddy et al. (2020).

Snoddy et al. (2020) used open access computer software (FIJI/ImageJ® v1.52) to develop a method of quantifying IGD to reduce the subjectivity and observer error of previous techniques (Mellanby 1928, Isokawa et al. 1963, D'Ortenzio et al. 2016, Veselka et al. 2019) (see Section 2.5.2). This thesis expanded on Snoddy et al.'s (2020) methodology. Where Snoddy and colleagues (2020) used an area of interest, this thesis examined the entire dentine area of the tooth to quantify IGD and presented a percentage of IGD relative to the surrounding unaffected dentin. It involved (as described in Chapter 3), imaging each section of tooth at 200x magnification and stitching the images together using the software provided on the Keyence® (VHX-2000) digital microscope, in order to have a whole picture of the tooth without losing the resolution needed to identify IGD. This is the first study to quantify the entire dentine area within a tooth cross section and not just a region of interest, as done by both Snoddy et al. (2020) and D'Ortenzio et al. (2016). As Snoddy and colleagues (2020) noted, the amount of IGD when calculated quantitatively can differ significantly than previous techniques as they likely over-estimate via visual analysis. For comparative purposes, Snoddy et al. (2020) used their protocol on the same region of interest of an image of a tooth used in D'Ortenzio et al. (2016). The researchers (D'Ortenzio et al. 2016) gave this region of interest a visual

estimate of 25-50% IGD coverage, while, using their own method, Snoddy et al. (2020) resulted in an IGD coverage of ~6.5%. The evidence presented in this thesis also agrees with Snoddy and colleagues (2020) assessment that a visual analysis may overestimate the percentage of IGD. Even when quantifying the entire tooth (instead of a region of interest), the highest amount of IGD observed in any of the teeth in this thesis was 26.22% (MAD40) of the total dentine area. Whereas a visual estimate would likely consider this tooth to have 75%+ of IGD coverage. The technique used in this thesis further demonstrates that previous techniques based on visual estimates are not accurate or comparable to the quantitative results. Should a standard be developed for understanding the relationship between amount of IGD and expression of lesions of VDD, it is important to consider using a technique that prioritizes quantitative measurements over visual estimates, as the latter would not be accurate.

It is important to examine the tooth as a whole when quantifying the IGD as certain areas can give different results. As IGD develops during tooth formation, periods of "severity" or amount may be different during different months of growth. Without looking at the entirety of the tooth, researchers may have a biased interpretation of the "severity" of IGD as a whole. To eventually establish a threshold for an amount of IGD that would be pathognomic for VDD, only looking at a region of interest or short episode limits that ability. Using a method that can be replicated by other researchers with minimal bias would help with establishing that threshold.

5.5 Conclusion

The samples analysed were from a known group with recognized skeletal evidence of rickets, however the results and analysis of IGD data provide much more information than simply the presence or absence of VDD. With the IGD data my research is able to indicate when, and for how long VDD occurred. Interpretations of this information can provide possible cultural or biological explanations for the timing of the VDD in relation to the mother-infant nexus (i.e., maternal vitamin D status, breastfeeding, weaning, swaddling, environmental conditions). Using this histological evidence in combination with historical documentation allows bioarchaeologists to understand and interpret the mother-infant nexus at a specific time and location. A benefit to this type of analysis is its universality; it can be used with archaeological samples from other time periods and populations.

CHAPTER 6: CONCLUSIONS

6.1 Introduction

Within the framework of the mother-infant nexus, measuring interglobular dentine (IGD) within the developing tooth provides a better understanding the relationship between a mother and foetus or infant, especially as it pertains to vitamin D deficiency (VDD). This histological evidence allows us to see periods of deficiencies and make inferences about the health, environment and cultural practices of the individuals (i.e., both the mother/wet-nurse and the infant) under investigation. Accurate measurements of the amount and timing of IGD support those inferences.

6.2 Summary of Findings

By developing a methodology for quantifying IGD that improves on the subjectivity and limitations of existing practices, this thesis provides a novel technique to measure the amount of IGD in a tooth that is both more accurate and easily replicable by other researchers. Existing histological methods for quantifying IGD are typically based on an area of interest within the tooth that shows evidence of the mineralization defect. These areas of interest have no distinct or standardized selection process and introduce the possibility for biases and make replication inconsistent. As IGD can form in different areas of tooth dentine, methodologies that focus on an area of interest may not necessarily capture all of the IGD within a tooth even if the amount seems negligible (such as with developmental IGD). Since we know the timing of tooth formation and how it relates to information on the health of the mother and foetal and infant development, valuable information about the mother-infant nexus could be lost without considering the entire

tooth. By examining the whole tooth and determining the amount of IGD relative to the entirety of the dentine space, this thesis presents a methodology that uses measured percentages, rather than visual estimates or indeterminate categories/grades of IGD amounts. A visual examination of a tooth when overlayed with a grid (see Section 3.2.5) shows that the amount of IGD can vary significantly in different areas of the dentine, thus confirming that the existing practice of assigning a visual percentage amount or grade to a tooth based solely on an area of interest will not provide a comprehensive understanding of IGD development. This is particularly important when using IGD to understand the conditions experienced by an individual and to further emphasize the mother-infant nexus. Knowing exactly how much IGD occurred and when, allows for the use of quantitative measurements to extrapolate the conditions faced during an individual's development and the health of their mother during that time period.

In addition to the development of a methodology, this thesis analyzed the timing, length and age of onset of episodes of IGD in deciduous and permanent teeth from a sample from 19th century Spain within the framework of the mother-infant nexus. This data presents the opportunity for understanding dietary, lifestyle and environmental conditions affecting the individual and their nutritional status, specifically concerning the timing of VDD. With that information, further implications were made regarding the mother-infant nexus in past populations. The results of the investigation of this sample of teeth provide insight into the association between the timing of VDD and their correlation to infant care (e.g., breastfeeding, swaddling) and the weaning process. Current literature on infant care during the 19th century Europe is wanting, specifically in Spain. Historic

documents do not provide enough specific information on the lifestyle of the mother's (or wet-nurse surrogate) care practices of infants and young children. Much of these practices can only be inferred from existing literature, such as censes, advertisements and records from religious institutions. However, historic literature and its interpretations are also prone to biases, whether purposefully or not. The identification and analysis of the timing, amount and number of IGD episodes within this sample of teeth for this thesis, provides physical evidence to support the literature-based inferences of maternal health and infant care during 19th century Spain.

This thesis shows that by using teeth to analyze VDD (as evidenced by IGD), more information can be amassed on timing, duration, and magnitude of the episodes of that deficiency. Using skeletal evidence in isolation to discuss VDD does not provide the same amount of detail that the analysis of IGD in teeth can. Particularly when using the methodology developed for this thesis. The skeletal evidence of VDD for the Trinitarias sample may provide a binary answer for the occurrence of VDD (yes/no), whereas the tooth analysis answers the questions of when, how much, and for how long an individual experienced VDD as well as the implications for the mother-infant nexus.

6.3 Directions for Future Research

The current research is lacking in IGD patterns between different tooth types (deciduous and permanent). It often focuses on samples of permanent teeth, neglecting the valuable information on both mother and infant health that can be found in deciduous teeth. This thesis presents a picture of IGD patterns using both types of teeth. Future

research can be better informed on IGD presence and expression with the use of different types of teeth. The use of both deciduous and permanent teeth, when analyzing VDD, gives a more robust picture of the mother-infant nexus.

The time and effort necessary to map out and measure the amounts of IGD for this thesis with the quantification method mentioned above, had its limitations. With a larger sample size, this methodology could prove prohibitively time consuming. Opportunities for software development and equipment that make the measurement process faster, would allow for large quantities of tooth samples to be analyzed.

There is currently no standard for the amount of IGD that correlates with the severity of a rickets diagnosis. Future work on this sample could examine the degree of expression of rickets on the skeletons and correlate that with the amount of IGD observed in the teeth. The ability to accurately measure IGD in teeth of individuals with known skeletal evidence of rickets across multiple archaeological samples from various time periods and geographic locations would help establish such a standard as would faster and more effective equipment and software. With such a standard for dental samples, bioarchaeologists might be able to infer the presence of metabolic conditions, including VDD, in the absence of the skeleton.

The enduring nature of teeth in the archaeological record allows bioarchaeologists to understand the health (or lack thereof) of past peoples who skeletons may have been improperly preserved, incomplete or completely absent. By having a method to quantify the temporal data of IGD, researchers can use the teeth to distinguish the timing of

vitamin D deficiencies and within the framework of the mother-infant nexus, establish the conditions experienced by both the mother and infant. The methodology developed for this thesis allowed for the analysis of the teeth from the Trinitarias sample to be used in conjunction with existing literature to make inferences on the health of women and children in 19th century Spain. This thesis can be used as a catalyst for determining the amount and timing of IGD that would correlate with different expressions of skeletal evidence of rickets. It could also perhaps establish an amount of IGD that is pathognomonic for VDD.

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