Lifeways and frailty experience of the population of Santiago de Chile during the 19th and 20th centuries: the *Colección Osteológica Subactual de Santiago*

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"La muerte es un hábito colectivo"

- Nicanor Parra

Abstract

This thesis explores the biological and socio-cultural variability in the experience of frailty of a population that inhabited Santiago de Chile, Latin America, during the late 19th and early-mid 20th centuries. This population, whose remains comprise the *Colección Osteológica Subactual de Santiago*, lived in low socioeconomic status communities affected by economic rural-to-urban migration and social inequalities. Very little attention has been focused on non-adults and older adults in the sample, with the great majority of studies focusing on the adult population. This is in line with the broader context of the bioarchaeological field, were early and late life-course experiences are often relegated due to issues with sample size and methodologies.

The purpose of this study was to conduct a comprehensive analysis of the morbidity and mortality patters of non-adult (<21 years of age) and older adult (>40 years of age) individuals. This research integrates conventional bioarchaeological evidence, documented antemortem data (age-at-death, sex and cause of death of each individual from cemetery documentary sources), as well as biomedical records from that era to enhance our current knowledge of skeletal evidence related to exposure to stressors during different stages of life.

Findings show that the effects of physiological stress on growth and development, pubertal timing, and survival are present within and across the samples. The results suggest that this population suffered from health assaults during the early stages of life, increasing mortality and morbidity during childhood, while also causing deficient immune and frailty phenotypes that marked their skeletal response to environmental and cultural stressors in later life.

This study has developed an interdisciplinary biocultural approach to the study of the lifeways of a modern population of Santiago, increasing our understanding of the health experiences and physiological responses to life events such as migration, social inequality, and urbanisation during this period.

Resumen

Esta tesis explora la variabilidad biológica y sociocultural en las condiciones de salud de una población que habitó Santiago de Chile, América Latina, a finales del siglo XIX y la primera mitad del siglo XX. Los restos de esta población integran la Colección Osteológica Subactual de Santiago, que corresponde a comunidades de bajo nivel socioeconómico afectadas por la migración económica del campo a la ciudad y desigualdades sociales. Históricamente, se ha prestado muy poca atención a los niños y los adultos mayores de la colección osteológica, y la gran mayoría de los estudios se centran en la población adulta. Esto concuerda con el contexto más amplio de la bioarqueología, donde las experiencias tempranas y tardías del curso de la vida a menudo se relegan debido a problemas con los tamaños muestrales y las metodologías existentes.

El objetivo de este estudio fue realizar un análisis exhaustivo de los patrones de morbilidad y mortalidad de individuos infantiles (<21 años) y adultos mayores (>40 años). Esta investigación integra evidencia bioarqueológica convencional, datos antemortem documentados (edad al morir, sexo y causa de la muerte de cada individuo provenientes de fuentes documentales del cementerio), y registros biomédicos de la época para mejorar nuestro conocimiento actual de la evidencia esquelética relacionada con la exposición a factores estresantes durante las diferentes etapas de la vida,

Los hallazgos muestran que el crecimiento y el desarrollo, el desarrollo puberal y la supervivencia de los individuos se vieron afectados por el estrés fisiológico. Los resultados sugieren que esta población sufrió alteraciones de salud durante las primeras etapas de la vida, generando un aumento en la mortalidad y morbilidad durante la infancia, y causando, asimismo, fenotipos inmunológicos y de fragilidad deficientes que marcaron la respuesta esquelética a los estresores ambientales y culturales en la vida adulta posterior.

Este estudio utilizó un enfoque biocultural interdisciplinario para estudiar los estilos de vida de una población moderna de Santiago, aumentando nuestra comprensión de las condiciones de salud y respuestas fisiológicas a eventos como la migración, la desigualdad social y la urbanización durante este período.

This PhD is dedicated to my grandparents,
Hugo Escobar, María Magdalena Arias, Juan Meza, and Ofelia Ortega,
living proof of how early life stressors
can affect health outcomes during the life course.

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List of Abbreviations

AMTL – Antemortem tooth loss
BLOS – Bone loss
Bow – Bowing of the long bone of the legs
CA – Chronological age
CAR – Caries
CF – Cribra femoris
CO – Cribra orbitalia
CoD – Cause of death
COSS – *Colección Osteológica Subactual de Santiago*
DA – Dental age estimation
DAD – Documented antemortem data (age-at-death and sex)
DEN – Dental diseases
DOHaD – Developmental Origins of Health and Disease
GWA – Gestational weeks of age (*intra utero*)
HR – Hazard ratio
LCT – Life Course theory
LEH – Linear enamel hypoplasia
MS – Metaphyseal swelling
OA – Osteoarthritis
PA – Physiological age
PGP – Porosity of the growth plate
PH – Porotic hyperostosis
PHV – Peak high velocity
PNBF – Periosteal new bone formation
RIC – Rickets
SA – Skeletal age estimation
SA-F – Age estimation through maximum femoral length
SA-P – Age estimation through pars basilaris measurements
SCU – Scurvy
SES – Socioeconomic status
SFI – Skeletal Frailty Index
SGC – Santiago General Cemetery (*Cementerio General de Santiago*)
WA – Weeks of age

Declaration

I, Camila Ofelia Meza Escobar, the author, confirm that this Thesis is my own work. I am aware of the University's Guidance on the Use of Unfair Means (www.sheffield.ac.uk/ssid/unfair-means). This work has not previously been presented for an award at this, or any other, university.

One peer-reviewed publication arose from this thesis before its submission:

Meza-Escobar, O., Galimany, J., González-Oyarce, R. and Barreaux Höpfl, N. (2023) 'The Colección Osteológica Subactual de Santiago: Origin and Current State of a Documented Skeletal Collection from Chile, Latin America', *Forensic Sciences*, 3(1), pp. 80–93.

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Chapter 1

Introduction

1.1 Introduction

Since its emergence during the 1970s and 80s, bioarchaeology has sought to comprehend the interconnected biological and cultural experiences of the lives of individuals from past populations (Larsen, 2015). This thesis shares this common goal of bioarchaeological research. Its aim is to critically examine the lifeways and frailty experience of a low socioeconomic status (SES) community that lived in Santiago, capital city of Chile, during the late 19th and early 20th centuries. In particular, this thesis explores the extent to which physiological stress impacted growth and survival of these individuals, and whether it is possible to use skeletal stress indicators and frailty to understand the life experience of this population. To further current understanding regarding the skeletal evidence for early and later life exposure to stressors, this thesis combines traditional bioarchaeological evidence, documented antemortem data (i.e., sex, age-at-death, and cause of death of each individual, known from antemortem documentary sources) and biomedical records of the time. Thus, this research can be seen as a interdisciplinary biocultural approach to the physiological response to life experiences that were impacted by processes of migration, social inequality, and urbanisation.

The broader research questions articulated in the study design of this thesis, before the development of more detailed analyses, were as follows:

- To what extent does this skeletal assemblage reveal the effects of migration and resulting poverty on the Santiago population?
- How does documented antemortem data of age-at-death and sex can help to further explore bioarchaeological issues?
- What are the possible etiological, pathogenic, and contextual explanations of the evidence of growth disruption and mortality risk in this population?

In order to answer these research questions and to achieve the intended research aim, this thesis defined four primary research objectives:

- To capitalise on the availability of documented age-at-death, sex and other antemortem data (years of birth and death, and cause of death) for this population.

- To contextualise bioarchaeological evidence with clinical data using a bio-historical approach to the life course.
- To analyse how patterns of frailty affect morbidity and mortality over the life course.
- To investigate the relationships between indicators of growth disruption and/or mortality risk while considering the contextual information associated with the individuals under analysis.

Each research chapter explores a specific life course stage: foetal life and infancy, childhood, adolescence, and older age. Although the documented skeletal collection studied here includes all ages and is composed in its majority by adults, individuals between 21 and 40 years of age (who characterise the life stage of adulthood) were not studied. The decision was made on the basis that there are already several existing studies covering the adult life stage in the Santiago population (summarised in Chapter 2), and the fact that fewer bioarchaeological studies have focused on this collection's individuals in the very early and later stages of life. Moreover, the focus on early and later life in this thesis addresses the lack of studies of both these life stages in Chilean bioarchaeology and their relatively scarcity across the wider field of bioarchaeology.

1.1.1 Thesis structure

The thesis is presented in a publication format, incorporating a collection of manuscripts that are formatted in a structure suitable for future publication in a peer-reviewed journal, in accordance with the regulations of the University of Sheffield.

The thesis begins with two chapters that set the scene for the study. Chapter 1 introduces the aims and research questions, outlines the structure of the thesis, presents a critical review of bioarchaeological approaches to palaeopathology, and a survey of the historical and geographical context to the study. Chapter 2 characterises the skeletal sample and describes all methods used across the research chapters, providing more detailed information than that included in each subsequent manuscript. These sections provide an overarching intellectual context for the research presented in the chapters that follow. The

thesis ends with an overall summary discussion and conclusions which draw together the findings of each manuscript to address the overarching aim of the project.

Each research chapter is intended as a self-contained manuscript with its own research questions, devised to form the basis of a future publication. Each research chapter, and the complete thesis, also includes two abstracts, one in English and one in Spanish (*Resumen*), in an effort to make the research accessible to a broader audience and to reflect the fact that the author and materials used in this thesis come from a Spanish-speaking country. In order to achieve the intended research aims and objectives, the manuscripts are focused on the following research questions:

Chapter 3. Growth disruption and physiological stress of foetal-infant individuals of documented chronological age from mid-20th century Santiago, Chile.

- Is it possible to explore growth disruption in foetal, perinatal, and infant individuals up to one year of age through discrepancies between chronological age and skeletal estimation of age?
- Is there evidence of growth disruption between different skeletal elements of each individual? If so, which anatomical element seems to be more resilient to physiological stress assaults?
- Does physiological stress affect growth and development? If so, how is that reflected on skeletal pathological changes?

Chapter 4. Piececitos de niño: patterns of physiological stress and survival among non-adults from 20th-century Santiago, Chile.

- Does physiological stress impact the survival of individuals during childhood? Is an age group more at risk of death than others?
- Are skeletal indicators of stress associated with increased risk of death? If so, which pathological changes are related to decreased survival and what are the possible causes of this?

Chapter 5. Descomedidos y chascones: pubertal timing and frailty in a modern documented adolescent skeletal sample from Santiago, Chile (1960–1986).

- Is the method proposed by Lewis and Shapland (2013, 2014) and further defined by Lewis, Shapland and Watts (2016) a reliable way to assess pubertal timing in this sample of skeletal remains?
- How does the timing of puberty in this sample compare to clinical data for the same population and time period? Is this sample of adolescent individuals showing signs of pubertal delay/advancement?
- Was pubertal development affected by physiological stress during childhood and adolescence? If so, what are the possible explanations for pubertal disruption?
- What are the potential implications of physiological stress related pubertal disruption on the pubertal timing method defined by Lewis and Shapland (2013, 2014) and further defined by Lewis, Shapland and Watts (2016)?

Chapter 6. Mortality risk and physiological stress in older adults of documented chronological age from mid-20th century Santiago, Chile.

- Is it possible to segregate older adults into biologically meaningful subgroups following senescence stages proposed by Roksandic and Armstrong (2011)?
- Is mortality risk associated with early or later life stressors in older adults? If so, which skeletal indicators of physiological stress are associated with a decreased survival in older adults in this sample?
- Are there differences in mortality risk between biological sexes and age stages in this sample? If so, what are the possible explanations behind this?

Although each research chapter includes its own introductory background, the remainder of this chapter will provide discipline-specific and historical information to contextualise the overall research project. The first subsection will describe key bioarchaeological terminology used in this thesis, as well as theoretical frameworks within which this research exists. The final subsection succinctly introduces the necessary Chilean historical context to understand the lived experience of this population.

1.2 Approaches in Bioarchaeology

The human skeleton has long been seen in the field of bioarchaeology as both a cultural and a biological entity (Larsen, 2015). This duality is at the basis of bioarchaeological research, as morphological variation related to genetic, epigenetic, and sociocultural lifeways is present across individuals, populations, and time periods. To explore how genetic and environmental changes influenced the life experience of an individual or population, bioarchaeology relies on palaeopathology to reconstruct, contextualise, and compare skeletal indicators of pathological change. To interpret macroscopic skeletal indicators in order to discern between pathological and normal markers in the bones, palaeopathologists must understand the process by which bones growth, develop and remodel over time (Ortner, 2003; Buikstra, 2019). To achieve this, bioarchaeology has adopted a broader and interdisciplinary approach to contextualise findings on disease in the past, using data and insights from fields such as biomedical sciences, epidemiology, evolutionary medicine, sociology, historiography, among others (Roberts and Manchester, 2010; Baker and Agarwal, 2017; Buikstra et al., 2022). The following subsections will present the theoretical frameworks used as a basis to interpret paleopathological changes in this research, summarise key definitions within palaeopathology that will be used throughout this thesis, and highlight the importance of a biocultural approach to understand these changes.

1.2.1 Theoretical frameworks

Two theoretical frameworks play a crucial role in shaping and guiding the research conducted in this thesis: the Life Course theory and the Developmental Origins of Health and Disease approach. They provide a conceptual structure that helps to frame the study, define key concepts, and establish relationships between variables, as the overall arching aim of this thesis focuses on physiological stress experience framed in life course terms. By drawing upon established theories and models within the bioarchaeology field, this subsection creates a roadmap for investigating the research questions and interpreting the findings.

The Life Course Theory (LCT) emphasises the importance of individual and social experiences over the course of an individual's life in shaping health outcomes (Elder, Johnson

and Crosnoe, 2003). The LCT proposes that social and historical context, as well as individual experiences, interact to influence health and wellbeing over time. This framework recognises that factors such as genetics, social circumstances, and environmental exposures can have both immediate and long-term effects on health; thus, health outcomes are the result of complex interactions between multiple levels of influence, including individual, family, community, and societal factors (Halfon *et al.*, 2014). It also recognises that experiences in early stages of life can have a cumulative impact on health outcomes in later life; as early life is recognised as a highly sensitive phase during which even temporary experiences of stress, such as infectious diseases or malnutrition, can have long-lasting effects that endure throughout an individual's entire life course (Gluckman, Hanson and Beedle, 2007; Hodson, 2021).

The issue of early childhood stress and its impact on later life health outcomes has received considerable critical attention within biological anthropology and bioarchaeology during the last decades. The Developmental Origins of Health and Disease (DOHaD) approach is a theoretical framework that proposes that early life experiences, such as fetal and early childhood development, can have a long-term impact on health and disease risk in later life (Gluckman, Buklijas and Hanson, 2016). The framework suggests that exposure to certain environmental factors during critical periods of development can result in changes to the physiology, metabolism, and structure of the body that increases the risk of chronic diseases such as obesity, cardiovascular disease, and diabetes in adult life (Fall and Sachdev, 2006; Barker, 2012). In particular, socioeconomic status (SES) has been a key topic of interest, as it affects social and biological life trajectories for longer periods of time (Gagnon and Bohnert, 2012). The DOHaD framework highlights the importance of the prenatal and early childhood periods as critical windows of development during which environmental exposures can have lasting effects on health outcomes (Miller, 2020). It also emphasises the need for public health interventions and policies that address the root causes of these exposures (Fall and Sachdev, 2006).

Biological anthropology studies have also tackled the concept of developmental plasticity to understand how early life exposure to stress impacts adult disease. Defined as the capacity of an organism to adapt and modify its phenotype or developmental trajectory in response to environmental cues or stimuli (Bateson *et al.*, 2004), developmental plasticity

allows organisms to adjust their developmental processes and phenotypic traits in order to optimise survival and reproduction in varying environmental conditions. It involves changes in gene expression, cellular processes, and morphological, physiological, or behavioural traits, changes that are particularly notable during early developmental stages when the organism is highly sensitive and responsive to environmental signals (Gluckman *et al.*, 2007). The plasticity observed can lead to variations in traits such as growth rate, size, morphology, metabolism, and behaviour, allowing organisms to adapt to diverse ecological niches or changing environmental conditions. For bioarchaeology, the adaptive nature of bone morphology and the skeletal system's capacity to preserve indications of environmental exposure, including those influenced by culture, have been pivotal in understanding the interplay between the human body and society in historical contexts (Roberts and Manchester, 2010). These aspects have played a central role in interpreting how societal factors and environmental influences shape the skeletal structure and provide insights into the interactions between individuals and their social environment in the past (Gowland, 2015). Bioarchaeology has studied skeletal indicators of developmental disruption (such as long bone length, linear enamel hypoplasia, among others) to estimate how stress affects the body, identifying developmental adaptations that correlate with younger ages at death in adulthood and therefore suggest heightened mortality risk among individuals who have had to adapt to environmental and cultural stressors in early life (Newman and Gowland, 2017; Temple, 2019).

The LCT and DOHaD frameworks intersect in several ways, as they both emphasise the importance of early life experiences and the role of social and environmental factors in shaping health outcomes over the lifespan. The LCT framework takes a broader view, highlighting the importance of individual and social experiences across the entire life course in shaping health outcomes. It recognises that experiences at different stages of life can have cumulative effects on health, and that the social and historical context in which individuals live also plays a significant role (Cheverko, 2020). The DOHaD framework focuses specifically on how early life experiences can lead to changes in physiology and metabolism that increase the risk of chronic diseases later in life. It highlights the critical windows of development during which environmental exposures can have lasting effects on health outcomes. Together, the LCT and DOHaD frameworks suggest that an individual's health is the result of a complex interplay between early life experiences, social and environmental

factors, and individual behaviours and choices. For epidemiology and public health, they highlight the need for interventions and policies that address the root causes of poor health outcomes, including improving maternal and child health, reducing exposure to environmental toxins, promoting healthy lifestyles, and addressing social determinants of health across the entire life course (Alvarado *et al.*, 2008). For bioarchaeology, they assert the need to recognise human skeletal remains as a reflection of temporal significance. Moreover, it also means an increased focus on investigating infant palaeopathology and its implications for maternal wellbeing, with the impact of non-proximate stressors, such as early life experiences and ancestral environments, on the presence of health indicators. These evolving perspectives and research directions broaden our understanding of the complex interplay between biology, environment, and social factors in shaping health outcomes across different time periods and populations (Gowland, 2015).

1.2.2 Stress, frailty and health

In broad terms, stress can be defined as the disruption of physiological homeostasis as a consequence of an external influence (Temple and Goodman, 2014). Biological stress, also known as physiological stress, refers to the impact that external or internal factors have on an organism's biological systems. Biological stress can arise from a variety of sources, such as physical injury, exposure to toxins, infections, psychological or emotional strain, and environmental changes. When confronted with a stressor, the body initiates a complex series of physiological responses aimed at adapting to the challenging situation and maintaining somatic stability. These responses involve the activation of various bodily systems, including the endocrine, immune, cardiovascular, and nervous systems. While short-term or acute stress can be adaptive and necessary for survival, chronic or excessive stress can lead to a range of negative effects on health, including increased vulnerability to diseases, impaired immune function, hormonal imbalances, and mental health issues (Dhabhar, 2014).

The concept was first proposed by Hans Selye, a pioneering researcher in the field of stress physiology. Selye's work, spanning the mid-20th century, laid the foundation for our understanding of stress as a physiological response. He defined stress as the nonspecific response of the body to any demand or stressor placed upon it, regardless of the nature of the stressor (Selye, 1959). Selye's stress theory, known as the General Adaptation Syndrome

(GAS), postulated that the body goes through a consistent pattern of physiological responses when faced with stress, with three stages of stress response: the alarm stage, the resistance stage, and the exhaustion stage. During the alarm stage, the body mobilises its resources to respond to the stressor. This involves the activation of the sympathetic nervous system and the release of stress hormones, such as cortisol and adrenaline. The resistance stage follows, where the body attempts to adapt and cope with the ongoing stressor by maintaining elevated physiological responses. However, if the stress continues for an extended period, the body enters the exhaustion stage. In this stage, the body's resources become depleted, and it becomes more susceptible to physical and psychological health issues (Selye, 1976). Selye's concept of stress as a general response of the body to any stressor has greatly influenced the field of stress research and has contributed to our understanding of the physiological mechanisms involved in health and developmental adaptation.

Chronic physiological stress during both resistance and exhaustion stages can lead to lesions on the skeleton, specific or non-specific to the origin of the stressors, which can in turn be studied in human remains using palaeopathological approaches. However, it's important to note that contemporary research has expanded our understanding of stress, recognising its multidimensional nature, including psychological, social, and environmental factors, in addition to the physiological responses identified by Selye (Temple and Goodman, 2014; Clukay *et al.*, 2018). The stress model commonly used in bioarchaeology (Figure 1.1) is significant because it takes into account environmental and external factors that can both protect individuals from stressful circumstances and create them (Temple and Goodman, 2014). It has been observed that social and cultural practices can serve as both stress mitigators and stressors when adopted (Clukay *et al.*, 2018). Additionally, the way individuals perceive and respond to stress can vary, leading to individual variations in physiological stress responses, which can be identified through skeletal lesions. Therefore, there is no universal response to stress, nor a single universal stressor.

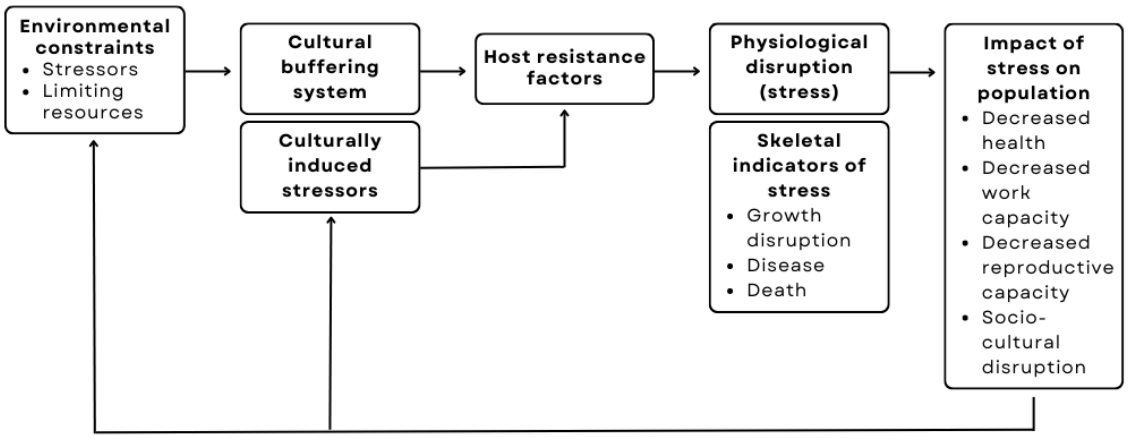

Figure 1.1 General model for the study of stress in skeletal populations revised by Goodman and Armelagos (1989) (in Temple and Goodman (2014), p.188).

A second key concept, frailty, refers to a state of increased vulnerability and decreased physiological reserve in an individual, characterised by a decline in various physical and cognitive abilities, increased susceptibility to illness and functional decline, and a reduced capacity to recover from stressors or adverse events (Bortz, 2002). Albeit greatly associated with ageing, frailty is not merely a result of chronological age but rather a complex interaction of biological, psychological, and social factors, being influenced by and coexisting between them (Fried *et al.*, 2004). A multidimensional syndrome that reflects an individual's overall health and resilience, frailty can be understood as a phenotype that rules an individual's present and future capacity to withstand or cope with stressors, resulting in reduced resilience (Fried *et al.*, 2004; Gluckman, Hanson and Beedle, 2007).

In bioarchaeology, a frailty phenotype is conceptualised as the skeletal changes associated with reduced muscle mass and strength (sarcopenia), decreased bone density (osteopenia), impaired homeostasis, and a decline in overall physical functioning (Marklein, Leahy and Crews, 2016). While some bioarchaeology studies have equated the presence of a frailty phenotype to an increased risk of death (Usher, 2000; DeWitte and Hughes-Morey, 2012), this thesis takes the epidemiological approach by which frailty phenotype is separated from the risks associated with morbidity and mortality, although it may exhibit correlations with both. In this sense, the development of a frailty phenotype in an individual is a reflection of the fluctuating and cumulative physiological state of functional loss that increases with age (Marklein and Crews, 2017). Skeletal frailty, therefore, it is not intrinsically associated

with ageing, but with a cumulative burden of chronic stress and life events, also known as allostatic load (Guidi *et al.*, 2020): individuals more susceptible to life's stressors, who's bodies suffer from allostatic overload, would exhibit more skeletal indicators of frailty, which in turn would mean that those who live into older adulthood will tend to concentrate more skeletal biomarkers of stress (Wood *et al.*, 1992). When reflected upon bioarchaeological research and the findings of this thesis, frail non-adult individuals died at young ages unable to cope with physiological stress, while frail individuals that lived into older age were more resilient to these health assaults, corroborating the idea that the frailty phenotype and morbidity/mortality risks are distinct concepts.

A last key term, health, is defined by the World Health Organization (WHO) as a "state of complete physical, mental, and social well-being, not merely the absence of disease or infirmity" (World Health Organization, 1946). Although greatly disseminated through different scholarly fields, this definition has not been without critics, particularly during the last decades. Critics have pointed out that the definition lacks operational value and is more political than scientific, as a "complete well-ness" is unachievable in a world were social inequality, oppression, war, violence, and discrimination, among other factors, are the reality (Leonardi, 2018). WHO's 1946 definition seemed to fall short to the complex interaction between biological, social, and cultural factors that permeates the health experience of individuals and populations. In 1984, a refined definition arose from WHO's Working Group on Concept and Principles of Health Promotion, stating that health is "the extent to which an individual or group is able to realize aspirations and satisfy needs and to change or cope with the environment […] Health is a resource for everyday life, not the objective of living; it is a positive concept, emphasizing social and personal resources, as well as physical capacities" (World Health Organization, 1984). Similarly, Halfon *et al.* (2014, p.355) define health as "a developmental capacity that allows an individual to interact successfully with his biological, physical and social environments". In this context, health refers to the individual's ability to maintain homeostasis and recover from adverse events, permeated by the social and cultural influences of their environment (Beckie, 2012).

Is it possible to assess health in past populations?

The ordeal of disease and injury is a recurring topic for all human populations, not only because as animals, human beings will never be free of pathologies, but also because the experience of illness is one that profoundly affects the physical, psychological, and social dimensions of human life (Chrisman, 1977; Waitzkin, 1981). Within bioarchaeological studies, the concept of health has served as the baseline for comparison with stress and frailty, with the presence of skeletal indicators of stress and the definition of a frailty phenotype in an individual more or less associated to ideas of "unhealthy" individuals or those with "decreased health" (de la Cova, 2011; DeWitte, 2014; Agarwal, 2021). However, this use of the concept has proven problematic.

Temple and Goodman (2014) have argued that health includes physiological status and individual perception, which is impossible to attest from skeletal remains, while categorisation of populations into levels of relative health might end up being a misleading approach. The definition of health still poses a problem for bioarchaeological research, as skeletal analyses will never be able to investigate and resolve the social and cultural factors associated with archaeological populations. Notwithstanding the importance of the biological aspect of health, environmental factors such as SES, availability and access to healthcare, nutritional intake, exposure to disease, and sanitation also playing a key role in the wellbeing of an individual (Halfon *et al.*, 2014). What bioarchaeology can do is to take into consideration the potential social and cultural factors a population might have endured, including the ability of these individuals to adapt, cope, and maintain a wellbeing state that allows them to survive and thrive, and use that understanding to interpret skeletal data as how likely a certain quality of life the individual enjoyed (Reitsema and McIlvaine, 2014).

Yet another problematic arises from the use of a biomedical term in bioarchaeological research: the absence of pathological indicators in the skeleton is not a synonym of good health or good wellbeing, because health and disease are not contrasting ends of a spectrum. Audy (1971, p.142) states that health "does not disappear during an illness to return on recovery but continues, even though it may drop in level while the organism is adapting to the current insult", indicating that health is a dynamic continuum, not a static state. As outlined by the Osteological Paradox, analysis of health in skeletal remains might prove challenging due to selective mortality (deceased individuals are inherently *unhealthy*, which

can lead to an overestimation of the occurrence of skeletal lesions within each age group) and hidden heterogeneity (the challenge of describing individuals who have survived long enough to exhibit skeletal stress markers as unhealthy, when in fact their survival could be indicative of their resilience and *good health*) (Wood *et al.*, 1992; DeWitte and Stojanowski, 2015). In addition, the biocultural context of health reflects on the dissimilar perceptions of what is considered a healthy individual today when compared to past populations, making a direct comparison between modern clinical ideas of health and archaeological data a highly subjective issue (Roberts and Manchester, 2010).

The bioarchaeological understanding of the health status of an individual or population is based on the construction of mortality distributions, analysis of growth and its potential disruption, and the presence of a multitude of non-specific indicators of stress (Goodman and Martin, 2002). As all these factors are mediated by the body's response to stressors, which is in turn permeated by genetic and environmental influences, health status derived from physiological response to stress is a highly individual process that varies through the life course and is dependant of sociocultural background. Thus, even though health is a key aspect of every human life, the concept itself has been a tricky one to pinpoint, in living and dead populations alike. Because of the multifaceted nature of the term, it might be impossible to ever ascertain with certainty what health meant for individuals in archaeological populations. Hence, although important to the discussion as part of this thesis' conceptualisation, each research chapter will not analyse "health", but body responses to physiological stress.

1.2.3 Growth, development, and senescence

A brief introduction to bone growth.

Human growth is defined by "progressive changes in size and morphology during the development of an individual" (Scheuer and Black, 2004, p.2), encompassing the physical, cognitive, emotional, and social development that occurs from infancy through adulthood. The growth process is controlled by both intrinsic and extrinsic factors, with an interplay of genetics and environmental influences affecting development (Bogin, 2020). Generally correlated with age, changes in size and maturity of skeletal elements are not linear, with

growth rates varying between populations and between individuals of the same population (Tanner, 1978; Scheuer and Black, 2004). As the human body is highly plastic, several developmental pathways coexist within the blueprint of development, which are triggered by environmental events that will induce a response in growth and maturation (Bateson *et al.*, 2004). Thus, the growth process will adapt to internal and external constraints either in a positive or negative way via developmental plasticity.

Skeletal growth is a complex process that occurs primarily during childhood and adolescence and refers to the development and maturation of the skeletal system, which includes bones, cartilage, and other connective tissues. As shown in Figure 1.2, longitudinal bone growth in long bones starts with the formation of a "cartilage template" around 49 to 52 days *intra utero*, which serves as the blueprint upon which primary centres of ossification appear and commence laying down a collar of bone around the midshaft between 7 to 8 gestational weeks via endochondral ossification. This formation of the initial periosteum via intramembranous ossification gives way, after birth, to the appearance of secondary centres of ossification that will form the epiphyses and epiphyseal plates, at both ends of the long bone. The epiphyseal plates, also known as growth plates, connect epiphyses and metaphyses, and are the site of proliferation, differentiation, and finally resorption of the chondroblasts, in a progressive dynamic of cartilage replacement by new bone. This process increments the length of the long bone and, in turn, increases the individual's stature. When the rate of cartilage proliferation in the growth plate is surpassed by the rate of deposition of new bone in the metaphysis, the growth plate decreases size and new bone forms connecting the epiphysis and the diaphysis, until no more cartilage is present. Then, longitudinal bone growth ceases, and bones can no longer increase in length (Scheuer and Black, 2004).

Alongside longitudinal growth, appositional growth results in increasing bone diameter (width). Osteoblastic action, via intramembranous growth, produce new bone tissue, which is added to the periosteal (outer) surface of existing bone, just below the fibrous periosteum (Figure 1.3). At the same time, osteoclastic activity on the endosteal (inner) surface triggers bone resorption; the balance between both periosteal and endosteal dynamics relies on a balanced process by which the medullary cavity increases medullary and cortical bone widths, assuring biomechanical stability during longitudinal growth (Scheuer and Black, 2004). This mechanism continues through childhood until the onset of puberty, when

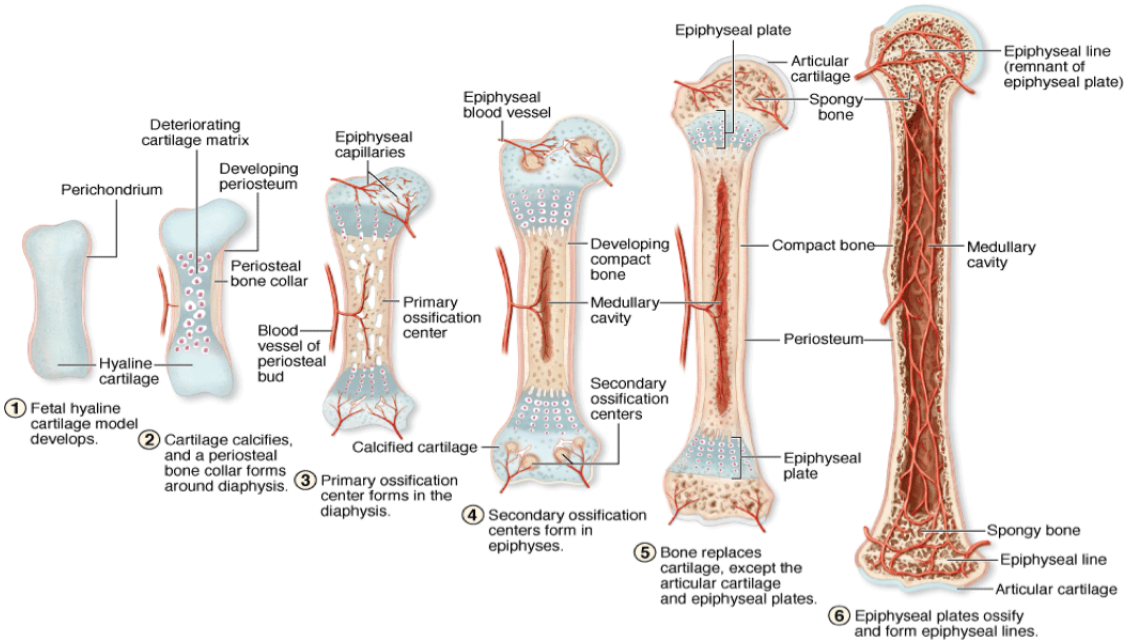

Figure 1.2 Longitudinal bone growth process, from foetal hyaline cartilage template to epiphyseal ossification and closure (in Mescher, 2016).

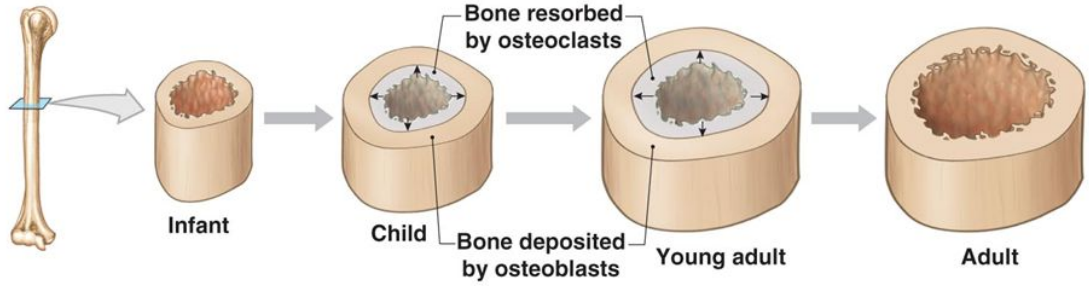

Figure 1.3 Appositional bone growth process. Osteoclast action increases medullary width via bone resorption, while osteoblastic action increases total bone width by bone addition (Copyright © 2010 Pearson Education, Inc.).

cortical bone apposition replaces bone resorption on the endosteal surface of bones (White, Black and Folkens, 2011). Hence, the process of appositional growth helps to strengthen and support the skeleton as the individual grows (Currey, 2006). After skeletal maturity has been achieved and growth ceases, bone still undergoes growth in diameter. Bone remodelling is a lifelong dynamic process that helps maintain the strength and integrity of the skeletal

system, playing a crucial role in several physiological processes, including fracture healing and maintenance of calcium homeostasis. It involves resorption of old or damaged bone on the same surface where osteoblasts lay new bone to replace that which is resorbed, helping to maintain bone strength, adapt to mechanical stresses, and repair micro-damage. Trauma, exercise, and other activities lead to remodelling (White, Black and Folkens, 2011).

Stages of the life course.

As the individual ages, they pass through different stages of growth and development, which are highly influenced by internal and external factors. Age can refer to the length of time that has passed since an individual's birth (chronological age), to the stage of maturation or the period during which certain physiological processes occur (physiological or biological age), or to the individual's position or status within a social group based on their perceived level of maturity, behaviour, and societal expectations (social age) (Lewis, 2007; Halcrow and Tayles, 2008). Both physiological and social age are linked, as age estimations made through bioarchaeological methods can be highly influenced by social and environmental factors (Adams and White, 2004; Gowland, 2007). By considering the interplay of these three perspectives on age, the trajectory of an individual's life is often roughly divided into distinct stages of "infant", "child", "adolescent", and "adult" (Table 1.1). These stages serve as frameworks for categorising skeletal data, which are then interpreted in relation to the expected norms for each stage in the individual's life (Duren *et al.*, 2013). During the growth process, several life stages are particularly important and determine the categories used by this thesis to understand the development of an individual. Four key processes are discussed in the research chapters of this thesis, as they can be studied through bioarchaeological research: the maternal-infant nexus during pregnancy and breastfeeding, growth and development during childhood, puberty and the pubertal growth spurt, and the process of senescence.

Bioarchaeology has recognised that the study of childhood, which starts during foetal and perinatal life and extends beyond the first decade of life, is of paramount importance within the field, as early life physiological plasticity is highly labile to environmental factors, providing much information regarding health, behaviour, and living environments within past populations (Goodman and Armelagos, 1989). The very earliest stages of life (i.e., the

Table 1.1 Age terminology used in this thesis, adapted from Lewis (2007), Sawyer *et al.* (2018), and Halcrow *et al.* (2021). The purpose of these categorisations is solely to facilitate the discussion pertaining to the data gathered in this thesis and might differ from other categorisations within bioarchaeological research.

Age terminology	Age range
Foetal	<40 gestational weeks of age (GWA), including preterm births
Perinatal	Between full term delivery to 28 days of life (40 GWA to 43 WA)
Infant	From 44 weeks of age (WA) to 1 year of age (92 WA)
Foetal-infant	From gestation to 1 year of age
Childhood	Foetal to 11 years of age
Adolescence	12 to 21 years of age
Non-adult	Foetal to 21 years of age
Adult	>21 years of age
Older adult	>40 years of age, including a subcategorisation into mature and senile adults (for a detailed description, see Chapter 6)

first 1000 days of life from conception to infancy) have been recent focus of research within bioarchaeology (Gowland and Halcrow, 2020), with the field drawing upon research from other related areas to explore the intricate relationship between maternal environment, foetal and infant growth, and breastfeeding that can be affected by diet, immunity, and stress in both the mother and her child (Thorsell and Nätt, 2016). *Intra utero* growth and development is characterised by a fast increase in body size, which reflects the dynamic and complex relationship between the mother and the foetus, which in turn reflects the child and their mother's health experience (Halcrow, Tayles and Elliott, 2017). They also reflect growth strategies that can be mediated by the foetus' biological sex or environmental factors affecting the mother during pregnancy (Eriksson *et al.*, 2010). Current research in bioarchaeology regarding the unique nexus between mother and child is advancing our understanding of infant and maternal stress, developmental plasticity, and the association between early life stressors and adult health outcomes (Lewis, 2017a; Hodson, 2018; Gowland and Halcrow, 2020). Much of this research has been conceptualised in light of the DOHaD framework, and includes studies on macroscopic palaeopathology to assess health, osteometric comparisons to assess growth, and isotopic analyses to explore stress timing to consider infant and maternal health, and their unique nexus (Halcrow, Tayles and Elliott, 2017).

The period of childhood is one of steady growth rate with decelerating velocity. Although postnatal growth usually follows a reasonable similar pattern for all individuals and no greatly significant differences in the grow curves between boys and girls of a population are usual, as sex differentials mediated by sex hormones does not start until the onset of puberty, variation in size and maturity is expected between individuals and populations (Bogin, 2020). Skeletal maturation tends to be more advanced in girls than boys, although this is at the expense of their bone density (Scheuer and Black, 2004). During this phase of growth and development, children are highly vulnerable to changes in climate, economic conditions, diet, disease exposure, and psychosocial stress (Figure 1.4) (Lewis, 2007). Infant mortality and morbidity are largely dependent of the physical environment the child was born into and lived, which makes their sensitive organisms an excellent window into study past population health. The stage of childhood ends with the onset of puberty, which generally starts earlier in girls than boys (Sawyer *et al.*, 2018), with the beginning of the adolescent stage of growth being another critical window of development.

The study of another key phase of the life course, adolescence, is of paramount importance due to the unique and transformative nature of this life stage. Adolescence is a critical period of rapid physical, cognitive, and psychosocial development that bridges the gap between childhood and adulthood (Sawyer *et al.*, 2018). This transitional phase is characterised by significant changes in brain structure, hormonal regulation, social interactions, and identity formation, all of which are permeated by childhood experiences and will in turn put through adult health outcomes (Dorn *et al.*, 2019). Onset of puberty leads to a rapid acceleration of the growth curve, reaching peak high velocity (PHV) associated with the pubertal growth spurt, and then decelerating until full maturity has been reached (Marshall and Tanner, 1986; Gluckman and Hanson, 2006). Understanding and exploring the intricacies of adolescence is crucial for several reasons, as adolescence plays a pivotal role in shaping long-term health trajectories (Gluckman and Hanson, 2006). Health behaviours and habits established during this period tend to persist into the adult life, significantly influencing future well-being (Dahl, 2004). At the same time, an intriguing paradox regarding adolescent health emerges: in modern populations, adolescence is often regarded as one of the healthiest stages of life but it is accompanied by a staggering increase of over 200% in morbidity and mortality rates compared to childhood (Dahl, 2004). Notably, this rise in morbidity and mortality is not primarily attributable to chronic or infectious

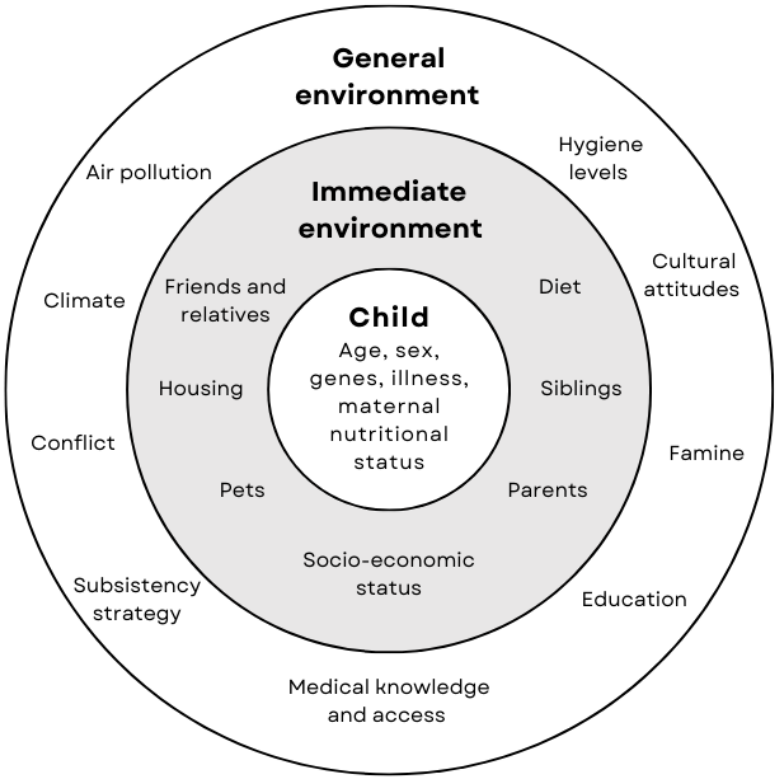

Figure 1.4 Socioeconomic factors that influence a child's health and their life experience by the World Health Organization (1993) (in Lewis, 2017b, p.2).

diseases, though it stems from challenges in regulating behaviour and emotions, leading to engagement in risky behaviours (Dahl, 2004). By studying adolescence, researchers can identify critical factors that contribute to healthy development and address potential risk factors that may impact long-term health outcomes, while also enabling a deeper comprehension of the societal and cultural influences that shape young individuals (Dorn *et al.*, 2019).

Despite the importance of this period, bioarchaeological research on adolescence is currently limited. Studying adolescence in archaeological context is challenging as there are fewer individuals being found on archaeological sites when compared to fully mature adults. This leads to poor representation in the funerary record (Lewis, 2007). Another reason is what Avery *et al.* (2022) call "the intangibility of adolescence": the methodological division of the life course between non-adults and adults in biological anthropology and bioarchaeology. Although the categorisation itself is based on developmental differences between childhood (when growth is still happening in the bones) and adulthood (when growth has ceased and skeletal degeneration starts), it leaves adolescents without clear

methodological or conceptual approaches in the osteological record (Halcrow and Tayles, 2008). Another source of limitations within the bioarchaeological study of adolescence is the division between biological and social dimensions of the individual (Avery *et al.*, 2022). Not only the risk of considering either dimension in isolation is present, but the definition of adolescence is in itself complex and responds to the idiosyncrasy of a population and time period. As to study adolescence in bioarchaeology needs for a biological age to be defined within a wider cultural context, Lewis (2022) notes that characterising adolescence into phases might benefit research in past populations (Table 1.2).

Table 1.2 Adolescent physical and psychosocial characteristics associated with different phases (in Lewis, 2022, p.521, adapted from State Adolescent Health Resource Center (SAHRC), nd).

Phase	Age*	Physical characteristics	Psychosocial characteristics
Early	10-14	Rapid physical growth and sexual maturation. Uneven growth results in awkward appearance. Often tired	Worries about being normal. Shyness, blushing, modesty. Emerging sexual feelings. Social contact with opposite sex often in groups
Middle	15-17	Continued physical development. Excessive activity alternating with extreme lethargy. Increase appetite. Increased need for sleep	Adapt sexually and establish sexual identity. Personal sense of masculinity or femininity. Learn psychosocial rules surrounding sexual behaviour
Late	18-24	Physical and sexual maturation mostly complete. Greater acceptance of physical appearance	New sense of physical self. Defined sense of identity. Developed stable and productive relationships. Meet demands of increasing roles and responsibilities

*Chronological age in years.

The extension of the adolescent years well into the third decade of life (i.e., 24 years of age) (Sawyer *et al.*, 2018) serves bioarchaeology as it allows the development of research into how physiological stress and frailty experiences might affect onset and progression of puberty, while also allowing space to note variation between sexes, populations and time periods (Arthur, Gowland and Redfern, 2016; Lewis, Shapland and Watts, 2016; Henderson and Padez, 2017; Doe *et al.*, 2019, 2022; Blom *et al.*, 2021; DeWitte and Lewis, 2021; Avery *et al.*, 2023).

The last life course stage pertinent to this thesis, senescence, is maybe the least explored of all the life stages in bioarchaeology. Senescence refers to the biological process of aging, characterised by a gradual decline in physiological function and an increased

vulnerability to diseases and, ultimately, death (Thomas *et al.*, 2003). At the cellular level, senescence is marked by a progressive loss of cellular division and replication potential. Cells in a state of senescence undergo changes in gene expression, telomere shortening, altered metabolism, and increased secretion of certain molecules, including pro-inflammatory factors (Muñoz-Espín and Serrano, 2014). Senescent cells can accumulate in tissues over time, contributing to age-related diseases and further impairing tissue function (Kuilman *et al.*, 2010).

The process of ageing is influenced by both genetic and environmental factors (Thomas *et al.*, 2003). While some aspects of aging are genetically determined, environmental factors such as lifestyle, diet, toxin exposure, and chronic physiological and psychosocial stress can accelerate the aging process. However, the exact mechanisms of senescence are complex and not fully understood. Several theories have been proposed to explain the underlying causes of aging, including the accumulation of cellular damage over time (e.g., DNA mutations and oxidative stress), the progressive shortening of telomeres, and the decline in the function of mitochondria (Kuilman *et al.*, 2010). While senescence itself is a natural process, it can contribute to age-related diseases and conditions, such as cardiovascular disease, neurodegenerative disorders, and certain types of cancer (Muñoz-Espín and Serrano, 2014). Understanding the mechanisms and processes of senescence is an active area of biomedical research, aiming to developing strategies to slow down or delay the aging process, promote healthy aging, and improve the overall well-being and quality of life in older individuals (Hayes, 2023).

Although sociocultural recognition of old age and ageing is an integral part of every society, (bio)archaeological research on the subject has been scarce (Appleby, 2010). Albeit neglected in comparison with other identities (e.g., gender, childhood, among others), archaeological studies of old age have explored how aging was perceived, experienced, and represented in different societies and time periods by examining archaeological evidence such as burials, skeletal remains, artifacts, and written records (Appleby, 2018). However, bioarchaeological research has been limited by the difficulty in accurately estimate age in older adults through skeletal remains (Buckberry, 2015). As noted by Appleby (2018), the difficulties related to estimating age in older adult skeletal remains might reflect physiological variability in the process of bone degeneration, rather than just methodological

imprecision. The correlation between chronological and physiological age during the life course decreases with ageing, as the degeneration processes are highly variable between individuals and skeletal age indicators are influenced by a variety of genetic factors, such as the inherent tendency towards bone formation and hormonal and reproductive factors (Mays, 2015). Additionally, environmental factors that have been observed to impact skeletal aging include biomechanical load of exercise and physical labour, nutritional and socioeconomic status, climate, medication and drugs, and body mass index (Márquez-Grant, 2015).

The complexity of assessing the process of ageing using bioarchaeological methods might never be overcome. However, new approaches to skeletal age categories using the life history and biology of senescence might help bioarchaeology in the quest for the study of old age: the life history model of Roksandic and Armstrong (2011) and the "functional age" categorisation of Tayles and Halcrow (2015). Both models propose to create biologically meaningful categories for ageing, particularly for older adulthood, bridging the gap between biological and social status. However, both models rely on a somewhat hazy differentiation of age-related changes into different stages that are based on sociocultural contexts or might be particular to the population in study. Thus, as Appleby (2018, p.147) argues, "rather than a 'one size fits all' approach to skeletal ageing of older individuals, we may have to take different approaches according to the research question being asked". Rather than discouraging, this means that the bioarchaeological study of old age needs to engage with the complexities of each sociocultural context, challenging stereotypes and assumptions about aging in the past by providing a nuanced understanding of the diversity of experiences among elderly individuals in different cultures and time periods. By examining how societies accommodated and valued their older members, bioarchaeology can gain a more comprehensive perspective on the human life course and the complex dynamics of aging within past communities.

Growth disruption.

As physiological growth is a highly plastic process influenced by the interplay of genetic and environmental factors, bioarchaeology has relied for decades on the assessment of growth and its alteration to explore the effects of health assaults on development (Boas, 1912; Hoppa, 1992; Larsen, 2015). Using a biocultural perspective, bioarchaeological studies on growth

disruption explore delays in maturation rates considering genetic factors in conjunction with environmental onslaughts from varied sources, such as prenatal and maternal stress, illness and disease during life, trauma and abuse, social inequalities, pollution and climate, access to healthcare, among others. Within this framework, "critical periods" of human growth (i.e., the first 1000 days of life and puberty) are of great significance as they can inform the how physiological stress and health assaults disrupt or alter normal growth rates of skeletal and dental elements. Two main lines of growth disruption will be presented further: alterations on longitudinal growth and dental development and eruption.

Longitudinal growth disruption refers to a disturbance in the normal progression of growth over time, typically involving the impairment or interruption of long bones' linear growth (Bogin, 2020). Several factors can impact the process of skeletal growth, with some genetic conditions and disorders resulting in stunted or excessive growth. Certain genetic disorders can cause disruptions in longitudinal growth, such as achondroplasia, Turner syndrome, or Marfan syndrome; they can affect bone growth, leading to short stature or abnormal proportions (Ergun-Longmire and Wajnrajch, 2020). Hormones play a crucial role in regulating growth, thus imbalances in hormone production or function, such as growth hormone deficiency or excess, thyroid disorders, or pituitary gland abnormalities, can interfere with longitudinal growth (Robson *et al.*, 2002; Dattani and Preece, 2004). Although maximum adult stature is largely dependent on genetic hereditability, studies show that environmental influences can heavily influence the expression of an individual's genetic potential (Duren *et al.*, 2013). Both longitudinal and appositional growth have been found to be impacted by environmental assaults (Mays, Ives and Brickley, 2009; Gooderham *et al.*, 2019), influencing cellular division and differentiation, and maturation of osteoblasts, osteoclasts, and chondroblasts that can lead to growth alterations and retardation. Chronic illnesses that affect overall health and nutrition can also impact longitudinal growth. Conditions such as malnutrition, kidney disease, or gastrointestinal disorders may hinder the body's ability to grow and develop properly (Swolin-Eide, Hansson and Magnusson, 2013; Budzulak *et al.*, 2022). Certain medications, particularly those used long-term or during critical windows of development might retard skeletal growth; for instance, corticosteroids prescribed for conditions like asthma or autoimmune disorders have an impact on growth velocity rates (Mushtaq and Ahmed, 2002; Pruteanu *et al.*, 2014). Physical injuries to the growth plates, such as fractures or damage from trauma during childhood, can disrupt the

normal growth process and potentially lead to limb length discrepancies or abnormal bone development (Perron, Miller and Brady, 2002; Xian *et al.*, 2004).

Dental development disruption refers to abnormalities or disturbances in the normal growth, formation and eruption of teeth (Nelson, 2014). Although several factors have been linked to contribute to the disruption of dental development, teeth have been found to be less sensitive to environmental assaults than bones, thus retaining a somewhat normal growth pattern when compared to skeletal elements (Cardoso, 2007). Despite the resilience of dental development to physiological stress, dental anomalies arise due to intricate interplay among genetic, epigenetic, and environmental factors throughout the extensive course of dental development (Riga, Belcastro and Moggi-Cecchi, 2014). This developmental process is multifactorial, operating at multiple levels and dimensions, and unfolding progressively over time (Brook, 2009). Genetic disorders or mutations can affect the development of teeth, as conditions such as amelogenesis imperfecta, dentinogenesis imperfecta, or ectodermal dysplasia can cause abnormalities in tooth enamel, dentin, or tooth structure (De Coster *et al.*, 2009; Chhabra, Goswami and Chhabra, 2014). Some developmental disorders, such as Down syndrome or cleft lip and palate, are also associated with dental abnormalities affecting the number, size, shape, or structure of teeth (Moraes *et al.*, 2007; Akcam *et al.*, 2010). Physically traumatic events involving the mouth, mandible and maxilla during the developmental stages can disrupt tooth development, as injuries that damage tooth buds, deciduous teeth, or the supporting structures can result in malformation or delayed eruption of teeth (Nelson, 2014). Nutritional deficiencies and inadequate intake of essential nutrients, particularly during early childhood, can affect tooth development. Deficiencies in minerals like calcium, phosphorus, or vitamin D can lead to weakened enamel, delayed tooth eruption, or enamel hypoplasia (King, Humphrey and Hillson, 2005; Martelli *et al.*, 2014; Rj *et al.*, 2021). Certain medications taken during pregnancy or early childhood can interfere with tooth development; for instance, tetracycline antibiotics, when used during dental development stages, can cause tooth discoloration and mineralisation defects (Bjorvatn and Olsen, 1982; Kameli *et al.*, 2019). Finally, exposure to environmental toxins or substances can disrupt dental development, such as prenatal exposure to tobacco smoke, excessive fluoride intake during tooth development, or exposure to heavy metals such as lead or mercury (Fejerskov *et al.*, 1994; Billings, Berkowitz and Watson, 2004).

1.2.4 Biocultural perspectives of disease

Palaeopathology explores the experience of health and disease in the past through the analysis and contextualisation of pathological changes in skeletal human remains, in an effort to better understand epidemiological trends throughout history (Kyle *et al.*, 2020). Despite the limitations outlined in previous sections, is of paramount importance for assessing health experiences in past populations to consider a biocultural approach to disease (Parkinson and Talbot, 2017). Recognising the extent to which modifiable environmental risks contribute to disease and health outcomes that can be evidenced in skeletal remains is crucial for increasing the value of bioarchaeological studies on past health. As Penny-Mason (2020, p.28) so clearly summarises, "the environment helps to determine the health status of an individual or a population – health and disease are mediated throughout the life course based upon biological temporality".

At the basis of the findings in this thesis lies the impact of the environment on growth and survival. Social and historical background for this population points towards migration, social inequality, and urbanisation as the key environmentally-related processes that affected the health outcomes of this sample.

Migration.

There is a complex relationship between migration and health that can have both positive and negative impacts on the health of individuals and populations. Differential prevalence of certain diseases or illnesses and related health outcomes due to migration are the result of complex interactions; for example, the spread of environmentally-limited diseases such as vector-borne illnesses (e.g., malaria, Chagas' disease, yellow fever, among others) is determined by environmental conditions (Gushulak and MacPherson, 2006). Other disparities in environmentally-related but non-communicable diseases also exist, including geographical exposure risks, such as health outcomes associated with high altitudes (Gassmann *et al.*, 2019), and deficiencies in micronutrients in the diet across populations (El-Ghannam, 2003). Disease transmission is also affected by the movement of people, impacting the epidemiology in the receiving region and on the health outcomes of the local population (Gushulak and MacPherson, 2006). It is important to note that health

experiences of migrants can vary widely depending on factors such as their migration status, socioeconomic background, the conditions in their countries/locations of origin and destination, and the policies and support available to them.

Over the past century, urbanisation (i.e., the increase in the proportion of people living in cities relative to rural areas) has emerged as a significant global demographic transformation, fundamentally altering the way the majority of the world's population has lived for thousands of years (Clark, 2003). The field of urban health explores the potential influence of urban environmental characteristics on population health (Galea and Vlahov, 2005), a topic bioarchaeology has studied for decades. In their extensive article on the subject, Betsinger and DeWitte (2021) note that bioarchaeological research on urbanisation can be grouped into three broad categories: comparison between urban and rural populations, exploration of urbanisation at a particular point in time, and studies examining the effects of urbanisation over time. The particular scenario pertinent to this thesis involved economic migration within the same country (see Chapter 1.3), as this population faced two dynamics closely associated with migration: the change from rural to urban living conditions, and the adjustment to the process of urbanisation.

Research focused on the rural/urban dichotomy has brought important insight into the demographic differences in mortality and morbidity of living in urban and rural environments, examining skeletal indicators of stress, health, frailty, survival, patterns of growth and stature, among others. Findings of these studies are not consistent, as some indicate that rural environments lead to worse health conditions (Rohnbogner and Lewis, 2017; Gamble, 2020), others suggest urban life was more detrimental for health in the past (Redfern *et al.*, 2015; Walter and DeWitte, 2017; Nagaoka *et al.*, 2019; Ives and Humphrey, 2020), while some found no differences between settings (Mays and Brickley, 2018). However, comparing rural and urban living environments has its own limitations, as it assumes homogeneity of each environment, when in reality urban and rural settlements are also impacted by contextual factors such as socioeconomic differences and migration (Kowalewski, 2020). Another way in which research has focused on this subject has been through the exploration of patterns of health and disease in urban settings through variables such as sex differentials, social status, age-at-death, migration, among others (Cho and Stout, 2011; Hagg, Van der Merwe and Steyn, 2017; Newman and Gowland, 2017; Ives and

Humphrey, 2020; Casna *et al.*, 2021; Newman and Hodson, 2021; Casna and Schrader, 2022). These kinds of studies show particularly well the internal heterogeneity of urban settings, highlighting how the variability of the life experiences of an urban population might be related with other variables, including socioeconomic status (Betsinger and DeWitte, 2021).

Socioeconomic status.

The connection between socioeconomic status (SES) and health outcomes has been extensively studied, with numerous studies demonstrating that lower SES is associated with poorer health outcomes across various dimensions (Glymour, Avendano and Kawachi, 2014). As SES is typically measured by factors such as income, education level, occupation, and wealth, individuals with lower SES are more likely to experience limited access to quality healthcare, including preventive services, regular check-ups, timely treatments, or live in areas with inadequate healthcare infrastructure (Babones, 2008). These factors contribute to disparities in healthcare access and use, and can result in delayed or suboptimal care, leading to negative health consequences. Studies suggest that worldwide, age-adjusted mortality for individuals of low SES are double or triple that for those in high SES (Phelan, Link and Tehranifar, 2010). In addition, lower SES is often associated with increased exposure to environmental hazards, such as pollution, unhealthy living conditions, and limited access to nutritious food options (Phelan, Link and Tehranifar, 2010). Prolonged exposure to stressors can negatively impact physiological systems and increase the risk of developing various health problems, including hypertension, diabetes, immune system dysfunction, and mental health issues (Glymour, Avendano and Kawachi, 2014).

However, the generalisability of the impact of SES on health should be taken into account. Although it might seem an obvious connection, studies have found that the strength of the link SES-health fluctuates across and within populations and time periods (Adler *et al.*, 1994). For example, an interesting observation in Swedish research is that the inverse association between SES and coronary heart disease risk is not universally consistent across all contexts. Studies found higher risks of chronic rheumatic valvular heart disease in immigrants from outside Europe in Sweden, even after living in the country for an extended period of time (Wändell *et al.*, 2022). In contrast, the health gradient based on SES tends to

be less pronounced between Swedish nationals (Feinstein, 1993). Furthermore, no social gradient was found in the mortality patterns of older adults (>60 years of age) from 19th century pre- and industrialised Sweden (Edvinsson and Broström, 2012). This suggests that although behaviour-specific health outcomes can vary over time, the connection between SES and health in childhood is maintained into adulthood, in line with the DOHaD framework.

The health-SES gradient has been extensively studied in bioarchaeology, ranging from studies comparing dental diseases of elite and lower classes in Classic Maya settlements in Mexico, studies comparing health status of monastic and nonmonastic communities in England, effects of SES on health in African- and Euro-American males of low SES in 19th century USA, to the implications of differential SES related to the processes of industrialisation and urbanisation in England, to name a few (Cucina and Tiesler, 2003; de la Cova, 2011; DeWitte, Boulware and Redfern, 2013; Newman and Gowland, 2017; Yaussy, 2019; Godde, Pasillas and Sanchez, 2020; Mathena-Allen and Zuckerman, 2020; Casna and Schrader, 2022; Marklein and Crews, 2022). These studies suggest that the complex interplay between SES and health outcomes can be influenced by a range of individual, social, and structural factors, and might be carried throughout the life course. Understanding these variations in different contexts provides valuable insights into the underlying mechanisms and social determinants that contribute to health disparities related to SES.

Access to healthcare and treatment.

Another issue to consider regarding the population studied in this thesis, is the access to healthcare and treatments during this time period. The relationship between treatment, healthcare, and health status is complex and multifaceted (Roberts and Manchester, 2010), and although it is in part related to an individual's or community SES, is also contingent on the existence of a treatment for a certain disease and the health policies related to its distribution (Apouey, 2013).

Existence and availability of treatments has changed over time. Vaccination efforts, from early variolation to fast response vaccines against COVID-19 in recent years, have been

around for more than 300 years, improving health outcomes against bacterial and viral infectious diseases (Plotkin, 2014; Chong et al., 2022). However, when prophylactic (i.e., preventative) vaccines were developed, the turn from them to therapeutic (i.e., once the illness has already affected the individual) vaccines was not immediate and was highly dependent on governmental policies, particularly in countries in the Global South (Kayser and Ramzan, 2021). Following the discovery of penicillin in 1928 by Alexander Fleming, antibiotics have revolutionised the fight against infectious diseases worldwide, giving way to development of more than a hundred different antibiotics (Nigam, Gupta and Sharma, 2014). This success, however, has been marred by the rise and spread of drug resistance, a process attributed to evolutionary selection against antibiotics and high human mobility across continents (Levy and Marshall, 2004). Other types of treatments (such as antibody treatments for passive immunity) have been overshadowed by the success of antibiotics and vaccination, but continue to cater for diseases that mainly affect the Global South, such as diphtheria, tetanus, and botulism (Keller and Stiehm, 2000). Moreover, the identification of genetic and environmental drivers of chronic diseases (such as coronary heart disease, type 2 diabetes, breast cancer, among others) has led to the development of public health policies oriented to prevention of risk factors during early life (Barker, 2012).

Access to quality healthcare services through all stages of the life course, including prevention and health promotion, early detection and treatment, and chronic disease management, is essential for maintaining and improving health (World Health Organization, 1984). Public health studies suggest that population's health and national income inequality are highly related, with governmental health policies directly related to indicators such as average life expectancy, infant mortality rate, and chronic diseases rate (Babones, 2008). Thus, not only individual level SES plays a significant role in access to healthcare and treatment, but country level socioeconomic inequalities, that fluctuate though time periods, can mark the health experience of a population. Because of these issues, socio-political contextual information should be taken into consideration when conducting bioarchaeological analyses on skeletal remains, because even as non-survivors these individuals are a reflection of the biocultural response of their society to health and disease.

1.3 Chilean historical background

The individuals analysed in this thesis are part of a documented skeletal collection from 20th-century Santiago de Chile (see Chapter 2.1 for details about the collection). They lived in low socioeconomic status areas of the city, inhabited at the time predominantly by economic migrants and working-class communities. To fully appreciate the social and political background of the population studied in this research, a brief summary of the history of the social and demographic changes in Santiago between 1850 and 1990 will be given.

1.3.1 Chilean rural-to-urban migration (1885-1952)

During the 19th and 20th centuries, the Chilean population experienced strong growth compared to previous centuries, rising from 1,010,336 inhabitants in 1835 (first official census) to 5,023,539 in 1940 (Republic of Chile, 2009). Population growth was accompanied by major changes in the country's demographic distribution, with large numbers migrating from the countryside to urban centres in the Norte Grande (Far North) and the Zona Central (Central Chile) regions (Figure 1.5). Throughout the first half of the 19th century, Chile was a predominantly rural country; only 30% of the Chilean population lived in cities in 1850 (Bauer, 2014). However, during the last third of the 19th century and the first half of the 20th century, the country experienced a major migratory phenomenon to urban centres, particularly to the country's capital, Santiago.

The Chilean rural-to-urban migration (1885-1952) was a consequence of several macroeconomic factors. Firstly, from 1850, the primary exporting system of the rural economy, based on the production of wheat, began to change as mining activities (mainly saltpetre and copper extraction) in the Far North region began to surpass agricultural production. For the exploitation of saltpetre, a large part of the rural population migrated to small urban centres in the north. To counteract the decline in agricultural exports, the owners of the central valley *latifundios* (landed estates) began a process of expansion and modernisation, at the expense of small and medium producers, whose already precarious living conditions worsened. Both phenomena of economic transformation caused a growth in foreign and national investments, allowing the increase in capital accumulation to expand the domestic market. The state increased its size and functions, and by the beginning of the

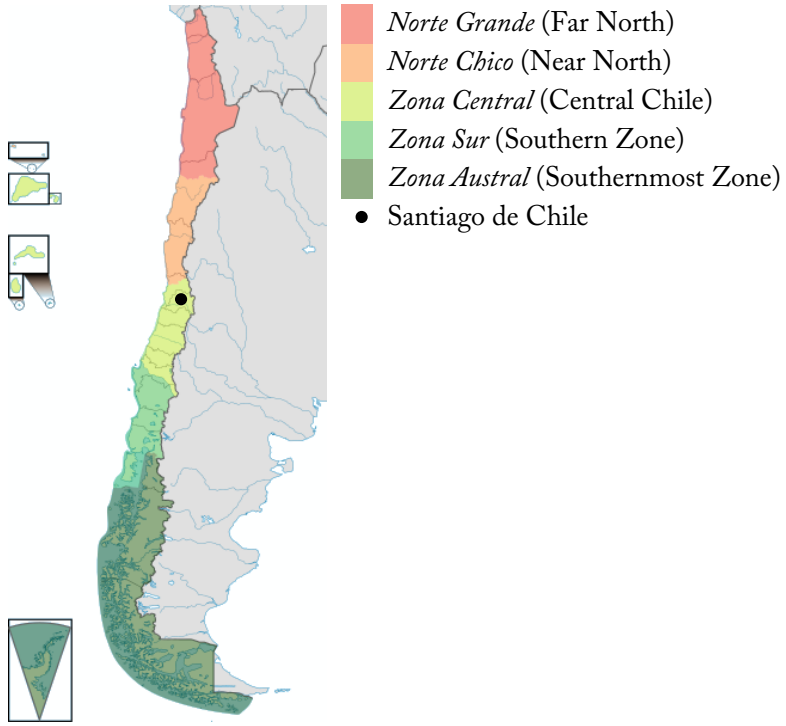

Figure 1.5 Map of the natural regions of Chile (image adapted from Janitoalevic, CC BY-SA 3.0 CL).

20th century, an industrialisation process started in some provinces, homologous to that of the Industrial Revolution in Great Britain (1760-1820/1840). Large urban centres, especially the capital of Santiago, accumulated new wealth and began to grow at a higher rate than the rest of the country; the demand for skilled labour rose in these cities in activities such as construction, domestic service, public employment, and military service (Coeymans, 1982).

By 1952, 1,755,000 people were living in the capital Santiago, 567,000 (32.3%) of whom were natives of other provinces. Of these economic migrants, 506,000 (89%) were born in the Central Chile region (Herrick, 1966); with its mediterranean climate, this region accommodated the largest part of the country's agricultural activities, and thus, the majority of Santiago's new inhabitants had been born and raised in the pre-industrial countryside in agricultural communities. The majority of the rural migrants that settled in Santiago came from six provinces in the Central Chile region: Valparaíso, O'Higgins, Talca, Ñuble, Concepción, and Cautín (Gómez, Arteaga and Cruz, 1981). The reasons why most rural migrants came from nearby regions can be explained by two main considerations; firstly, transportation costs increased dramatically with distance as there was no railway network to the south (just airplane and boat trips available, both expensive), while train transportation

to the north was better but did not reach the Far North. Secondly, the relative rates of growth of employment possibilities were higher in Santiago for migrants from nearby regions, as migrants from provinces in the north tended to move within the area due to the employment opportunities in the copper and nitrate mining industry and processing plants existing there. Southern provinces were less limited in their economic expansion (as the area is known for several extraction and collection of natural resources processes, such as farming, forestry, and fishing), enabling populations to find opportunities closer to home, but also had fewer inhabitants (roughly half the population of the north of Chile), so proportionally fewer individuals that could become migrants.

These demographic changes profoundly influenced Chilean social structure and were accompanied by an accelerated process of urbanisation and territorial redistribution, which transformed a society of marked rural character into a predominantly urban one (Figure 1.6). The heterogeneous and fluctuating nature of migration caused overpopulation and saturation of urban infrastructure, especially in the capital. When estimating the impact that internal migration had on the population of Santiago, authors have stated that between 1860 and 1950 the majority of population growth in the city was the result of migration rather than any increase in birth rate (Alberts, 1977). Governmental records show that only from 1970 the city's growth can be explained by a natural increase (calculated subtracting the crude death rate from the crude birth rate of the given region) rather than migration (Rodríguez, 1993).

During the first half of the migratory process, women made up the bulk of the migrants to urban centres (Rodríguez, 1993). These were young and unmarried women between 15 and 35 years of age whose working opportunities in agricultural production were scarce. Women were the first to migrate to urban areas, finding employment in the growing domestic service as servants, laundresses, cooks, and seamstresses (Milanich, 2010). By 1875, just a few years after the canonical beginning of the rural-to-urban migration, domestic work provided 85% of female employment in Santiago (Milanich, 2010). After 1960, census information shows an increase in male migration towards Santiago, outnumbering women migrants, although the total migration did not account for the majority population growth, as births did (Rodríguez, 1993). It is important to note the shift between female/male migrants during this period, as the individuals analysed in this study might reflect this change;

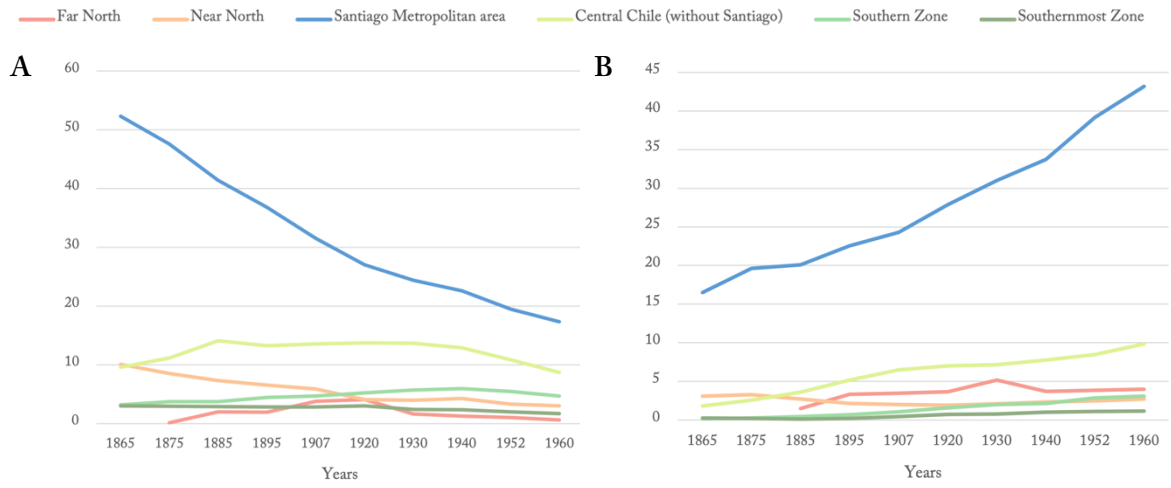

Figure 1.6 Distribution (%) of rural (A) and urban (B) Chilean population between 1865 and 1960 by region (adapted from Hurtado Ruiz-Tagle, 1966, p.145).

for the non-adult sample used in this study, which includes foetal, perinates, infant, children and adolescents, cemetery archives record years of death between 1960 and 1986. It is then likely that the mothers of these non-adult individuals might have been economic migrants or first generation *santiaguinas*. The older adult sample analysed (40-96 years of age), with years of death between 1957 and 1986, might be representing the migrants themselves. And if not migrants, their life experience would have been greatly similar to that of the economic migrants that came to inhabit Santiago, who settled in poverty-stricken areas of the city, which were devoid of public services and in poor material conditions, such as a lack of health care, poor hygiene, and limited access to basic services (Eyzaguirre and Errázuriz, 1903).

1.3.2 Social marginality and health experience in Santiago (1960-1990)

The internal migration of the late 19th and early 20th centuries in Chile resulted in poor living conditions for the new inhabitants of major cities, particularly Santiago. The highly mobile growing population was attracted to new employment opportunities in the urban centres, increasing the number of inhabitants in cities that were not prepared for the migratory movement, meaning the newcomers often endured inadequate, overcrowded living spaces and poor sanitation. In many ways, the processes that shaped Santiago during the early 20th century mimics those of industrial revolutions occurring in various periods across the world (Engels, 1950).

Although Santiago was utterly unprepared for the growing number of new inhabitants that followed the rural-to-urban migration, authorities have been trying to enforce some improvements in its sanitary infrastructure since Colonial times (1600-1810). In this area, the Bourbon Reforms, Spain's official program for introducing Enlightenment ideas into the administration of its empire, had committed to apply the goals of urban medicine. As described by Foucault, the notion of urban medicine was that "not of men or of the body, but of things and of living environmental conditions" (Foucault and Rabinow, 2002). These ideas developed during the 18th century, and from them emerged a century later the concepts of salubrity, a notion related to cleanliness and the condition of the environment and its elements, and to public hygiene, related to the political and scientific control of the environment and its hazards (Ibarra, 2015). The idea was that illnesses spanned beyond the individual sphere into an institutional structure that could, and should, be controlled collectively was implemented by the Bourbon Reforms through the newly created administrative units called *intendencias*, that watched over the implementation of the three main aspirations of urban medicine:

- To contribute to the circulation of air and water, as these elements were key to the miasmatic theory of the time.
- To organise the adequate distribution of the urban infrastructure that allowed collective life, such as water fountains, drainage and drinking water.
- To survey, recognise and ameliorate the effects of large-scale contact between people that could generate illnesses and expand epidemic or endemic phenomena, such as slaughterhouses, public buildings, and cemeteries.

The Colonial authorities in the major urban centres in the Captaincy General of Chile enforced these precepts during the 18th and the beginning of the 19th centuries with varied success. European Modern medicine came to Latin America during late Colonial times and resulted in the proliferation of public health physicians, or *higienistas*, that pushed for hygiene and public health to be part of the urban agenda. After the Independence period (1810-1823), the new Republican authorities continued along the path set by the Bourbon Reforms, at least on matters of salubrity and public hygiene. A number of authorities, scholars and professionals envisaged urban projects based on the ideals of *higienismo*,

considering the need for an institutional sanitary framework to deal with illnesses and diseases that could arise faster and prove deadlier in poor living conditions (Ferrer, 1911). One of the most prominent of these was the proposal made by the *Intendente* of Santiago, Benjamín Vicuña Mackenna, who submitted to the National Congress in 1872 a 20- project plan to improve the urban and sanitary landscape of the city. Among his ideas were the improvement of water supply, the canalisation of the River Mapocho, the opening of new and blocked streets, and the enhancement of existing and new school infrastructure, claiming that buildings were determinant for the overall sanitation of the city's urban setting (Vicuña Mackenna, 1874). All efforts culminated in the foundation of the *Instituto de Higiene* (Hygiene Institute) in 1892, which took charge of research on public health conditions in Santiago, as well as the management of the technical equipment and personnel assigned to the disinfection of tenements and houses through the *Desinfectorio Público* (Public Disinfectorium) (Figure 1.7).

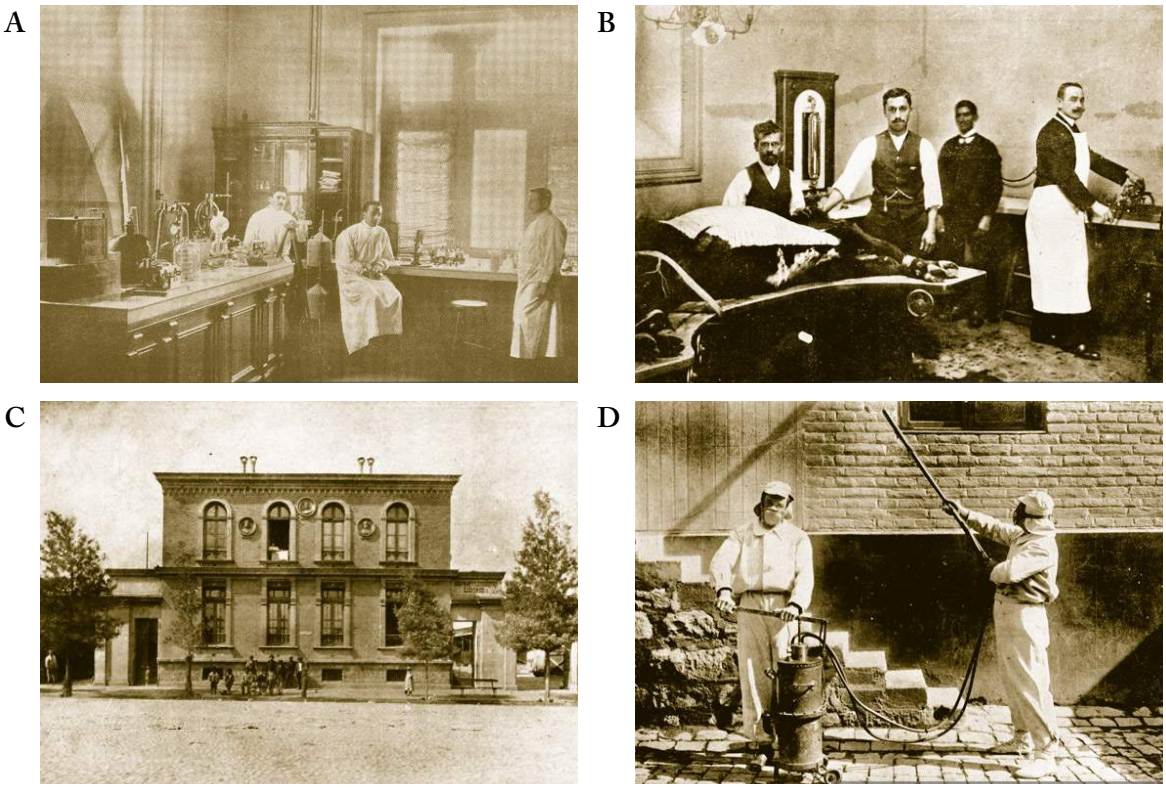

Figure 1.7 Governmental efforts to improve public health in Santiago, circa 1910. Bacteriology division at the *Instituto de Higiene* (A), harvest of vacciniferous lymph (B), building of the *Desinfectorio Público* (C), and disinfectors at work (D) (from Ferrer, 1911).

However, actions taken by the authorities to improve public health during the previous century were not prepared to withstand the relentless expansion of the city's population which characterised the end of the 19th and beginning of the 20th centuries. The city's continuous growth was not matched by an equally fast and efficient expansion of basic living and sanitary infrastructure. Newcomers and working-class inhabitants of Santiago had little choice in housing: they could choose to live in *conventillos*, unventilated rooms, usually overcrowded, situated along a narrow interior passage, within the limits of the city, or at the rapidly proliferating tenements at the outskirts of Santiago (Figure 1.8). But wherever they settled, transportation, water supply, electricity, health infrastructure and sanitary facilities were overstretched or scarce (Eyzaguirre and Errázuriz, 1903).

The controversy of the housing problem was the centre of public debate during the late 19th century. Discussions about public hygiene even transcended the country's boundaries and reached the American Sanitary Congresses held in several Latin America cities from 1888 onwards. The first three of these Pan-American meetings, in particular, addressed the problem of urban hygiene and its relation to the population's health, emphasising high rates of infant mortality and the deficient housing available to the working class. The latter would be a source of intense debate for medical professionals, architects, urban planners, and authorities until the 20th century, with the First Pan-American Congress of Architects in 1920 stating that "it is preferable to live under the trees as the primitive man than in the filth-infected tenements" (Ibarra, 2015, p.187). Three years later, at the Second Pan-American Congress of Architects, its members urged for more discussions of "issues of true social importance" (Ibarra, 2015, p.191), which they identified as working-class housing, sanitation, and public health.

In Chile, the issue of working-class housing had been left aside during the second half of the 19th century, as the country faced economic recession from 1876. The economic downfall aggravated the already pressing matter of urban sanitation, yet regulations only started to be imposed after the cholera outbreak of 1886 with the creation of the Commission of Public Health (renamed Superior Council of Public Hygiene a year later). By 1906, in the midst of the surge of migrants that arrived in the capital looking for new working opportunities, regulation number 5950 ordered the demolition of dwellings located in unhealthy areas and constructed with unhygienic materials, such as the slums that had arisen

A

B

Figure 1.8 The two faces of low socioeconomic status living conditions in Santiago during the 20th century: interior of a *conventillo* in the city centre (Brasil Avenue) on October 20th, 1920 (A), and a house in Maipú, at the outskirts of the city c. 1958 (B) (image A from CHILECTRA (2001); image B from researcher's personal archive).

on the periphery and the *conventillos* in the city centre. However, this regulation was left in the hands of local government and its ability to enforce such ordinances was limited; *conventillos* and slums continued to be in use until at least the 1970s (Ibarra, 2016).

The high mortality rates that were the norm in Santiago during this period of migration disproportionately affected the working class, whose living conditions were seen by authorities and the media as both the source and the catalyst of their poor health. The idea of "civilisation versus barbarism" (Chávez Zúñiga, 2018) permeated the accounts and political debates around what to do with the working-class housing problem; those supporting this idea alleged that not only the lack of hygiene and sanitation was responsible for the high mortality rates, but also and more importantly, responsibility lay with the social context of the population. The labouring population was seen as having intrinsically lesser morale and being inherently susceptible to social ills that prevented them from achieving a full 'civilised' state. Political authorities, with the support of sanitary professionals such as medical doctors, claimed that reforming and improving social housing was the only way "to eradicate the most powerful causes of infant mortality, degeneration of the race, alcoholism, criminality, and many other moral ills that affect [working-class] families" (Chávez Zúñiga, 2018, p.274). An economically deprived social context, embodied in the *conventillo* and its deficient sanitary condition, was regarded by authorities and elites of the time as the reason for ill health in infants, and therefore the men and women that they were to become could not be the healthy and fit citizens that would take part in the economic development of the country. The idea that unhealthy environments shaped the morale of future citizens and led them to criminality and alcoholism was key to the governmental decision to alleviate the social housing problem. From a modern perspective, the vision that the political and economic elites had of the working class and their health experience can be seen through the paternalistic and patronising prism that the intellectual classes, worldwide, had when dealing with impoverished people at the time. The perspective of ill health and higher mortality as intrinsic characteristics of the less wealthy portion of the population, that reflects their environment but that can be changed and improved only when authorities and elites reach out to 'save' them, is one that continues to permeate modern ideas of poverty and economic mobility. The issue is, however, much more complex; the DOHaD approach, used as theoretical framework throughout this study, highlights the importance of the mother-infant nexus, childhood health insults, and environmental factors in adult health outcomes (Fall

and Sachdev, 2006). In this sense, many of the 'uncivilised' traits attributed to the working-class population (e.g., higher infant mortality, heart disease, and alcoholism) have a root in previous generations. By proposing the improvement of housing and living conditions of the low socioeconomic areas of the city, authorities thought they would immediately remediate the 'vices of the poor', but that was not the case (Chávez Zúñiga, 2018, 2019).

The overcrowded and unhygienic living conditions in the *conventillos* and peripheral slums were such that common diseases developed and spread much faster than any response taken by the sanitary authorities to fight them. By 1880, infant mortality exceeded 300 per thousand live births and life expectancy at birth for a working class man was not more than 28 years old (Illanes, 1993). Infant mortality would remain high during the early 20th century, with a steady but slow descent over the next decades (Figure 1.9). Parasitic plagues such as ringworm and scabies were common among the entire working class, while a number of illnesses such as smallpox, chickenpox, scarlet fever, measles, typhoid fever, tuberculosis, whooping cough, and diphtheria were always present and periodically developed in epidemic outbreaks. The 1886-1887 outbreak of cholera in Santiago had epidemic features that killed up to 5% of the population and was followed in later years by several more outbreaks that flourished in working class neighbourhoods. In addition, just between 1890 and 1895, smallpox killed 24,618 people; even though a prophylactic vaccine was available in the country, immunisation programmes were irregular, and the policy was to provide it to the population affected after an outbreak (i.e., therapeutic vaccine) as a governmental measure of economic austerity (Borgoño, 2002).

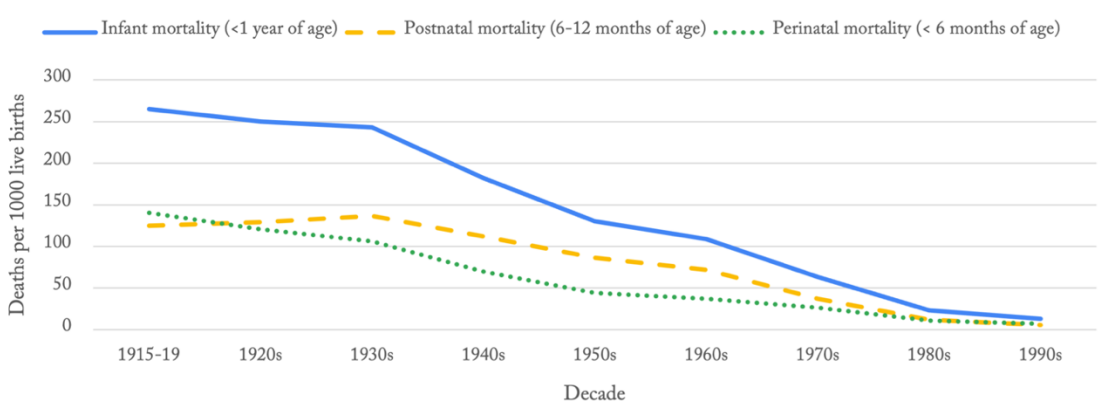

Figure 1.9 Infant mortality in Chile, yearly mean age from 1915 to 1990s (adapted from Llorca-Jaña *et al.*, 2021).

Among those most affected by illnesses and epidemics were children and old people. Although the city's population steadily increased during this period due to internal migration from the countryside, natural population growth stalled as a consequence of the high infant mortality the city experienced (Chávez Zúñiga and Soto-Lara, 2018). By the end of the 19th and the beginning of the 20th centuries, authorities dubbed infant mortality in Santiago as a "demographic catastrophe" (Murillo, 1896, p.7). Not just the harmful environment of poverty was blamed by authorities and elites, but also the maternal capabilities of impoverished women; as an extension of their view of "uncivilised" population, working-class women were seen as not able to care for their children in the same nurturing way women from high socioeconomic class could (Chávez Zúñiga, 2019). However, following the ascension of the Radical Party to the government (starting in 1938 with President Pedro Aguirre Cerda), new governmental efforts were launched to increase sanitation in low-income housing areas and the implementation of various initiatives to improve nutrition of working-class children. Between 1930 and 1960, infant mortality in Santiago dropped to 100 per thousand live births. From 1960, a sustained decrease in the city's infant mortality meant that by the 1980s, Santiago registered less than 20 infant deaths per thousand live births (Rodríguez, 1993).

Senescence was impacted by lacking living conditions during this period, but also by the cultural view of old age itself. During early and mid-20th century, the medical opinion was that an individual became senile between 50 and 60 years of age (Correa Gómez, 2013), age after which they were liable of losing their legal autonomy. Although young for our modern standards, this senile age threshold reflected the Chilean population life expectancy at the time; by 1960, life expectancy was 58.02 years of age (55.27 for men, 60.95 for women) (Republic of Chile, 2009). Although life expectancy would rise through the 20th century, sex differences continued to exist (Table 1.3). Older adults in low SES communities during the period continued to work in hard labouring jobs when they were able to do so, without retiring (Espinoza, 1988). In the best of cases, they would remain in their own houses, but more often than not they were confined to charitable institutions that provided the bare minimum living conditions, and housed together those individuals with mental and physical disabilities as well as old people (Correa Gómez, 2013). The experience of old age in low SES communities of Santiago during the 20th century was one of invisibility; not included in public policies and at the margin of the economically active population.

Table 1.3 Life expectancy in Chile between 1960 and 1990 (adapted from Republic of Chile, 2009).

Year	Chilean population	Chilean males	Chilean females
1960	58.05	55.27	60.95
1965	60.64	57.64	63.75
1970	63.57	60.46	66.80
1975	67.19	63.94	70.57
1980	70.70	67.38	74.16
1985	72.68	69.59	75.89
1990	74.34	71.45	77.35

In addition to the hardships of economic migration and social inequalities in an ever-changing capital city, the health and well-being of the population of Santiago was vastly affected by the military dictatorship that ruled Chile for seventeen years from 1973 to 1990. Socio-political unrest, a severe economic crisis, and a set of regressive measures rooted in neoliberalism taken by the authoritarian government to insert health into the market economy (including the privatisation of the healthcare system) meant a stop to the many positive changes promoted in the country during the previous decade (Eliana, 2002). Social inequality in Chile deepened during the dictatorship years, and the devastating economic crisis of 1982 hit the already impoverished communities the hardest, as the military rule focused on rescuing private corporations at the sacrifice of social policies (Ffrench-Davies and Stallings, 2001).

At the very simplest level, the massive effect that economical migration and social inequalities had on the lifestyle and health affected every man, woman, and child's health and well-being. Although the transition from rural life to living in the country's urban centres was not done in one single step, the impact of the alteration of lifeways, diet, physical activity, and sanitary living conditions was such that it was vast. These lacking living conditions were shared by first generation of *santiaguinos* and the working-classes who already lived in the city, shaping their experience of health and life. These would not greatly improve in the years after, even hardening during the dictatorship period. At the core of this research are the contrasting health outcomes this population underwent on the basis of their SES. Social inequality in Chilean society, exemplified here by those who lived in Santiago around the end of the 19th and the beginning of the 20th centuries (Figures 1.10, 1.11, and 1.12), take roots and nurtures from several socio-political events, being the rural-to-urban migration one of the major episodes in Central Chile. Social stratification that emerged in the

landscape of the Greater Santiago around this time played, and still does today, a preponderant part in the population's access to healthcare, sanitary conditions, quality education, and ultimate likelihood of securing permanent and well-paid employment. This scenario, sketched ever so briefly, can be still appreciated in the modern population of Greater Santiago, where the children and grandchildren of those examined in this thesis inhabit today, and who still, in their great majority, live in the same area or in nearby neighbourhoods as their ancestors.

Figure 1.10 Children in Santiago during the 20th century, circa 1935 in Santiago Centro (A), 1954 in Maipú (B), 1960 in San Miguel (C), 1966 in Conchalí (D), 1967 in Barrancas (now Lo Prado) (E) and Barrancas (now Pudahuel) (F), and 1974 in La Cisterna (now Lo Espejo) (G) (images A to E from researcher's personal archive; images F and G from *Archivo Nacional de Chile*).

A

B

C

D

Figure 1.11 Adolescence in Santiago during the 20th century. "Imperio" and "Real Imperio" youth clubs celebration in 1962 in La Cisterna (now Lo Espejo) (A), schoolboys visit the "Concha y Toro" vineyard in 1968 in Pirque (B), "Club Holanda" sports club circa 1971 (C), and neighbourhood friends in 1972 in Lo Barnechea (D) (images A and D from *Archivo Nacional de Chile;* images B and C from researcher's personal archive).

A

B

Figure 1.12 Older adults in Santiago during the 20th century. 1977 in La Legua, San Miguel (A), and 1978 in La Cisterna (now Lo Espejo) (B) (image A from researcher's personal archive; image B from *Archivo Nacional de Chile*).

1.4 Summary

The effects of physiological stress on developmental homeostasis can disrupt the normal process of growth and lead to pathological changes in the skeleton. For children and adolescents, the experiences and living conditions encountered during critical developmental periods have long-lasting effects on their adult health and potentially their life course, as research suggests that childhood events lay the foundation for future health outcomes and can influence factors such as physical stature, susceptibility to diseases, immune resistance or deficiency, vulnerability to future health problems, and even cognitive development (Barker, 1997; Gowland, 2015; Bogin, 2020). Thus, growth disruption, mortality, and morbidity in children can be used as proxy for the overall health and wellbeing experiences within their population. Older adulthood health outcomes can also be explored using skeletal evidence of pathological conditions, informing on the potential interaction between early and later life stressors. For older adults, physiological stress can act upon an already frail phenotype which adds to the already heavy burden of illness, disease and degenerative changes in their skeleton.

This study attempts to offer a nuanced understanding of the interwoven biological and cultural factors that characterise bone response to physiological stress by focusing on the examination of the skeletal remains individuals from Santiago de Chile. There are myriad ways in which the body can respond to environmental (extrinsic) assaults, but only a few ways in which those assaults can show in the skeleton, leaving traces in developmental trajectories (i.e., disruption or delay of growth) and/or presence of skeletal pathological changes (i.e., osteoblastic or osteoclastic activity, or a combination of both). Exploring bioarchaeological evidence in light of contextual information (i.e., a biocultural approach) provides a better understanding of the multitude of factors that influenced growth, development, frailty and risk of death in this population, contributing to the field by reconstructing these individuals' response to stress from a life course perspective.

Chapter 2

Materials and Methods

2.1 Materials

The bioarchaeological sample selected for this study derives from a skeletal collection from Chile, known as the *Colección Osteológica Subactual de Santiago* (COSS). Sometimes translated as Modern Collection of Santiago or Santiago Subactual Osteology Collection, and formerly known as *Cementerio General* skeletal collection, it is curated in the Department of Anthropology at the University of Chile (UChile) and originates from exhumations undertaken at the *Cementerio General de Santiago* (Santiago General Cemetery). The COSS comprises both human skeletal remains and an archive of documentary information from the SGC, representing individuals from low SES living in the capital of Chile during the late 19th and 20th centuries (Meza-Escobar *et al.*, 2023).

The main objective of the remainder of this section is to introduce the COSS collection and describe its use as material for the present study. This section first describes SGC as a site. Second, the origin and current curatorial state of the COSS. Finally, the sources that contributed to the integrated database in terms of their origin and data composition are introduced.

2.1.1 The site

Santiago General Cemetery (SGC) is located in the north of Santiago (Figure 2.1), in an area regarded as the outskirts of the city during the 19th century. It represents the first urban and the second secular cemetery in Chile, established on December 9th, 1821, soon after the country gained its independence from the Kingdom of Spain (February 12th, 1818). The SGC was conceived as the model for the new republican cemeteries, devoid from religious background and with carefully arranged distribution of the burial plots, reflecting the power relations of the society of the living (Asenjo *et al.*, 2004; Bustos Stears, 2018). Its original layout was designed following two trends popular in urban cemetery landscapes at the time: the French pantheon, designed to serve as the burial place of the famous dead of the nation, and the Spanish monumental cemetery, of narrow alleys, grand architectural constructions, and sumptuous vegetation (Rugg, 2000). Cheaper burial plots, not associated with the aristocracy and bourgeoisie, were usually relegated to lateral roads and the most remote sections to the north, with almost non-existent vegetation. Although the SGC layout

has changed over the years, it is still possible to appreciate the original patterns of segregation today (Figures 2.2 and 2.3).

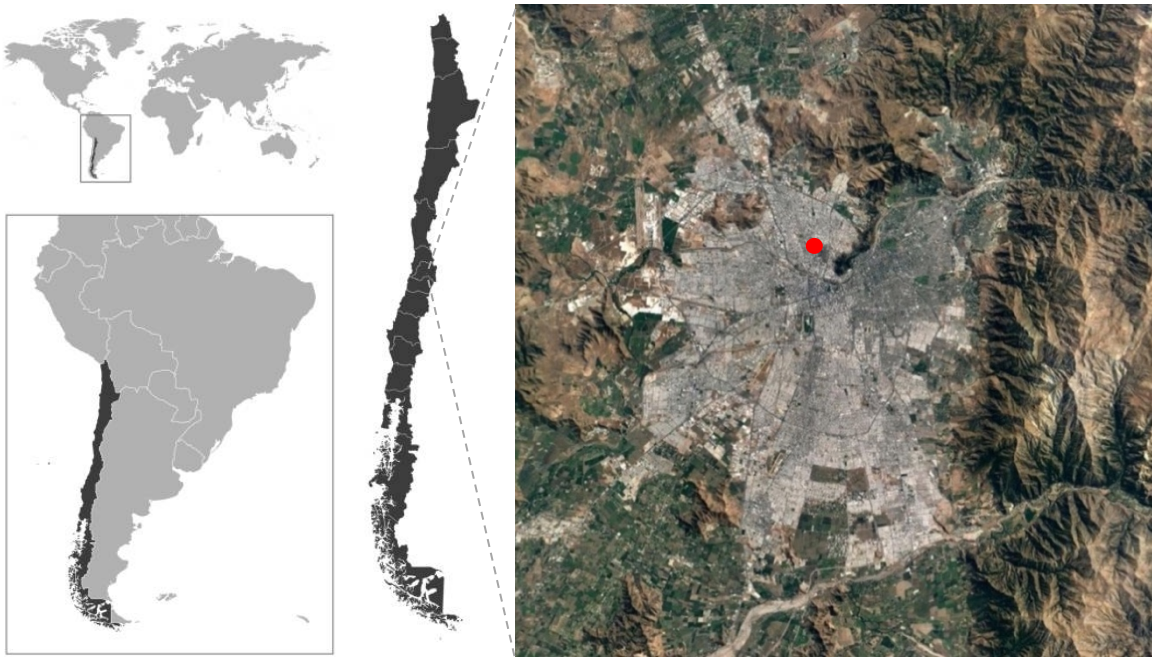

Figure 2.1 Santiago General Cemetery (red dot) is located in Santiago, the capital city of Chile, South America (Google Earth, 2023).

Figure 2.2 Present day satellite image of Santiago General Cemetery (Google Earth, 2023).

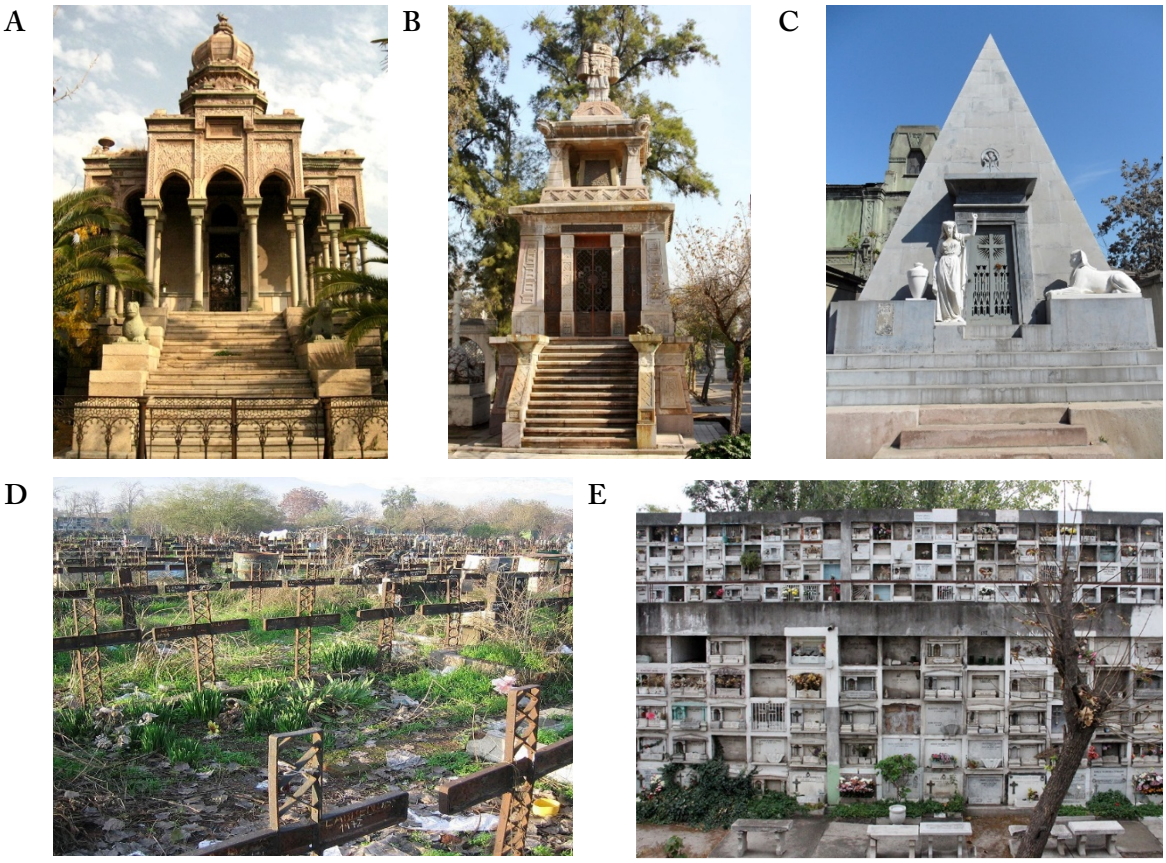

Figure 2.3 Moorish (A), Aztec (B) and Egyptian (C) styled mausoleums contrasting with more affordable grave plots (D) and niches (E) in the Santiago General Cemetery (images by the researcher).

2.1.2 The skeletal collection

Origin of the collection.

Efforts to create a skeletal collection of forensic interest in Chile date back to the 1960's. The majority of the COSS skeletal remains were originally transferred to the UChile during the 1970's, with a smaller portion of them entering the Department of Anthropology in 1993. When Professor Juan Munizaga and university staff interceded for the remains to be entrusted to them and become part of their teaching resources (Aspillaga, 1995), there was no legal background covering the donation of human remains to any sort of institution beside a legally recognised cemetery. Thus, Chilean legislation had to be modified to create a route through which the material could be legally donated and curated (Paredes, Hagn and Constantinescu, 1993). By means of Supreme Decree No. 254 (Ministerio de Salud (Chile) and Subsecretaría de Salud (Chile), 1992), two earlier Decrees issued by the Ministry of

Health were modified. The donation of the human remains was only possible as Chilean legislation recognised the existence of long and short-term graves. Decree 357 of 1970, Title III Of Graves, Article 33 states that "short-term temporary graves are those that give the right to the burial of a single corpse, for a minimum period of 5 years, with right to its renewal for equal and successive periods of up to 20 years [...]" (Ministerio de Salud (Chile), 1970). If short-term graves are not renewed, cemetery staff are entitled to reuse them, as stated in Article 38 of said decree: "once the term of occupation of a temporary burial has expired, the cemetery, if no one claims the remains existing in it, may remove them to transfer them to the common grave or to proceed with their incineration, in cases where the establishment has a crematorium, without any responsibility for the Cemetery Management". The latter decree was modified to authorise a third option: entrusting those unclaimed human remains to universities (Table 2.1).

Given the nature of the activities in most burial grounds, cemeteries are also subject to Chilean Health Code, regulations related to the promotion and protection of health and safety of the inhabitants of Chile. The Chilean Health Code's Ninth Book, Article 147, regulates the use of human remains of any kind for scientific research; it details that remains could be entrusted to scientific institutions "when the spouse or, in the absence of the latter, first-degree relatives of direct or collateral consanguinity do not express their opposition [to the exhumation and handing over] within the [agreed upon and paid] period and in the manner indicated in the regulations" (Ministerio de Salud (Chile), 1968). The drafting of the code left space for ambiguity, thus Decree 240 of 1983 was published to regulate its application (Table 2.1).

Table 2.1 Legal modifications allowing for universities to receive unclaimed human remains from cemeteries in Chile (adapted from Ministerio de Salud and Subsecretaría de Salud (Chile), 1992).

Decree modified	Section modified	Text modified or added
D. 357 of 1970	Title III, Article 38	*"In the same way, said remains may be entrusted free of charge by the Cemetery to public or private Universities that teach careers in the health area, for the purposes of teaching or research of said entities."*
D. 240 of 1983	Article 16	*"Unclaimed human remains, which are exhumed by the authority of the Cemetery of temporary burials once their lease has expired, may also be used for university teaching and research purposes."*

Through these legal modifications, the UChile and SGC agreed for unclaimed human remains from certain short-term burials to be exhumed by cemetery staff and relocated to the Department of Anthropology dependencies. Two kinds of short-term graves were chosen: traditional or lawn graves from *patios* (Figure 2.3D), and niches from *galerías* and *pabellones* (Figure 2.3E). Exhumations took place in two stages and were performed by non-specialised diggers (Abarca, 2011): stage A during the 1970´s, which resulted in the exhumation of the majority of remains from the COSS, and stage B in 1993 under government funded project FONDECYT 1028-91, with the exhumation of *patio* 134 (Figure 2.4). Cemetery information provided to university staff consisted of burial origin (location within cemetery grounds) and administrative records containing some individual biographical information.

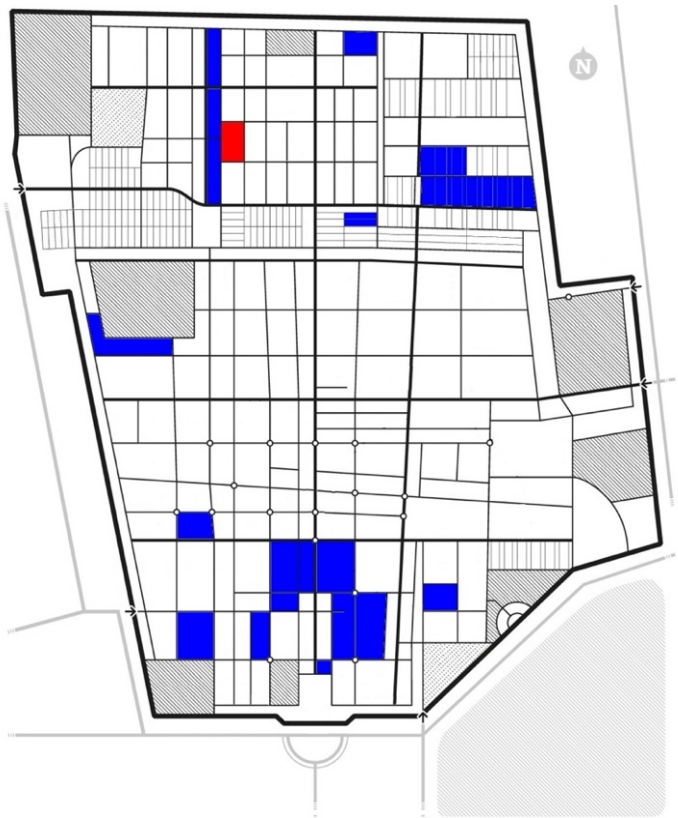

Figure 2.4 Burial areas exhumed during stages A (blue) and B (red). Main entrance at the bottom, secondary entrance on right side (map adapted from Wayfinding Consultores ©).

In addition to SES segregation, burial areas within the SGC are organised into *párvulos* (children) and adult burial areas, regardless of the type of grave. However, the SGC lacks specific regulations on age limits for the children's area, which is governed by customary

use and the personal decision of cemetery staff in charge of approving the burial. Based on personal communication with current cemetery authorities and staff, it appears that they regard *párvulos* as children younger than 12 years of age. Yet, this threshold may be subject to the current cultural context and not coincide with the concept of childhood during the early 20th century when some of these burials were created. Whatever the case, several *párvulos* were exhumed from adult burial areas, showing that regulations were sometimes overlooked, for unknown reasons.

Management and current state of the COSS.

After the remains entered the custody of the UChile, their management was assigned to the Department of Anthropology. However, due to the military dictatorship that ruled Chile for seventeen years (1973-1990), the UChile suffered great political constraints and the Department of Anthropology was heavily impacted, with its staff numbers and resources reduced. During this time, no funds were allocated to curate the osteological collections, which resulted in partial loss and damage of the remains due to lack of adequate infrastructure, and no measures to control and prevent physical deterioration. Physical records containing antemortem information produced when the remains entered the UChile's custody were also misplaced or lost, and it wasn't until the 2000's that a bigger effort was made to improve physical conditions and retain and digitise individual archival data. Different projects have been launched; a 2001 project, co-financed by the Andes Foundation, focused on conservation measures and treatments to improve physical condition of the remains, while at the same time facilities and laboratories were adapted to bioarchaeological research (Lemp *et al.*, 2008). This project did not, however, generate any new osteological information and the majority of the COSS remained stored in lacking conditions and unavailable for research.

In 2010 a university funded project (*Iniciativa Bicentenario Juan Gómez Millas*) invested in a new building for the Faculty of Social Sciences, including new laboratories and facilities for the Department of Anthropology and a new space for the archaeological collections. The initiative also funded a 2014 project to systematise and improve the conservation conditions of the COSS, the "Bicentennial Project" (*Puesta en Valor de la Colección Osteológica Subactual de Santiago, Trayectorias de Patrimonialización, Proyecto*

Bicentenario). Its focus was on the improvement of the COSS for continuous research and teaching purposes, including further application of conservation measures, macroscopic analyses, and documentation of antemortem information (Figure 2.5). The Bicentennial Project involved major restructuring of the collection itself; it was discovered that throughout the years of teaching and research, remains belonging to single individuals had been disassociated to create small collections of skeletal elements (such as skulls, long bones, vertebrae, mandibles, among others), in line with the idiosyncrasy of the time. As many bones were labelled (with annotations on the periosteal surface made with India ink covered with clear nail polish) with an inventory number given to them upon entering the University's custody, the team managed to reassociate some remains with their original individual. When remains were not possible to reassociate, they were left grouped together by skeletal elements, organised as ossuaries. After reassociation of skeletal remains, the new COSS comprised 1635 individuals, of whom 1629 are skeletonized and eight are semi-mummified with presence of soft tissue in at least one anatomical segment (Meza-Escobar *et al.*, 2023). Of these 1635 individuals, 1603 are associated with cemetery records about their original inhumation areas within the SGC. After efforts by the Bicentennial Project and this research project, currently 1198 individuals have documented antemortem information (Table 2.2).

Figure 2.5 State of the COSS materials before (A) and after (B) the Bicentennial Project of 2014 (image A courtesy of Nicole Barreaux, image B by researcher).

Table 2.2 Original areas in the Santiago General Cemetery from where COSS individuals were exhumed (summary by researcher with up-to-date information).

Burial area*	Age type	Children? **	Years in use	Total	With DAD
Galería Capilla Blanca (Patio 102)	Adult	No	1969	1	1
Galería Crematorio and Pabellón letra H (Patio 83)	Adult	No	1972	5	5
Galería 17 (Patio 164)	Adult	No	1970s	4	4
Galería 20 (Patio 144)	Adult	No	1972	1	1
Galerías 18, 22 and 23 (Patio 123)	Adult	No	1962-1972	16	16
Galerías 24, 25 and 26 (Patio 154)	Adult	No	1971-1972	14	14
Pabellón Provasoli (Patio 114)	Adult	No	1971	2	1
Pabellones letras A, B, F, and 16 (Patio 121)	Adult	No	(1967) 1971-1972	5	5
Pabellón 26 (Patio 132)	Adult	No	Unknown	1	1
Patio 6	Adult	Yes, 1	1973	52	49
Patio 10	Adult	Yes, 4	1962-1970	197	184
Patio 13	Children	-	1970-1979	26	25
Patio 15	Children	-	1970-1979	13	11
Patio 16	Children	-	1973	31	31
Patio 25	Adult	Yes, 7	(1891) 1960-1968	605	309
Patio 26	Unknown	-	1960-1964	3	0
Patio 28	Adult	Yes, 1	1960-1977 (1986)	278	208
Patio 29	Unknown	-	Unknown	2	0
Patio 31	Adult	No	1962-1970	26	22
Patio 36	Adult	Yes, 4	1970-1971	230	225
Patio 134	Adult	Yes, 3	1986	91	86
Total				**1603**	**1198**

DAD: documented antemortem data (sex and age-at-death).

* Burial areas in the original Spanish.

** Individuals <12 years of age (*párvulos*).

2.1.3 Accessible records and data

Several types of information were available and gathered during the documentation phase of this research, previous to the collection of osteological data. These records can be associated with three main sources: the UChile database, cemetery records, and previous studies using the COSS as a main or comparative sample.

UChile database.

The main source of administrative and osteological information for the COSS belongs to the database constructed and managed by the Department of Anthropology at the UChile. Using skeleton number, the database allows to search all the administrative, osteological, and biographical information documented for the individual. Estimations of sex and age-at-death are also available in the database; these estimations were conducted by physical anthropologists and university staff involved in the management of the collection and the Bicentennial Project, using macroscopic methods listed in Table 2.3. Osteological data is organised using OsteoWare, a free database application software developed from Buikstra and Ubelaker (1994) by the Smithsonian Institution (Washington, USA) for recording observations on a range of skeletal markers, covering age and sex estimation, osteometrics, and pathology (Engel, Schlager and Wittwer-Backofen, 2015).

Table 2.3 Methods used to estimate sex and age during the Bicentennial Project (personal communication with Sebastián Espinoza and Jacqueline Galimany).

Feature	Subgroup	Methods
Age-at-death	Non-adults	Epiphyseal closure (Schaefer, Black and Scheuer, 2008)
	Adults	Cranial suture closure (Meindl and Lovejoy, 1985) Pubic symphysis morphology (Brooks and Suchey, 1990) Auricular surface morphology (Lovejoy *et al.*, 1985; Osborne, Simmons and Nawrocki, 2004) Sternal end of the 4th rib morphology (İşcan, Loth and Wright, 1984 and 1985)
Sex	Adults	Cranial and pelvic morphology (Krenzer, 2006)

Pathological changes are also recorded for the collection, although the detail of macroscopic analyses varies between individuals. Records taken during earlier stages of the Bicentennial Project contain larger amounts of information per individual, including a detailed account of skeletal pathologies, with their respective photographic documentation. As the project proceeded, funding and time made it impossible to continue with the same level of detailed recording, and the team decided to reduce the pathological descriptive analysis and discontinue the photographic process (Barreaux *et al.*, 2015; Jacqueline Galimany, personal communication 2022). The team based their paleopathological analysis

on differential diagnosis using traditional bioarchaeological literature regarding pathological conditions (Aufderheide and Rodriguez-Martin, 1998; Ortner, 2003; Waldron, 2009).

The Bicentennial project also involved recording of skeletal completeness, a written and photographic inventory, as well as the recording of osteobiographic profiles for the individuals (Barreaux, 2015; Barreaux *et al.*, 2015). The team used a 3-point scale to assess completeness: *complete, semi-completo* or *incompleto*, adapted from Buikstra and Ubelaker (1994) (Table 2.4). Along with osteological information, the database also contains inventory data to locate the remains within the underground storage.

Table 2.4 Inventory code for completeness used in the UChile database and its counterpart in Buikstra and Ubelaker's (1994) Standards.

UChile code	Standards code	Meaning
Completo	Complete	At least 75% of the bone is present
Semi-completo	Partial or Damaged	25 - 75% of the bone is present
Incompleto	Fragmentary	Less than 25% is present

Cemetery records.

The Bicentennial project also involved the recovery of antemortem biographical information from cemetery records. Since the creation of the SGC in 1821, its Archive department has gathered legal and administrative information from each individual buried in the cemetery (Radio Recoleta, 2019). Information available for each individual varies through time, as the administrative needs of the cemetery have changed over the years (Rodríguez and Salinas, 2015). All burial records are registered in four different types of books, with information sometimes overlapping or missing. Each book type contains different data: Index (*Libros Índices,* with individual biographical information such as name, age, sex, dates of birth and death and cause of death), Location (*Libro de Ubicación,* regarding the use over time of each burial plot, when multiple burials have occurred), Area (*Libros de Patios*, with the exact location of each burial plot within the cemetery) and Transfers (*Libros de Traslados*, regarding moving and transfers of human remains within the cemetery areas or between other burial places). As of 2023, only burial information for deaths after 1989 had been digitised and could be accessed online or at the SGC's Archive office, with no current plans to extend the

database. All information prior to 1989 could only be accessed through physical records available on request. Unfortunately, only some of the paper files are arranged in a coherent way (according to the year of burial) within the SGC Archive.

For individuals in the COSS, information available normally consists of proof of cemetery fees payment (known as exclusive right of burial), and a burial authorisation certificate (*pase de sepultación* or *autorización de sepultación*) issued by the Civil Registry and Identification Service of Chile (*Servicio de Registro Civil e Identificación*, SRCeI). Some biographical information (such as name, sex, age-at-death, as well as date, place, and cause of death) can be found within the burial authorisation certificate, whose template designs varied depending on the year and office they were issued from (Figure 2.6). Due to differences in the design of burial authorisation certificates, information filled out by Civil Officers was variable and is not the same for every individual in the COSS; of the certificates inspected, only a few had more information than strictly necessary (as exemplified in Figure 2.6A). For some individuals, sex was not stated but could be deduced from the person's first name, relying on the researcher's knowledge of the Spanish language and Chilean idiosyncrasy. In addition to the information Civil Officers provided, cemetery staff would on occasions add some information on the certificates reverse, adding information such as last known address, next of kin in charge of arranging the funeral and burial, and mortuary fee and its reduction, if applicable.

During the Bicentennial Project, antemortem information was sought from the SGC, but the search was limited by the loss of some books or previous damage that made them unreadable. The reuse of the burial plots also limited the extent to which each individual could be paired with its identity, as documentation provided by the SGC at the time of the exhumation only detailed the burial plot number recorded in the Index Book, which could not be cross referenced with the Transfer Book. As the graves were heavily reused and bureaucracy often delayed the exhumation after the occupancy time limit has passed, Index Books record several successive occupations around the time of the COSS conformation; without information regarding transfer to other plots or cemeteries, in some cases it's impossible to discern which individual belonged to which occupancy. When faced with this issue, estimation of skeletal sex and age was contrasted with the records to determine identity; this was only done when there was absolute certainty of exclusion (e.g., adult female

estimation versus non adult male identity). Recovery of antemortem information carried out during the Bicentennial Project resulted in 645 individuals with documented sex, 573 with documented age-at-death, and 525 with cause of death (Galimany, 2020).

Previous studies.

In addition to the UChile database and SGC burial records, past scholarly work has used the COSS either as a main resource or as a comparative sample. Ranging from dissertations, peer-reviewed articles, to book chapters, most of it has been led by Chilean researchers in Spanish language (Table 2.5). Varying in size and reported information, these datasets derive from macroscopic osteological analyses produced for different research projects. Mainly taking a forensic approach, analyses include assessments of metric and non-metric traits, enthesis robusticity, pathological conditions, and evaluation of several biological profile estimation methods. Two of these datasets were requested to their authors to explore if they may contain additional osteological data for the collection that could be used to enhance the data collected for this research. The larger of the two datasets, gathered by Abarca (2011) as part of her undergraduate dissertation, explored the effects of nutrition on sexual dimorphism expressed through stature, with a sample of 96 individuals (53 males and 43 females) between 20 and 49 years of age. A second dataset, gathered by Urrutia (2018) for her undergraduate thesis, explored infant and adolescent morbidity patterns and growth profiles, comparing modern non-adults to archaeological populations that inhabited Chile's Central Zone. For the modern sample, 50 individuals from the COSS were selected; contrary to the work of Abarca (2011), Urrutia did not utilise documented antemortem data on biological sex or age-at-death, but decided to estimate age via macroscopic analysis and divided the sample into age categories based on the work of Roksandic and Armstrong (2011).

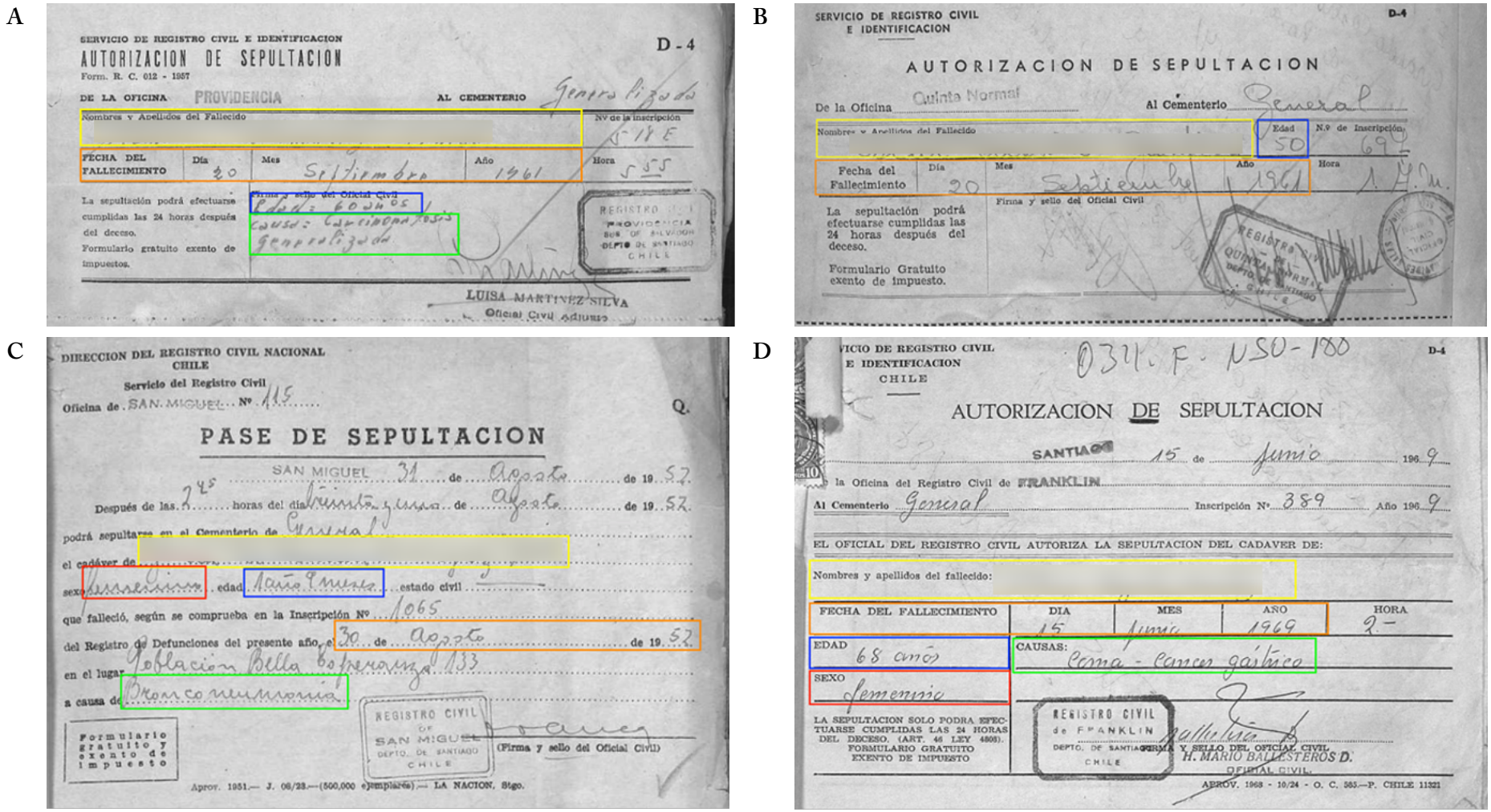

Figure 2.6 Burial authorisation certificates issued by the Civil Registry and Identification Service of Chile in 1952 (C), 1961 (A and B), and 1969 (D), showing differences in template design. Note biographical information in coloured squares: name (yellow), sex (red), age-at-death (blue), date of death (orange), and cause of death (green) (reproduced with permission from the Santiago General Cemetery, coloured highlights and blurring by researcher).

Table 2.5 Scholarly work using the COSS as a main or comparative sample (summary by researcher).

Topic	Title*	Author(s)	Year	Type of publication and language
Biological sex estimation	Effects of intentional cranial deformation on the expression of sexual dimorphism in adult skulls	Retamal	2004	Undergraduate dissertation, in Spanish
	Predictive value of the anatomical features of the skull used in the visual estimation of sex in a Chilean population: a geometric morphometry analysis	Díaz	2010	Undergraduate dissertation, in Spanish
	Metric parameters for sex determination in modern Chilean skeletal remains	Garrido-Varas, Thompson and Campbell	2014	Peer-review article, in Spanish
	Sex estimation from the scapula in a contemporary Chilean population	Peckmann, Logar and Meek	2016	Peer-review article, in English
	Is "Latin American" population-specific? Testing sex discriminant functions from the Mexican tibia on a Chilean sample	O'Bright, Peckmann and Meek	2018	Peer-review article, in English
	Sex estimation using the proximal end of the femur on a modern Chilean sample	Carvallo and Retamal	2020	Based on undergraduate dissertation by Carvallo (2018), in English
	Evaluation of sexual dimorphism from cranial and postcranial metric variables using binary logistic regression analysis and discriminant function analysis in a modern population of Santiago, Chile	Maldonado	2020	Undergraduate dissertation, in Spanish
	Estimation of sex from the shape of the upper second molar in a sample of adult individuals from the COSS, using geometric morphometry	Millán	2020	Undergraduate dissertation, in Spanish
	The informative value of the pubic symphysis for age-estimation in a Chilean skeletal sample using transition analysis	Galimany	2020	Master dissertation, in English
	Reconsidering the Age-Informative Value of the Pubic Symphysis. A Comparison with TA3 Skeletal Traits	Galimany and Getz	2022	Based on master dissertation by Galimany (2020), in English

Age estimation and growth	Identification: determination of age in pubic symphysis	Paredes, Hagn and Constantinescu	1993	Peer-review article, in Spanish
	Evaluation of Three Methods of Adult Age Estimation Based on Root Translucency Height, Periodontosis Height and Root Height in a Chilean Sample	Retamal and Ubelaker	2011	Peer-review article, in English
	New identification criteria for the Chilean population: Estimation of sex and stature	Ross and Manneschi	2011	Peer-review article, in English
	Evaluation of the age estimation method through the auricular surface of the ilium in a modern Chilean sample	Herrera	2012	Undergraduate dissertation, in Spanish
	Evaluation of the method of estimating age-at-death on the auricular surface in a modern Chilean population sample	Espinoza	2015	Undergraduate dissertation, in Spanish
	Reliability of age estimation from iliac auricular surface in a modern Chilean sample	Herrera and Retamal	2017	Based on undergraduate dissertation by Herrera (2015), in English
Stature and body size	Effects of nutrition on the Sexual Dimorphism expressed in the Stature (SSD) of a sample of the modern Chilean population	Abarca	2011	Undergraduate dissertation, in Spanish.
	New identification criteria for the Chilean population: Estimation of sex and stature	Ross and Manneschi	2011	Peer-review article, in English
	Juvenile Stature Estimation: A Chilean Perspective	Sutphin and Ross	2011	Book chapter, in English
Dental pathologies	Caries prevalence and tooth missing of an adult Chilean 19th century population	Urzúa, Huberman, Delgado, Pacheco and Retamal	2009	Peer-review article, in Spanish
Sex-related differences	Sexual division of labour in three skeletal collections from Chile: an exploratory study based on postcranial biomechanics	González	2019	Undergraduate dissertation, in Spanish.
Growth disruption and mortality	Infant-adolescent morbidity patterns and growth profiles: A diachronic comparison for different populations of the Central Zone of Chile	Urrutia	2018	Undergraduate dissertation, in Spanish.
Shape variation	An investigation into bilateral asymmetry of the appendicular skeleton of the adult human and its use in physical and forensic anthropology	Garrido-Varas	2013	Doctoral dissertation, in English

	Mandibular morphology in modern and archaeological populations that exert different intensities of masticatory loads according to their diet	Ugarte	2017	Undergraduate dissertation, in Spanish.
	Normal and altered masticatory load impact on the range of craniofacial shape variation: An analysis of pre-Hispanic and modern populations of the American Southern Cone	Eyquem, Kuzminsky, Aguilera, Astudillo and Toro-Ibacache	2019	Peer-review article, in English
	Dental malocclusions are not just about small and weak bones: assessing the morphology of the mandible with cross-section analysis and geometric morphometrics	Toro-Ibacache, Ugarte, Morales, Eyquem, Aguilera and Astudillo	2019	Peer-review article, in English
Trauma and violence	Characterization of perimortem traumas in a sample of the General Cemetery collection of Santiago de Chile	Olivares	2022	Undergraduate dissertation, in Spanish.

*Presented here in English; if original in another language and translated title not given, translated by researcher.

2.2 Methods

This section starts with an overview of the documentation phase of this thesis, followed by an explanation on the sample selection rationale for original data collection, and a contextualisation of the demography of the selected sample for this study. Next, the section provides a general introduction to all osteological methods utilised for the collection of original osteological data in this study, while also presents an overview of the statistical analyses selected for analysis of datasets resulting from combination of the original osteological data with the integrated database.

Varying combinations of osteological methods were selected from those introduced here to address specific objectives in each research manuscript, including skeletal and dental age estimations (Chapter 3, 4, 5), assessment of pubertal timing (Chapter 5), and assessment of physiological stress (Chapters 3, 4, 5 and 6). As many of these methods were applied in two or more manuscripts, they are all introduced once, in this chapter, rather than repeatedly in each manuscript to which they relate. Particular details of the selection and application of methods to address each research objective are, however, discussed in more detail in the relevant manuscript. No part of this research involved destructive analyses, with all assessments done macroscopically and undertaken in compliance with BABAO guidelines for the recording of human remains (Mitchell and Brickley, 2017), and BABAO's Code of Ethics and Code of Practice (BABAO, 2019a, 2019b). Institutional guidelines of the Department of Anthropology at the University of Chile were also followed during data collection.

Statistical approaches to data analysis were also variously selected and applied in each manuscript to best address the research objectives. Hedge's g analysis was employed to measure the effect size of the discrepancy between chronological age (CA) and age estimations derived from skeletal elements (SA) (Chapters 4, 5 and 6). Simple (symmetric) correspondence analysis was used to visualise the relative association between and within two groups of variables, growth disruption and physiological stress (Chapter 3). Finally, survival analyses were applied to CA and skeletal markers of stress to assess the effect of several risk factors on survival time (Chapter 4 and 6). The statistical methods are all introduced here, with more specific and unique details of their application to the research objectives appearing in each manuscript.

2.2.1 Construction of an integrated database

The COVID-19 pandemic interfered with timely fieldwork completion, resulting in UK and Chilean universities closures, inaccessibility to files and skeletal material, and international travel restrictions and delays. Due to this, part of the methodology used for data collection included a documentation phase led by the researcher from the UK, aiming to gather as much digital information as possible. This documentation phase resulted in an integrated database for the COSS, that not only includes osteological data from extant data gathered from different sources (UChile and private researchers' datasets) but also historical information about the origin and current state of the COSS to contextualise the population. The process of obtaining permission to use the datasets was lengthy but made possible by the researchers's personal connections with the university and colleagues in Chile.

From the private datasets that were assessed to determine the usefulness of adding them to an integrated database, in the end partial information of only two datasets were included. Relevant information that was incorporated into the integrated database from Abarca's (2011) research included documented antemortem data (documented identities, biological sex, age-at-death, date of death, and cause of death) only for individuals between 20 and 21 years of age; other administrative information, such as year of death and last known address of next of kin, was gathered to contextualise the population within time periods and socioeconomic status. From Urrutia's (2018) work, information regarding age estimations, pathological conditions (non-specific stress markers, trauma, and dental lesions) and metric data from non-adult individuals was included in the integrated database. Both researchers granted their permission to use their datasets in this research.

After travel restrictions were lifted, the first fieldwork period in Chile (mid-2021) was devoted to collecting information from the archive at the SGC, as access to the collection at the UChile was still not permitted to external researchers at the time. Documentation efforts made during this fieldwork period increased the number of documented antemortem data, with new information for reassociated but undocumented individuals, while it was also possible to corroborate information on the individuals identified during the Bicentennial Project. Thus, documented antemortem data (sex and age-at-death) is currently known for 1198 individuals; of them, 954 have recorded cause of death, while years of birth and death are documented in 1079 cases (Table 2.2). One particularly important achievement of the

documentation phase of this research was the identification of 31 previously undocumented non-adults, 19 of them corresponding to foetal–infant individuals (<1 year of age).

The integrated database, therefore, combines efforts carried out by the researcher and UChile colleagues (university staff and students in biological anthropology), regarding macroscopic osteological analyses (e.g., age-at-death and sex estimations, and recording of pathological changes) and cemetery documents regarding antemortem information (e.g., biological sex, age-at-death, years of birth and death, cause of death) compiled from the SGC's Archive. The construction of an integrated database was essential for the selection of the sample that was to be analysed to fulfil the aims of this thesis. Selection of the skeletal sample and planning of the original osteological analyses to be carried out were carefully outlined for a second fieldwork period in Chile (mid-2022), when COVID-19 restrictions were finally lifted.

Sample selection and rationale.

After combining information from the UChile dataset, SGC records reviewed by the researcher, and private datasets, a first version of the integrated database was compiled. Knowing what skeletal material was available and which individuals had documented antemortem data (biological sex and age-at-death, referred as DAD from now on) allowed the researcher to define the sample that would be analysed during the second fieldwork period (2022), targeting specific biological information needed to answer research questions outlined in Chapter 1. Considering the limited time available to analyse the sample, the goal of the second field trip was to gather as much information as efficiently as possible. Recording forms for foetal-infant, non-adult, and adult individuals were designed for this purpose (see Appendix A.1), with focus on age-at-death estimation for the foetal-infant subsample, assessment of pubertal stage for the non-adult sample, and/or pathological conditions in all individuals. Once the second data collection was completed, all new information was gathered to produce a single composite database of biographical and biological information for the human remains that comprise the COSS. From 1198 individuals with documented antemortem information, a sample was selected to be analysed in this research; although the final integrated database includes more information than the one used in this dissertation, the following section will only discuss information regarding

the selected sample. The complete integrated database will be handed over back to the UChile for their management, after the completion of this dissertation.

A sample of 946 individuals (164 non-adults and 782 adults) was selected to answer research questions within this dissertation. Individuals were selected on three grounds: availability of DAD, skeletal completeness, and overall bone preservation. To be included, all individuals had to have at least documented age-at-death and documented sex, but might also have documented cause of death, and years of birth and death. Skeletal completeness was required to be good or moderate (*completo* or *semi-completo* in previous UChile assessments) to maximise the quantity of osteological data that could be obtained. Good overall preservation was also necessary to ensure the accuracy and reliability of the data. Although it was possible to include individuals without documented antemortem data in some parts of the analytical work using only skeletal age estimations, the research questions were better answered when biological sex and age-at-death were also documented. Positively, the sample with documented data was large and therefore skeletons without documented data could be excluded without any significant impact on sample size.

As the skeletal remains in the COSS derive only from short-term graves which are recognised to be temporary in Chilean law, and for which no relatives objected to the exhumation, there is a socioeconomic bias in the resulting collection and selected sample for this study. Short-term graves were the cheapest burial plots available at the time, and therefore disproportionately represent the burials of residents of poorer areas of the city. This assertion can be further supported by information found in cemetery documentation, where the address of the person that paid burial fees could be found alongside other antemortem data. This address usually corresponded to the next of kin (e.g., wife, husband, parent) but could also list neighbours or hospice representatives, as many individuals were sent to the SGC directly from hospices or hospitals and ultimately provided burial free of charge. The majority of recorded addresses point to residential areas of low income status, such as Santiago Centro (particularly to *conventillos*, urban communal housing usually without running water or electricity and shared bathroom facilities, where families would rent one room), Barrancas (currently Lo Prado and Pudahuel, an area created and inhabited by rural-to-urban economic migrants during the 19th century), and Renca (an agricultural area of the city at the time) (Meza-Escobar *et al.*, 2023). Although these addresses may not be those of

the deceased, it seems reasonable to assume that they are of relatives and friends who lived nearby, or in the case of the hospital locations, that patients originally lived in close proximity.

Ages-at-death ranged from 5 gestational months (*intra utero*) to 96 years of age (Figure 2.7). The non-adult sample (5 gestational months to 21 years of age) was 40% females (n=66) and 60% males (n=98), while the older adult sample (40 to 96 years of age) was 42% females (n=327) and 58% males (n=455). Years of birth span between 1942-1980 for the non-adult sample and 1864-1941 for the older adult sample, while years of death were between 1960-1986 for non-adults and 1950-1986 for older adults (Figure 2.8).

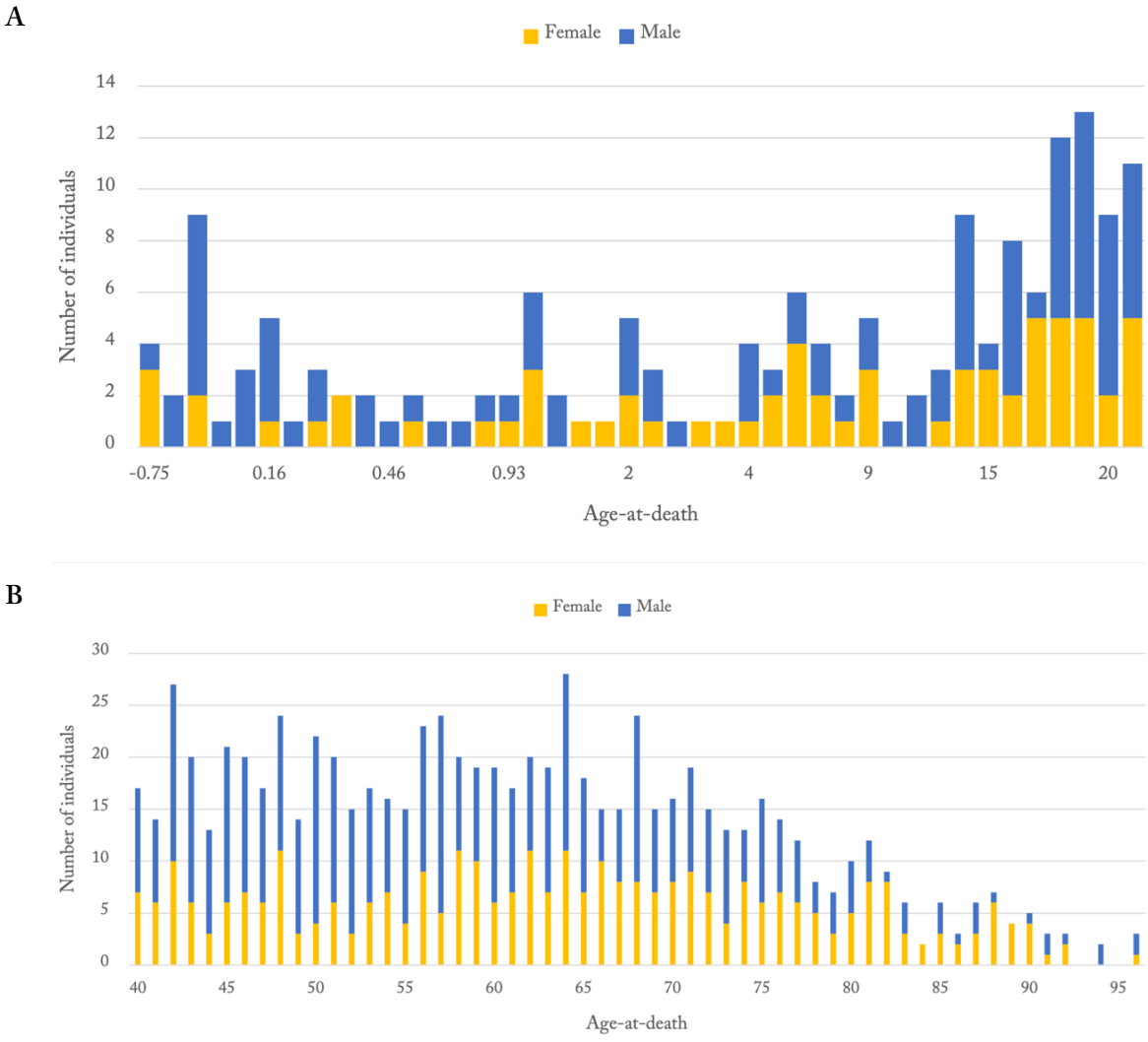

Figure 2.7 Distribution of age-at-death and sex of non-adult (A) and older adult (B) age groups.

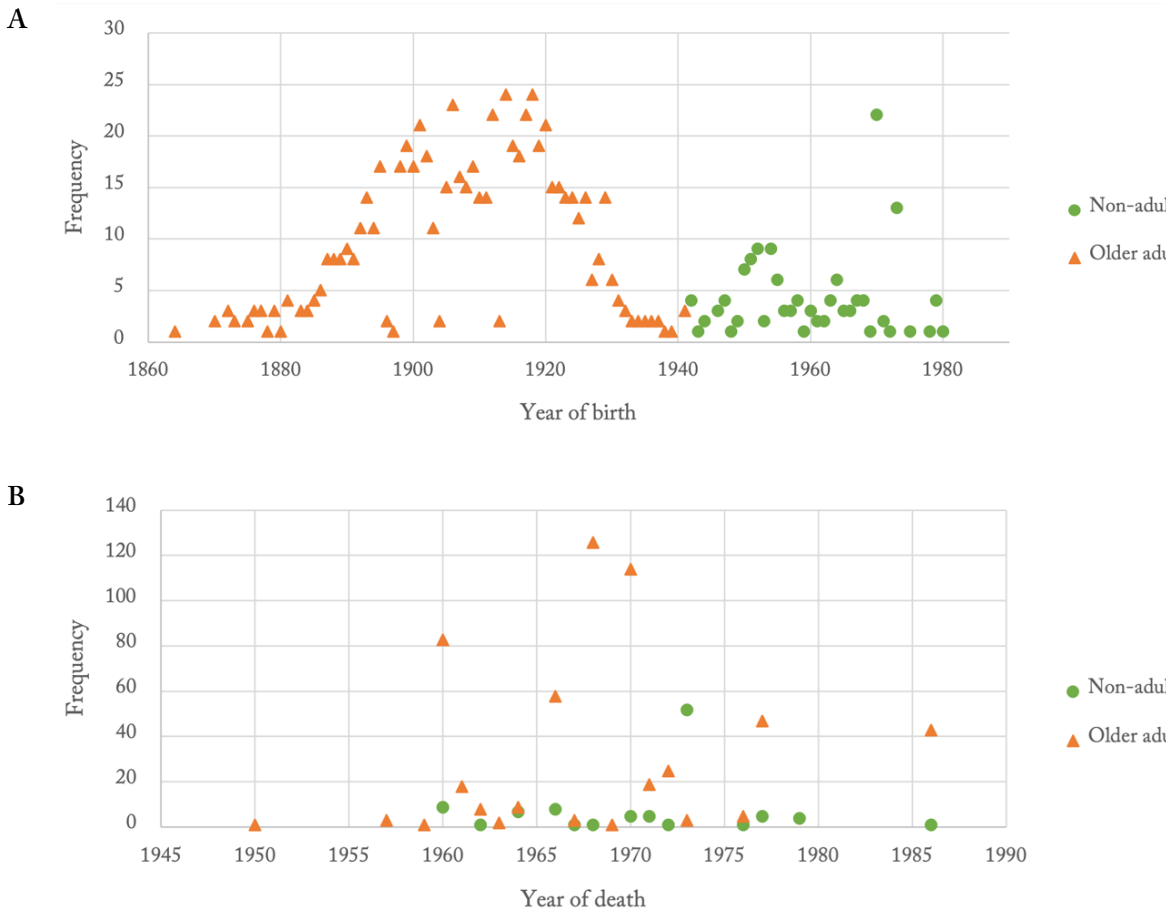

Figure 2.8 Frequency of years of birth (A) and death (B) for non-adults and adults.

2.2.2 Estimation of age-at-death

Estimation of age-at-death from skeletal remains relies on the characterisation of the progression of growth and development in immature life followed by assessment of the processes of skeletal degeneration which appear progressively from the point of skeletal maturity to old age (White, Black and Folkens, 2011). As a result of the distinctive skeletal changes that accompany development and degeneration, methods for assessing age-at-death are conventionally separated into those applied to immature individuals and those applied to mature individuals, with slight overlap in application around late adolescence when the final stages of maturation are often accompanied by the early stages of osteologically-visible degenerative change (Cox, 2000).

Methods used to estimate physiological age employed dental and skeletal elements. However, as preservation of the remains varied from complete to semi-complete, some

elements were not present or presented damage that prevented recording of measurements. Hence, physiological age estimations were made whenever anatomical elements were present and well preserved.

Dental age estimation.

Assessment of dental physiological growth and development is one of the most commonly used method in bioarchaeological and forensic studies to estimate age-at-death in non-adult individuals (Lewis, 2007). Previous studies have shown that dental development follows a predictable sequence from gestation to around 20 years of age (Moorrees, Fanning and Hunt, 1963; Braga *et al.*, 2005; AlQahtani, Hector and Liversidge, 2014) and due to their highly mineralised composition, teeth have been found to be minimally affected by physiological stress derived from environmental and nutritional causes (Cardoso, 2007, 2009; Conceição and Cardoso, 2011). Deciduous dentition has also been described as more resilient to environmental insults than permanent teeth (Lewis, 2007).

Deciduous (primary) dentition starts to form around the 6th to 7th gestational week of embryonic life, when the dental lamina appears (Scheuer and Black, 2004). Tooth mineralisation, occurring from the crown cusps to the apex closure of the root, happens around the 15th gestational week, with all deciduous teeth completely mineralised and erupted into the oral cavity by the time a child reaches 4 years of age (Lewis, 2007; Nelson and Ash, 2010). Permanent (secondary) dentition begins its development around birth with the initial calcification of the upper and lower first molars, and continues throughout childhood until the upper second molar's root completion around 14-16 years of age, and the variable eruption of the third molar, nominally occurring around 17-18 years of age (Lewis, 2007; Nelson and Ash, 2010). The sequential nature of tooth development allows the use of formation of deciduous and permanent dentition as a means to assess maturity and estimate age-at-death in non-adult individuals.

Dental age was estimated assessing development of dentition, recording formation stage for each tooth in bespoke recording forms developed for this study. Scores were recorded for all available dentition following dental development scores by Moorrees, Fanning and Hunt (1963); their work illustrates the growth and development of the tooth,

from the formation of the dental crown to the root and apex. Each tooth was assigned a stage and age-at-death estimation using the London Atlas of Human Tooth Development and Eruption produced by AlQahtani (2012). If dental development fell between two age categories, a mid-point was recorded, as well as lower and upper boundaries for error range comprising both age categories.

Skeletal age estimation.

Although typically regarded as less precise than dental development, age estimation in non-adults through the linear growth of the skeleton is one of the traditionally used methods in bioarchaeology (Buikstra and Ubelaker, 1994). Deciduous and permanent dental pieces in early stages of mineralisation are often lost in archaeological settings, due to the small size of dental buds in younger non-adults as foetal, perinates and infants (Hodson, 2018). Skeletal elements usually have better overall preservation and are more likely to be identified during archaeological excavation (Lewis, 2017a; Utczas *et al.*, 2017).

Linear growth and maturation of the skeleton was used to estimate skeletal age (SA); measurements of the pars basilaris of the occipital and long bones (humerus, ulna, femur, and tibia) were taken for each individual when available. As the base of the skull is the most stable area during growth and development (Redfield, 1970), previous studies have found that morphological development and size growth of the pars basilaris of the occipital is strongly correlated to age thresholds and can be used as indicators of age-at-death (Redfield, 1970; Scheuer and MacLaughlin-Black, 1994). Long bone diaphyseal lengths have been also correlated with age-at-death (Cardoso, Abrantes and Humphrey, 2014); although both upper and lower limb long bones were measured, only maximum length of femur diaphysis was used to derive age estimations. Femoral diaphyseal length has been found to be the most accurate long bone to estimate age-at-death, particularly in foetal and perinatal individuals (Scheuer and Black, 2004; Carneiro, Curate and Cunha, 2016).

All direct measurements were recorded in their individual recording form to the nearest millimetre using a Mitutoyo AOS digital calliper (accuracy of +/-0.02mm according to manufactory guidelines), twice by the researcher to assess intra-observer error. Pars basilaris measurements (maximum width, sagittal length, and maximum length) were taken

following guidelines outlined by both Fazekas and Kósa (1978) and Schaefer, Black and Scheuer (2008). Age estimates from the pars basilaris (SA-P) were calculated comparing the measurements with age ranges provided by Scheuer and MacLaughlin-Black (1994) for pre and postnatal groups; Fazekas and Kósa (1978) estimates only include estimations for prenatal individuals and were therefore not used. Age estimates given by Scheuer and MacLaughlin-Black (1994) do not provide error levels or standard deviations; when measurements fell into more than one age category, a mean age was plotted with both minimum and maximum age categories used as upper and lower boundaries for the age estimation.

Age estimation using the maximum length of femur diaphysis (SA-F) was derived from charts given by Scheuer, Musgrave and Evans (1980) for foetal individuals, and Maresh (1970) for postnatal individuals. Following Feldesman (1992), correction factors were applied to age estimations made derived from Maresh (1970), to reduce any error related to radiographic enlargement. Due to completeness issues of the skeletal remains, measurements used to estimate age represent either side, that is, left femur preferred, but right if left was not available or complete.

2.2.3 Assessment of pubertal timing

Chapter 5 focused on the assessment of maturation indicators of pubertal timing in all individuals with documented antemortem data between 8 to 21 years of age, analysing developmental markers and the physical changes of puberty that are known to occur in a particular sequence. To achieve this, the manuscript followed the method devised by Shapland and Lewis (2013, 2014), who used clinical methods to study skeletal morphological changes to estimate pubertal stage in human skeletal remains. In a later article, Lewis, Shapland and Watts (2016) summarised the previous findings and formally proposed a six pubertal stage approach.

In their first study, Shapland and Lewis (2013) assessed pubertal stage in 79 individuals aged between 10 and 19 years of age from St. Peter's Church, Barton-upon-Humber, England. Serving a small market town located in Lincolnshire, St. Peter's churchyard was in use during medieval and early post-medieval periods, between AD 950

and 1700 (Rodwell and Rodwell, 1982). They explored skeletal areas such as the mandibular canine root, wrist and hand, iliac crest epiphysis, and distal radius. A following article on the same topic (Shapland and Lewis, 2014) explored cervical vertebrae maturation to assess pubertal stage. For this study, they analysed 594 individuals between 10 to 21 years of age from the St. Mary Spital collection in London, England. Dating from AD 1100 to 1539, the collection derives from the cemetery associated to the St. Mary Spital hospital, located on the outskirts of the medieval city. Both studies included the estimation of biological sex and age-at-death for the individuals.

Lewis, Shapland and Watts (2016) outlined six pubertal stages based on clinical literature (Hewitt and Acheson, 1961; Grave and Brown, 1976; Marshall, 1978; Chertkow, 1980; Hägg and Taranger, 1982; Morrissy and Weinstein, 2006): initiation, acceleration, transition (which includes peak high velocity), deceleration, maturation, and completion (end of growth spurt or post-puberty). These osteological pubertal stages propose morphological changes in skeletal elements that range from the onset of puberty (when the adolescent growth spurt starts) leading to peak high velocity (PHV, the period of time in which a child grows the fastest during their adolescent growth spurt) and continue after into deceleration (when menarche occurs, around a year after PHV) and maturation stages until the end of the growth spurt, when adult phenotype is reached (Lewis, 2022). Each stage includes seven anatomical areas of analysis: permanent mandibular canine, humeral capitulum, radial distal epiphysis, hamate hook, hand phalanges and metacarpals, cervical vertebrae, and iliac crest (Lewis, 2022). Assessment of pubertal timing carried out in Chapter 5 included one further anatomical area, ulnar proximal epiphysis. A visual chart was developed by the researcher from Shapland and Lewis (2013, 2014) and Lewis, Shapland and Watts (2016), with the aim of using drawings in conjunction with descriptions of the morphological changes associated with pubertal development for each skeletal element (Figure 2.9). A pubertal stage was assigned where three or more features could be observed, with morphological changes associated to each skeletal area as follows:

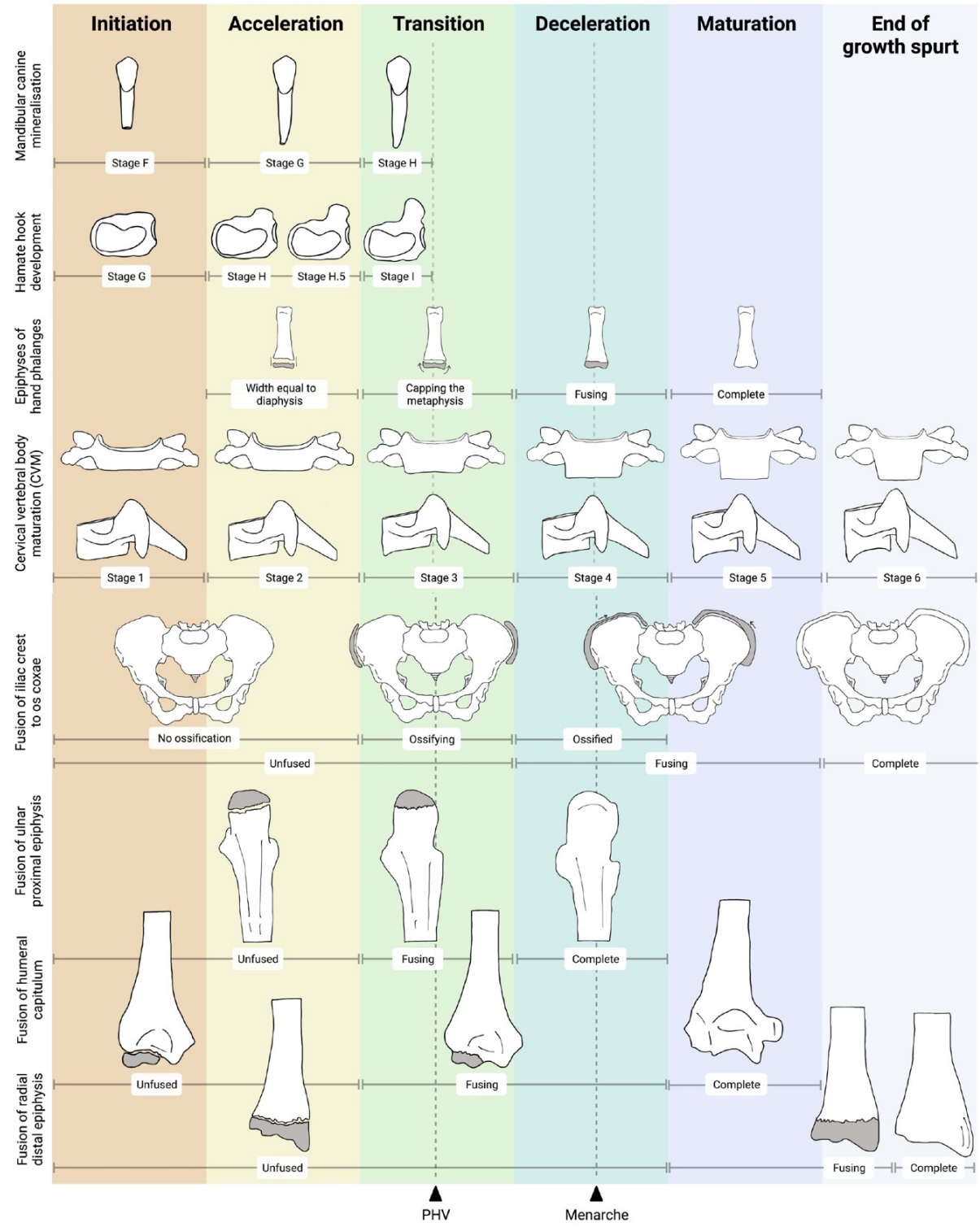

Figure 2.9a Front of the "Osteological assessment of pubertal stages" visual chart, developed by the researcher from Shapland and Lewis (2013, 2014) and Lewis, Shapland and Watts (2016) (diagram created with BioRender.com).

Use diagrams and description of morphological changes
Assign pubertal stage when 3 or more features are in accordance

	Morphological changes	Pubertal stage
Mineralisation of the permanent mandibular canine root	Stage F: ¾ of the canine root completed, with an open end	Initiation
	Stage G: canine root length complete, the end remains open	Acceleration
	Stage H: a completely mineralised mandibular canine root	Approaching PHV
Hamate hook development	Stage G: hook not developed yet	Initiation
	Stage H: hook is appearing	Acceleration
	Stage H.5: hook is developing	
	Stage I: hamate hook is completely formed	Approaching PHV
Hand phalanges morphology	Epiphyses unfused to the diaphysis	Initiation to Transition
	Epiphyses grow until they are as wide as the diaphysis	Acceleration
	Epiphyses start to cap the phalangeal metaphysis	Transition
	Fusion occurring in a distal to proximal direction; distal phalangeal epiphyses start fusing around 1.5 to 1.8 years after PHV; middle and proximal phalangeal epiphyses fusing 2.1 to 2.8 years after PHV	Deceleration
	Fusion of distal phalanges complete	Menarche achieved
Morphological changes in cervical vertebrae maturation **(Analyse C3 or C4)**	Stage 1: inferior border flat, vertebral body wedge shaped	Initiation
	Stage 2: concavity appearing in inferior border, body nearly rectangular in shape	Acceleration
	Stage 3: concavity developing in inferior border, body rectangular in shape	Transition
	Stage 4: distinct concavity in inferior border, body nearly square in shape	Deceleration
	Stage 5: accentuated concavity in inferior border, body square in shape	Maturation
	Stage 6: deep concavity in inferior border, body taller than it is wide	End of growth spurt
Ossification and fusion of the iliac crest epiphysis	Ossification of iliac crest epiphysis starts	Acceleration
	Iliac crest epiphysis remains unfused	Until after PHV
	Partial fusion of the incomplete epiphysis	Menarche achieved
	Fusion of partially ossified epiphysis to the iliac blade starts	Deceleration
	Complete ossification of the iliac crest epiphysis	Maturation
	Completely fused iliac crest epiphysis	End of the growth spurt
Fusion of ulnar proximal epiphysis	Partial or complete fusion of the proximal epiphysis of the ulna	Transition
	Completely fused ulnar proximal epiphysis	Deceleration (before menarche)
Fusion of humeral capitulum	Initial fusion of the capitulum to the distal end of the humerus	Transition
	Complete fusion of the epiphysis	Deceleration
Fusion of radial distal epiphysis	Unfused radial distal epiphysis	Deceleration
	Fusion of the epiphysis starts	Maturation
	Completely fused epiphysis	End of growth spurt

Adapted by Ofelia Meza-Escobar from:
- Shapland, F. and Lewis, M.E. (2013) 'Brief communication: A proposed osteological method for the estimation of pubertal stage in human skeletal remains', American Journal of Physical Anthropology, 151(2), pp. 302–310.
- Shapland, F. and Lewis, M.E. (2014) 'Brief communication: A proposed method for the assessment of pubertal stage in human skeletal remains using cervical vertebrae maturation', American Journal of Physical Anthropology, 153(1), pp. 144–153.
- Lewis, M., Shapland, F. and Watts, R. (2016) 'On the threshold of adulthood: A new approach for the use of maturation indicators to assess puberty in adolescents from medieval England', American Journal of Human Biology, 28(1), pp. 48–56.

Figure 2.9b Back of the "Osteological assessment of pubertal stages" visual chart, developed by the researcher from Shapland and Lewis (2013, 2014) and Lewis, Shapland and Watts (2016).

Mineralisation of the permanent mandibular canine root.

Following Demirjian *et al.* (1985), the mineralisation stage of the permanent mandibular canine was estimated. Three stages of root mineralisation were used to assess pubertal timing, from onset of puberty to just before PHV. Stage F shows ¾ of the canine root completed, with an open end, indicating imminent growth spurt. In stage G the canine root length is completed, but the end remains open, indicating acceleration of the growth spurt. Stage H shows a completely mineralised mandibular canine root, and indicates the individual is approaching PHV. Thus, the degree of mineralisation of the permanent mandibular canine root can be used to assess the first part of the pubertal growth spurt, from onset of puberty to just before PHV.

Hamate hook development.

Adapting a system developed by Tanner *et al.* (2001) for radiographic examination of the hamate, Shapland and Lewis (2013) proposed using an extra stage when analysing skeletal remains. Stage G, where the hook of the hamate has not developed yet, indicates a pre-pubertal individual. Stage H, when the hook is appearing, and the extra stage H.5, when the hook is developing, both indicate an acceleration in the growth spurt. Finally, stage I signals the reaching of PHV soon after the hamate hook is completely formed. As with the permanent mandibular canine root, the development of the hamate hook can be used to assess the first three stages of pubertal development proposed by Lewis, Shapland and Watts (2016).

Hand phalanges morphology.

Widely used in modern paediatrics, Shapland and Lewis (2013) categorised the appearance and ossification of phalangeal epiphyses into their pubertal stages by analysing two components: shape and fusion. Before PHV, the focus is on changes in epiphyseal shape: during the acceleration stage, epiphyses grow until they reach an equal width than the diaphysis, when they start to cap the phalangeal metaphysis during the transition stage. During this time, the epiphysis remains unfused to the diaphysis, with fusion occurring during the deceleration stage of pubertal growth spurt in a distal to proximal direction; distal

phalangeal epiphyses start fusing around 1.5 to 1.8 years after PHV, followed by middle and proximal phalangeal epiphyses, fusing 2.1 to 2.8 years after PHV. Fusion of the distal phalanges are a sign that menarche has been achieved.

Morphological changes in cervical vertebrae maturation.

Analysing the third or fourth cervical vertebrae (i.e, C3, C4) to assess the shape of the vertebral body and the concavity degree of the body's inferior border, Shapland and Lewis (2014) defined six stages of cervical vertebrae maturation (Table 2.6). The inferior border of the vertebral body gradually becomes more concave, while the vertebral body changes from a wedge rectangular shape to a square, with the height surpassing the width.

Table 2.6 Maturation changes to assess pubertal timing in the third or fourth cervical vertebrae.

CMV stage	Description	Pubertal stage
Stage 1	Inferior border flat, vertebral body wedge shape	Initiation
Stage 2	Concavity appearing in inferior border, body nearly rectangular in shape	Acceleration
Stage 3	Concavity developing in inferior border, body rectangular in shape	Transition
Stage 4	Distinct concavity in inferior border, body nearly square in shape	Deceleration
Stage 5	Accentuated concavity in inferior border, body square in shape	Maturation
Stage 6	Deep concavity in inferior border, body taller than it is wide	End of growth spurt

Ossification and fusion of the iliac crest epiphysis.

Using the "Risser signs" of iliac crest ossification, Lewis, Shapland and Watts (2016) defined the morphological changes associated with each pubertal stage. Ossification of the epiphysis starts during the acceleration stage, and it remains unfused until after PHV. Deceleration marks the start of the fusion of the partially ossified epiphysis to the iliac blade, with a complete ossification achieved during maturation, while a completely fused iliac crest epiphysis marks the end of the growth spurt. Although ossified but unfused iliac crest epiphyses are not usually recovered during archaeological excavations, partial fusion of the incomplete epiphysis indicated PHV has passed, and that menarche has been achieved in girls.

Fusion of ulnar proximal epiphysis.

As with the humeral capitulum, the partial or complete fusion of the proximal epiphysis of the ulna was briefly mentioned by Lewis, Shapland and Watts (2016) to occur during the transition stage, when PHV is achieved. A completely fused ulnar proximal epiphysis is expected during the deceleration stage, before menarche in girls.

Fusion of humeral capitulum.

Briefly mentioned by Lewis, Shapland and Watts (2016), initial fusion of the capitulum (capitate epiphysis) to the distal end of the humerus occurs during PHV, with a complete fusion of the epiphysis during the deceleration stage.

Fusion of radial distal epiphysis.

Marking the last part of the adolescent growth spurt, the distal epiphysis of the radius remains unfused until the deceleration stage; fusion of the epiphysis starts during the maturation stage, with a completely fused epiphysis being found in post-pubertal individuals (Lewis, Shapland and Watts, 2016).

2.2.4 Assessment of physiological stress

In order to explore physiological stress, selected skeletal stress markers were used on each manuscript, depending on each research objective. Macroscopic signs of pathological changes were recorded following recommended standard techniques (Mitchell and Brickley, 2017); severity of the lesion was documented but not included within the final statistical analyses in each research chapter, where only presence or absence was analysed. Radiographic assessment of skeletal stress markers, that could have been further helped diagnose bone loss and periosteal new bone formation, was not undertaken due to limitations of cost and time.

Brief summaries of the principal aetiologies and macroscopic signs for each skeletal indicator of physiological stress are as follows.

Bone loss (BLOS).

Progressive bone loss is a normal part of ageing and can lead to increased morbidity and mortality (Agarwal, 2021). Bone loss is defined as a metabolic and degenerative disease characterised by the imbalance between bone formation and resorption, decreasing bone quality and density (van Spelde *et al.*, 2021), leading to compromised bone strength that predispose individuals to higher risk of fracture from low-impact trauma and chronic pain (Tomasevic-Todorovic *et al.*, 2018). Early stages of bone loss and decreased bone density are known as osteopenia, where there is increased structural weakening of skeletal elements, but the condition is not as severe to expect higher risk of pathological fractures. Age-related bone loss occurs in both sexes, but females are more frequently affected due to hormonal changes triggered during menopause; oestrogen is essential to the maintenance of healthy bone metabolism, and its fall after the menopause heavily impacts bone density (Leboime *et al.*, 2010).

Bone loss was recorded when signs of osteopenia were found in the skeletal remains (Figure 2.10). As structural changes related to age-related osteoporosis are not macroscopically visible until they develop into pathological fractures, osteoporosis was recorded when vertebral body compression, distal radius (Colles' fracture) and/or the femoral neck fractures were found. Osteoporosis was also listed as cause of death in some individuals, in which case it was also recorded as present. Osteopenia was recorded when general lightness, cortical thinning and prominent vertical trabeculae was found on the bones, including translucency of the ilia, diffuse porosity of the ectocranium, severe kyphosis, and thinning and deformities of parietal bones and ribs (Buikstra, 2019; Brickley, Ives and Mays, 2020). Both osteoporosis and osteopenia were blended together into a single variable, BLOS, that was analysed in Chapter 6 in the older adult sample.

Cribra femoris (CF).

Also known as cribra femoralis, CF is a porotic kind of pathological lesion, and it has been linked to certain diseases such as iron-deficiency anaemia and malaria, as well as other nutritional deficiencies during intense growth periods (Smith-Guzmán, Rose and Kuckens, 2016; Brickley, 2018; Buikstra, 2019; Schats, 2021). It has been traditionally associated with

Figure 2.10 Examples of bone loss: bilateral femoral neck fracture in a 61 years of age female (B0203) (A), and vertebral compression fracture of C6 in an 81 years of age female with recorded osteoporosis as cause of death (B1791) (B).

younger individuals, with its presence decreasing with age, and it has been found to be more prevalent in females (Radi *et al.*, 2013; Smith-Guzmán, Rose and Kuckens, 2016; Gomes *et al.*, 2022).

Its presence was defined in this study when porosity was found on the anterior side of the femoral neck, in a unilateral or bilateral occurrence (Figure 2.11). It is important to note that presence of CF was distinguished from the non-metric trait known as Allen's fossa, a normal variant of the femoral neck found at the same location, differentiated from CF by the absence of cortical bone margins. Allen's fossa presents as a concave area with clear sclerotic margins, while CF lacks said boundaries and expresses only as cortical discontinuity in the form of porosity on a periosteal level (Radi *et al.*, 2013; Göhring, 2021). Severity was recorded following Radi *et al.* (2013), using numeric values to record absence (0), clustered porosity of 1 mm or more on the cortical surface (1) and cortical erosion with trabecular exposure and possible depression of the surface (2). However, only presence/absence was analysed in Chapter 3.

Cribra orbitalia (CO).

One of the most common pathological lesions found on skeletal remains and reported in bioarchaeological literature, CO is a non-specific indicator of physiological stress (Lewis, 2007; Zariía *et al.*, 2016; Buikstra, 2019). Several aetiologies have been proposed for the presence of CO, the most traditional of them linked to anaemia, including iron deficiency,

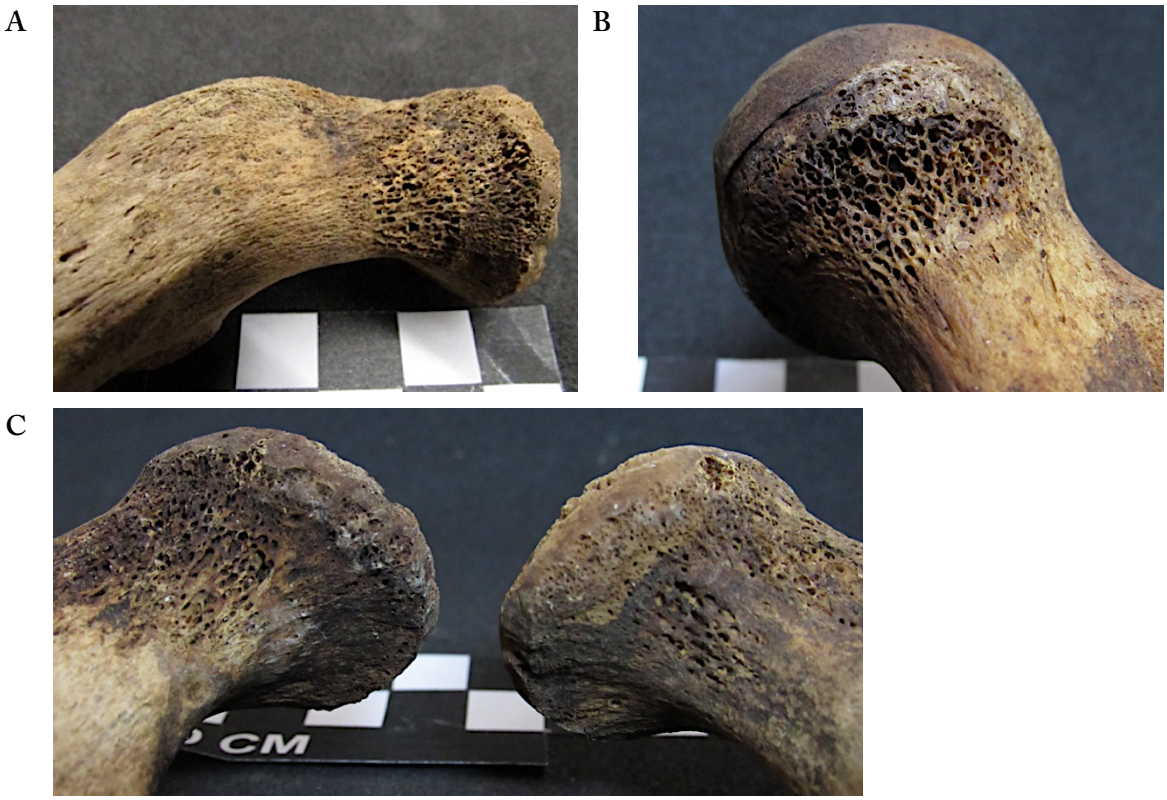

Figure 2.11 Examples of cribra femoris: score-1 lesion on the proximal right femur in a 7 years of age female (B0234) (A), score-2 depressed lesion on the proximal left femur in an 18 years of age female (B0236) (B), and lesions of different severity in a 9 years of age female (B0758) (C).

megaloblastic, and haemolytic anaemias (Buikstra, 2019). However, Stuart-Macadam (1992) argued that nutritional intake was not the direct cause of iron deficiency anaemia, but iron insufficiency was to be seen as an adaptive strategy, a defence mechanism against diseases. In addition, Brickley (2018) warns that a wide range of conditions can produce CO and that anaemia might not be the sole cause behind porous lesions in the orbital roofs. Other potential aetiologies proposed include but are not limited to malaria (Rabino Massa, Cerutti and Marin D. Savoia, 2000), leprosy (Moller-Christensen and Sandison, 1963), renal failure (Rivera and Mirazón Lahr, 2017), scurvy (Rivera and Mirazón Lahr, 2017), congenital heart conditions (Brickley, 2018; O'Donnell *et al.*, 2020), bacterial or viral infections (O'Donnell *et al.*, 2020), parasitic infections (Godde and Hens, 2021), and localised inflammation (Ortner, 2003).

It is worth noting that there are also differences in bone response to hematopoietic disorders during childhood that affect the presentation of CO (Lewis, 2017b). The transforming immune system in children includes the replacement of red to yellow bone

marrow in the frontal bone as age increases (Kricun, 1985). This conversion is reported to happen between 10 and 11.5 years of age in boys and girls, respectively (Simonson and Kao, 1992), meaning there should not be active CO lesions after this age. Thus, presence of CO in older non-adults and adults indicate episodes of physiological stress in childhood, rather than marking new episodes during later life.

CO was found present when porous lesions with a trabecular organisation appearance were observed on one or both orbital roofs, with variable severity impacting the expansion of the diploë and outer table. A five-grade protocol was used to record severity of CO, following Rivera and Mirazón Lahr (2017) and in accordance with Stuart-Macadam (1985) and Buikstra and Ubelaker (1994) (Figure 2.12). Said protocol ranges severity from presence of isolated porosity in milder cases, to severe cases where changes related to trabecular growth beyond the outer table in a honeycomb-like structure are found. Only stages 1 to 4 were used to describe presence of CO as a sign of nutritional deficiency, as they describe lytic lesions only; the last stage of severity includes bone deposition changes, a type of lesion that has been linked to infection and scurvy (McFadden and Oxenham, 2020). In addition, the stage of healing was noted for each case. Although severity of CO was recorded, only presence/absence was analysed in Chapters 3, 4, 5, and 6 (Figure 2.13).

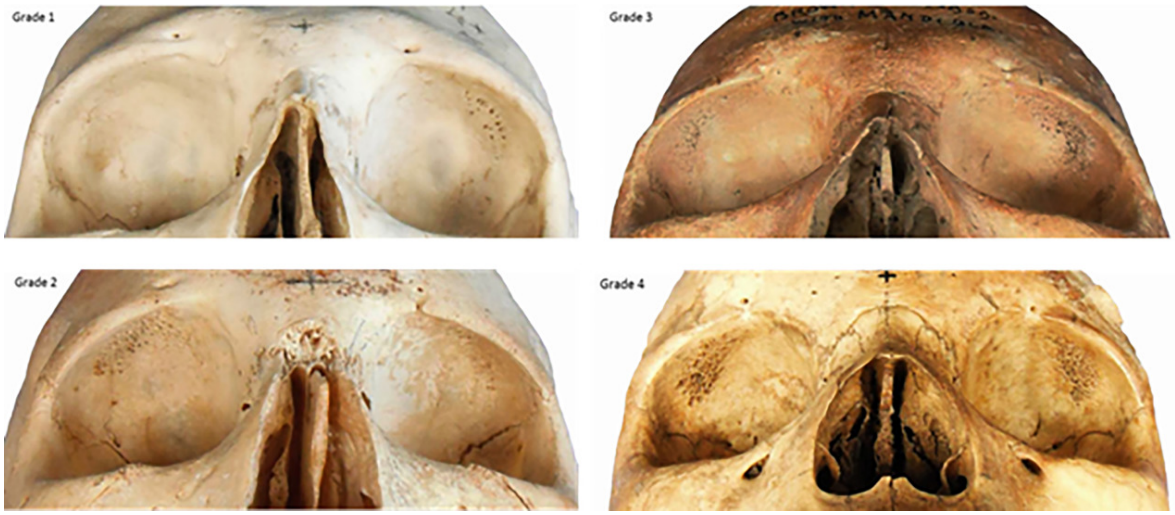

Figure 2.12 Grades of development of cribra orbitalia as defined by Rivera and Mirazón Lahr (2017).

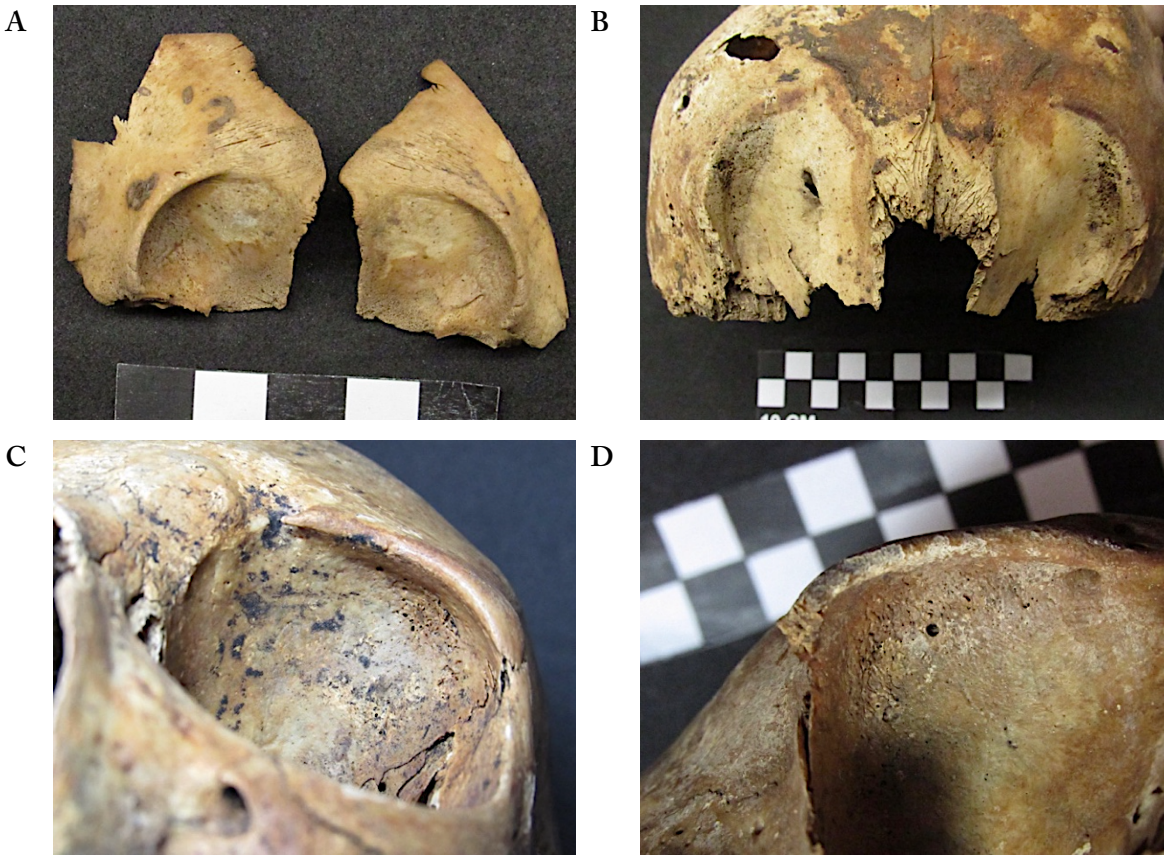

Figure 2.13 Examples of cribra orbitalia: mild lesions on the left orbit in a 7-month of gestation male (B2054) (A), marked lesions on the left orbit in a 13 months of age male (B2092) (B), moderate lesions on the left orbit in a 70 years of age female (B0861) (C), and moderate lesions on right orbit of a 71 years of age male (B0054) (D).

Dental diseases.

Healthy periodontal and dental tissues are key to maintain dentition stability and retention, affecting individuals in both physical and psycho-social levels (Watson and Tuggle, 2019). Several stressors during the life course such as bacterial infections, chronic gingivitis, and bone resorption affect oral health and can inform about an individual's lifeways (Hillson, 2014; Larsen, 2015; Temple, 2015).

This study examined dental diseases such as dental caries (CAR), antemortem tooth loss (ATML), periodontal disease, and linear enamel hypoplasia (LEH) (Figure 2.14); the latter will be discussed separately. Presence of CAR was analysed by itself in Chapter 4, while three markers (CAR, AMTL and periodontal disease) were grouped as DEN and analysed together in Chapter 5. CAR, AMTL, and periodontal disease share an infectious aetiology, and have been characterised as chronic diseases (Larsen, 2018). Dental caries is

associated with the focal demineralisation of enamel and loss of tooth substance (dentine) caused by the organic acids resulting from acidic by-products of bacterial metabolism (Gussy *et al.*, 2006). Its presence is used in bioarchaeology and paleodemography to explore changes in diet between populations, during major cultural transitions, susceptibility of tooth/host, and socioeconomic status (Temple, 2015). Diagnosis of CAR in bioarchaeological materials is done primarily by visual and visual-tactile examination of carious lesions but can also be done using radiographs. Secondly, ATML can be the result of several causes, such as diet, nutritional deficiency, intentional removal, and/or trauma, and has been linked to higher rates of systemic infection as a result of low socioeconomic status, lacking living conditions and impaired access to healthcare (Russell *et al.*, 2013). Finally, periodontal disease – also known as periodontitis – is an infectious disease of the periodontal soft tissues caused by acidic metabolic waste from bacterial accumulation, resulting in infection and inflammation of the gums and bone that surround and support the dentition (Larsen, 2015). The inflammatory response in the alveolar bone can lead to AMTL. Both CAR and periodontal disease have been found to be more prevalent among females, and their presence has been linked to the changes in oral environment associated with pregnancy (Lanfranco and Eggers, 2012).

Linear enamel hypoplasia (LEH).

A dental defect characterised by transverse lines, pits, and grooves on the tooth crowns surfaces, resulting from insufficient enamel deposited during the secretory stage of amelogenesis (Hillson, 2014). Physiological stress impacts ameloblasts production of enamel matrix, creating bands of disruption on the crown, increasing the space between perikymata (Nelson, 2014). When stress is systemic, matching bands of LEH can be found across different teeth with overlapping developmental timing; when stress is localised, due to infection or trauma, enamel defects are not matched across the dentition. The condition is often used as indicator of stress related developmental disruption during foetal life and childhood (King, Humphrey and Hillson, 2005), as initial calcification starts as early as 14 weeks *intra utero* for deciduous teeth (upper and lower central incisors) and continues to 7/10 years of age for permanent teeth (upper and lower third molar) (Nelson, 2014). Contrarily to skeletal indicators of stress, the advantage of analysing LEH relies on the fact that enamel

Figure 2.14 Examples of dental diseases: dental caries in the first and second lower left molars of a 19 years of age male (B0128) (A), edentulous maxilla in a 70 years of age female (B0861) (B), and antemortem tooth loss and periodontal disease in the maxilla a 69 years of age male (B1533) (C) and the mandible of a 54 years of age female (B0889) (D).

does not remodel and provide precise timing of the stress event, as it is possible to quantify the frequency, age of occurrence and its duration (King, Humphrey and Hillson, 2005).

Presence of LEH was recorded for all dentition, deciduous or permanent, across the sample (Figure 2.15). Time constraints during data collection allowed only for macroscopic diagnosis of presence or absence of LEH, which was analysed in Chapters 3, 4, 5 and 6.

Osteoarthritis (OA).

The most common form of arthritis, OA is a degenerative disorder of the articular cartilage in synovial joints associated with hypertrophic bone changes (Buikstra, 2019). It can involve several tissues of a joint and multiple joints in the body, with its prevalence increasing with age and disproportionately affecting women (Hunter, March and Chew, 2020). Several

A  B 

Figure 2.15 Examples of linear enamel hypoplasia: on incisors of an 11 years of age male (B1497) (A), and on incisors and premolars of a 19 years of age male (B0128) (B).

causes have been proposed for OA and it is now accepted the disease is multifactorial in its aetiology (Domett *et al.*, 2017); some known causes of OA include genetic predisposition, lifestyle factors (activity, overuse, obesity), advancing age, and immunological response to inflammatory processes (Jones, Glimcher and Aliprantis, 2011; Larsen, 2015).

OA was found to be present when signs of "wear and tear" were observed on articular surfaces of the long bones, scapulae, ox coxae, vertebrae, patellae, carpals, and tarsals (Figure 2.16). Lesions included lipping (marginal proliferation of pathological new bone resulting on changes in shape of the joint surface or contour), pitting (porous lesions with or without joint surface erosion), surface osteophytes (proliferative changes associated with bone growth along the edges of the articular surface), and eburnation (polished subchondral bone result of mechanical scoring of the joint surface against other bone, usually related to late stages of OA). Presence of eburnation alone, or in combination with other lesions, was sufficient to record OA, as this is a strongly diagnostic lesion (Buikstra and Ubelaker, 1994; Calce *et al.*, 2018). When eburnation was not found, two other lesions had to be present for a positive diagnosis (Rogers and Waldron, 1995). As an age-related pathological change, OA was analysed in Chapter 6.

Periosteal new bone formation (PNBF).

Areas of new bone formation on the external surface of bones have been traditionally associated with non-specific inflammation of the bone surface, the periosteum (Roberts and Manchester, 2010). Recorded in bioarchaeology as periosteal reaction, periostitis and/or

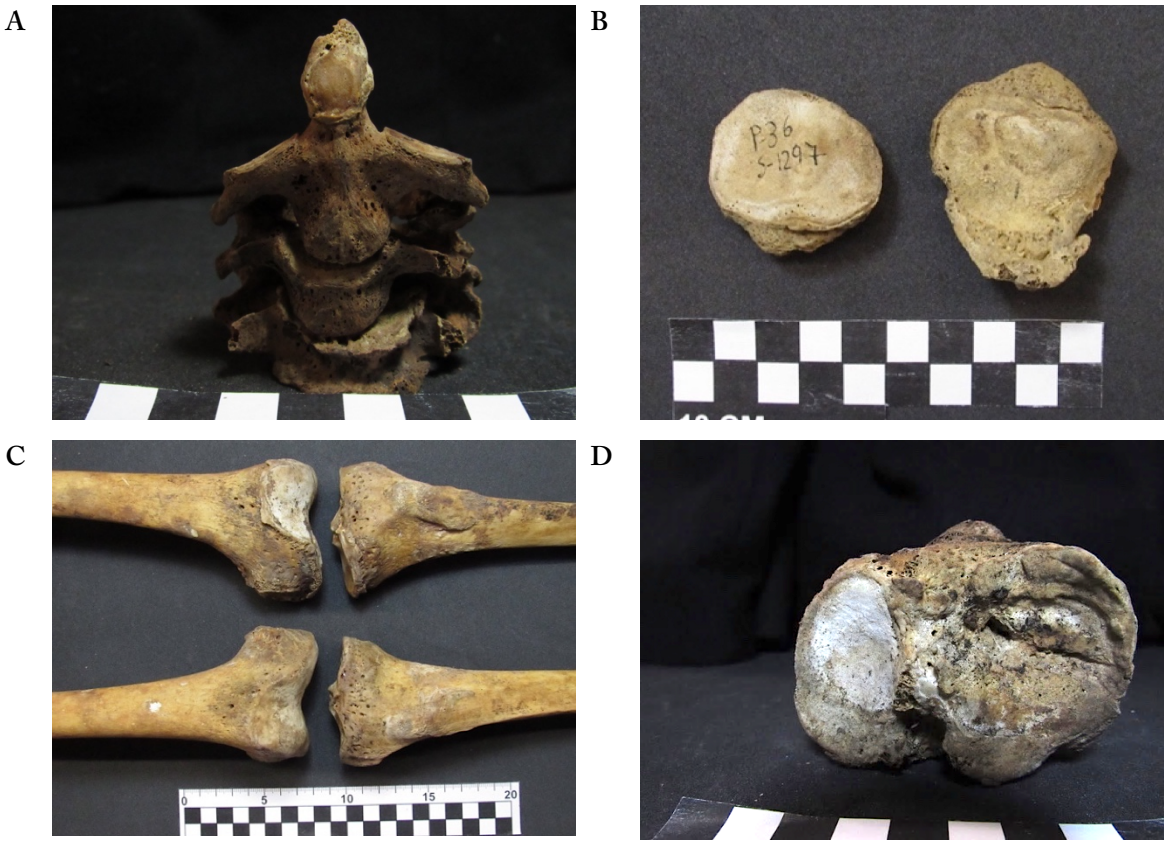

Figure 2.16 Examples of osteoarthritis: contour changes and eburnation on the odontoid process of C2 in a 69 years of age female (B1505) (A), osteophytes and pitting on right patella in a 67 years of age female (B0823) (B), left distal femur eburnation and lipping in a 73 years of age male (B0822) (C), and eburnation, lipping, erosion, and severe destruction of articular surface of the distal end of the left femur in a 71 years of age male (B0814) (D).

periostosis, manifestations of PNBF include fine porosity and pitting, longitudinal striation, and plaque-like formations of bone (Buikstra, 2019). PNBF can be seen as woven or lamellar bone; woven (or fibrous) bone is characterised by a disorganised collagen apposition and weaker structure, being a faster forming bone response to stress, while lamellar bone is characterised by regular, parallelly aligned collagen fibres bone that is mechanically stronger and develops more slowly, replacing woven bone in time (Currey, 2006). As bone remodelling, including substitution from woven to lamellar bone, is a continuous process, PNBF lesions indicate processes occurring at or during the time around death. Being one the most commonly seen non-specific pathological bone changes (Roberts and Manchester, 2010), the aetiology of PNBF has been typically linked to non-specific stress, including infection, trauma, and metabolic disturbances (Lewis, 2017b; Buikstra, 2019).

Important to note is the major limitation in analysing PNBF in non-adults, particularly those in the foetal-infant age group in this study. Differentiating pathological from normal new bone formation in young individuals is challenging, as bone reaction to both pathological insults and normal healthy growth is almost indistinguishable at this stage of life (Lewis, 2017a). Regardless of a normal or pathological origin, newly formed bone will be secreted as woven bone, an immature and highly disorganised type of bone (Lewis, 2017b; Buikstra, 2019). This new bone is macroscopically identical in both normal and pathological scenarios, and presence of woven periosteal new bone in individuals under 4 years of age should be expected as part of the normal growth process (Ortner, 2003). Moreover, juveniles present a rapid remodelling rate of the expression and repair of pathological skeletal indicators (Lewis 2017b), which might make the analysis and recording of PNBF even more complex.

All evidence of PNBF was recorded (Figure 2.17) providing type (woven/lamellar), severity (mild/moderate/severe) and location of the lesion, but only presence/absence was analysed in Chapters 3, 4, 5 and 6. For the foetal-infant age group, lesions with the distinctive grey appearance woven bone were recorded as presence of PNBF when they were found to be asymmetrical and/or bilaterally differing in severity (Hodson, 2018).

Porotic hyperostosis (PH).

As other porous lesions such as CO and CF, PH is a commonly found lesion in bioarchaeological remains (Aufderheide and Rodriguez-Martin, 1998). Lesions associated with PH are described as ectocranial porosities with diploic expansion on the cranial vault as a result of hypercellularity and hyperplastic processes (Rivera and Mirazón Lahr, 2017; Buikstra, 2019). As with CO, presence of PH in older non-adults and adult individuals should not be taken as evidence of new episodes physiological stress, as there is no bone response resulting in marrow expansion due to hematopoietic disorders after 10-11 years of age (Lewis, 2017b). Traditionally, most studies have linked these kinds of lesions to anaemia, either from a congenital haemolytic origin or associated with nutritional deficiencies, such as iron deficiency (Stuart-Macadam, 1985, 1992; Oxenham and Cavill, 2010; Brickley, 2018). There has been considerable debate over the past decade over the aetiology of porous lesions and their traditional association with anaemia, particularly on the association – or

Figure 2.17 Examples of periosteal new bone formation: right mandible of a 1 month old male (B2027) (A), tibiae of a 5.5 months of age male (B2020) (B), femurs with PNBF associated to osteomyelitis of a 13 months of age male (B2042) (C), right ilium with PNBF associated with tuberculosis of a 6 years of age female (B0217) (D), left tibia of a 15 years of age female (B0016) (E), and left tibia of a 53 years of age male (B0840) (F).

lack thereof – between CO and PH (Rivera and Mirazón Lahr, 2017). Some studies suggest it can also represent an age-related response to common pathological conditions (Oxenham and Cavill, 2010) or infections (O'Donnell *et al.*, 2020), while a recent study using ancient DNA from Neolithic individuals suggests that anaemia and low bone mineral density as the main cause for PH (Ferrando-Bernal, 2023).

When porosity and/or pitting on the cranial vault was observed (Figure 2.18), particularly on the frontal and parietal bones, PH was recorded as present. This lesion was analysed in Chapters 3, 4, 5 and 6.

Figure 2.18 Examples of porotic hyperostosis: mild lesions on a 59 years of age female (B0189) (A), moderate lesions on an 18 years of age male (B0357) (B), and severe lesions on an 18 years of age female (B0236) (C) and 64 years of age male (B0034) (D).

Rickets (RIC).

An inadequate bone mineralisation due to abnormal bone metabolism often caused by vitamin D deficiency, RIC is characterised by softening and weakening of bones in children (Brickley, Ives and Mays, 2020). Vitamin D is a pro-hormone fat-soluble secosteroid responsible for increasing intestinal absorption of calcium, magnesium, and phosphate, and is essential for bone health, immune response, mineral metabolism, and normal cell growth (Holick, 2006). Its deficiency interacts with the normal deposition of calcium in cartilage and bone osteoid during growth and development, triggering insufficient mineralisation at sites of endochondral growth and leaving the bone susceptible to deformation. Although the most common cause of RIC is vitamin D deficiency, it is also caused by genetic predisposition, having dark skin, insufficient sun exposure, exclusive breastfeeding without supplementation, coeliac disease, and nutritional deficiency of calcium or phosphorus (Thacher *et al.*, 2006).

In adults, the milder form of RIC is known as osteomalacia. Osteomalacia was not diagnosed during data collection, as this skeletal indicator of stress was only used in the manuscript relating to non-adults (Chapter 4). Following Brickley, Ives and Mays (2020), macroscopic features related to RIC and assessed within this sample were medial angulation of the mandibular ramus, alteration of rib neck angle, lateral straightening of the rib shaft, enlargement of costochondral rib junctions, exaggerated medio-lateral curvature of the ilium, acetabulae protruding anteriorly, flaring of long bone distal metaphyses, porosity and cupping deformities of growth plate and metaphyses, bending of the long bones and angulation of the femoral neck with *valgus* or *varus* changes (Figure 2.19). A diagnosis was only made when the overall pattern of changes across the skeleton was suggestive of RIC (Vlok, 2023).

Scurvy (SCU).

Long term and/or chronic vitamin C deficiency results in scurvy, a potentially fatal disease characterised by musculoskeletal pain and weakness, lethargy, and haemorrhagic episodes that can lead to sub-periosteal inflammation and AMTL (Mays, 2018; Brickley, Ives and Mays, 2020). Vitamin C deficiency is also linked to decreased osteoblastic activity, with abnormal osteoid deposition and structurally faulty mineralised bone leading to osteopenia (Fain, 2005). As both RIC and SCU are diseases caused by nutritional deficiencies, there is considerable overlap between their presence among individuals (Mays, 2018).

Following Brickley, Ives and Mays (2020), presence of SCU was recorded when particular macroscopic lesions associated with scurvy in non-adults were found: abnormal pores of <1 mm across and penetrating the cortex in the skull (particularly on the sphenoid, mandible, maxilla, and orbits), PNBF on orbits and the cranial vault, fracture and/or enlargement of rib costochondral ends (scorbutic rosary), abnormal porosity on supraspinous and infraspinous areas of the scapulae, and PNBF near the end of long bones (Figure 2.20). Presence of these lesions was assessed in all individuals within this sample, and a diagnosis was only made when the overall pattern of changes across the skeleton suggested SCU (Vlok, 2023).

Figure 2.19 Examples of skeletal lesions associated with rickets: flaring of femoral distal metaphyses in a 1 year of age female (B2053) (A), mild bowing of tibiae in a 14 months of age female (B), angulation of femoral necks in *coxa valga* (C) and severe bending of femurs and fibulae in a 21 years of age female (B0212) (C and D).

Skeletal scorbutic lesions in adults are rare, with little bone changes that can be directly associated with the condition and their diagnostic features often overlapping other non-specific origins including infectious, traumatic, or inflammatory conditions (e.g., PNBF on the end of long bones, such as those illustrated in Figure 19B, can be attributed to scurvy in said individual only through antemortem data). Thus, only non-adult scurvy was analysed in Chapter 4.

Figure 2.20 Examples of skeletal lesions associated with scurvy: marked porosity above the right mandibular foramen in a 7-month GA male (B2054) (A), periosteal new bone formation on the distal end of the right tibia in a 21 years of age female (B0188) (B), and abnormal porosity of the external surface of the greater wings of the left sphenoid bone in a 21 years of age female (B0212) (C) and right sphenoid bone in a 64 years of age male (B0828) (D).

2.2.5 Statistical analyses

Different statistical analyses were used to answer particular research questions within each chapter. A brief description of each method follows, with details of why they were chosen. All statistical analyses were performed using RStudio 2021.09.0 Build 351.

Lin's concordance correlation coefficient.

To assess the degree of agreement between two measures of the same variable, Lin's concordance correlation coefficient (ρc) was used to determine the accuracy of metric assessments. Like a correlation, it ranges from -1 to 1, with perfect agreement at 1, and it cannot exceed the absolute value of ρ (Pearson's correlation coefficient between Y and X) (Lin, 2000; Steichen and Cox, 2002; McBride, 2005). This statistical method was used to

calculate intra- and inter-observer error on measurements of maximum length of femur diaphysis and pars basilaris undertaken in Chapter 3. This method was chosen because of the small sample size analysed in said chapter, as Lin's concordance correlation coefficient can be calculated to provide a way to evaluate reproducibility and inter-rater reliability on as few as ten observations (Steichen and Cox, 2002).

Effect size.

Also known as measure of association, effect size statistics inform how meaningful the relationship between variables is or how much one group differs from another, indicating the practical significance of a result. The selected measure of effect size, Hedges' g, is designed to compare two groups, taking the difference between two means, and expressing it in standard deviation (SD) units, informing how many SDs are between said two means (Kline, 2004; Fritz, Morris and Richler, 2012). Although Hedges' g is similar to other measures of effect size (e.g., Cohen's d), Hedges' g outperforms other methods when used in very small sample sizes (<20) (Hedges and Olkin, 1985). Hedges' g was used as measure of effect size to calculate the difference between chronological age (CA) and age estimations derived from skeletal elements (SA).

To interpret results from effect size statistics, three outputs are reported: Hedges' g, confidence intervals (CI), and Common Language Effect Size (CLES). Reporting confidence intervals is strongly recommended to explore the precision of the estimates while also allowing for related studies to compare their results and explore stability across population parameters (Thompson, 2007). It is often suggested to follow Cohen's d "rule of thumb" to interpret Hedges' g, where effects size can be small (around or below 0.2), medium (around 0.5) and large (around or above 0.8) (Cohen, 1988). However, Cohen himself and later researchers have argued against the use of set benchmarks as immobile criteria to interpret effect size (Dunlap, 1994; Thompson, 2007); current guidelines suggest to consider the source of the research, compare results across similar other research conditions, and to consider the findings' clinical or practical significance (Thompson, 2007). In addition, to convey results in a more intuitive way, results output also include CLES values, which are representative percentages of Hedges' g calculated values (Lakens, 2013). Calculated directly from Hedges' g, CLES expresses the probability that a randomly sampled age-at-death from

one group (CA) will have a higher observed measurement than a randomly sampled age-at-death from the other group (SA). To effectively interpret the contextual magnitude of the differences between CA and SA, the research manuscripts used results > 0.60 (CLES > 65%) as significant.

Simple (symmetric) correspondence analysis.

A data visualisation technique, simple correspondence analysis (SCA) falls within classical multivariate statistical methods of dimension reduction based on the singular value decomposition (Greenacre, 1993). It is conceptually similar to principal component analysis (PCA), with the difference that it applies to categorical and ratio-scale data rather than continuous data. The data is organised in a contingency table, with rows representing one categorical variable and columns representing the other categorical variable, with the entries in the table representing the frequency or proportion of the joint occurrence of the two variables. SCA first calculates the row and column marginal totals, which are the sums of the entries across rows and columns, respectively. These marginal totals are then used to calculate the expected values of the joint distribution under the assumption of independence between the two variables. Next, SCA calculates the residuals, which are the differences between the observed and the expected values and uses these residuals to calculate the chi-square distance between each pair of categories. The distances are then used to produce a geometric representation of the data on a low-dimensional map; as a geometrical approach to visualising data, the total variance of the variables and the total decomposed optimally along "principal axes" are defined; the larger the percentage of total variance accounted for by the first two principal axes, the stronger the relationship between the components (Greenacre, 2002, 2010).

SCA was used in Chapter 3 to produce a map of the relative relationship between and within two groups of variables, growth disruption and physiological stress (evidenced by the presence of skeletal stress markers in this sample). Biplots generated using SCA show the first two dimensions on both x and y axes, with proximity in the plot space indicating a positive association between the variables. In addition, the closer the angle between two groups is to 90°, the less association there is between them; a weak association between variables indicates little deviation between the observed and expected values.

Survival analysis.

A type of regression problem, survival analysis is statistical technique used to analyse the time it takes for an event of interest to occur, thus estimating the probability of said event occurring in a particular time while identifying factors that may influence the time until the event occurs. The analysis describes and models data using two related probabilities, survival (the probability that an individual survives from the time of origin to the event of interest) and hazard (the event rate for an individual who has already survived to the time of the event of interest). Thus, survival reflects the cumulative non-occurrence while hazard indicated said event rate. One of its unique features is that it takes into account censoring, which occurs when the event of interest has not occurred for some subjects by the end of the study, allowing for a more accurate analysis of the time to event data (Clark *et al.*, 2003). Although often used in medical research to study survival rates of patients with a particular disease or exposed to certain treatments, its use in bioarchaeology over the last decade has increased as it allows to use age-at-death as proxy for death as the event of interest.

Two statistical techniques in survival analysis were used in Chapters 4 and 6: Kaplan-Meier estimator with a log rank test and Cox proportional hazard models. The use of survival analysis across chapters differed depending on the research question, but general concepts of these statistical techniques remained the same. Time-to-event or serial time, which in clinical studies is the variable period of time between entering the study and the occurrence of the event of interest, was defined here by the entire duration of the life of each individual in the sample. Skeletal stress indicators were used as risk factors, recording its presence (coded 1) or absence (coded 2) while also censoring non-observable lesions (coded 0) to prevent overestimation of absence. Predictor variables such as sex and age group were also used to compare survival functions within each population.

Kaplan-Meier survival curves were used to analyse survival probability according to one risk factor, ignoring the impact of other factors. A univariate method, Kaplan-Meier estimator is used when the predictor variable is categorical. For this analysis, each individual was characterised by three variables: age-at-death as serial time, presence/absence/censored of each skeletal stress marker as status at the end of the serial time, and the group they belonged to, either biological sex or age group. Serial time end-point time scale in bioarchaeological studies is always assumed to be age-at-death, point where death as the

event of interest occurs and the known survival is terminated; this period is known as interval and is illustrated as a horizontal line in Kaplan-Meier curves (Rich *et al.*, 2010). Censored data is graphed as tick marks, as they do not interfere with the interval. Vertical lines do not represent survival time, with the distance between one interval line and the next indicating the cumulative probability as the curve continues, as seen on the Y-axis. To quantify the difference between two survival curves, a log rank test was applied calculating the chi-square (X^2) for each event time for each group, summing the results; the summed results for each group are then added to calculate the overall chi-square for the full curve of each group (p-value). For all analyses, p-values less than 0.1 are statistically significant and considered suggestive of a trend.

Cox proportional hazard models were used to simultaneously assess the effect of several risk factors on survival time. Essentially a regression model, Cox models work with both quantitative and categorical predictor variables to model the hazard rate of an event. The model assumes that the hazard rate is proportional across different levels of the covariates, hence the name "proportional hazards". In this study, Cox models allowed to examine how certain predictor variables (risk factors) influence the hazard rate of death happening. Differently from Kaplan-Meier analysis, the predictor variables (also called covariates) in Cox models are the risk factors themselves (e.g., skeletal stress markers), as each model analyses a different group (e.g., biological sex or age group) separately. To interpret the results from the Cox proportional hazards model, one of the main parameters of interest is the hazard ratio (HR) (Clark *et al.*, 2003). HR measures the effect size of each covariate in any given model, this is, the relative risk of an event occurring while simultaneously controlling for all the covariates in the model. An HR greater than 1 indicates an increased risk of the event occurring, while an HR less than 1 indicates a decreased risk. An HR of 1 indicates no difference in the risk of the event occurring, representing the baseline of survival. HR results are accompanied by a confidence interval (CI), which gives an estimate of the precision of the HR estimate. The p-value is another measure of statistical significance that indicates whether the effect of the covariate on the event of interest is likely to be due to chance; a p-value less than 0.05 is typically considered statistically significant. To facilitate communication about results that are quantified using HR, results are discussed in percentages adapted from Spruance *et al.* (2004)'s approach, deriving the probability of dying (or risk of death) from the hazard ratios (HR) assuming $HR = \frac{P}{(1-P)}$ and $P = \frac{HR}{(1+HR)}$.

2.3 Summary

The population selected for analysis within this study is representative of low socioeconomic status communities that inhabited Santiago de Chile during the late 19th and early 20th centuries. Data collected from this population included extant osteological data, cemetery records and original osteological evidence, to offer the unique opportunity to work with skeletal remains of documented age-at-death, sex, cause of death, and years of birth and death.

The methodologies outlined here enabled the combination of data into a comprehensive database tailored to addressing the research objectives of each of the four research chapters that comprise Chapters 3, 4, 5 and 6. Each manuscript aims to address different dimensions of the impact of physiological stress on health throughout the life course, in accordance with the research questions outlined in Chapter 1. Though the overarching purpose of exploring the effects of physiological stress on frailty experience, Chapter 3 examines growth disruption in foetal-infant individuals, Chapter 5 explores pubertal timing and its delay during adolescence, while Chapters 4 and 6 investigates survival in non-adults and older adults, respectively. The findings of these four research chapters are then summarised and discussed as a whole in Chapter 7.

Chapter 3

Growth disruption and physiological stress of foetal–infant individuals of documented chronological age from mid-20th century Santiago, Chile

Abstract

Objectives: This paper explores growth disruption in foetal, perinatal, and infant individuals of documented chronological age (CA) that lived in low socioeconomic status communities in mid-20th century Santiago, Chile.

Materials and Methods: 47 foetal-infant individuals of documented CA and biological sex were examined. Physiological age (PA) estimations from skeletal (pars basilaris measurements and femoral lengths) and dental elements were compared to CA to explore discrepancies. Kruskal-Wallis tests ($p<0.05$) were used to identify growth disruption. Pathological changes were analysed through 8 skeletal stress markers. Evidence of growth disruption in combination with skeletal changes was analysed through correspondence analysis to examine the relationship between growth and physiological stress.

Results: *Age estimation*. Dental age estimates were the most accurate for this sample. Femoral length underestimated CA, while pars basilaris dimensions randomly over and underestimated CA regardless of age group or sex. *Physiological stress*. Correspondence analysis with symmetrical normalisation showed growth disruption scenarios were related to the presence of skeletal stress markers. Growth disruption in this sample is particularly associated with presence of cribra orbitalia and periosteal new bone formation.

Conclusions: This study supports the idea that growth disruption due to physiological stress affects growth and maturation and can bias age estimations made using dental and skeletal elements. The pars basilaris method, argued to be an accurate age assessment method for European individuals, performed poorly on this South American sample. As the effects of physiological stress causing growth disruption are present both pre- and postnatally (and before weaning), this indicates elevated maternal physiological stress in this population.

Keywords: maternal nexus, documented age-at-death, age estimation, developmental stress

Alteración del crecimiento y estrés fisiológico en fetos, perinatos e infantes de edad cronológica documentada de mediados del siglo XX de Santiago, Chile

Objetivo: En este trabajo se explora la alteración del crecimiento en fetos, perinatos y lactantes de edad cronológica documentada (CA) provenientes de comunidades de estrato socioeconómico bajo que vivieron a mediados del siglo XX en Santiago, Chile.

Materiales y Métodos: Se examinaron 47 individuos de edad cronológica y sexo biológico documentados. Las estimaciones de edad fisiológica (PA) a partir de elementos dentales y esqueléticos (medidas de pars basilaris y longitudes femorales) se compararon con CA para explorar posibles discrepancias. Se utilizaron pruebas de Kruskal-Wallis ($p<0,05$) para identificar la interrupción del crecimiento. Los cambios patológicos se analizaron a través de 8 marcadores de estrés esquelético. La evidencia de interrupción del crecimiento en combinación con cambios esqueléticos se analizó mediante un análisis de correspondencia para examinar la relación entre el crecimiento y el estrés fisiológico.

Resultados: *Estimación de edad*. Las estimaciones dentales de edad fueron las más precisas en esta muestra. El largo femoral subestimó CA, mientras las dimensiones del pars basilaris entregaron edades inconsistentes, sobre y subestimando CA sin importar grupo etario o sexo. *Estrés fisiológico*. El análisis de correspondencias con normalización simétrica mostró que los escenarios de alteración del crecimiento se relacionan a la presencia de marcadores de estrés esqueletal, particularmente criba orbitalia y parches periósticos.

Conclusiones: La evidencia presentada revela que la alteración del crecimiento debido al estrés fisiológico afecta el crecimiento y la maduración ósea, pudiendo afectar las estimaciones de edad basadas en elementos dentales y esqueléticos. Las dimensiones del pars basilaris, usadas con éxito para estimar edad en individuos europeos, fueron deficientes en esta muestra sudamericana. Dado que los efectos del estrés fisiológico relacionados a una alteración del crecimiento están presentes tanto antes como después del nacimiento (y antes del destete), se sugiere un estrés fisiológico materno elevado en esta población.

Palabras clave: nexo materno, edad de muerte documentada, estimación de edad, estrés del desarrollo

3.1 Introduction

Human growth is a dynamic process by which individuals increase in size and mass over a specific unit of time (Decrausaz and Cameron 2022). This increase is regulated by genetic and environmental influences, and both intrinsic and extrinsic factors play a role in the physiological changes the body can endure while maintaining a balance between growth and internal homeostasis (Bogin, 2020). Developmental (physiological) plasticity, or the ability to adapt to certain degrees of modification in response to external factors during development (Temple, 2019), is highly critical during periods of rapid growth, such as during foetal development and the months immediately after birth. Some physiological assaults to the growing body might translate to the skeleton, expressing the imbalance between development and homeostasis, and illustrating the body's effort to preserve steady internal, physical, and chemical conditions while dealing with physiological stress. Any imbalance leads to growth disruption, which can be observed skeletally in the discrepancy between an individual's documented chronological age (CA) – a measure of time since conception or birth – and estimated physiological age (PA) – a measure of skeletal development and maturity.

Rate of skeletal maturation is affected by various factors including disease, hormonal imbalance, environment conditions, nutritional status, and genetics. Bioarchaeologists regularly assess biological growth in non-adults by estimating age-at-death from osteometric, morphological, or radiographic methods. Methods to estimate age-at-death of non-adults create a standard that describe the maturation level achieved by a group of normal individuals at any given chronological age (Buikstra and Ubelaker, 1994). Usually developed from 20th century populations or archaeological skeletal materials of European descent, previous studies have found that some of these methods are population specific, particularly during the foetal period of development (Lim *et al.*, 2000; Allen, Hulsey and Hulsey, 2005; Unterscheider *et al.*, 2013; Sletner *et al.*, 2015). In addition, these methods operate under the assumption of favourable ("normal") living conditions experienced by the individual, allowing for method reproducibility on samples of different contextual origin. However, this notion is incomplete as it does not consider how the individual's ontogenetic trajectory – or even that of their mothers – might be affected by external factors (Niel, Chaumoître and Adalian, 2022).

Adverse conditions during intrauterine life and in the first few months after birth can have a lasting impression on skeletal growth, compromising the ability of an individual to achieve their full genetic potential. Framing foetal-infant growth (or its disruption) within the Developmental Origins of Health and Disease (DOHaD) paradigm, helps us to understand how early life environmental stressors can impact health, growth, morbidity, and mortality in later stages of the life course (Agarwal, 2016). Long-term detrimental outcomes arising from adverse influences during these early stages of life affect individuals themselves but can also serve as a glimpse into overall maternal health and environment during pregnancy, puerperium, and until breastfeeding is over. As previous studies have shown, foetal, perinatal, and infant individuals' health outcomes and their susceptibility to suffer growth disruption is maternally regulated (Barker, 1997; Gowland, 2015). Exploring the link between physiological stress and patterns of skeletal and dental growth can help understanding the effect that external factors and maternal buffering have over foetal-infant individuals (Hodson, 2021).

This study examines growth disruption in foetal-infant individuals of documented CA of low socioeconomic status that lived during the mid-20th century in Santiago de Chile. Pathological changes were recorded and analysed in combination with growth disruption to explore how physiological stress affected these individuals. This study also provides new insights into the use of methods to estimate age-at-death across different foetal-infant populations, and their reliability when applied to populations of different geographical origin than the reference sample.

3.1.1 Child health in mid-20th century Chile

Several lines of evidence suggest the multicausal nature of child morbidity and mortality in mid-20th century Chile. During this time, the lower socioeconomic classes in the capital of Santiago were mainly formed by a mass of economic migrants for rural areas of the country and their descendants (Cambiaso *et al.*, 2001). Migrants came to the capital in search of better opportunities but found themselves dealing with a new kind of poverty: where before they lacked access to education, technology and luxury goods, now in their new urban homes they lacked sanitary living conditions, and access to sufficiently nutritious food and healthcare (Espinoza, 1988). The rapid urbanisation process, often poorly planned and

uncontrolled, impacted the entire population's health and well-being, but was particularly hard on children, who are more susceptible to environmental changes than adults.

Yet, child health during the period 1950-1980, particularly of those living in Santiago, saw crucial changes. After starting the 20th century with infant mortality of 300 deaths per thousand live births, the trend fell at its fastest rate in the 1940s and 1950s, due to several vaccination campaigns and the introduction of a national healthcare system with new institutions (Program of Complementary Feeding (PNAC) and National Commission for Child Nutrition (CONIN), most notably) (Sater, 2003; Paluzzi, 2004; Mardones-Restat and Azevedo, 2006). From mid-1960 onward, Chile experienced a notable decrease in child mortality; Taucher (1978) ascribe this trend to improvements in mother-infant public policies that included pre- and postnatal medical care for both mother and child, support and encouragement of breastfeeding (either by the mother or a wet nurse), hospital admissions, births in hospitals, vaccinations, and malnutrition control programs, among others. Detailed examination of the downward trend in morbidity and mortality for the period by Kaempfer and Medina (1982), showed that it was not associated with the economic situation in the country nor the changes in national organisation (i.e., the USA-mediated economic collapse during Allende's presidency followed by a military coup d'état in 1973). Like Taucher (1978), they attribute this decline to the change in number of children because of family planning programs and the introduction of the contraceptive pill in 1962.

Notwithstanding the general decline in child mortality and morbidity, day-to-day living conditions inside impoverished communities in Santiago remained more or less the same. Although respiratory and infectious diseases decreased in relative importance from 58% to 25% of total children deaths in the country, perinatal problems (e.g., preterm birth) increased from 17% to 30%, concentrating especially on low SES areas of the capital (Taucher, 1978). Any impact public measures could have on children's health and well-being were limited by the already poor health of the adults that would become parents to the next generation (Chávez Zúñiga, 2019). Indeed at the time, women's health was not viewed as impacting the health outcomes of their offspring; infant mortality was viewed as the fault of mothers' personal suitability rather than their physical fitness (Cavieres, 2001).

3.2 Materials and Methods

3.2.1 Skeletal sample

A skeletal sample of 47 foetal, perinatal, and infant individuals between 32 gestational weeks and 1 year of age-at-death from the COSS was analysed. Documented sex, age-at-death (with exact dates of birth and death) and cause of death were available for all the individuals. Burial records also offered supplementary biographical information, such as annotations of preterm births and their gestational age; this was taken into consideration when assigning an age group to each individual and when discussing the results of the analyses.

All individuals in the foetal-infant sample died between 1970 and 1979, and although they would have endured severely diminished living conditions, they also benefited from improvements in public health and new social care policies enforced during previous decades. Their mothers, on the other hand, would have been economic migrants, first generation *santiaguinas*, and/or working-class women; all these potential origins share in common belonging to poverty-stricken communities (see Chapter 1.3). Thus, individuals were pooled together on the basis of the degree of homogeneity of their inferred life experience (low SES, impoverished living conditions, and shared child-rearing cultural practices).

3.2.2 Age estimation

Based on reliability in previous studies and the relative contemporaneity between the reference samples used to develop these standards and the sample in this study, three methods were used to estimate physiological age. A detailed description of each method is given in Chapter 2.2, and this section will only briefly identify the methods used. Due to preservation issues, not all skeletal elements were present or complete for each individual, thus physiological age estimations were made whenever possible.

Dental age (DA) was estimated analysing tooth development and eruption sequence, identifying developmental formation stage for each tooth according to Moorrees, Fanning and Hunt (1963) and matching teeth stages using the London Atlas of Human Tooth

Development and Eruption by AlQahtani (2012). DA estimations are regarded as one of the best methods to estimate age in non-adult individuals as dentition is highly resilient to environmental stressors (AlQahtani, Hector and Liversidge, 2014; Lewis, 2017a).

Metric assessment of non-adult skeletal remains was also used, as skeletal elements are more easily identifiable in archaeological settings and usually better preserved than developing teeth (Lewis, 2017a; Utczas *et al.*, 2017). Skeletal age (SA) estimations were made from two anatomical elements: measurements of the pars basilaris of the occipital (to assess maturation) and maximum femur length (to assess growth). Previous studies show that morphological development and size growth of the pars basilaris correlates with certain ageing thresholds, being strongly associated with age-at-death (Redfield, 1970; Scheuer and MacLaughlin-Black, 1994). Age estimations using the pars basilaris (SA-P) were calculated by directly comparing measurements of maximum width, sagittal length, and maximum length with those given by Scheuer and MacLaughlin-Black (1994) for pre and postnatal groups; these reference measurements were generated from archaeological English samples, namely St Bride's and Spitalfields collections (London, United Kingdom). Another widely used method (Fazekas and Kósa, 1978) was not used as it only includes estimations for prenatal individuals. In addition, SA-P was also calculated using novel regression formulas proposed by Irurita and Alemán (2017), who generated their formulas from a late 20th century Spanish sample (Granada, Spain). Results from this method, however, were only generated for reference and comparison, and all SA-P estimates discussed further reflect age estimations derived from the method by Scheuer and MacLaughlin-Black (1994).

Finally, femur length age estimations (SA-F) were calculated using Scheuer, Musgrave and Evans (1980) charts for individuals of prenatal origin, and Maresh (1970) for the postnatal subsample. When possible, measurements were taken from the left femur. If left femur was incomplete or not available, the right femur measurements were used for age estimation.

3.2.3 Skeletal indicators of stress

Assessing physiological stress on non-adult skeletal remains is particularly problematic (Lewis, 2017a). Using macroscopic pathological changes to derive differential

diagnosis is always challenging, but more so in non-adult samples, due to their immature immune systems and the fast response their bones have to pathological conditions (Newman and Hodson, 2021). To assess the potential impact that physiological stress has on growth disruption of foetal-infant individuals, presence/absence of eight skeletal stress markers was recorded as indicative of physiological stress (Marklein, Leahy and Crews, 2016) (Table 3.1). In the case of PNBF, presence was only recorded when lesions with a distinctive grey appearance of woven bone were found to be asymmetrical and/or bilaterally differing in severity (Hodson, 2018). This decision was made to address the limitations of analysing PNBF in non-adults (Lewis, 2017a; Hodson, 2018).

Table 3.1 Skeletal stress markers recorded for the sample, their diagnostic features and possible aetiologies.

	Abb.	Diagnostic features	Possible aetiologies	Reference
Bowing of long bones	Bow	Angulation deformities of the long bones, following characteristic patterns (e.g., in the femur bowing occurs laterally, and in the tibia it occurs anteriorly)	• Vitamin D deficiency • Rickets • Metaphyseal chondrodysplasia • Osteogenesis imperfecta • Infantile cortical hyperostosis • Treponemal diseases	Buikstra and Ubelaker, 1994; Brickley, Ives and Mays, 2020
Cribra femoris	CF	Porous lesions displaying a trabecular organisation appearance on the inferior surface of the femoral neck	• Traditionally associated with anaemia • Physiological stress periods or dietary deficiencies during postcranial development • Malaria	Ortner, 2003; Brickley, 2018
Cribra orbitalia	CO	Porous lesions displaying a trabecular organisation appearance on the orbital roofs, resulting in the expansion of the diploë and resorption of outer table	• Traditionally associated with anaemia (including iron deficiency, megaloblastic, and haemolytic anaemias) • Localised inflammation • Rickets	Roberts and Manchester, 2010; Brickley, 2018
Linear enamel hypoplasia	LEH	Irregularities such as pits, grooves, or missing enamel of varying size on the dental surface. Defects arise due to the disturbance of enamel	• Associated with metabolic diseases, nutritional disorders/deficiencies (vitamin A, C, D, and calcium)	Ortner, 2003; Roberts and Manchester, 2010; Hillson, 2014

		formation in the developing teeth	• Also thought to be associated with social risk factors (including socioeconomic inequalities, rural environments, and young maternal age), preterm birth and low birth weight	
Metaphyseal swelling	MS	Enlargement of metaphyseal plate in non-adult skeletal remains	• Active vitamin D deficiency • Early stages and active rickets • Metaphyseal chondrodysplasia • Infantile scurvy (rare)	Ortner, 2003; Brickley, Ives and Mays, 2020
Porosity of the growth plate	PGP	Porous growth plate surfaces due to abnormal mineralisation of osteoid	• Active vitamin D deficiency • Rickets	Brickley, Ives and Mays, 2020
Porotic hyperostosis	PH	Porous and hypertrophic lesions on the cranial vault	• Traditionally associated with anaemia, particularly iron deficiency • Infectious diseases • Metabolic disorders • Cancer	Buikstra and Ubelaker, 1994; Roberts and Manchester, 2010; Brickley, 2018
Periosteal new bone formation	PNBF	Woven and/or lamellar new bone formation on the periosteal surface. Only asymmetrical bone formations were recorded as pathological	• Nonspecific infections. • Might indicate inflammatory processes associated with trauma or metabolic disease (vitamin C deficiency, scurvy, infantile cortical hyperostosis)	Ortner, 2003; Lewis, 2017b

Abb: abbreviation.

3.2.4 Analytical approach

Adjustments to the data were made to better analyse the results. Age estimations were made in gestational weeks (GWA) or months of age, depending on the individual's CA and method used to estimate PA. To ease the comparison between individuals and methods, all postnatal estimations were transformed into weeks (GWA prenatally and WA

if post-partum); 0 GWA reflects conception, individuals over 40 GWA can be considered as post-partum and the first birthday is equivalent to 92 WA. In addition, and following current guidelines (World Health Organization, 2006), trends in mortality were explored by dividing the sample into three age groups: foetal, perinatal, and infant. The groups were defined based on the physiological development each individual had achieved at their given chronological age, as outlined in previous bioarchaeological works. The foetal group included all preterm births, as these individuals' developmental level should be equated to gestational weeks, regardless of when they were born (<40 GWA). The perinatal group included all individuals from full term delivery to 28 days of life (40 GWA to 44 WA), while infants included individuals up to the first year of life (44 to 92 WA). The sample upper boundary for age was adopted based on the widespread cultural practice during the period of breastfeeding infants until the first year of age (Zárate Campos, 2010; Halcrow *et al.*, 2021). Within the foetal and perinatal groups, eight cases of preterm birth and stillbirths are documented; statistical approaches were made to analyse the effect of physiological stress in the completion of normal pregnancy.

To examine growth disruption, two approaches were followed. Firstly, to visually compare PA estimations (DA, SA-P, and SA-F) to CA, a scatter plot with custom error bars was constructed examining each individual. As PA are estimated ranges, error bars allowed to plot the variability in Y and X corresponding to each mean, with different deviations given by the different methods used. Secondly, comparisons of discrepancies were carried out between documented CA and PA estimates (CA/DA, CA/SA-P, and CA/SA-F). As the data did not follow a normal distribution, a nonparametric test (Kruskal-Wallis test, $p < 0.05$) was performed to assess the significance of the differences between groups.

To assess the role physiological stress had on growth disruption on this sample, a simple (symmetric) correspondence analysis (SCA) statistical test was performed. The test, an extension of principal components analysis, is suited to explore relationships between qualitative variables, quantifying categorical data by assigning each variable a numerical value and computing factor scores (coordinates) between them (Greenacre, 1993). The analysis constitutes a geometric approach for visualising the relative relationship between and within two groups of variables: growth disruption and skeletal stress markers. All statistical analyses were conducted using RStudio 2021.09.0 Build 351.

3.3 Results

3.3.1 Age estimation

Of the 47 foetal-infant individuals of documented chronological age in the COSS, 28 had dental elements suited to explore dental development, 10 individuals had pars basilaris present to measure, and 29 had either a left (n=21) or right (n=8) femora present and in good conservation state to measure. Thus, 43 individuals had dental elements and/or at least one skeletal element available to measure; for these 43 foetal-infant individuals, dental and/or skeletal age-at-death estimates can be directly compared to documented chronological age.

Intra and inter-observer error was analysed using Lin's concordance correlation coefficient (McBride, 2005). Measurements were taken three times by the researcher and twice by an external researcher with expertise in biological anthropology. No significant differences ($\rho c > 0.9$) were found for intra and inter-observer analysis of each measurement; results indicate a high degree of concordance between the measurements, allowing the rejection of random similarities.

Figure 3.1 plots all individuals' ages (in GWA/WA), in ascending order according to CA, with their respective DA, SA-P and SA-F estimates where available. As each method has its own deviation degrees regarding error levels, all age estimations were plotted with custom error bars and given as a range. By doing so, comparison between age-at-death estimates generated from each of the methods used can be observed against CA, highlighting the difference in physiological age estimates between anatomical elements.

Dental age estimations are the most accurate of the three PA estimates. Of the 28 cases, 18 overlap with the individuals' chronological age, with the remaining cases all producing older estimates. In contrast, only two of the pars basilaris estimates are consistent with the individuals' chronological age (B2054 at 34 GWA and B2046 at 36 GWA). Four SA-P results overestimate CA, B2008 (36 GWA), B2011 (40 GWA), B2030 (40 GWA), and B2051 (48 WA), while four underestimate CA, B2058 (40 GWA), B2036 (52 WA), B2024 (65 WA), and B2035 (86 WA). In general, age estimations given by femoral length underestimate CA, with 17 cases giving younger estimates. Ten cases accurately predict CA, with only 2 cases (both male foetal individuals) producing slightly older estimates.

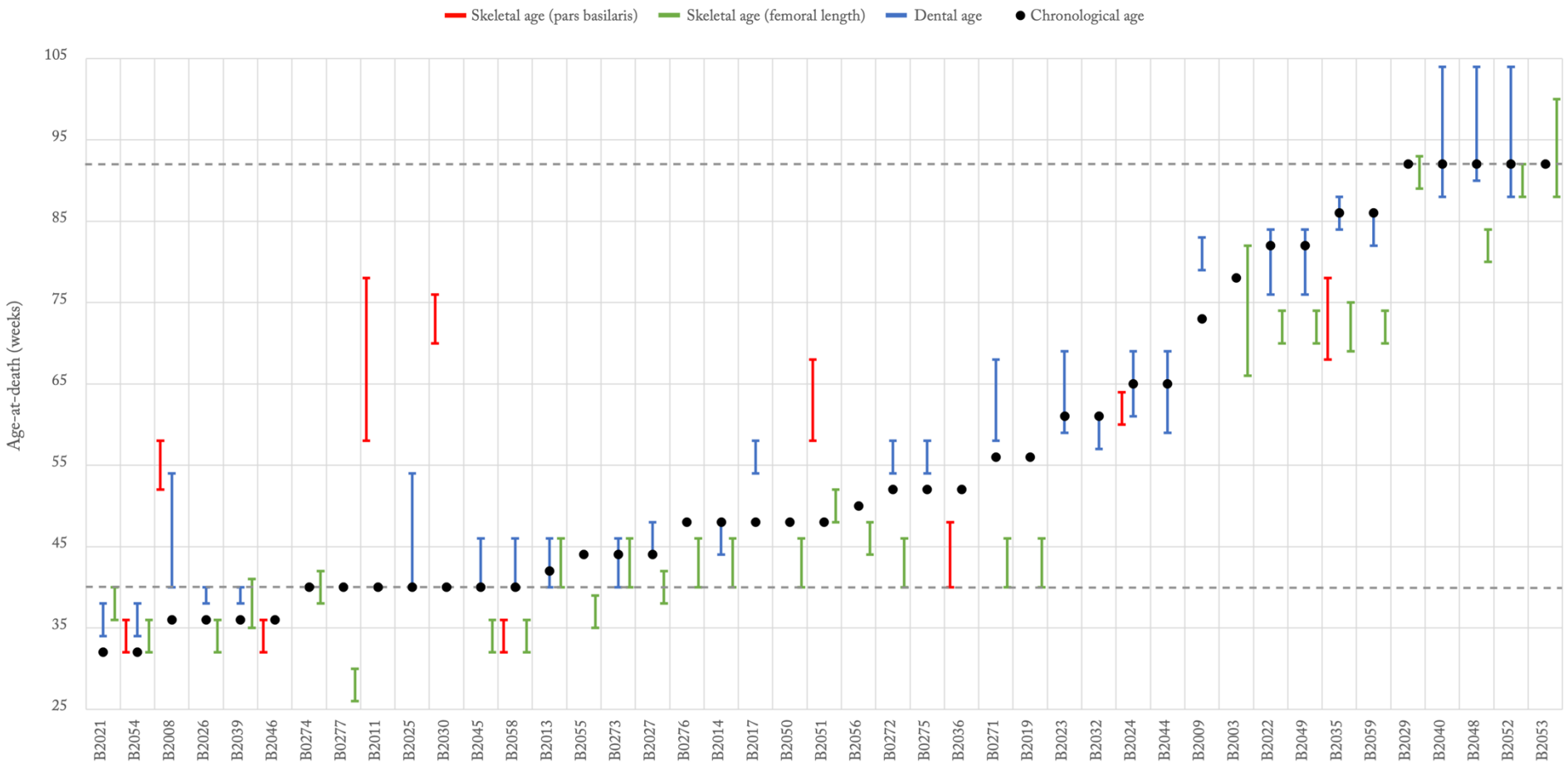

Figure 3.1 Comparison between physiological age-at-death estimates and chronological age per individual. Error bars for each age estimation given per error ranges provided by Scheuer and MacLaughlin-Black (1994) for pars basilaris estimates, Scheuer, Musgrave and Evans (1980) and Maresh (1970) for femoral length estimates, and AlQahtani et al. (2010) for dental age. Dashed lines denote birth (40 GWA) and 1 year of age (92 WA).

In addition to SA-P estimations derived from Scheuer and MacLaughlin-Black (1994), regression formulas created by Irurita and Alemán (2017) were applied to the sample (Figure 3.2). Generated age estimations are highly accurate, even if usually falling on the upper boundary of each confidence interval. Only one individual (B2035) age-at-death is not accurately predicted, estimating a younger age than the documented age-a-death. SA-F for the same individual also shows a younger skeletal age estimate (Figure 3.1), contrary to the finding of DA; these results might be indicating growth disruption.

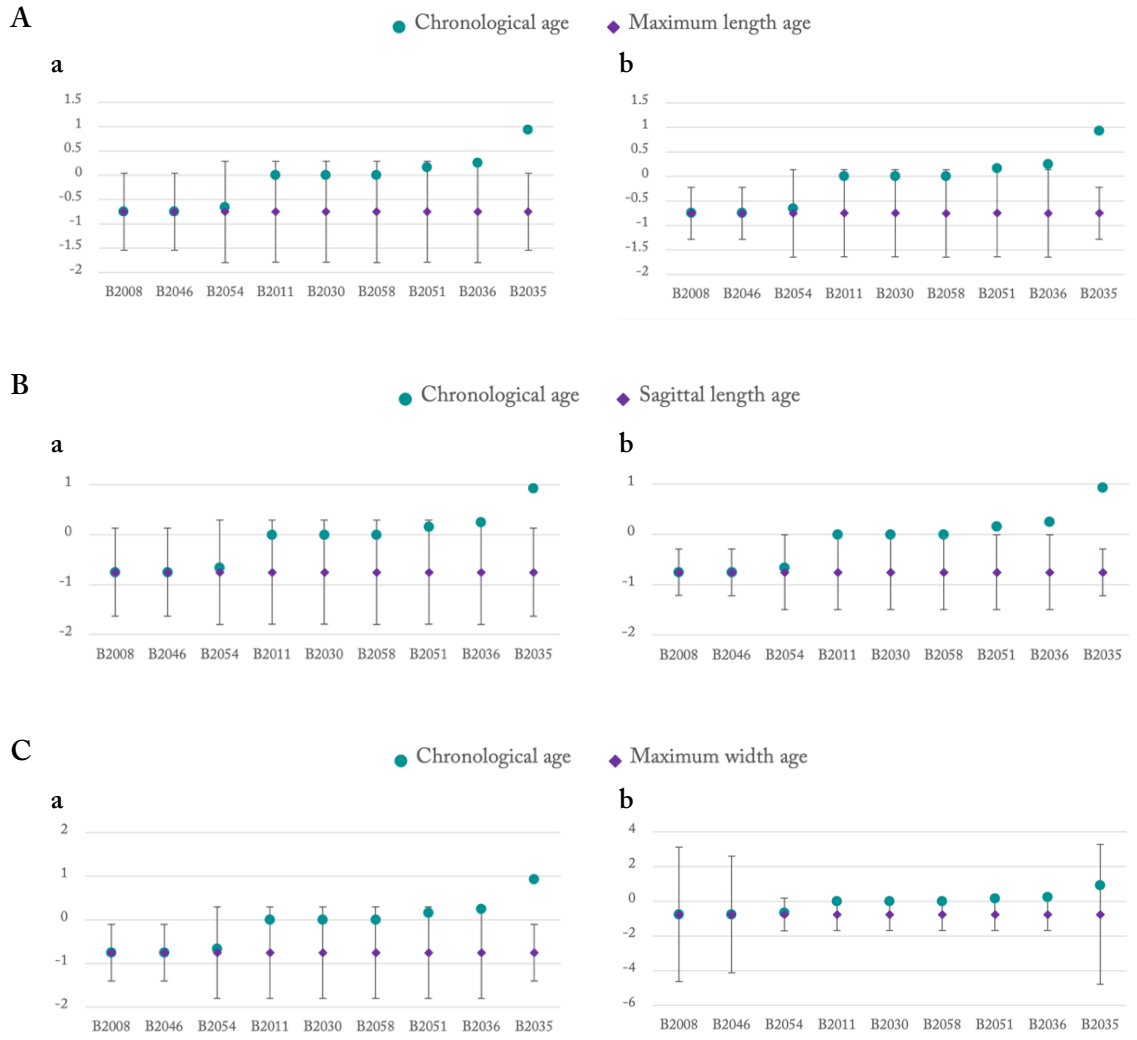

Figure 3.2 Comparison between age-at-death and age estimations from the pars basilaris according to regression formulae by Irurita and Alemán (2017). Age estimations derived from maximum length (A), sagittal length (B) and maximum width (C) measurements are reported when using 50% (a) and 97.5% (b) confidence interval errors. All ages presented in years; sex specific regression formulas used for each individual.

CA-PA discrepancies show that skeletal elements typically produce younger age estimates than that of dentition (Figure 3.3). When looking at the complete sample, DA estimates are in average advanced by 3 weeks, SA-P has a slight average advancement of 1 week and SA-F shows an average deficit of 5 weeks (Kruskal-Wallis, chi-square: 7.815, df = 3, p = 0.160). When segregating by sex, there is no statistical difference between estimates for DA and SA-F; however, SA-P shows a discrepancy of 1 week between male (advanced) and female individuals (deficit).

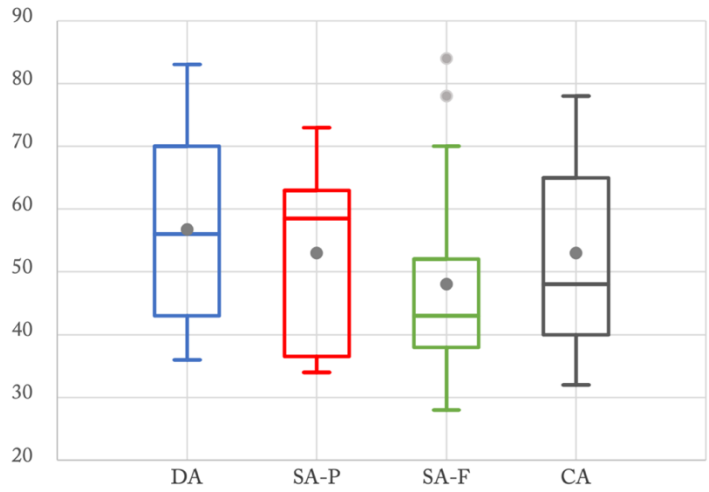

Figure 3.3 Comparison of distribution (in weeks) of physiological age estimations (DA = dental age, SA-P = skeletal age given by the pars basilaris, SA-F = skeletal age given by femur length) and chronological age (CA), for the complete sample. Box height represents differences in the first and third quartiles. Middle band in each box represents the median. Whiskers represent the minimum and maximum differences within 1.5 times the interquartile range of the lower and upper quartiles. Dots represent outliers with age differences exceeding 1.5 times the interquartile range.

Across the different age categories, CA-PA discrepancies tend to remain the same when using DA and SA-F estimates; SA-P estimates, however, show marked differences between foetal, perinatal, and infant individuals (Figure 3.4). For the foetal individuals, DA and SA-P tend to estimate older ages, while SA-F tends to be more accurate or slightly underestimate age. For the perinatal and infant individuals, DA tends to slightly overestimate age, while SA-F tends to slightly underestimate. For this sample, methods to estimate age through dental development and femoral length tend to proportionally over and underestimate CA, respectively. In contrast, SA-P overestimates age in foetal individuals and underestimates age in infant individuals.

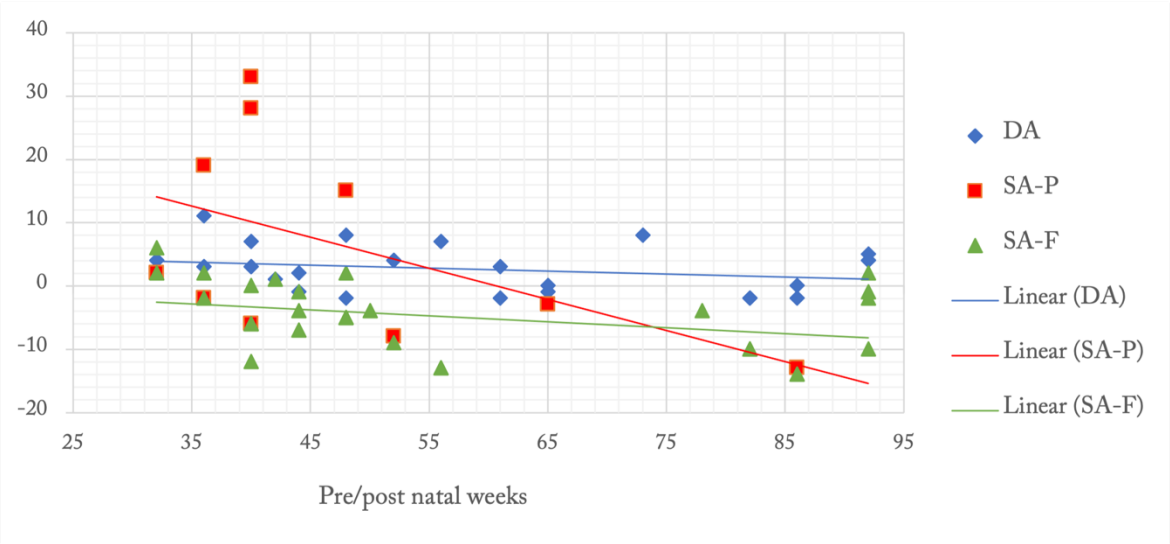

Figure 3.4 Age distribution of discrepancies (in weeks) between chronological age and physiological age estimations (DA = dental age, SA-P = skeletal age given by the pars basilaris, SA-F = skeletal age given by femur length). Lines mark tendency for each physiological age estimate.

Discrepancies between documented CA and PA estimates, where estimated age ranges did not overlap CA, were explored as evidence of growth disruption, due to their value as indicators of stress exposure and delay in physical growth and maturation (Lewis, 2007).

3.3.2 Physiological stress

Macroscopic analysis of pathological changes showed high prevalence of stress markers for the overall sample and when segregated by biological sex (Table 3.2a) and age groups (Table 3.2b). Bow, CO, LEH, and PNBF were among the most prevalent skeletal markers of physiological stress for this sample, generally affecting 50-80% of the individuals.

Co-occurrence of stress skeletal markers was also explored, with limited statistically significant associations between stress markers. Only two co-occurrences were determined: porotic hyperostosis (PH) and cribra orbitalia (CO), and cribra femoris (CF) and bowing of the long bone of the legs (Bow). PH and CO co-occurrence was found in the overall sample (Spearman's $\rho = 0.655$, p-value = 0.046), but was particularly high in the infant subsample (Spearman's $\rho = 1.000$, p-value = 0.003). In addition, the same levels of CF and Bow co-occurrence were found between the foetal and perinatal subsamples (Spearman's $\rho = 1.000$, p-value = 0.017).

Table 3.2a Crude prevalence of the skeletal stress markers recorded for this study, when describing the complete sample and by biological sex. Shaded cells denote a prevalence equal or higher than half of the individuals.

	Overall			Females			Males		
	N	**n**	**P**	**N**	**n**	**P**	**N**	**n**	**P**
Bow	40	20	50	12	6	50	28	14	50
CF	30	17	57	9	4	44	21	13	62
CO	18	15	83	5	4	80	13	11	85
LEH	26	14	54	9	5	56	17	9	53
MS	39	14	36	12	4	33	27	10	37
PGP	37	6	16	11	1	9	26	5	19
PH	14	6	43	4	1	25	10	5	50
PNBF	43	32	74	14	10	71	29	22	76

N: total number of individuals with the skeletal elements present for analysis; *n*: number of individuals exhibiting the skeletal stress marker; P: prevalence in %.

Table 3.2b Crude prevalence of the skeletal stress markers recorded for this study by age group. Shaded cells denote a prevalence equal or higher than half of the individuals.

	Foetal			Perinatal			Infant		
	N	**n**	**P**	**N**	**n**	**P**	**N**	**n**	**P**
Bow	6	2	33	7	4	57	27	14	52
CF	5	2	40	5	2	40	20	13	65
CO	3	2	67	4	4	100	11	9	82
LEH	5	3	60	4	2	50	17	9	53
MS	6	1	17	7	2	29	26	11	42
PGP	6	2	33	6	1	17	25	3	12
PH	2	1	50	3	0	0	9	5	56
PNBF	6	5	83	8	6	75	29	21	72

N: total number of individuals with the skeletal elements present for analysis; *n*: number of individuals exhibiting the skeletal stress marker; P: prevalence in %.

Correspondence analysis was used to statistically analyse and graphically display the relationships between growth disruption scenarios (rows) and skeletal stress markers (columns) (Figure 3.5). Variables for stress markers included bowing of long bones of the legs, cribra orbitalia, cribra femoris, linear enamel hypoplasia, metaphyseal swelling, porotic hyperostosis, porosity of growth plates, and periosteal new bone formation. Inertia values (Table 3.3) quantify the amount of variation that is accounted for by the corresponding principal dimensions.

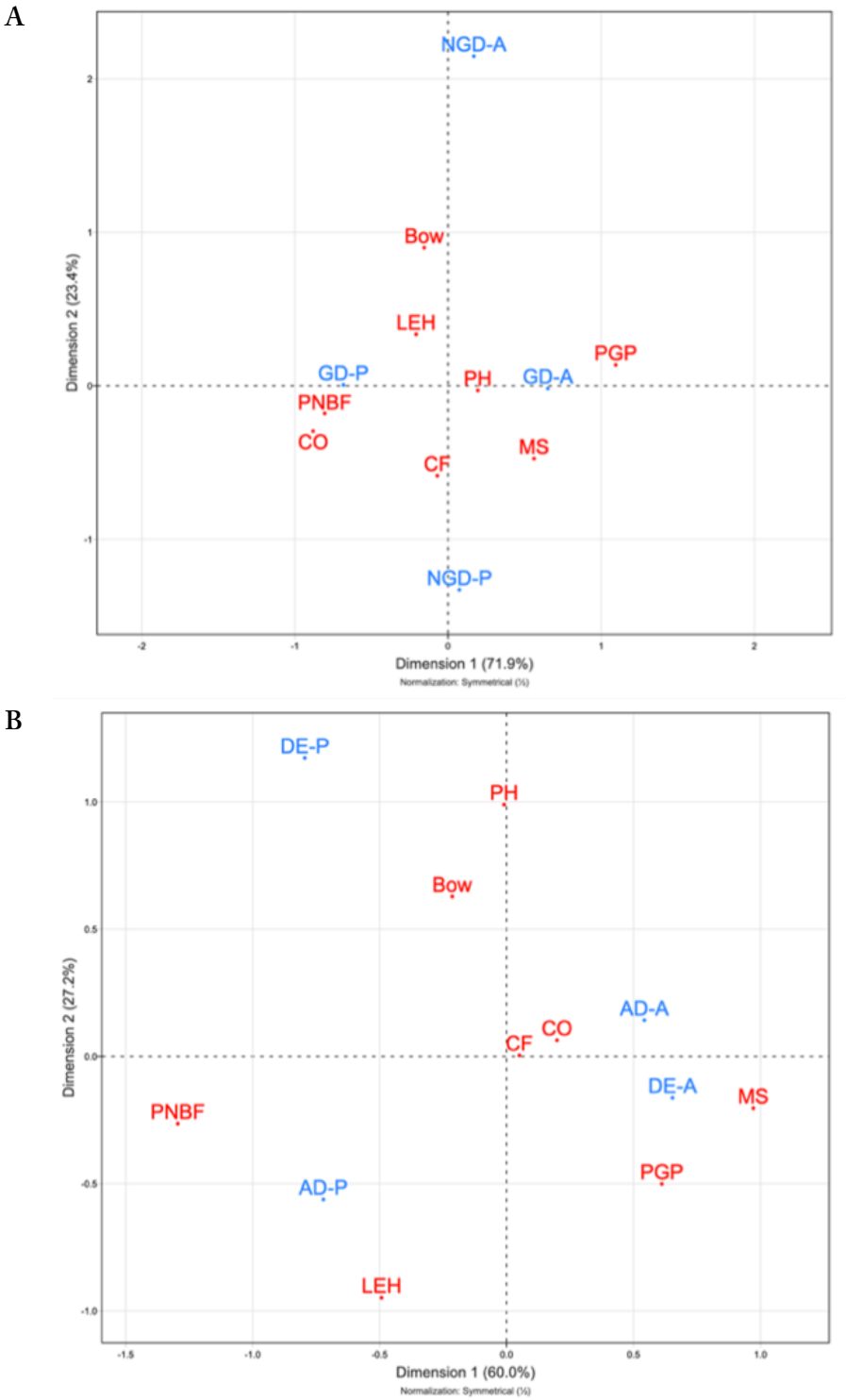

Figure 3.5 Correspondence analyses with symmetric normalisation for the complete sample (A) and the preterm-stillborn subsample (B). Plots depicts relative relationship between growth disruption scenarios in blue (GD-P: growth disruption when stress marker is present, GD-A: growth disruption with stress marker is absent, NGD-P: no growth disruption when stress marker is present, NGD-A: no growth disruption with stress marker is absent, AD-P: growth advancement when stress marker is present, AD-A: growth advancement when stress marker is absent, DE-P: growth delay when stress marker is present, and DE-A: growth delay when stress marker is absent) and skeletal stress markers in red (Bow: bowing of long bones, CO: cribra orbitalia, CF: cribra femoris, LEH: linear enamel hypoplasia, MS: metaphyseal swelling, PH: porotic hyperostosis, PGP: porosity of growth plates, and PNBF: periosteal new bone formation).

Table 3.3 Adjusted principal inertias for CA dimensions (Dim) based on the eigenvalues of the Burt matrix.

	Overall sample			Preterm-stillborn subsample		
	Dim1	Dim2	Dim3	Dim1	Dim 2	Dim 3
Eigenvalue	0.145	0.046	0.010	0.192	0.087	0.041
Inertia %	72.385	22.807	4.807	59.979	27.248	12.773
Cumulative %	72.385	95.193	100.000	59.979	87.227	100.000

Growth disruption (or its absence) was assessed against the presence of skeletal stress markers, creating four scenarios: growth disruption when stress marker is present (GD-P), growth disruption when stress marker is absent (GD-A), no growth disruption when stress marker is present (NGD-P), and no growth disruption when stress marker is absent (NGD-A). Further scenarios were tailored to a small subsample of documented preterm and stillborn individuals (n=8); as all individuals were found to have growth disruption, the scenarios focused on delay or advancement of age estimates: growth advancement when stress marker is present (AD-P), growth advancement when stress marker is absent (AD-A), growth delay when stress marker is present (DE-P), and growth delay when stress marker is absent (DE-A).

For the complete sample, SCA with symmetrical normalisation shows that growth disruption scenarios are not independent but associated with the presence of the aforementioned skeletal stress markers (chi-square = 36.415, df = 24, p= 0.000, total inertia = 0.2). The symmetric correspondence graph for the different contemplated variable categories of dimensions 1 and 2 accounts for approximately 95.19% of the explained inertia. Figure 5A illustrates relative relationships between stress markers and growth disruption scenarios in the complete sample (overall), with clustering of the variables indicating the existence of associations through closeness and similarities; clusters that form around the four growth disruption scenarios allow differentiation of the levels of associations. The plot shows that GD-P is associated with CO and PNBF; this means the presence of these skeletal markers is related to growth disruption in this sample. Other skeletal indicators align more loosely with the three remaining scenarios, with PH, MS and PGP relating to GD-A, and CF relating to NGD-P; even in the presence of these stress markers, growth was not affected. Bow and LEH presence did not relate to growth disruption for this sample.

The subsample of documented preterm (B2021, B2039, B2050, B2046, and B2077) and stillborn (B2008, B2026, and B2054) individuals SCA dimensions 1 and 2 account for

approximately 87.2% of the explained inertia, with Figure 5B illustrating relative relationships between stress markers and growth delay or advancement scenarios (chi-square = 32.671, df = 21, p = 0.590, total inertia = 0.321). The presence of PNBF and LEH are associated with advancement in age estimations, while PH and Bow are associated with delay in age estimates. Both CO and CF are the least distinct (i.e., not associated with growth delay or advancement) skeletal stress markers for this subsample.

3.4 Discussion

Human development is plastic, resulting in changes to the way biological processes behave when impacted by adverse environmental influences (Bogin, 2020). Appreciating the complex and sometimes diffuse relationship between biological growth and environmental factors is essential to explore the impact that contextual influences have over development. For foetal, perinatal, and infant individuals this assertion encompasses not only their own response to external stressors, but that of their mothers. Maternal health has an incredible impact on the development of their offspring during gestation and postanal life; while still in the womb, maternal buffering against environmental conditions shields the foetus, while at the same time conferring the foetus with passive immunity and nutritional needs met via transplacental exchange (Thorsell and Nätt, 2016). Birth marks the verge of a multitude of new biological, physical, and environmental changes for the individual (Lewis, 2007), with passive immunity and nutrition still being maternally provided via breastfeeding (Thorsell and Nätt, 2016). However, multiple external stressors such as exposure to pathogens (McDade, 2005), reduction on nutrient supply from the mother, and social and cultural detrimental factors, can negatively affect the growth and development of an individual, as well as their health outcome in the moment and later in life. This study explored foetal-infant individuals' growth patterns and health status, providing insight into the complex period of gestation and postanal life up until the first year of age.

3.4.1 Age estimation methods

The findings of this study reveal that skeletal and dental age-at-death estimation methods had mixed accuracy outcomes when compared to documented chronological age.

When considering skeletal versus dental age estimation methodologies, DA estimations produced broader age ranges, but more accurately predicted CA than their skeletal counterparts. Overall, results support the assumption that dental development is more buffered against environmental stressors than skeletal development (Cardoso, 2007; Spake et al., 2021). Results of this study can be discussed through a double lens: the potential population specificity of age estimation methods, and the underlying health of both foetal-infant individuals and their mothers.

Population specificity.

The suitability of applying methods developed from a specific population, into a sample from different temporal and geographical origin has been extensively discussed in recent years. A multitude of attempts to evaluate methods to estimate biological profile (i.e., biological sex, age-at-death, stature) and develop new ones specific to a particular population populate our field. However, age-at-death estimation in non-adults is usually undertaken worldwide using the same set of methods, with dental development utilised in this study, in particular, being praised for its accuracy across populations (AlQahtani, Hector and Liversidge, 2014).

Some studies have suggested an acceleration in the rate of dental maturation during the past 50 years due to secular trends (Holtgrave, Kretschmer and Müller, 1997; Nadler, 1998), but dental age methods which do not take this into account remain in use in bioarchaeology, forensic anthropology, orthodontics, among other fields. Modern probabilistic dental methods have been shown to be inaccurate in archaeological samples, but less so in modern populations, which can be attributed to differences in living conditions and secular trends (Braga et al., 2005). Delays in dental development have been reported in non-adults from modern impoverished communities, but the discrepancies seem to affect older children (>6 years of age) rather than foetal-infant individuals (Cardoso, 2009). Results of this study show lower discrepancies between CA and DA compared to SA estimates, with dental development accurately predicting CA or overestimating it by only a few weeks. This shows that, even when accounting for potential acceleration in dental maturity, dental age methods used in this study are a good fit for this particular population, and that any disagreement between CA and DA estimates would be more likely due to growth disruption as a consequence of physiological stress.

Skeletal methods for estimating age-at-death have been subject to sustained, sometimes extensive, critique. Results for SA-P estimations in this sample attest this issue. Some studies have praised the use of the pars basilaris of the occipital as a good estimator of age-at-death (Nagaoka, Kawakubo and Hirata, 2012; Thornton, Edkins and Hutchinson, 2020), while others report estimations of mixed accuracy (Irurita and Alemán, 2017). When using Scheuer and MacLaughlin-Black (1994) method, SA-P did not accurately estimate age-at-death for this sample. Although poor SA-P results are likely to be impacted to some extent by the small sample size for this element (n=10), it is worth noticing the particularly inconsistent results provided by this method. While a small but consistent overestimation is observed in DA method and a consistent underestimation in the SA-F method, SA-P over and underestimates age-at-death at random, seemingly regardless of biological sex or age group. Based on the results, traditionally used methods to estimate age-at-death using pars basilaris are not accurate estimators in this population and should be used on South American populations with extreme caution. However, other methods might serve better when using the pars basilaris to estimate age in this population, such as the regression formulas derived by Irurita and Alemán (2017) from a sample from Granada, Spain. Several studies suggest that linear regression methods are strongly correlated to the demographic profile of the sample used to develop them, and therefore impose biases proportional to the differences in population structure between the sample the method was developed on and that it was applied to (Bocquet-Appel and Masset, 1982, 1996; Aykroyd et al., 1999; Lim et al., 2000; Gowland and Chamberlain, 2002; Allen, Hulsey and Hulsey, 2005; Lewis and Gowland, 2007; Unterscheider et al., 2013; Sletner et al., 2015). In this case, population specificity might be at the basis of the highly accurate age-at-death estimates generated by Irurita and Alemán (2017) regression formulas. The association between Chilean and Spanish skeletal samples is to be expected, as both populations share ancestral affinities due to a complex history of colonialism and *mestizaje* (Ross and Williams, 2021).

Underlying health.

Sherwood et al. (2000) demonstrated that certain pathological conditions influence age estimation methods, resulting in high levels of inaccuracy and even significant bias. This study used maturation (dental development and pars basilaris size) and growth (femoral length) to

estimate age-at-death, with results showing a mixed accuracy for both approaches. Particular caution should be taken with age estimations based on femoral length for this sample, as between 33-57% of the individuals presented bowing of the long bones, which could directly affect estimations made. That none of the methods provided the same accuracy for pre- and postnatal individuals in the sample suggest the individuals were at risk of growth and health disruption (Hodson, 2017). A possible explanation for this might be that physiological stress factors were affecting both child and mother; maternal health must have been impacted and reduced during pregnancy as growth disruption is evident in both intra- and extrauterine life. As the majority of the sample exhibited some degree of physiological stress, it can be suggested that it impacted growth and maturation rates, thus biasing results from age estimation methods. However, when comparing accuracy levels between methods, dental development seems to be less influenced by growth disruption than femoral length.

Methods based on linear growth, like femoral length, are regarded as reliable across populations, although their accuracy has been shown to be significantly affected by environmental growth disruption (Buikstra and Ubelaker, 1994; Scheuer and Black, 2004; Lewis and Gowland, 2007; Cardoso, Abrantes and Humphrey, 2014; Carneiro, Curate and Cunha, 2016). Results from this sample agree, with length accurately predicting (n=10) or underestimating CA (n=19), with the latter likely reflecting growth disruption. Genetic or contextual factors can alter long bone morphology and cause developmental conditions that will result in growth delay or growth advancement (Niel, Chaumoître and Adalian, 2022). As evidence during the macroscopic analysis, the individuals in this sample had a high prevalence of pathological changes, with only 12% not showing any of the eight skeletal stress markers recorded for this study. The high prevalence of pathological changes in this population might indicate that a consistent underestimation of CA based on femoral length is associated with stress-related growth delays. These findings suggest that dental development remains the best methodology to estimate age-at-death for this population.

3.4.2 Physiological stress

This sample showed high prevalence of skeletal pathological changes, suggesting that they were exposed to chronic physiological stress. Presence of PH and CO was found to co-occur in all individuals overall and in the infant subsample. Both skeletal markers are frequently

recorded in bioarchaeology, even found in hominins and non-human primates, and have been traditionally associated with iron-deficiency anaemia (Brickley, 2018). Some studies have suggested different subjacent aetiologies (related to different types of anaemia) and, more recently, a potential association with respiratory infection diseases (O'Donnell et al., 2020). In addition, CF and Bow co-occurrence was found in the foetal and perinatal subsamples. Both pathologies share possible dietary deficiencies as aetiologies, particularly that of vitamin D. While bowing of the long bones of the legs is a diagnostic feature of rickets, related abnormal porosities can also occur in other bones. Moreover, mechanical overload (e.g. due to bowing of the femoral diaphysis) can change the morphological structure of the anterior aspect of the femoral head-neck junction (Radi et al., 2013). However, it is possible that CF does not have a pathological origin, as normal growth may also cause porosities on the femoral neck (Brickley, Ives and Mays, 2020). Ultimately, although it is not possible to determine whether any of these pathological changes and their co-occurrence shared an underlying cause (Rivera and Mirazón Lahr, 2017), they suggest increase susceptibility to severe morbidity and/or mortality due to a weakened immune response (Newman and Hodson, 2021).

When examining the relatedness of physiological stress and growth disruption scenarios, the SCA illustrates the positive association between growth disruption and the presence of CO and PNBF in this sample.

In non-adults, CO is used to determine the prevalence and risk of death associated with active lesions and stress (McFadden and Oxenham, 2020). Although sometimes associated with rickets, the traditional aetiology attributed to CO is anaemia (Lewis, 2017b), from several possible sources: dietary deficiencies, parasitic infection, genetic influences, or other underlying causes (Godde and Hens, 2021). Growth disruption in this sample is not associated to other traditional markers for rickets (bowing of long bones, porosity of growth plate, or metaphyseal swelling), thus its presence in this sample could be more closely associated with anaemia, which would be in line with historical records for the period. In spite of public efforts to provide the infant population with better nutrition, by the start of the 1970's, 30% of the children under 12 months of age in Chile suffered from iron deficiency anaemia (Hertrampf et al., 2009). Infant anaemia was thought to be linked to malnutrition, and although private efforts to supply free infant formula to children were already in existence in the country, their coverage was low; from 1970 the government boost the free distribution of formula, targeting half a litre of milk

per child, reaching 650 thousand infants per year in 1971 and expanding to 3,600,000 per year by 1974 (Olivares et al., 1989). However, milk formula by itself fell short to the nutritional needs of children in impoverished communities. Although several attempts to measure the efficacy of fortified formulas were made during the 70's and 80's, it would not be until 1999 that milk would be fortified with iron, vitamin C, zinc, and copper (Hertrampf et al., 2009). CO findings in this study, supported with historical records, show that iron deficiency anaemia was prevalent among foetal-infant individuals, likely reflecting maternal iron deficiency, causing growth disruption and potentially being a mortality risk factor for this population.

As a sign of nonspecific infection, PNBF is widely recorded in human skeletal remains. From infections and metabolic diseases to nutritional deficiencies of vitamin C, vitamin D, calcium, and iron, PNBF has been traditionally presented as the biological response to non-specific physiological stressors and as evidence of generalised frailty (DeWitte, 2014). However, Lewis (2017b) suggests caution about its use as a pathological marker in non-adults, as long bone appositional (normal) growth involves the deposition of immature disorganised bone on the cortical surface before the age of four. Macroscopically, normal bone apposition looks identical to bone formation associated with pathological responses and can be misleading to even the most experienced researcher. Ortner (2003) states that before the age of four, presence of woven periosteal new bone should be expected, with Lewis (2017b) adding that diffuse symmetrical and thin deposits are also part of the normal growth process. To address this limitation, this study only recorded PNBF as present when there were signs of asymmetrical bone formation, with woven bone indicating active lesions, and lamellar bone deposits indicating initial stages of healing (Ortner, 2003). Thus, asymmetry or differential severity between sides was used as an indicator of pathological change rather than normal growth. Of the 43 individuals, 30 showed growth disruption and the presence of PNBF, mainly of asymmetrical woven lesions with the vast majority located on the long bones. Although the exact aetiology of PNBF is impossible to assert, the presence of these active pathological lesions is highly suggestive of poor intra- and extrauterine environments, and the result of constant stress events that cause energy to shift from growth to maintenance/survival. As growth disruption and PNBF presence can be found across the sample, these results are consistent with generalised physiological stress.

In addition, these findings broadly suggest a low level of maternal buffering for foetal-

infant individuals in this sample. Maternal health modulates foetal growth and development in a complex balance between the mother, the placenta, and the child (Harding and Johnston, 1995). As growth disruption and pathological changes can be traced across the sample and particularly in the documented preterm-stillborn subsample, in utero maternal buffering might have been diminished due to disease, limited access to food sources or food of poor nutritional value, as well as social and cultural constraints as poverty and lack of healthcare. Although small, the subsample of documented preterm and stillbirths allows to explore into the maternal health conditions for this population. All eight cases suffered growth disruption and showed signs of heavy physiological stress with high prevalence of PNBF and PH. Maternal malnutrition and disease can lead to anaemia and maternal haemorrhage and can be related to outcomes such as low birth weight, stillbirths, and preterm birth. According to Molina, López and Muñoz (1980), 17% of the 240,463 births registered in Chile in 1977 can be considered preterm; for the same year, only 4-7% of births were preterm in the USA and Europe. As foetal development is intrinsically related to maternal nutritional provision and in utero protection, any environmental health stress the mother might suffer during pregnancy negatively affects her ability to do so (Bateson et al., 2004). In addition, intrauterine stress exposure has been linked to a reduced immune function in the offspring postnatal life (McDade, 2005), which adds on to the physiological stress the new environmental stressors of extrauterine life. In this sample, maternal health most likely played a role in the individuals' physiological response to stress, and the growth disruption evidenced in their skeletons.

In contrast, the presence of CF was associated with a scenario of no growth disruption (NG-P) in the complete sample (and was found non distinctive for the preterm-stillborn subsample). Although traditionally associated with anaemia, as CO, it has also been linked to physiological stress periods or dietary deficiencies during postcranial development. One possible explanation for this apparent paradox, is to argue that rather than be viewed as an indicator of frailty, CF could be interpreted as an indicator of resilience in particular contexts (Kyle et al., 2018). Further work is required to explore these findings.

3.5 Conclusion

This study is the first to compare age estimations and chronological age in foetal, perinatal, and infant skeletal remains from Chile. Detailed analysis of the foetal-infant

individuals within the COSS supports the use of dental development as a recommended biomarker for prenatal and postnatal age-at-death estimations in Chilean population but reveals that methods using dimensions of the pars basilaris as a maturity marker show problematic levels of population specificity, at least when applied to a modern South American sample. In addition, femoral length measurements should take into consideration the effect physiological stress can have on long bone diaphyseal mechanics and the impact this might have on age estimation methods.

Growth disruption in this sample, explored through age-at-death discrepancies and physiological stress markers, links biological plasticity and social factors by showing how environmental stressors affected the way in which these individuals developed. Particularly, cribra orbitalia and periosteal new bone formation and its association with growth disruption emerge as supportive evidence that these skeletal stress markers can be used as evidence of physiological stress in this population. Using only asymmetrical lesions of periosteal new bone formation as indicative of pathological changes seems to be a better guideline to differentiate it from normal bone apposition in non-adults.

Taken together, these findings also suggest a maternal-infant nexus evident in skeletal growth and development, and their biological response to physiological stress. Although caution is necessary to avoid reducing skeletal biology and its response to stressors to the basic assumption that environmental circumstances determine certain health outcomes (Gowland, 2015), the bioarchaeological analysis of the frailty experience of this sample of foetal-infant individuals offers the unique opportunity to address physiological stress in utero and during the first year of life in a modern population. Hardships of migration, the strains of urbanisation and deep-rooted systemic inequalities are still contexts that afflict many worldwide, particularly influencing the health experience of this population. Thus, understanding the complex interaction between sociocultural and economic factors and biological responses to physiological stress in non-adults remains critical.

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Chapter 4

Piececitos de niño[1]*: patterns of physiological stress and survival among children from 20th-century Santiago, Chile*

[1] Excerpt from the poem "*Piececitos*" (Little feet) by Chilean poet-diplomat, educator, and Nobel laureate Gabriela Mistral. The last stanza of the poem reads *"Piececitos de niño / dos joyitas sufrientes, / ¡cómo pasan sin veros / las gentes!"* (Little feet of children, / two tiny suffering jewels, / how can people pass / and not see you!).

Abstract

Context: Within the life course, childhood is a period highly sensitive to physiological stressors, with factors such as biological sex, chronological age or socioeconomic status affecting the way frailty relates to mortality risk.

Objectives: This study examines the impact of physiological stress in non-adults using survival analysis to explore the hazards associated with the experience of childhood in low socioeconomic status communities in mid-20th century Santiago, Chile.

Materials and Methods: A sample of 164 non-adult individuals of documented sex and age-at-death between 5 gestational months and 21 years of age, was categorised into three age groups: foetal-infant (<1 year of age), childhood (1-11 years of age), and adolescence (12-21 years of age). Presence or absence of five non-specific skeletal stress markers (dental caries, cribra orbitalia, linear enamel hypoplasia, periosteal new bone formation, and porotic hyperostosis) and two health conditions (rickets and scurvy) were recorded to explore physiological stress. Patterns of survival associated with physiological stress were assessed via Kaplan-Meier estimators and Cox proportional hazard models.

Results: Survival analysis showed that presence of cribra orbitalia and porotic hyperostosis was significantly associated with greater mortality risk in foetal-infant males compared to females. Evidence of greater risk of death during childhood was associated with the presence of rickets in all individuals and in combination with linear enamel hypoplasia in females and caries in males.

Conclusions: Decreased survival is likely to be associated with malnutrition and subsequent inadequate immune response during early stages of life. Results differentials depending on age group and biological sex might reflect variation in sensitivity to stressors, as well as selective mortality, or some combination of these factors.

Keywords: early life-course experience, risk of death, physiological stress, selective mortality, low socioeconomic status

Piececitos de niño: **patrones de estrés fisiológico y supervivencia en niños de Santiago de Chile durante el siglo XX**

Contexto: La infancia es un período altamente sensible a los estresores de origen fisiológico. Factores como el sexo biológico, la edad cronológica o el nivel socioeconómico juegan un papel preponderante en la forma en que la fragilidad se traduce en riesgo de mortalidad.

Objetivo: Este estudio examina el impacto del estrés fisiológico en individuos infantiles utilizando análisis de supervivencia para explorar los peligros asociados con la experiencia de la niñez en comunidades de bajo nivel socioeconómico a mediados del siglo XX en Santiago, Chile.

Materiales y Métodos: Una muestra de 164 individuos infantiles de sexo y edad documentados entre 5 meses de gestación y 21 años, se clasificó en tres grupos etarios: etapa prenatal-infancia (<1 año), niñez (1-11 años) y adolescencia (12-21 años). Para evaluar el estrés fisiológico se registró la presencia o ausencia de cinco marcadores de estrés esquelético no específicos (caries dental, cribra orbitalia, hipoplasia del esmalte, parches periósticos e hiperostosis porótica) y dos condiciones de salud (raquitismo y escorbuto). Los patrones de supervivencia asociados al estrés fisiológico se evaluaron mediante estimadores de Kaplan-Meier y modelos de riesgos proporcionales de Cox.

Resultados: El análisis de supervivencia mostró que la presencia de cribra orbitalia e hiperostosis porótica se asoció significativamente con un mayor riesgo de mortalidad en fetos e infantes de sexo masculino en comparación con las niñas. El riesgo de muerte durante la infancia en todos los individuos estudiados incrementó ante la presencia de raquitismo, en combinación con otros indicadores esqueléticos: hipoplasia del esmalte en niñas y caries en niños.

Conclusiones: Es probable que la menor supervivencia de estos individuos haya estado asociada a la desnutrición y una respuesta inmune inadecuada durante las primeras etapas de la vida. Los resultados diferenciales según el grupo etario y sexo biológico podrían reflejar una variación en la sensibilidad a factores estresantes, así como la mortalidad selectiva o alguna combinación de estos factores.

Palabras clave: experiencia de vida temprana, riesgo de muerte, estrés fisiológico, mortalidad selectiva, bajo nivel socioeconómico, sobrevivencia

4.1 Introduction

It is now widely acknowledged within the field of bioarchaeology that the study of childhood is fundamental to our understanding of health experience through the life course. Studies of non-adult skeletal remains continue to offer a wealth of information about mortality, morbidity, development, and growth disruption within these samples (Lewis, 2007). As sensitive indicators of biocultural change, they serve as proxy for the health status of the whole population (Buikstra and Ubelaker, 1994). Children are particularly vulnerable to the effects of physiological stressors; development and growth disturbances are a reflection of trade-offs in the allocation of energy within the body, reducing investments in growth to maintain essential tissue function (Klaus, 2014). As biological systems are highly plastic, reallocation of energy in early stages of growth and development is more flexible than in adulthood, so being a child might prove to be a protective factor against mortality from an early life stress event (Temple, 2019).

Bioarchaeological evidence framed within the Developmental Origins of Health and Disease (DOHaD) approach shows that, although individuals can be genetically predisposed to certain physiological stressors, detrimental environmental factors not only have an immediate effect on growth and development, but also contribute to long-term outcomes on health and mortality risk (Gluckman and Hanson, 2006; Bogin, 2020). Although such long-term impacts are usually examined in adult remains using skeletal lesions as evidence of childhood stress, the effect of physiological stress in non-adults can be studied as patterns of frailty and related mortality risks when the sample shares a particular environmental context (Gowland, 2015; Garland, 2020).

Stress exposure related to socioeconomic status (SES) has been well documented on bioarchaeological samples from different time and places (Marklein and Crews, 2022), showing that economic and socio-political factors affect prevalence of disease, morbidity, mortality, and frailty, particularly among low SES communities (Newman and Gowland, 2017). Non-adults living in poverty-stricken areas of Santiago, Chile, during the mid-20th-century experienced an urban environment that has long been known to create and recreate social inequality (Cambiaso *et al.*, 2001; Chávez Zúñiga, 2018). The impact of rapid urbanisation of Santiago at the end of the 19th century and the beginning of the 20th, meant low SES communities experienced reduced access to nutritional resources, an elevated risk of physiological stress due

to widespread infections, metabolic diseases as a consequence of malnutrition and early life infections, increased risk of death, as well as growth and development disruptions (Garland, 2020). Children living in these areas would be particularly affected by social and health inequalities, but would also be the target of public policies created to fight the elevated infant mortality and morbidity rates present in this population (Chávez Zúñiga, 2020). Exploring this dichotomy through skeletal indicators of stress, thus combining biocultural context with palaeopathology, can offer a way to better understand how health experience might be affected by heterogeneous frailty and selective mortality (DeWitte and Stojanowski, 2015).

As the presence of skeletal stress markers is not always related to poor health, and under some circumstances can actually indicate a healthier individual that has been able to adjust energetic trade-offs to fight stress while maintaining essential tissue function (Wood *et al.*, 1992), the interpretation of skeletal stress markers must be undertaken with caution. The sample studied here represent a cohort of short duration (26 years, or about one generation), with a shared socioeconomic living experience, which somewhat lessens concerns about demographic nonstationary and provides a better understanding on how other factors, such as age-at-death, biological sex, and health status, can be associated with risk of death. By controlling potential sources of heterogeneity, this study might overcome the notion of unknown variation in susceptibility to disease and death within individuals in this population, thus ameliorating – although not disregarding – concerns about heterogeneity in frailty (DeWitte and Stojanowski, 2015). Selective mortality, or the elevated likelihood of those most biologically vulnerable to die, can be then explored with respect to skeletal lesions —that is, whether individuals with lesions are at higher risks of dying than their peers without lesions. This study, however, does not make assumptions on whether the presence of skeletal indicators of physiological stress indicate good or bad health, but only explores if there is a difference in the risk of death and survival between individuals with or without skeletal stress markers.

In this context, this study examines patterns of stress, frailty, and survivorship in non-adult individuals of documented biological sex, documented chronological age-at-death, and well-recorded sociocultural context. This study makes a substantial contribution to overcoming the conceptual challenges inherent to the interpretation of health in past populations, such as those outlined by the "osteological paradox" (Wood *et al.*, 1992).

4.2 Materials and Methods

4.2.1 Skeletal sample

This study analysed non-adult individuals (n = 164) from the COSS who died between 1960 and 1986. All individuals have documented sex and chronological age (CA) and were between the ages of 5 gestational (*intra utero*) months to 21 years of age. The sample also had documented cause of death (CoD), which was categorised, when possible, into cardiovascular (e.g., cardiac arrest, heart failure), infectious (e.g., bronchopneumonia, sepsis and septic shock, acute diarrhoea, meningitis), metabolic (e.g., malnutrition, cachexia), neoplastic (e.g., leukaemia, brain tumour, cancer), or traumatic (e.g., traumatic brain injury, drowning, asphyxiation).

Skeletal age (SA) estimations for this sample were retrieved from the UChile database, while antemortem data on CA and markers of skeletal frailty were recorded by the researcher. Using CA, individuals were grouped for analysis into three age categories: foetal-infant (n=47), childhood (n=42), and adolescence (n=75). Foetal, perinatal, and infant individuals from 5 gestational months to 1 year of age were grouped together as their health outcomes are mostly maternally regulated through *in utero* nutrition and breastfeeding practices (Barker, 1997; Gowland, 2015; Vatanen *et al.*, 2022). Childhood was defined broadly from the moment of weaning around 1 year of age (Zárate Campos, 2010), when food is introduced in the individual's diet and the maternal buffering of breast milk stops (Vatanen *et al.*, 2022), to 11 years of age, with adrenarche marking the end of this stage. During this time, no significant sex differentials are found in growth curves between females and males; although females tend to reach puberty before than their male counterparts, their growth curve during childhood follows a similar pattern and velocity (Rogol, Roemmich and Clark, 2002). Individuals between 12 and 21 years of age were grouped together in the adolescence stage due to the great number of morphological and physical changes in bodily composition and function that begin to happen from the onset of puberty to the acquirement of a fully mature sexually dimorphic adult body (Gluckman and Hanson, 2006). These age categories were defined using a broad understanding of the underlying biological development of the individual and were not intended to necessarily reflect cultural identity or societal roles.

4.2.2 Skeletal markers of physiological stress

To analyse physiological stress and its relationship with risk of death and survival, macroscopic analyses for presence/absence of five non-specific skeletal stress markers and two health conditions were recorded. Stress markers included caries (CAR), cribra orbitalia (CO), linear enamel hypoplasia (LEH), periosteal new bone formation (PNBF), and porotic hyperostosis (PH), while rickets (RIC) and scurvy (SCU) were also diagnosed within the sample. These skeletal stress markers, although non-specific in nature, were selected to explore physiological stress due to a wide range of potential aetiologies, including metabolic diseases, nutritional disorders/deficiencies, famine exposure, infectious diseases, and inflammatory processes associated with trauma or metabolic disease. In addition, rickets and scurvy, determined by following Brickley, Ives and Mays (2020) diagnostic criteria for juvenile individuals, were included as conditions related to nutritional deficiencies (vitamin D and C, respectively). These conditions are also associated with social risk factors such as socioeconomic inequalities, urban environments, and young maternal age (Roberts and Manchester, 2010).

The impact of physiological stress, evidenced in human remains as skeletal stress markers, on survival was tested in this study according to two scenarios: with skeletal stress markers as independent factors and with stress markers as interdependent risk factors. Results of both analyses will be explored together, as the nature of skeletal stress markers is that they are discernible macroscopically only when the individual has been exposed to stress for enough time for it to be visible on the skeleton. In this sense, it is likely that the younger individuals in the sample (e.g., foetal-infant group) exhibit less skeletal stress markers; for this reason, prevalence alone was not used, and survival analysis was selected as statistical tool to analyse this sample. The goal is to assess if individuals saw their risk of death increased by the presence of a particular set of skeletal markers (covariates), when assessed as independent and interdependent risk factors.

4.2.3 Statistical analysis

Crude prevalence (i.e., individuals presenting pathologies as a proportion of total of individuals) was calculated for each skeletal stress marker segregating by biological sex and age group. The effect of presence of skeletal stress markers on survival depending on sex and age

group was assessed using survival analysis techniques, namely Kaplan-Meier estimator with a log rank test and Cox proportional hazard model. Both analyses enabled the impact of early life stressors on survival and risk of death to be explored among individuals who died during each of the three age categories but took two different approaches: while the Kaplan-Meier estimator shows sex differentials in mortality risk (describing the survival time of members of the group), Cox proportional hazard model compare changes in risk of death associated with different predictor variables (describing the effect of categorical variables on survival). Each skeletal stress marker was recorded with a binary variable outcome of presence (coded 1) or absence (coded 2); censored data (when a skeletal element was not available to study, therefore it was not possible to assess presence/absence of a skeletal stress marker) was included in the study and coded independently as 0, to prevent overestimation of absence. All analyses were performed using RStudio 2021.09.0 Build 351.

Kaplan–Meier estimator.

To explore how each factor (skeletal stress marker) independently affected survival, a Kaplan-Meier estimator with a log rank test was applied to each skeletal stress marker using chronological age-at-death as time scale, and sex and age category as predictor variables. This enables the disaggregation of the effect of each skeletal stress marker on survival depending on sex and age group. For all analyses, p-values less than 0.1 are considered suggestive of a trend.

Cox proportional hazard model.

To explore how survival is affected by presence/absence of several skeletal markers simultaneously, Cox proportional hazard models were used to examine the effect of multiple variables (skeletal stress markers) on the time of death (chronological age-at-death) depending on age group (all, foetal-infant, childhood, adolescence) and biological sex (pooled, female, male). To measure effect size of each covariate in each model (Clark *et al.*, 2003), hazard ratios (HR) of the covariates are given, with HR>1.0 indicating an increased risk of death, and HR<1.0 indicating a reduced risk of death as the value of the covariate increases. Hazard ratios results will be discussed in percentages, as outlined by Spruance *et al.* (2004).

4.3 Results

4.3.1 Demography

Age-at-death category and biological sex distribution within the sample is shown in Figure 4.1; of the 164 individuals, 60% (n = 98) were males and 40% were females (n = 66). Infectious diseases were the most prevalent CoD in all groups except males during Childhood and Adolescence, when traumatic injuries were more frequent (Figure 4.2).

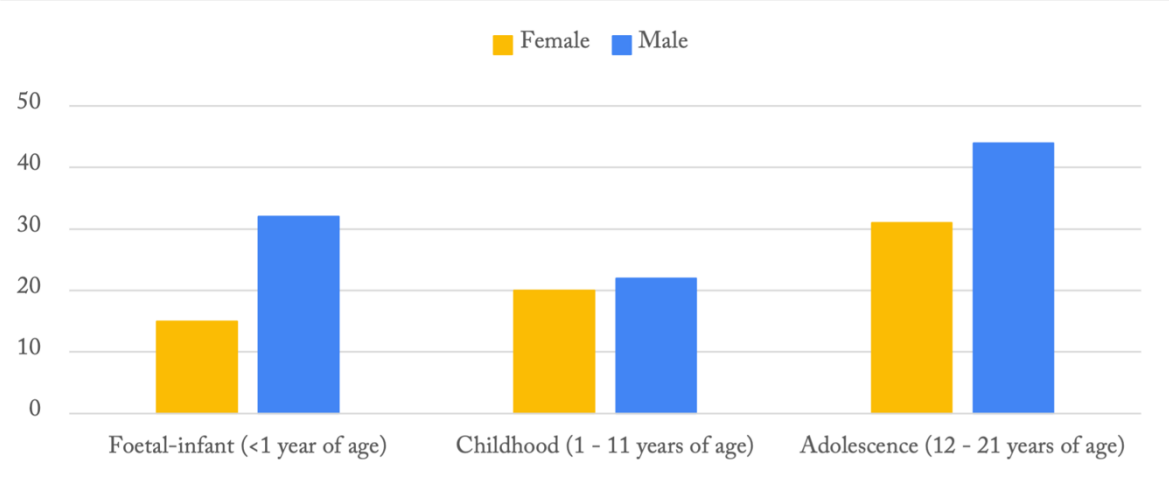

Figure 4.1 Distribution of non-adult individuals per sex and age category.

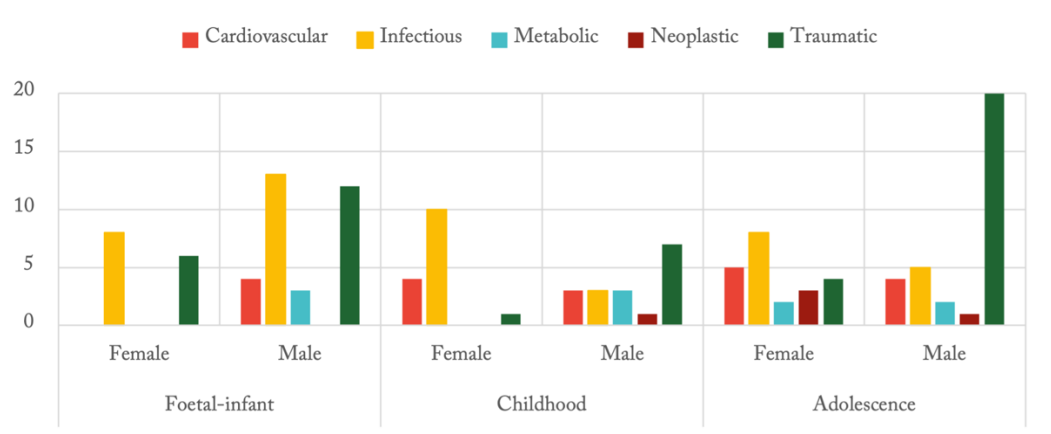

Figure 4.2 Frequency of cause of death (CoD) type segregated by age group and biological sex.

As precise age-at-death is not often known when studying archaeological populations, CA and SA mean ages for each age group were compared to explore how the use of SA could impact results of this study (Table 4.1). Effect sizes (Hedges' g) of the discrepancy between

CA and SA in each age group were calculated (Table 4.2). As expected, no significant discrepancies were found. Bioarchaeological methods of age estimation are more accurate in non-adults than in adults, as they focus on known stages of growth and development, as opposed to morphological changes linked to degeneration and wear (Scheuer and Black, 2004). While CA was used for survival analyses here, these data show that the study could be replicated using only SA.

Table 4.1 Descriptive statistics for chronological and skeletal ages segregated by age group and sex.

	Females					**Males**				
		CA		SA			CA		SA	
	n	MA	SD	MA	SD	*n*	MA	SD	MA	SD
Foetal-infant	15	0.27	0.64	0.40	0.65	32	0.24	0.45	0.26	0.65
Childhood	20	5.20	2.53	5.68	2.64	22	5.39	3.21	5.53	3.47
Adolescence	31	17.52	2.59	17.85	6.97	44	17.59	2.81	18.83	5.17

CA: chronological age; SA: skeletal age; n: sample size; MA: mean age; SD: standard deviation.

Table 4.2 Effect size difference between documented chronological age and skeletal age estimations.

	Effect size Females (CA v. SA)			**Effect size Males (CA v. SA)**		
	g	CI	CLES	g	CI	CLES
Foetal-infant	-0.19	-0.91 – 0.53	44.66	-0.04	-0.53 – 0.45	48.87
Childhood	-0.18	-0.80 – 0.44	44.94	-0.04	-0.63 – 0.55	48.87
Adolescence	-0.06	-0.56 – 0.44	48.31	-0.30	-0.72 – 0.12	41.60

g: Hedges' g; CI: confidence interval (95%); CLES: common language effect size (in %).

4.3.2 Statistical analysis

In general, there was a high prevalence of all skeletal stress markers within this sample, across age groups and biological sex (Table 4.3). Prevalence of CAR was increased for all individuals during childhood, while CO was higher in the foetal-infant age group, irrespective of biological sex. Both LEH and PNBF were highly prevalent in all the individuals, while observations of PH tended to peak during adolescence in both females and males. Prevalence of rickets was stable in females, while males exhibited more cases per total of the sample in the foetal-infant sub-group. Scurvy tended to follow a similar pattern, with a tendency to a lower prevalence during adolescence in females as sole difference than rickets.

Table 4.3 Crude prevalence of skeletal stress markers by biological sex and age group. Shaded cells denote a prevalence equal or higher than half of the individuals.

		Foetal-infant			Childhood			Adolescence		
		N	**n**	**P**	**N**	**n**	**P**	**N**	**n**	**P**
Females	CAR	11	1	9	17	7	41	25	2	8
	CO	7	5	71	16	12	75	25	8	32
	LEH	9	4	44	16	11	69	26	17	65
	PNBF	15	8	53	19	15	79	30	21	70
	PH	4	0	0	19	3	16	25	14	56
	RIC	14	5	36	16	6	38	29	9	31
	SCU	15	5	33	20	6	30	31	5	16
Males	CAR	13	0	0	22	9	41	34	2	6
	CO	14	10	71	18	10	56	37	12	32
	LEH	17	9	53	21	15	71	36	19	53
	PNBF	32	26	81	22	15	68	43	18	42
	PH	12	6	50	19	5	26	31	21	68
	RIC	29	15	52	17	6	35	44	9	20
	SCU	32	14	44	22	4	18	44	6	14

N: total number of individuals with the skeletal elements present for analysis; n: number of individuals exhibiting the skeletal stress marker; P: prevalence in percentage (%).

Results of Kaplan-Meier estimators (mean survival times with their corresponding standard deviations and 95% confidence intervals) show a significant difference between males and females in the foetal-infant age group (Table 4.4). Presence of two skeletal stress markers, cribra orbitalia (CO) and porotic hyperostosis (PH), is associated with decreased male survival during the foetal-infant stage, with females generally having lower risk of death (protective predictor) when these non-specific stress indicators are present (Figure 4.3). Although the Kaplan-Meier curve for PH shows a decreased survival in female foetal individuals before birth, the difference compared to male foetal individuals is not statistically significant.

Significance of the computed Cox proportional hazard models was analysed using likelihood ratio test, as it has been found to behave better for small sample sizes (Xu, Shaw and Mehrotra, 2018). Three models were found significant for different sets of covariates, representing all individuals in the Childhood group (Childhood: Pooled, Childhood: Females, and Childhood: Males) (Table 4.5).

Table 4.4 Results of Kaplan-Meier survival analysis. Mean survival times (corresponding to mean ages-at-death) in years are shown for each skeletal stress marker for the complete sample and segregated by age group.

		Females		Males		p-value
		MST (CI)	SD	MST (CI)	SD	
Foetal-infant	CAR	0.108 (0.000 – 0.382)	0.140	0.257 (0.047 – 0.467)	0.107	0.284
	CO	0.530 (0.150 – 0.910)	0.194	0.049 (0.000 – 0.293)	0.124	0.053
	LEH	0.167 (0.000 – 0.507)	0.174	0.275 (0.062 – 0.488)	0.109	0.830
	PNBF	0.255 (0.000 – 0.543)	0.147	0.244 (0.114 – 0.375)	0.066	0.292
	PH	0.333 (0.000 – 0.782)	0.229	0.071 (0.000 – 0.292)	0.113	0.046
	RIC	0.254 (0.000 – 0.539)	0.145	0.237 (0.113 – 0.362)	0.063	0.166
	SCU	0.169 (0.000 – 0.521)	0.176	0.273 (0.049 – 0.497)	0.112	0.984
Childhood	CAR	4.817 (3.841 – 5.793)	0.498	4.915 (3.882 – 5.947)	0.527	0.605
	CO	4.950 (3.854 – 6.046)	0.559	4.958 (3.750 – 6.167)	0.617	0.677
	LEH	4.574 (3.535 – 5.614)	0.530	4.516 (3.411 – 5.620)	0.564	0.812
	PNBF	5.163 (4.100 – 6.225)	0.542	5.442 (4.354 – 6.531)	0.555	0.544
	PH	5.139 (4.280 – 5.997)	0.438	5.213 (4.106 – 6.321)	0.565	0.523
	RIC	4.707 (3.919 – 5.495)	0.402	4.033 (3.248 – 4.817)	0.400	0.394
	SCU	4.574 (3.414 – 5.630)	0.528	4.516 (3.326 – 5.706)	0.595	0.782
Adolescence	CAR	17.455 (16.718 – 18.191)	0.376	17.771 (17.117 – 18.426)	0.334	0.371
	CO	17.524 (16.716 – 18.332)	0.412	17.984 (17.327 – 18.641)	0.335	0.455
	LEH	17.800 (16.954 – 18.646)	0.432	17.943 (17.223 – 18.664)	0.368	0.654
	PNBF	17.692 (16.906 – 18.478)	0.401	17.706 (17.047 – 18.364)	0.336	0.654
	PH	17.135 (16.236 – 18.034)	0.459	17.400 (16.451 – 18.349)	0.484	0.523
	RIC	17.449 (16.693 – 18.205)	0.386	17.620 (16.996 – 18.244)	0.318	0.636
	SCU	17.382 (17.630 – 18.134)	0.376	17.708 (17.040 – 18.376)	0.334	0.921

MST: mean survival time; SD: standard deviation; CI: confidence interval (95%). Highlighted are p-value <0.1

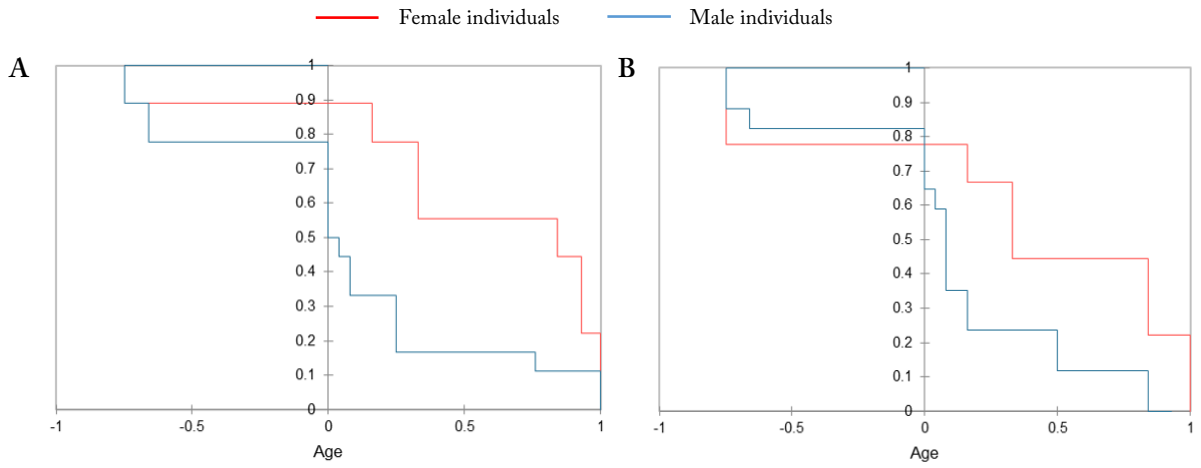

Figure 4.3 Kaplan-Meier survival distribution functions for cribra orbitalia (A) and porotic hyperostosis (B) in the foetal-infant group.

Informative covariates for each of the significant models display differential hazard ratios (Figure 4.5), pointing to differences in risk of death given the presence (positive B coefficient) or absence (negative B coefficient) of skeletal stress markers (Table 4.6). When holding all other covariates constant, the presence of rickets during childhood is associated with poor survival, with an increased hazard of 77.02% (HR = 3.351) in females and 79.67% (HR = 3.919) in males. In addition to rickets, presence of LEH increases the risk of death by 79.51% (HR = 3.88) in females, while presence of dental caries (HR = 5.166) is related to an increased risk of death by a ratio of 83.78% in males. Conversely, when all covariates are held constant, individuals during childhood show that absence of cribra femora (CF) in females (HR = 0.116) is a protective predictor associated with a 10.39% of improved survival.

Table 4.5 Statistical significance of Cox proportional hazards models.

	Pooled		Females		Males	
	-2 Log	**C index**	**-2 Log**	**C index**	**-2 Log**	**C index**
Foetal-infant	0.900	0.588	0.600	0.742	0.400	0.700
Childhood	<0.0001	0.775	0.040	0.778	<0.0001	0.858
Adolescence	0.300	0.629	1.000	0.598	0.500	0.663

-2 Log: likelihood ratio; C index: concordance index. Highlighted are models with p-values <0.05

Table 4.6 Results of significant Cox proportional hazards models.

Model	Covariates	B	SE B	HR/OR	CI	p-value
Childhood: Pooled	CF	-1.040	0.517	0.353	0.1283 – 0.9738	0.044
	LEH	1.299	0.462	3.667	1.4816 – 9.0809	0.005
	RIC	1.036	0.297	2.820	1.5736 – 5.0549	0.000
Childhood: Females	CF	-2.151	1.035	0.116	0.0152 – 0.8853	0.037
	LEH	1.355	0.646	3.880	1.0921 – 13.7871	0.036
	RIC	1.209	0.498	3.351	1.28345 – 8.7537	0.013
Childhood: Males	CAR	1.642	0.828	5.166	1.01912 – 26.188	0.047
	RIC	1.365	0.535	3.919	1.37192 – 11.198	0.010

B: beta coefficient; SE B: standard error of beta coefficient; HR/OR: hazard ratio/odds ratio; CI: confidence intervals; p-value: Pr(>|z|). Highlighted are covariates with p-value < 0.05 and HR > 1.0.

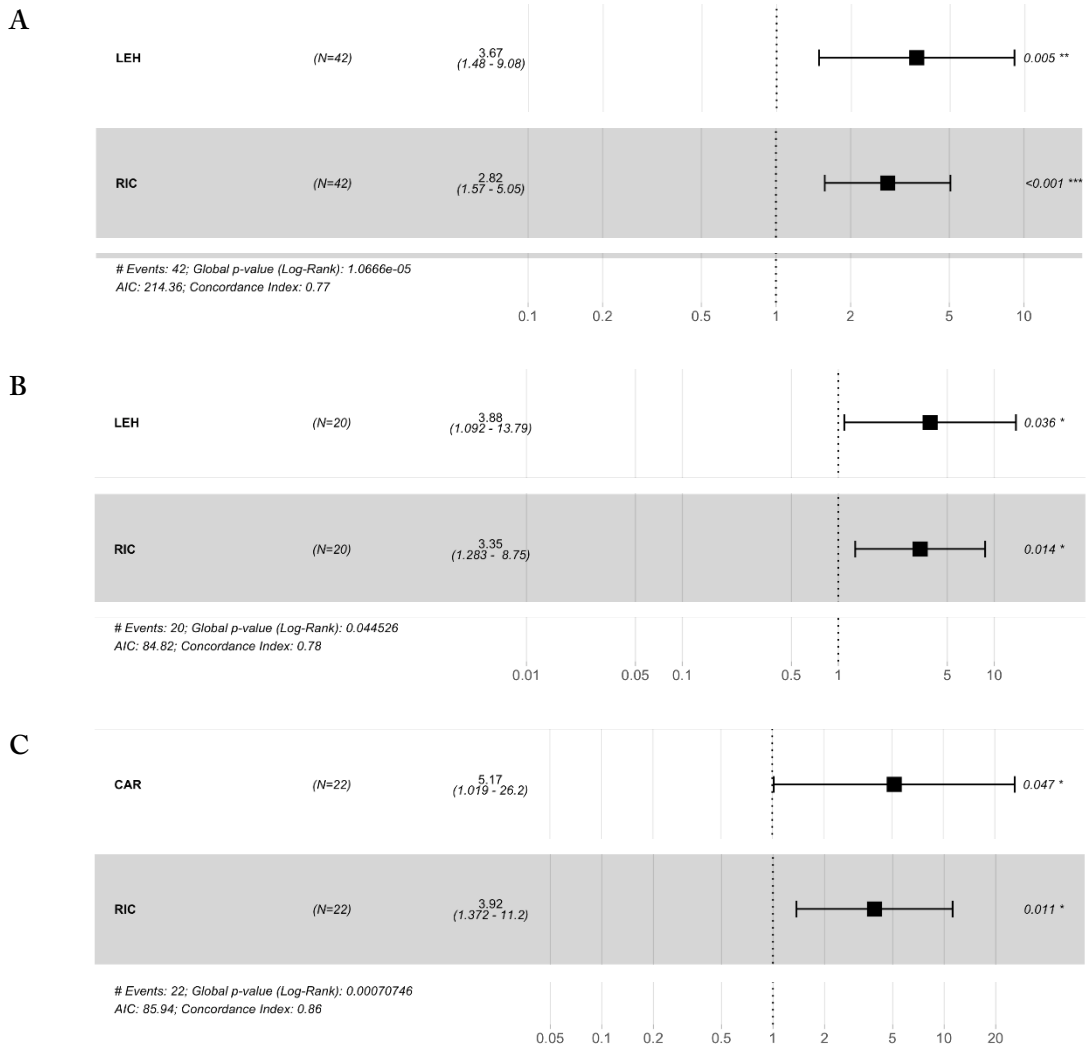

Figure 4.4 Hazard ratios for Cox proportional models Childhood: Pooled (A), Childhood: Females (B) and Childhood: Males (C). Only shown covariates whose presence increases risk of death (HR<1).

4.4 Discussion

Although all individuals in this sample can be considered non-survivors, as outlined by the "osteological paradox", this study offers the rare opportunity to explore the extent of how selective mortality operates within this population. The results of survival analyses indicate that overall, individuals in the foetal-infant and childhood groups were most susceptible to excess mortality, with distinct male vs. female patterns of survivorship. Increased risk of death was associated with different indicators of skeletal stress depending on the age group, which may suggest differences in the biological response to physiological stress depending on the individual's stage within the life course. In this sample, increased risk of death of foetal-infant

individuals was associated with being male. This is confirmed by the Kaplan-Meier survivorship analysis, which reveals decreased male survival when cribra orbitalia and porotic hyperostosis were present. In a later stage, during childhood, increased risk of death was associated with the presence of rickets in all individuals, in combination with linear enamel hypoplasia in females and dental caries in males.

Although the causes of porotic lesions of the skull (CO and PH) have been the centre of great debate over the past decades (Brickley, 2018), bioarchaeology usually identifies their presence as an indicator of nutritional stress, particularly of iron-deficiency anaemia (Buikstra, 2019). It is known that nutritional status is heavily related to immune competence and that malnutrition in the early stages of the life course can lead to long-term negative effects that increase the risk of mortality in adults (Calder *et al.*, 2006; Hughes and Kelly, 2006; Jones, Berkley and Warner, 2010; DeWitte and Hughes-Morey, 2012). Results for this sample show sex differential patterns of survivorship, with the presence of cribra orbitalia and porotic hyperostosis associated to an increased risk of death in males.

The differentials in survival between biological sexes might be explained by different strategies during gestation. In foetal-infant individuals, *intra utero* nutrition relies on the ability of the mother to provide the adequate amount of nutrients, a scenario that persists after birth through breastfeeding practices (Barker, 1997). Although in cases of mild or short-term deficiency maternal buffering prioritises the foetus' nutritional needs at the expense of the mother (Vatanen *et al.*, 2022), where deficiency is severe the biological response of foetal-infant individuals to under or malnutrition is to adapt to the extant offer of nutrients, at the expense of their own bodily structure and function (Martin-Gronert and Ozanne, 2006). Clinical studies have found that male individuals are more sensitive to environmental stress and nutritional deficiencies (Fogel *et al.*, 1983; McFadden and Oxenham, 2020), experiencing higher mortality than females from early life and independently of the cause of mortality (Balsara *et al.*, 2013). While *intra utero*, males' growth strategy is riskier than that of females (Eriksson *et al.*, 2010): male foetuses grow faster, investing less in placental growth which results in a reduction of placental area (Roseboom *et al.*, 2011). This strategy ensures larger body size proportions at birth, at the expense of efficient nutritional storage which puts them at danger of becoming undernourished when compared to females (Barker *et al.*, 2012). McFadden and Oxenham (2020, p.212) describe this female survival advantage as "indicative

of greater robustness in females or greater susceptibility in males". Anaemia, as nutritional iron-deficiency, has been observed to affect infants and children differently depending on their biological sex (Domellöf *et al.*, 2002; Wieringa *et al.*, 2007); clinical studies have found that before 2 years of age, males are at greater risk of developing iron-deficiency anaemia than females (Woodruff, 1958; Betke, 1970; DeWitte, 2018; McClorry *et al.*, 2018), pattern that is not as clear in older children (Fernandes-Costa *et al.*, 1984; Florentino and Guirriec, 1984). Increased susceptibility of male infants to malnourishment and iron-deficiency anaemia might explain why male foetal-infant individuals in this sample experienced decreased survival associated with the presence of CO and PH.

From a biocultural approach, it is interesting to note that social dynamics during this period in Chile might help contextualise the findings of this study. Historical records for the period show that iron-deficiency anaemia affected 30% of infants under 12 months of age (Hertrampf *et al.*, 2009) and was thought to be linked to malnutrition (Olivares *et al.*, 1989). Successful governmental efforts during the 1970's and onwards tried to tackle malnutrition by supplying free milk formula to new-borns and babies during their first year of life (Largo, 1982; Atalah *et al.*, 1985). Although malnutrition within this age group decreased during the next decades, high prevalence of anaemia in infants continued until the 1990's (Ríos *et al.*, 1983; Olivares *et al.*, 1989). This might have been due to the formulation of the milk itself; aimed particularly to poverty-stricken communities, these public policy tackled undernutrition, but not iron-deficiency anaemia, as fortification of free milk formulas with iron, vitamin C, zinc, and copper did not start until 1999 (Hertrampf *et al.*, 2009). Although studied in a different geographical region but sharing many of the same socio-political issues, recent bioarchaeological research in Industrial England shows that in poor living conditions, boys' health was particularly impacted, as female physiology during early life reserves nutritional resources that buffer them during times of stress (Reedy, 2020). On the other hand, cultural attitudes towards early nutrition in children during this time in Chile shows differences for boys and girls; the vast majority of children in low-SES communities were breastfeed for at least the first 6 months of life (Juez *et al.*, 1984), but girls were weaned earlier than boys and their nutrition depended on formula for longer than their male counterparts (Niño, Silva and Atalah, 2012). Interestingly, this cultural difference in food allocation preference might have impacted survivorship in these infants: exclusive breastfeeding protects infants from iron-deficiency anaemia only for the first four months of life, after which there is an increase in

anaemia rates, with odds of iron-deficiency increasing for each additional month of exclusive breastfeeding (Maguire *et al.*, 2013; Marques *et al.*, 2014). This correlates with the antemortem information available for these individuals: malnutrition, and any metabolic disease, was only listed as CoD for male infants. Thus, decreased survival for males in this group might be related not just to malnutrition, but to the specific combination of innate susceptibility and undernutrition and iron deficiency in theirs or their mother's diet (see Chapter 3).

During childhood, decreased survival was associated with the presence of rickets in all individuals, irrespective of their biological sex. Rickets is the condition that arises when growing bones fail to mineralise, resulting in softened and weakened bones in children, usually related to dietary deficiencies or genetic causes. Within the bioarchaeological literature, the most common cause attributed to rickets is an extreme and prolonged vitamin D deficiency (Mays, Brickley and Ives, 2006; Roberts and Manchester, 2010; Buikstra, 2019). Exposure to vitamin D during childhood is important for brain development (Siracusano *et al.*, 2020), bone acquisition and development Brickley, Ives and Mays, 2020), while also working as a protective factor for the immune response (Snoddy, Buckley and Halcrow, 2016). But as a dietary deficiency, clinical studies show that rickets can also be associated with an isolated calcium deficiency and, therefore, to malnutrition, particularly when found in individuals belonging to low SES communities (Jones *et al.*, 2018). Thus, nutritional rickets, as a multifactorial disease that exists in an aetiological range from solely vitamin D deficiency to isolated calcium deficiency, and all combinations in-between (Thacher *et al.*, 2006), should be understood as the tip of an "epidemiological iceberg" (Ginde, Liu and Camargo, 2009). Its presence in bioarchaeological remains represent the end of the spectrum of severity; the individual would be prone to other illnesses while suffering from rickets for enough time to make it macroscopically visible on the bones. While diagnosing skeletal manifestations of rickets is useful in itself - to explore how prevalent the condition was in the past - it also serves as the visible part of a multitude of potential comorbidities (Snoddy, Buckley and Halcrow, 2016), particularly those arising from nutritional deficiencies. This might explain why results for the increase in risk of death include skeletal markers associated with rickets within the childhood sample, but not the foetal-infant individuals.

For older children in the childhood age category in this study, increased risk of death of individuals showed to be multifactorial and the relationship between comorbidities differs

depending on biological sex. While some studies have reported that males at younger ages are more vulnerable to vitamin D deficiency rickets than females (AlQuaiz *et al.*, 2018), results in this study may be reflecting the spectrum of aetiologies associated to nutritional rickets as all individuals during childhood saw their risk of death increased when rickets was present, irrespective of sex.

Female risk of death increased during childhood when LEH was also present. Although LEH has many aetiological explanations, such as premature birth, childhood disease, viral and bacterial infections, and toxins consumption (Miszkiewicz, 2015), it is also related to weaning and malnutrition, particularly that of vitamin D deficiency (Sheetal *et al.*, 2013). Breast-feeding has been found to be protective against LEH in both sexes (Agarwal *et al.*, 2003) and, as mentioned, Chilean girls were weaned earlier than boys during this period (Niño, Silva and Atalah, 2012). Male survival during childhood was found negatively impacted when also suffering from dental caries. In bioarchaeology, caries is usually used as an indicator of diet and cooking technology (Lanfranco and Eggers, 2012), with its associations to systemic diseases rarely investigated in non-adults (Sabharwal, Stellrecht and Scannapieco, 2021). However, clinical studies suggest that caries of deciduous dentition is associated with early childhood malnutrition (Alvarez *et al.*, 1993; Psoter, Reid and Katz, 2005; Sokal-Gutierrez *et al.*, 2016; Folayan *et al.*, 2020), while also associated with iron-deficiency anaemia in children between 3 and 5 years of age (Folayan *et al.*, 2020). Both LEH and dental caries are related to the deciduous dentition in this sample; LEH affects deciduous teeth during dental development, which starts *intra utero* and continues through the first years of life, while dental caries appear after the tooth has formed and erupted into the oral cavity. Although the timing of both pathological changes is different within the life course, they might be pointing to a shared underlying cause in this population; many studies have found that developmental defects of the tooth and dental caries are significantly related (Li, Navia and Bian, 1996; Seow *et al.*, 1996; Milgrom *et al.*, 2000; Salanitri and Seow, 2013), as disturbances of the tooth germ during embryological development can result in an integrity loss of the enamel surface, causing LEH and allowing plaque accumulation that can lead to caries. These developmental disturbances have been linked to nutritional deficiencies, as well as premature birth, pre- and postnatal infection and even maternal practices such as smoking (Sabharwal, Stellrecht and Scannapieco, 2021). Combined with a decrease in the individual's innate immunity linked to rickets (Snoddy, Buckley and Halcrow, 2016), oral pathologies such as LEH and dental caries might be

reflecting a reduced immune function within these individuals, elevating the individual's risk of developing other diseases that would cause their premature deaths (DeWitte, 2010).

An inadequate or diminished immune response leaves the body more exposed to infections that otherwise would lie dormant in the body; this can be correlated to the documented CoD for the childhood group, which lists predominantly infectious diseases, mainly those affecting the respiratory tract with boys and girls dying mostly from bronchopneumonia and croup (laryngotracheobronchitis). As vitamin D deficiency plays a role in paediatric diseases from the respiratory tract (Tachimoto *et al.*, 2016), it would be expected that individuals that died of respiratory infections would be the ones exhibiting skeletal changes associated with rickets (Snoddy, Buckley and Halcrow, 2016), but no such correlation was found in this study. However, pathological changes associated with rickets in the skeleton mark, as mentioned before, the last stage of severity of this illness; it is highly probable that malnutrition and/or nutritional rickets have affected these individuals without leaving any skeletal sign. The uniqueness of this skeletal sample is that it is possible to explore both bioarchaeological evidence and documented antemortem information to assess this group of non-adults as a population, and not just as independent individuals, while also inserting them into their historical context.

During the 20th century in Chile, malnutrition was one of the leading causes of infant mortality (Medina and Kaempffer, 2007) and rickets prevalence was seen as a serious public health issue: from 0.45% of infants affected in 1909, almost half the children under 5 years of age were diagnosed with rickets in 1930 (Meneghello *et al.*, 1948). In the early 20th century, rickets was widely known as a "social disease", with clinicians and political scientists of the time denouncing poor living conditions and poverty as the main cause. Baeza (1931) pointed that rickets among Santiago's children was higher in low SES areas, associating the increase in the disease prevalence to malnutrition, infectious diseases, and the ongoing rural-to-urban migration, which caused a progressive surge of inhabitants in a city that was not ready to accommodate such bigger population. Despite having been made more than 90 years ago, Baeza's conclusions resonate with recent bioarchaeological studies: Lewis (2002) has reported that urbanisation was one of the major causes of increase prevalence of rickets in England, while Brickley, Mays and Ives (2007) suggest that vitamin D deficiency disease is an important socioeconomic indicator, usually associated to low SES communities. This also relates to the

individuals in this study: coming from poverty-stricken neighbourhoods of Santiago, they most likely lived in lacking housing conditions while suffering from nutritional deprivation.

Thus, the difference in nutritional intake in early life might be causing the sex differentials found in this study. Both female and male individuals were experiencing physiological stress in utero and during the early stages of infancy, while dependant on their mothers; boys were more prone to death from malnutrition (particularly related to iron deficiency) as infants, while girls were more likely to survive this stage, but developed LEH as a result. Later during childhood, both boys and girls would see their risk of death increased if undernutrition persisted, particularly that related to nutritional rickets. Malnutrition, as a quintessential marker of poverty, affected the children within this sample, with subtle differences in their associated risk of death depending on age-at-death and biological sex.

4.5 Conclusion

The experience of childhood in late 20th century Santiago, Chile was inseparable from the socioeconomic status of the children themselves. The estimated values of increased risk of death associated with presence of skeletal stress markers from this study suggest that exposure to physiological stressors was detrimental for the survival of non-adults living in low-SES communities of Santiago between 1960 and 1986.

Examining the health experience of non-adults of documented biological sex, chronological age and shared socioeconomic background has the potential to improve our understanding of the biological mortality bias in their population, to some degree allowing us to overcome the limitations of heterogeneity in frailty and selective mortality. Coming from a documented osteological collection, non-adults in this study can be placed within their historical context with more precision than that of other bioarchaeological remains; their sociocultural background, documented antemortem information, paired with palaeopathological data allows to understand their health outcomes. Future research on this population has the potential to continue to explore and expand our understanding of the link between childhood survival as a contextual experience mediated by a combination of environmental and genetic factors.

4.6 References

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Chapter 5

Descomedidos y chascones[2]*: pubertal timing and frailty in a modern documented adolescent skeletal sample from Santiago, Chile (1960-1986)*

[2] Translated as “unruly and shaggy”, the title references a 1972 documentary by Chilean filmmaker Carlos Flores del Pino, portraying contemporary young people from different social and political classes. It is one of the few, if not the only, audio-visual pieces to put Chilean adolescents as protagonists during the period, reflecting on the meaning of "being young" in a historical context of high social and political conflict (Ganter, Basulto and Mendoza, 2021).

Abstract

Objectives: This study explores pubertal timing of modern adolescents of documented biological sex and chronological age from a modern South American population.

Materials and Methods: 85 non-adult and young adult individuals aged between 8 and 21 years at death from low socioeconomic status communities in mid-20th century Santiago, Chile, were analysed. Pubertal timing estimations were made according to the method proposed by Shapland and Lewis (2013, 2014), assigning each individual to one of the pubertal stages outlined by Lewis, Shapland and Watts (2016a). Six skeletal stress markers and recorded causes of death were examined for each individual and pubertal stage to explore frailty during this period of rapid growth and development. Hedges' g tests for effect size were used to analyse differences between chronological age-at-death and skeletal age estimation.

Results: Onset of puberty happened at a similar age to that historically reported in adolescents for the period, but progression through puberty was slower and more prolonged. Delay in the acceleration towards the peak high velocity point and subsequent delay in age of menarche (17.46±2.25 years) compared to the clinical data was found in the females from this sample. Presence of frailty indicators might link disease exposure and susceptibility to disruption in pubertal timing.

Conclusions: This research is the first study of skeletal pubertal timing in South America and only the second ever study to apply pubertal timing methods to individuals of documented antemortem data (chronological age and biological sex), advancing our bioarchaeological understanding of puberty and adolescence from an individual and population wide perspective.

Keywords: pubertal disruption, developmental stress, adolescence, bioarchaeology

Descomedidos y chascones: desarrollo puberal y fragilidad en una muestra esquelética de adolescentes modernos con edad y sexo documentados de Santiago de Chile (1960-1986)

Objetivo: Este estudio explora el desarrollo puberal en adolescentes de sexo biológico y edad cronológica documentados de una población sudamericana moderna.

Materiales y Métodos: Se analizaron 85 individuos no adultos y adultos jóvenes con edades entre 8 y 21 años que vivieron en comunidades de bajo nivel socioeconómico en Santiago de Chile a mediados del siglo XX. Las estimaciones de desarrollo puberal se realizaron de acuerdo con el método propuesto por Shapland y Lewis (2013, 2014), asignando a cada individuo a una de las etapas puberales descritas por Lewis, Shapland y Watts (2016a). Se examinaron seis marcadores de estrés esquelético y la causa de muerte documentada para cada individuo y en cada etapa puberal para explorar la fragilidad durante este período de rápido crecimiento y desarrollo. Se utilizaron pruebas g de Hedges para analizar las diferencias entre la edad cronológica y la estimación de la edad esquelética.

Resultados: El inicio de la pubertad ocurrió a una edad similar a la históricamente reportada en adolescentes para el período, pero la progresión a través de la pubertad fue más lenta y prolongada. Las mujeres de esta muestra presentan un retraso en la aceleración del crecimiento en el punto máximo de alta velocidad, mostrando un retraso en la edad de la menarquia en comparación con datos clínicos (17,46±2,25 años). La presencia de indicadores de fragilidad podría vincular la susceptibilidad y exposición a enfermedades con la interrupción del desarrollo puberal.

Conclusiones: Esta investigación es el primer estudio sobre el desarrollo de la pubertad en restos óseos en América del Sur, siendo uno de los estudios pioneros en la aplicación de métodos para estimar desarrollo puberal en individuos de edad cronológica y sexo biológico documentados. Este estudio mejora nuestra comprensión bioarqueológica de la pubertad y la adolescencia desde una perspectiva individual y poblacional.

Palabras clave: retraso puberal, estrés del desarrollo, adolescencia, bioarqueología

5.1 Introduction

As the transition from childhood to adulthood, puberty is a key stage in the life course. Both a socio-cultural and biological process, this period is marked by the adolescent growth spurt, the emergence of secondary sex characteristics, somatic changes in body composition, and profound psychological changes (Rogol, Roemmich and Clark, 2002). Puberty affects the entire individual, triggering morphological changes in size and shape, and physical composition and function, that will transform the non-adult body into a sexually dimorphic adult one (Gluckman and Hanson, 2006). Although a normal part of growth and development, each individual's experience of puberty is unique; the sequence of pubertal changes is fairly predictable, but its timing is variable and responds to a multitude of life history factors (Farello *et al.*, 2019).

Onset of puberty and pubertal growth can be traced through growth velocity curves, measured in centimetres per year, plotted against age. During childhood and in pre-pubertal stages, no significant differences are found in growth velocity curves between boys and girls in any one population (Rogol, Roemmich and Clark, 2002). This changes around the end of the first decade of life and throughout the beginning of the second, when growth curves in girls rise before those in boys, indicating the start of the adolescent growth spurt (Gerver and de Bruin, 2003). Linear growth accelerates until reaching "peak high velocity" (PHV), after which growth decelerates while girls experience menarche and boys acquire their adult voice. In the same population, PHV usually starts earlier in girls, with boys experiencing it, on average, two years after their female counterparts (Tanner, 1953; Marshall and Tanner, 1986; Granados, Gebremariam and Lee, 2015; Pereira *et al.*, 2019). Growth eventually stops with the epiphyseal fusion of the long bones and the final changes of pelvic morphological maturation, indicating end of puberty and attainment of an adult body size and shape (Marshall and Tanner, 1986).

Pubertal development has been recognised as an indicator of overall health status during childhood (Hoyt *et al.*, 2020). Several factors have been associated to variation in pubertal timing, including nutritional and metabolic conditions (Soliman, De Sanctis and Elalaily, 2014; Soliman *et al.*, 2014; Duan *et al.*, 2021), chronic diseases (Lewis, Shapland and Watts, 2016b), genetic influences (Fernández *et al.*, 2019), endocrine disruptions (Mouritsen *et al.*, 2010; Parent *et al.*, 2016), and socio-cultural factors such as low socioeconomic status (SES), migration, parental education, and urban/rural environments (Muzzo *et al.*, 1988; van Jaarsveld

et al., 2007; Walvoord, 2010; Canelón and Boland, 2020). Variation in the timing of menarche, as a signal for the onset of fertility and reproductive ability, is also an important clinical threshold to measure a girl's physical maturation, nutritional status, developmental progress, and reproductive health (Sommer, 2013). In addition, early or delayed pubertal timing might be linked to adverse health outcomes in adulthood (Hoyt *et al.*, 2020), which makes studying the biological changes associated with this period in the life course in past populations an important area of research.

Clinical studies show that the pubertal growth spurt seems to set in motion some paradoxical biological processes: while it causes a brisk acceleration of the prepubertal growth rate, leading to an increase of 15-18% of adult height, it also triggers epiphyseal fusion and therefore, cessation of growth (Tanner and Whitehouse, 1976). The seemingly paradoxical progression of biological growth is in fact what allows bioarchaeology to study puberty, as epiphyseal fusion and morphological skeletal changes leading to the end of growth are possible to analyse in skeletal human remains. An increasingly important area in bioarchaeological research, methods for estimating pubertal timing from skeletal remains were introduced by Shapland and Lewis (2013, 2014). Since then, several bioarchaeological studies have explored pubertal timing estimations using skeletal populations from different time periods, however all have focused on European or North American populations (Shapland, Lewis and Watts, 2015; Arthur, Gowland and Redfern, 2016; Lewis, Shapland and Watts, 2016a; Fisk *et al.*, 2017; Henderson and Padez, 2017; Doe *et al.*, 2019; Goncharuk-Khomyn *et al.*, 2020; Blom *et al.*, 2021; Wright, 2021; Avery *et al.*, 2023). Of these, only three studies have used documented biographical information instead of estimations of skeletal age (SA), with Henderson and Padez (2017) being the only ones using osteological remains as sample, in contrast to panoramic x-rays (Goncharuk-Khomyn *et al.*, 2020) and CT scans (Wright, 2021).

This study examined pubertal timing in non-adult and young adult individuals of documented antemortem data (chronological age (CA) and biological sex) from a low SES population that lived during mid-20th century in Santiago, Chile. A major advantage of this study is the documented biological profile of each individual (also including year and cause of death when available), which allows two main topics to be explored: the bioarchaeological approach to timing of puberty and the association of pubertal timing with health experience and frailty. The first issue tackles two related problems: how estimations of pubertal timing are

affected by using documented CA or estimations of skeletal age (SA), and how pubertal timing for this sample compares to clinical data for the same population and time period. The second issue tackles the problem of how skeletally-visible markers of childhood frailty and clinically recorded causes of death relate to pubertal development, especially regarding individuals for whom the relationship between CA and pubertal stage suggests developmental delay.

5.2 Materials and Methods

5.2.1 Skeletal sample

A skeletal sample of 85 individuals derived from the COSS with documented age-at-death who died between 1960 and 1986 between the ages of 8 and 21 years of age was analysed. Upper and lower chronological ages included in the sample were defined following Lewis (2022) proposed biological ages of adolescence (Table 1.2); the lower boundary was extended from 10 to 8 years old to investigate a potential early onset of puberty, while the upper boundary was defined as 21 instead of 24 years of age due availability of skeletal remains of this ages. Skeletal age estimations retrieved from the UChile database were used to compare against CA, while all other data was recorded by the researcher.

Documented causes of death (CoD) were obtained from cemetery archival records. The data was used to explore whether any relationships could be discerned between pubertal timing, skeletal frailty, and CoD. Individual's CoD, when available, was categorised as either accidental/misadventure (e.g., traumatic brain injury, physical asphyxia, gunshot wounds, sharp and blunt force trauma), acute condition (e.g., scarlet fever, peritonitis, hepatitis), or chronic condition (e.g., cancer, tuberculosis, malnutrition, cachexia).

5.2.2 Clinical data

Clinical literature for the period was used to contextualise and compare findings from this study. The available clinical data reflect puberty experience in Santiago de Chile among living children of the time (Table 5.1). These studies focused on biological thresholds such as development of secondary sex characteristics and changes in anthropometric measurements, while also documenting the impact of environmental factors on the growth and development

Table 5.1 Clinical literature used in this study.

Data recorded	Location	Sample size	Sample age*	Dates	Source
Age at menarche	Chile	4600 (981 in Santiago)	Adult	NR	Díaz, 1886
Age at menarche, ethnicity	Santiago	354	10 – 17	1970	Rona and Pereira, 1974
Pubertal development, anthropometric measurements	Santiago	2046	6 – 20	1971-1974	Valenzuela and Avendaño, 1979
Age at menarche	Santiago	6337	Schoolgirls	1963-1973	Patri *et al.*, 1980
Pubertal development, anthropometric measurements	Santiago	225	6 – 20	1965	Avendaño and Valenzuela, 1988
Female pubertal development	Santiago	2328	8 – 15	1982-1984	Burrows et al., 1988
Pubertal development, adult height prediction	Santiago		6 – 20		Canals *et al.*, 1988
Male pubertal development	Santiago	2535	8 – 16	NR	Muzzo *et al.*, 1988
Age at menarche	Santiago	425	Adult	2002	Ponce and Risco, 2003
Female pubertal development, age at menarche, body mass index, SES	Santiago	758	5.8 – 16.1	2000-2003	Codner *et al.*, 2004
Age at menarche, body mass index, SES	Santiago	1302	7 – 19	NR	Hernández *et al.*, 2007
Age at menarche, nutritional status, ethnicity	South of Chile	8504	9 – 16	NR	Amigo *et al.*, 2010
Male pubertal development	Santiago	319	Schoolboys	NR	Gaete *et al.*, 2015
Age at menarche, psychosocial behaviour	Santiago	1944	12 – 19	2005-2010	Leal *et al.*, 2015
Early pubertal development, ethnicity, body mass index, SES	Chile	1003	4 – 20's	2006-2019	Fernández *et al.*, 2019
Female pubertal development, age at menarche	Chile	549	6 – 20's	2006-2019	Pereira *et al.*, 2019

* Sample ages in years; NR: not reported.

of the individual (Canals *et al.*, 1988; Muzzo, 2007; Leal *et al.*, 2015; Fernández *et al.*, 2019). In addition, the first studies specifically on menarche were undertaken at end of the 19th century (Díaz, 1886), with later reports focusing on secular trends and comparative studies between SES, body mass index and ethnicity, among other factors (Rona and Pereira, 1974; Valenzuela and Avendaño, 1979; Gaete *et al.*, 2002; Ponce and Risco, 2003; Gaete and Codner, 2006; Hernández *et al.*, 2007; Amigo *et al.*, 2010).

5.2.3 Pubertal stage estimation

Skeletal and dental markers of pubertal onset and development were assessed using the method proposed by Shapland and Lewis (2013, 2014). Mandibular canine root calcification, cervical vertebrae maturation (CVM), epiphyseal fusion of the elbow, wrist and bones of the hand, and maturation of the iliac crest were assessed using the "Osteological assessment of pubertal stages" visual chart developed by the researcher (Figure 2.9). When paired elements were present, both sides were scored, with the more advanced stage recorded in cases of asymmetrical development (Arthur, Gowland and Redfern, 2016). Pubertal stage was assigned to each individual only if three or more features from different anatomical areas produced consistent results. Following the analysis and recording of pubertal indicators, each individual was assigned to one of the six pubertal stages outlined by Lewis, Shapland and Watts (2016a): initiation (pre-puberty), acceleration, transition (PHV), deceleration, maturation, and end of growth spurt (completion) (Figure 5.1). Following previous studies, menarche was estimated to occur during the deceleration stage of pubertal growth, as it is correlated with commencement of fusion of the distal phalangeal epiphysis and the final stages of iliac crest ossification (Shapland and Lewis, 2013).

Osteological marker	Pubertal stage					
	Initiation	Acceleration	Transition	Deceleration	Maturation	End of growth spurt
Mandibular canine mineralisation	Stage F	Stage G	Stage H			
Hamate hook development	Stages G - H	Stage H.5		Stage I		
Fusion of hand phalanges and MC epiphyses	Unfused		Fusing		Complete	
Shape of hand phalanges epiphyses		Equal width	Capping		Fusion	
Ossification of iliac crest			Ossifying	Ossified		
Fusion of iliac crest to the os coxae	Unfused			Fusing		Complete
Cervical vertebral body maturation (CVM)	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Stage 6
Fusion of ulnar proximal epiphysis	Unfused		Fusing	Complete		
Fusion of humeral capitulum	Unfused		Fusing		Complete	
Fusion of radial distal epiphysis				Unfused	Fusing	

PHV Menarche

Figure 5.1 Skeletal markers recorded for this study to assess pubertal timing, based on Shapland and Lewis (2013, 2014), and the associated pubertal stages according to their stage of development, as outlined by Lewis, Shapland and Watts (2016). Mandibular canine calcification uses Demirjian, Goldstein and Tanner (1973) stages, while hamate hook development and CVM can be found in Shapland and Lewis (2013, 2014). Complete fusion of radial distal epiphysis occurs post puberty (not shown).

5.2.4 Skeletal markers of frailty

In addition to estimating pubertal timing, physiological stress during early childhood and adolescence was also explored using skeletal evidence. For this, frailty in this study was explored as a sign of increased mortality risk, in line with previous studies in bioarchaeology (DeWitte, 2014b), while also including the notion of cumulative physiological assault, the consequential reduced resistance to stressors and switch in body systems to procure survival over growth. In line with this definition, Marklein, Leahy and Crews (2016) proposed a method to assess frailty, the Skeletal Frailty Index (SFI), selecting a set of biomarkers to reflect clinical indicators of frailty such as growth disruption, nutrition and infection, physical activity, and trauma. The benefit of this approach is that it includes as much information about physiological stress experience as possible, covering different aetiologies together with the individual's personal lifeways.

To record frailty, this study used a modified approach to the SFI (Marklein and Crews, 2017). Macroscopic analysis of pathological changes was conducted for all individuals, recording presence/absence of six skeletal stress markers as indicatives of a wide range of physiological stress aetiologies: cribra orbitalia (CO), linear enamel hypoplasia (LEH), periosteal new bone formation (PNBF), porotic hyperostosis (PH), antemortem fractures (FRA), and dental disease (DEN) (Table 5.2). Alongside traditional markers of physiological stress such as CO, LEH, PNBF and PH, physical activity and trauma was explored through healed or healing antemortem fractures of different aetiologies (e.g., projectile injuries, puncture wounds, and surgical procedures such as trepanations) (FRA), and oral health was inferred from dental diseases such as dental caries, antemortem tooth loss, and periodontal disease (DEN). As nonspecific indicators of systemic stress, these skeletal markers are likely to have different aetiologies and relate to stress episodes experienced at varied periods of these individuals' life course. Thus, they were selected to serve as proxies for an increased susceptibility to morbidity and mortality, as well as state of cumulative stress.

Presence/absence of each skeletal stress marker was recorded, with the sum of the presences then divided by the number of observations made for each individual to create a numerical SFI. Individuals were then assigned as frail or non-frail depending on their SFI, with all equal or greater than 0.5 grouped as frail.

Table 5.2 Skeletal stress markers recorded for the sample and their possible aetiologies.

Skeletal change	Abb.	Possible aetiologies
Cribra orbitalia	CO	Traditionally associated with anaemia (including iron deficiency, megaloblastic, and haemolytic anaemias), but has also been linked to localised inflammation and rickets (Roberts and Manchester, 2010).
Linear enamel hypoplasia	LEH	Well-known indicator of systemic growth disturbances during childhood (King, Humphrey and Hillson, 2005). Its presence is not consistent with increased mortality or probability of survival but informs on physiological stress at an early age (O'Donnell and Moes, 2021).
Dental diseases	DEN	Dental caries, antemortem tooth loss (ATML) and periodontal disease have different aetiologies (Hillson, 2014). While dental caries is commonly associated with a high proportion of sugar in the diet, ATML is linked to dental and periodontal disease, trauma, high rate of attrition, among others (Temple, 2015; Larsen, 2018).
Antemortem fractures	FRA	Usually tied to interpersonal violence and conflict (Lewis, 2014; Ubelaker and Montaperto, 2014) but can also be caused by accident-related events (Lovell, 1997). The premise for inclusion relies on the idea that physical trauma can elicit an equivalent or potentially greater physiological stress response compared to psychosocial stressors (Sterling, 2004).
Periosteal new bone formation	PNBF	Traditionally used as an indicator of nonspecific infections, although it has been suggested it also indicates inflammatory processes associated with trauma or metabolic disease (vitamin C deficiency, scurvy, infantile cortical hyperostosis) in non-adults (Ortner, 2003; Lewis, 2017).
Porotic hyperostosis	PH	As other porous lesions such as CO, it is traditionally linked to iron deficiency anaemia (Brickley, 2018).

5.2.5 Statistical analyses

To compare mean CA versus mean SA of each pubertal stage, effect size was calculated using Hedges' g, as this statistical test accounts for small sample sizes (Cohen, 1988). As variables are not normally distributed, the test used an approximation of differences to explore sex-related associations and physiological stress impact for each pubertal stage. For a more intuitive interpretation of the results, common language effect size (CLES) was also reported, giving a representative percentage of each Hedges' g calculated value (Lakens, 2013). The commonly used interpretation for effect size is categorise it as small (g = 0.2), medium (g = 0.5), and large (g = 0.8), but these benchmarks have been criticised for their arbitrary designation (Thompson, 2007). To more effectively interpret the magnitude of differences observed, this study reported all effect sizes in full,

even the statistically nonsignificant ones, but explored in more depth results with g > 0.60 (CLES > 65%). All statistical analyses were carried out using RStudio 2021.09.0 Build 351.

To explore pubertal timing, frailty and CoD, line charts were constructed to compare trend over pubertal time for skeletal frailty (segregating by frail (SFI ≥ 0.5) and non-frail (SFI < 0.4) individuals) and CoD category (accidental/misadventure, chronic condition, and acute condition).

5.3 Results

5.3.1 Timing of puberty

Of the 85 individuals between 8 to 21 years of documented CA analysed, a combined pubertal score could not be determined for five (6%) cases, all of which had fewer than three scorable skeletal areas to assess due to limited completeness, fragmentation, or poor preservation of the remains. The remaining 80 individuals had three or more skeletal elements present to estimate pubertal development; no discrepancies were found between skeletal elements for pubertal estimation in any of these individuals. All pubertal timing data were recorded on two separate occasions by the researcher and intraobserver error calculated. This showed no significant differences at the 95% confidence level between the first and second recording of each feature (Wilcoxon signed-rank test = 0.17).

The pubertal stage for individuals divided into one-year CA cohorts is presented in Figure 5.2. Chronological and skeletal mean ages, with their respective standard deviations, were calculated for each pubertal stage for the complete sample (Table 5.3a) and for female and male individuals (Table 5.3b).

As bioarchaeological research often relies only on SA (as CA is unknown in the majority of archaeological populations), CA and SA mean ages for each pubertal stage were compared to test how the use of SA could impact the accuracy of assessment of pubertal timing. Thus, effect sizes (Hedges' g) of the discrepancy between CA and SA in female and male individuals were calculated (Table 5.4). A large effect size difference (>0.60), suggesting a significant discrepancy, was found in female individuals assigned to the first two pubertal stages: initiation and acceleration stages.

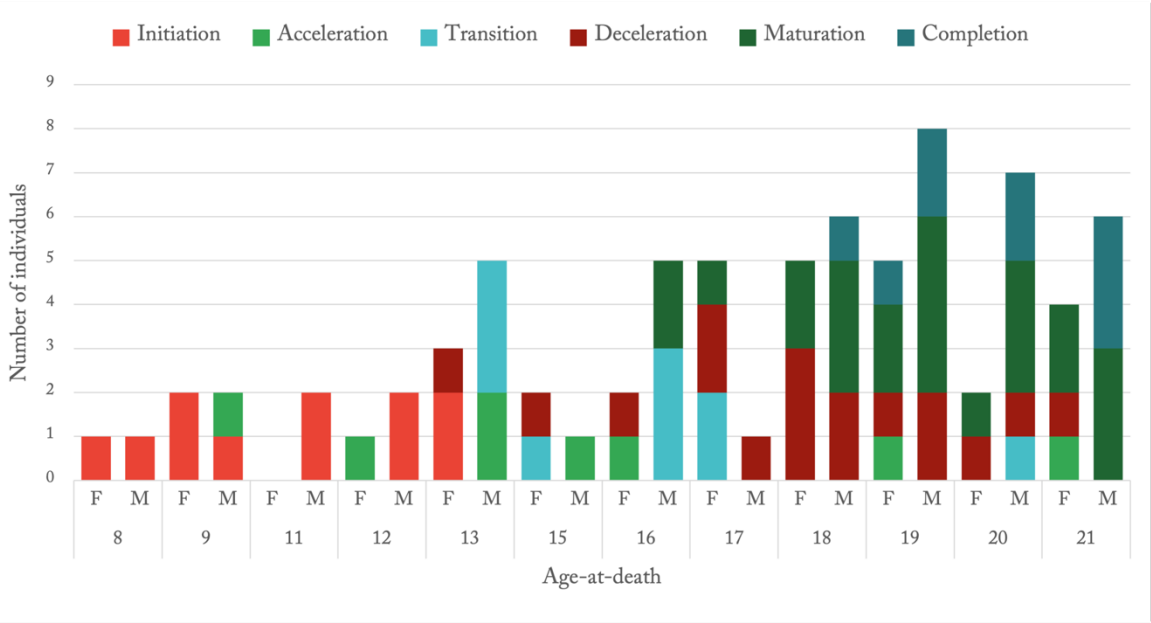

Figure 5.2 Distribution of individuals in each pubertal stage by documented sex (F: females, M: males) and age-at-death.

Table 5.3a Descriptive statistics for chronological and skeletal ages per pubertal stages for the overall sample.

		Chronological age		Skeletal age	
	n	MA	SD	MA	SD
Initiation	11	10.46	1.92	9.86	1.98
Acceleration	8	14.75	3.88	12.88	1.71
Transition	10	15.60	2.22	15.85	2.36
Deceleration	18	17.72	1.93	17.31	1.86
Maturation	23	19.04	1.52	20.13	2.74
End of GS	10	19.90	1.10	20.85	1.72

n: sample size; MA: mean age; SD: standard deviation.

Table 5.3b Descriptive statistics for chronological and skeletal ages per pubertal stages segregated by females and males.

	Females					Males				
		CA		SA			CA		SA	
	n	MA	SD	MA	SD	*n*	MA	SD	MA	SD
Initiation	5	10.40	2.40	9.10	1.25	6	10.50	1.64	10.50	2.35
Acceleration	4	17.00	3.91	12.88	2.40	4	12.50	2.51	12.88	1.03
Transition	3	16.33	1.16	16.33	4.04	7	15.29	2.56	15.64	1.65
Deceleration	11	17.46	2.25	17.09	2.12	7	18.14	1.35	17.64	1.46
Maturation	8	19.13	1.46	19.88	2.45	15	19.00	1.60	20.28	2.96
End of GS	2	20.00	1.41	20.00	1.41	8	19.88	1.13	19.88	1.27

CA: chronological age; SA: skeletal age; n: sample size; MA: mean age; SD: standard deviation.

Table 5.4 Effect size difference between documented chronological age and skeletal age estimations.

	Effect size Females (CA v. SA)			Effect size Males (CA v. SA)		
	g	CI	CLES	g	CI	CLES
Initiation	0.61	-0.66 – 1.88	66.69	0.00	-1.13 – 1.13	50.00
Acceleration	1.10	-0.38 – 2.59	78.17	-0.17	-1.56 – 1.22	45.22
Transition	0.00	-1.60 – 1.60	50.00	-0.15	-1.20 – 0.90	45.78
Deceleration	0.16	-0.67 – 1.00	54.50	0.33	-0.72 – 1.39	59.23
Maturation	-0.35	-1.34 – 0.64	40.23	-0.52	-1.25 – 0.20	35.66
End of GS	0.00	-1.96 – 1.96	50.00	0.00	-0.98 – 0.98	50.00

g: Hedges' g; CI: confidence interval (95%); CLES: common language effect size (in %). Highlighted are Hedges' g > 0.60 (CLES > 65%).

Means for both CA and SA for females and males in each pubertal stage generally displayed the expected pattern of progression in pubertal development with increasing age, with some variation between sexes and stages (Figure 5.3). Mean CA and SA for the next stages from transition to end of the growth spurt show similar ages for females and males. However, females in the acceleration stage show a drastic difference between their chronological and estimated ages: 17.00±3.91 and 12.88±2.40 years of age, respectively. In addition, mean CA for females in this stage (17.00±3.91 years) was higher than the mean age in the subsequent transition stage (16.33±1.16 years), which does not reflect the expected pattern of pubertal development. Both of these issues might be an unexpected consequence of the sample size for each pubertal stage and/or the fact that, as highly frail individuals, the females that were categorised in the acceleration stage were significantly older than expected for clinical data.

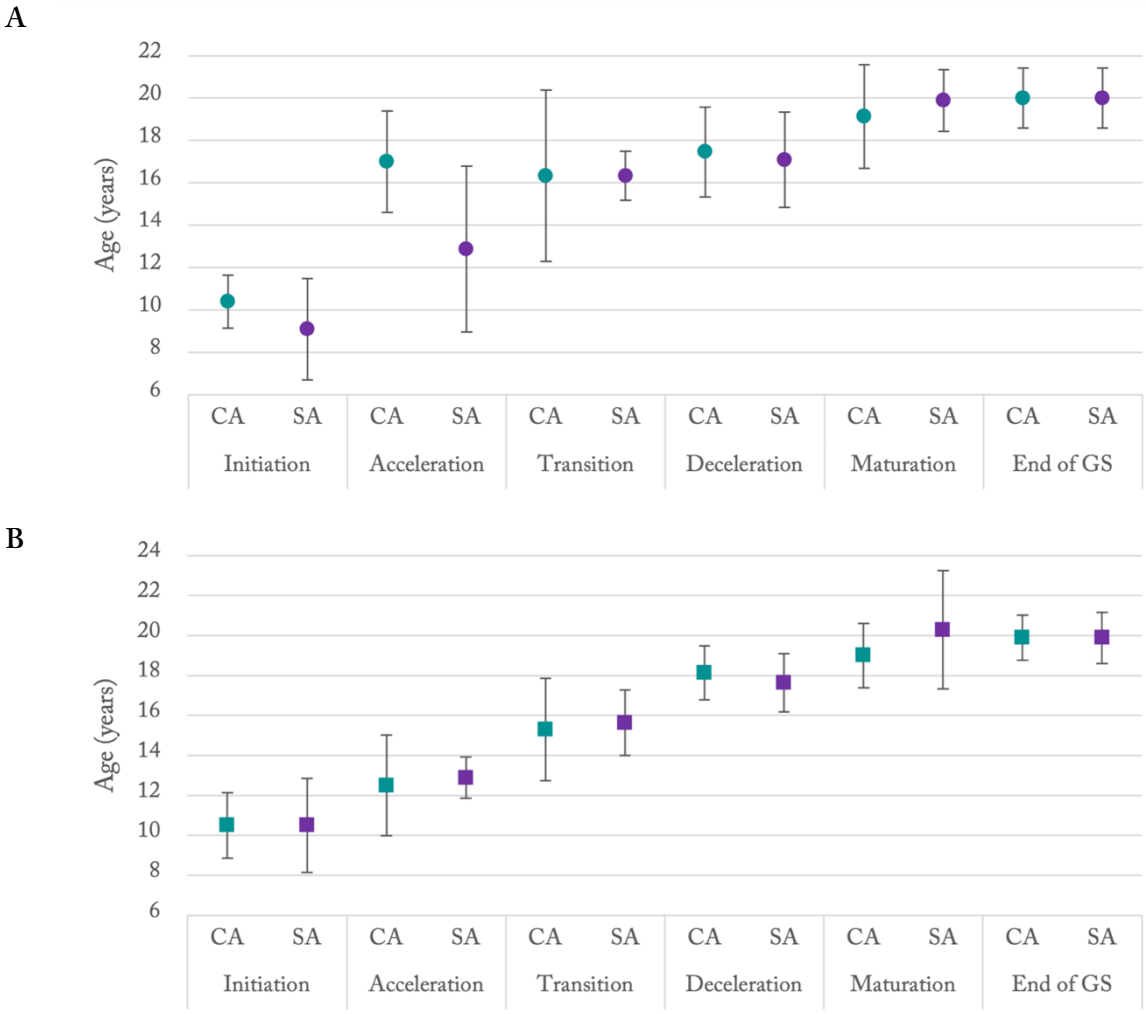

Figure 5.3 Female (A) and male (B) mean ages and standard deviations for each pubertal stage using chronological age (CA) and skeletal age (SA).

The deceleration stage of pubertal development in females is associated with menarche. Chronological age-at-death of female individuals assigned to the deceleration stage was plotted against recorded age of menarche for this population from the period between 1886 and 2017 (Figure 5.4) (Díaz, 1886; Rona and Pereira, 1974; Valenzuela and Avendaño, 1979; Patri *et al.*, 1980; Avendaño and Valenzuela, 1988; Ponce and Risco, 2003; Codner *et al.*, 2004; Gaete and Codner, 2006; Hernández *et al.*, 2007; Amigo *et al.*, 2010; Pereira *et al.*, 2019). Females in this stage presented similar mean CA and SA (17.46±2.25 and 17.09±2.12 years of age, respectively), but both were higher than age at menarche expected for the period. All but one female individual (B0192) assigned to the deceleration stage showed a significant delay; four cases (B0254, B0759, B1049 and B1566) experienced

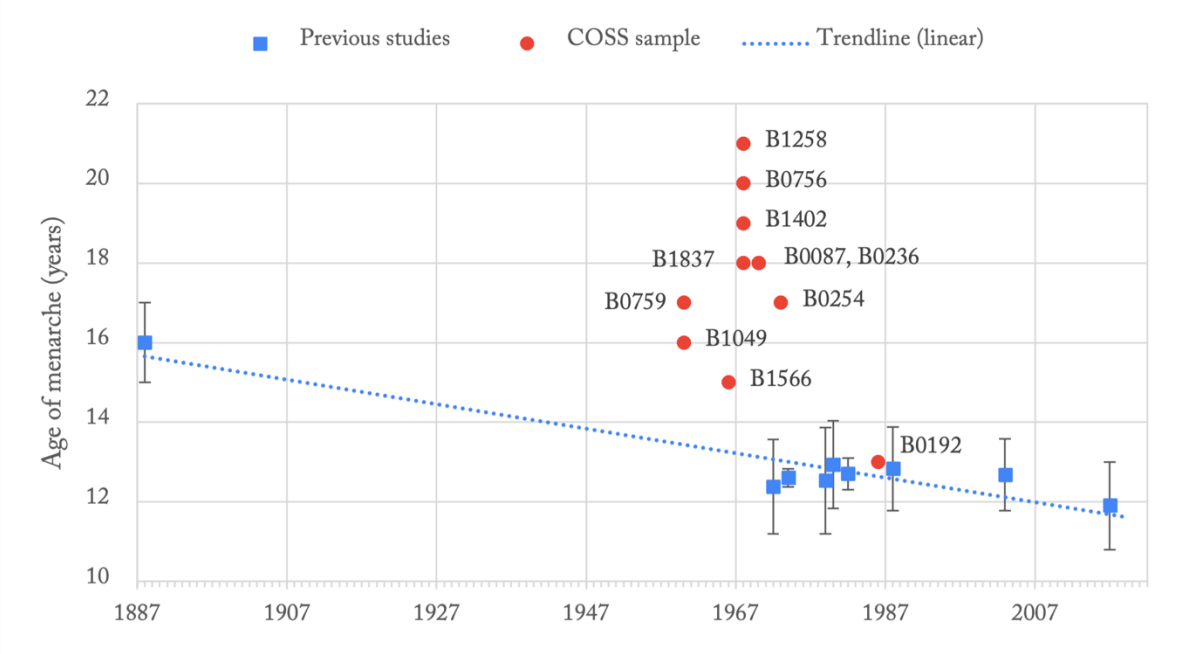

Figure 5.4 Age of menarche recorded in previous studies for Chilean girls from Santiago between 1887 and 2009 (blue squares, with linear trend in dotted blue line) and documented CA for female individuals in the deceleration stage from this sample (red dots).

menarche at an age expected for low SES population of Santiago during the 1880's (Díaz, 1886), with the remaining five cases showing a delay even when compared to girls that died a hundred years prior.

5.3.2 Pubertal timing and frailty

To explore the relationship between pubertal timing and skeletal frailty, presence/absence of skeletal markers of physiological stress were analysed through a modified SFI. Two approaches were calculated using SFI: visualising crude SFI differences between males and females within each pubertal stage, and segregating individuals between frail (SFI ≥ 0.5) and non-frail (SFI < 0.4) and then comparing the mean CA for each pubertal stage. To explore the first approach, separated charts for each pubertal stage showing CA versus SFI trend lines were generated for males and females of the sample (Figure 5.5). Assuming that if skeletal frailty was correlated with pubertal delay, CA would correlate positively with SFI within each stage; however, no clear trend was observed in any of the pubertal stages or between sexes. Thus, the second approach was tested, and line graphs were generated

illustrating mean CA and standard deviation of frail and non-frail individuals in each pubertal stage segregated by females and males (Figure 5.6). In males, this approach shows a general pattern of correlation between frail and non-frail mean CA across pubertal stages, suggesting frailty is not related to pubertal growth disruption within males. However, females in the acceleration stage show a visible difference when categorised as frail or non-frail. Frail females in the acceleration stage show a significant delay when compared to non-frail females, and all males, suggesting frailty delays early pubertal growth development.

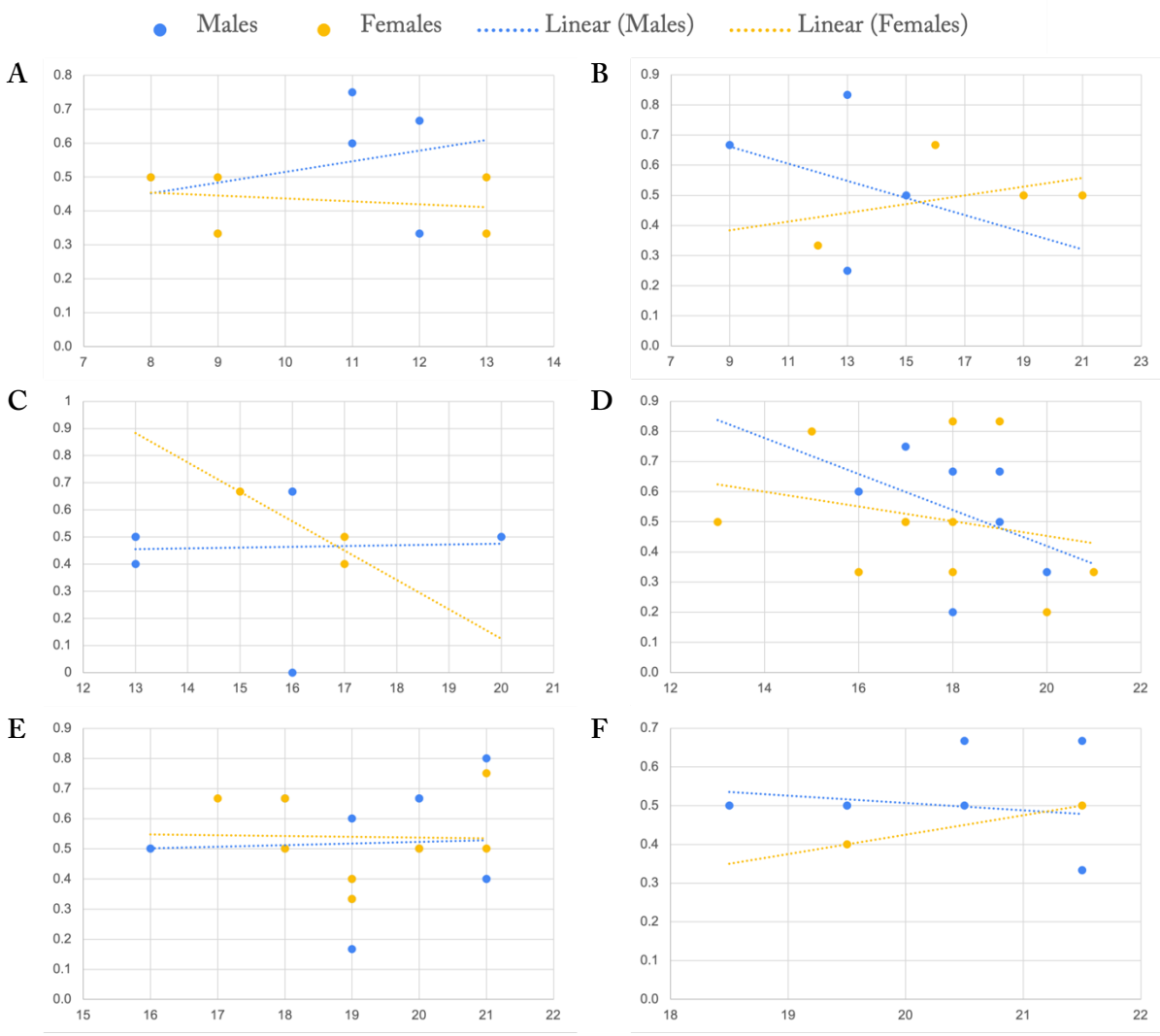

Figure 5.5 Chronological ages (x axis) across pubertal stages using crude SFI (y axis) segregated by female and male individuals in each pubertal stage: initiation (A), acceleration (B), transition (C), deceleration (D), maturation (E), and end of growth spurt (F).

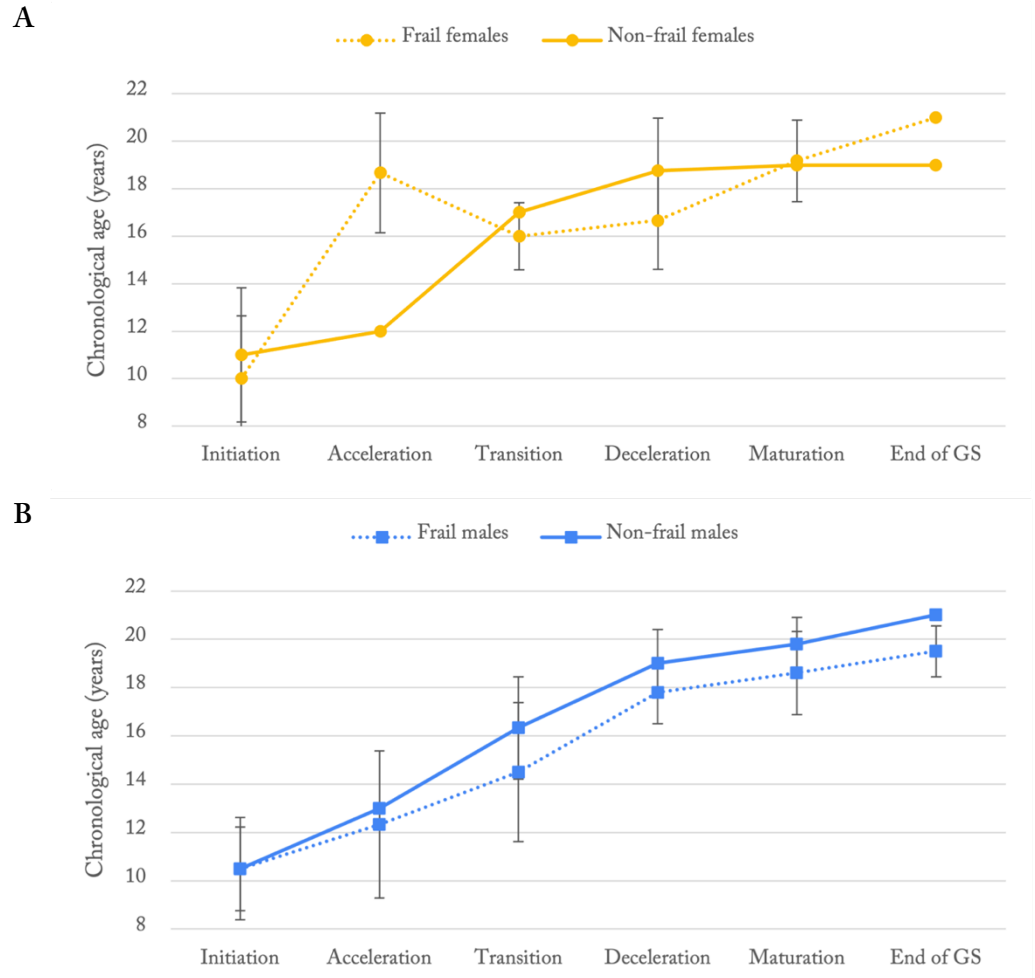

Figure 5.6 Chronological age (mean and standard deviations) across pubertal stages for frail (SFI ≥ 0.5) and non-frail (SFI < 0.4) female (A) and male (B) individuals.

The impact of different types of CoD on pubertal timing was also explored. CoD was available for 72.5% individuals (n = 58) and categorised into accidental/misadventure, acute conditions, and chronic conditions to account for the fact that different CoD, as a reflection of health experience during life, might indicate differences in the pubertal timing that skeletal frailty alone cannot show. Mean CA and standard deviations were generated for each biological sex and pubertal stage for the pooled sample (Figure 5.7) segregated by CoD group (Figure 5.8). When both sexes are analysed together, the pattern shows a slightly elevated delay during the acceleration stage if the individual CoD was listed as a chronic condition. When segregating by sex, the data shows that different CoD in male individuals is not impacting pubertal timing, while females that died of chronic conditions show a delay in the acceleration and transition stages leading to PHV; thus, differences observed when

pooling together males and females is an artefact of the greater delay observed in females when pooling together sexes in small sample sizes.

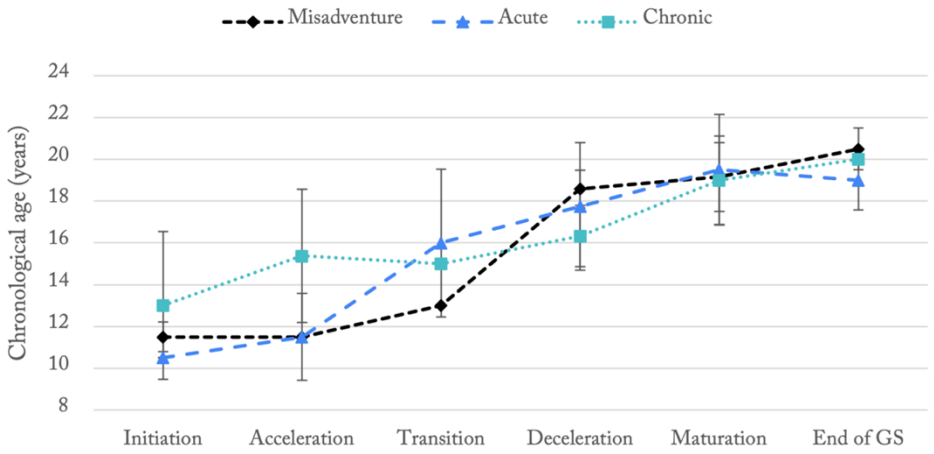

Figure 5.7 Chronological age (mean and standard deviations) across pubertal stages when segregated by cause of death (CoD).

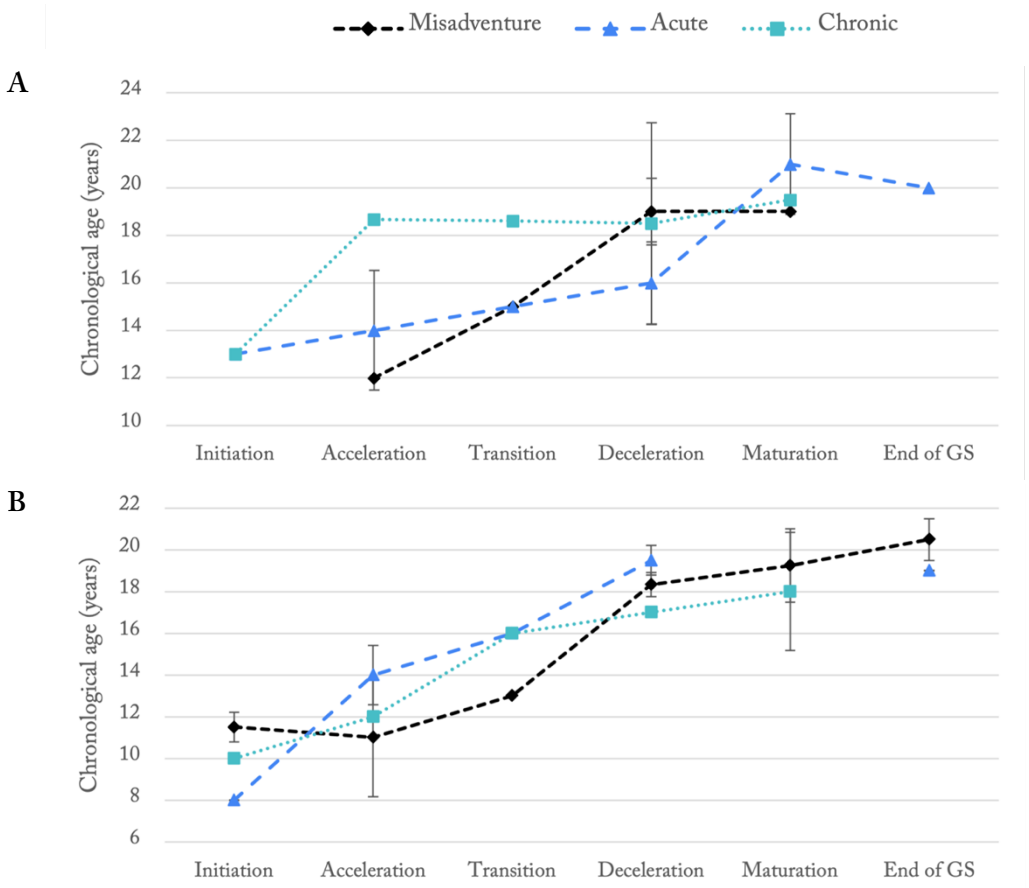

Figure 5.8 Chronological age (mean and standard deviations) across pubertal stages by cause of death (CoD) when segregated by females (A) and males (B).

5.4 Discussion

As bioarchaeologists do not often have access to biographical information such as biological sex, chronological age and cause of death, the study of osteological pubertal timing and its relationship with health has relied on comparing skeletal age with pubertal stage and pubertal delay with skeletal markers of frailty. However, estimates of pubertal timing rely on developmental stages of the dentition and skeleton that can also be used in age estimation. Although Shapland and Lewis (2013, 2014) used different developmental features from diverse anatomical areas, comparing those they suggest are more closely tied to puberty than age with those more closely tied with age than puberty, an underlying issue remains: all skeletal developmental markers are affected to some extent by both chronological age (growth) and hormonal development. In addition, modern clinical studies suggest general poor health during childhood probably leads to delayed growth and slower progression through puberty, while also following secular trends that change between time periods and geographical locations (Mouritsen et al., 2010; Parent et al., 2016; Duan et al., 2021). In line with the osteological paradox (DeWitte and Stojanowski, 2015), bioarchaeology operates under the assumption that individuals studied are likely the least healthy among their population, thus we might expect that the means for age at each pubertal stage from skeletal assemblages is likely to be biased to younger ages, which can in term bias conclusions made from osteological pubertal timing.

This study offers the rare opportunity to compare skeletal development related to puberty with documented antemortem data of biological sex, chronological age, and cause of death, while also evaluating pubertal timing estimation through skeletal markers when pathological changes are present.

5.4.1 Timing of puberty

When assessing osteological pubertal timing, two issues arise: differences between chronological (CA) and estimated skeletal age (SA) per pubertal stage, and differences between CA and contemporary clinical records. By exploring the first, this study has evaluated how the use of bioarchaeological age estimations might affect the accuracy of conclusions regarding pubertal timing in the past. As data were available for both CA and

calendrical year of death, this study has also been able to compare patterns of pubertal timing to geographically and temporally appropriate clinical records for age-appropriate cohorts to evaluate how osteological pubertal markers predict age of pubertal initiation and menarche among females.

Chronological versus skeletal age.

When comparing mean ages for CA and SA in each of pubertal stages, there is general agreement between both ages with the exception of two cases. Significant differences between CA and SA were observed for female individuals in two pubertal stages: initiation (g = 0.61, 95% CI = [-0.66, 1.88], CLES = 66.69%; CA 10.40±2.40 vs. SA 9.10±1.25 years of age) and acceleration (g = 1.10, 95% CI = [-0.38, 2.59], CLES = 78.17%; CA 17.00±3.91 vs. SA 12.88±2.40 years of age).

Clinical data gathered by Burrows et al. (1988) reported schoolgirls from low SES from Santiago were experiencing pubertal onset at 10.56±1.08 years of age. The mean CA at initiation stage reported among girls in the present study matches that from the clinical reports, whereas mean SA at initiation would have erroneously identified evidence of slight pubertal delay. Even though they were assessed as skeletally younger, these female individuals were chronologically older than their SA estimation. On the other hand, clinical studies indicate that males are expected to enter puberty after females, with progression through pubertal development differing a couple of years between sexes (Tanner, 1953; Tanner and Whitehouse, 1976). Muzzo *et al.* (1988) stated that middle and low SES schoolboys were experiencing the start of puberty at 11.4±1.7 years of age. Mean CA and SA for males in this stage (10.50±1.64 and 10.50±2.35 years of age, respectively) are slightly younger, but not statistically different, than that expected from clinical data.

These findings suggest that Shapland and Lewis' (2013, 2014) method for detecting pubertal timing in bioarchaeological remains using SA is accurate for all pubertal stages in males and most pubertal stages for females when compared to documented CA in this sample. However, the results also revealed females in the initiation and acceleration stages for whom SA and pubertal stage were out of line with CA. If assessed based on SA without knowledge of CA, this group would be considered to follow a normal pubertal progression,

but in light of CA it is clear that the individuals are in fact experiencing significant pubertal delay. Although this particular finding is highly specific to this population, the fact that SA and CA might not be in agreement in all individuals is a limitation that should be considered when assessing pubertal timing through skeletal markers, as it likely reflects an underlying relationship between skeletal markers of age-at-death and pubertal timing.

Bioarchaeological pubertal timing and clinical data.

Availability of clinical data for the same population and time period represented by the skeletal remains allows this study to compare pubertal timing based on skeletal methods with clinically-observed stages of pubertal expression. As clinical studies examine the attainment of two specific thresholds – onset of puberty and age at menarche – the two closest corresponding skeletal pubertal stages are examined: initiation and deceleration.

When comparing mean CA for females in the initiation stage to clinical data for schoolgirls (Burrows *et al.*, 1988), the data shows that onset of puberty in this sample was in agreement with that of the period. However, taking into consideration that CA is not often known in archaeological settings, the comparison between mean SA to clinical data should be done to simulate the common bioarchaeological scenario; this shows that mean SA at initiation would have erroneously identified evidence of pubertal delay in females if compared to clinical studies for the same time period and SES group. Conversely, in males both mean CA (10.50±1.64 years of age) and mean SA (10.50±2.35 years of age) are slightly younger than that expected from clinical data (11.4±1.7 years of age), which might be related to the small sample size or the SES origin of the individuals. Muzzo *et al.* (1988) provided data on the onset of puberty was recorded pooling together middle and low SES schoolboys, while also stating that high SES individuals were experiencing onset of puberty at a slightly older age (11.82±1.25 years of age) than their middle-low SES counterparts. Given that the sample studied here comes from a low SES group, a younger pubertal onset could be expected if clinical data were to separate low from middle SES individuals. However, Gaete and Codner (2006) warn about the limitations of clinical studies assessing pubertal onset in boys, as there is no uniformly recognisable threshold and studies evaluate bodily markers differently, hence the biomedical data presented might not accurately reflect pubertal onset.

Age of menarche was substantially delayed in this sample compared to the expected age derived from clinical data. Overall, female mean CA for the deceleration stage (17.46±2.25 years) presented a marked delay compared to the clinical data for age of menarche, with all but one individual falling outside the standard deviation of the clinical evidence. For this sample, no differences between CA and SA were detected for this stage, which suggests that pubertal delay was associated with frailty in life. A possible explanation of this will be discussed later.

5.4.2 Pubertal timing, frailty, and health experience

Comparing frailty from skeletal remains with clinical literature from the same population and time creates interpretive complications. It can serve to contextualise pathological changes seen in bioarchaeological remains, but the medical data itself reflects living people. However, the unique biographical information available for the COSS means causes of death are known for the majority of the individuals in this sample, allowing investigation of whether, and what combination of, skeletal frailty and CoD might be related to delays in pubertal timing.

One possible explanation for the significant discrepancy between skeletal maturity and chronological age in females in early stages of pubertal development might be that their overall health was impacted by their living conditions during childhood, making them appear skeletally younger and experience onset of puberty at a later time than expected for the period. Physiological stress during early infancy and childhood might affect onset and progression of puberty, although the links between stress and pubertal timing are still a matter of debate (Ellis and Giudice, 2014; Dorn *et al.*, 2019). During the time period of this sample, lower weight, stature, and body fat percentage is reported in girls from Santiago from low SES groups compared to high SES, which Burrows *et al.* (1988) attributes to differences in genetic and environmental factors. In contrast, no discrepancies in SA, CA and pubertal timing were seen among males from the same stages, although they most likely shared similar living conditions with the females in the sample. In fact, mean CA for the onset of puberty in males was found to be slightly younger than that clinical studies report (Muzzo *et al.*, 1988), although this is not considered overly precocious pubertal development for the time

standards (Gaete and Codner, 2006). In addition, initiation mean CA did not differ between frail and non-frail male individuals.

Given their similar genetic background and living conditions, the question arises why only females were experiencing a delay in skeletal growth and maturation, as evidenced by SA/CA discrepancies. Some evidence suggests that pubertal onset is sensitive to nutritional influence (Engelbregt *et al.*, 2000), but with different timing depending on biological sex: male puberty seems to be more sensitive to nutritional challenges during early stages of development, while female pubertal timing might be more vulnerable to nutritional stress during puberty itself (Sánchez-Garrido *et al.*, 2013). This might explain the fact that female skeletal maturation was delayed, but pubertal onset for the complete sample remained within what was expected for individuals at the time.

As the sample size upon which these observations are made is small, it is valuable to also consider the study sample at individual level. The delay at the acceleration stage for females might be related to their recorded causes of death: gastrointestinal haemorrhage (B0179, 12 years of age, SFI = 0.3 non-frail), stomach cancer (B1343, 16 years of age, SFI = 0.7 frail), left lung liposarcoma (B0755, 19 years of age, SFI = 0.5 frail) and bronchopneumonia due to severe malnutrition (B0212, 21 years of age, SFI = 0.5 frail). Only individual B0179 was found to have experienced the start of the growth spurt at an age in accordance with the pubertal development progression of their contemporaries among the clinical sample; her CoD and SFI also suggest she was not frail in life, or at least that she was not experiencing a chronic condition that might have affected her health enough to show skeletal signs of physiological stress. In the last three cases (individuals B1343, B0755, and B0212), it is possible that chronic diseases would have had a great impact on their overall health, subjecting their bodies to high levels of physiological stress that went on to be visible on the macroscopic analysis of these remains, as shown in their SFI. This is in line with known elevated susceptibility to chronic disease during adolescence, as the increase demand for resources during the pubertal growth spurt triggers trade-off dynamics between the immune system and physiological development (McDade, 2003).

One unanticipated finding was that of the only individual showing delay in the transition stage. Although high prevalence of PH is found in all the individuals in the transition stage, only one male individual (B1827, 20 years of age, cause of death recorded

as tuberculosis) also showed presence of PNBF, a commonly used indicator of a local or systemic nonspecific inflammation due to infection or traumatic injury, that can also be the result of nutritional imbalance (Roberts and Manchester, 2010; DeWitte, 2014a). Although by itself PNBF correlates with active or past physiological stress, in combination with the presence of PH these stress markers might indicate a more limited group of aetiologies. PH is traditionally associated with anaemia, particularly iron deficiency (Buikstra, 2019), but it has also been seen as a response to infectious diseases, such as chronic respiratory infections (Brickley, 2018). Called a 'social disease', tuberculosis incidence differed greatly among social classes, with low SES communities being deeply affected during the mid-20 century (Paluzzi, 2004) and in 1970, tuberculosis was the third leading cause of death in Chile, only surpassed by measles and diarrhoea (Medina and Kaempffer, 2007). Other studies have found such potential links; O'Donnell *et al.*, (2020) found an association between PH and respiratory infections in a modern sample from New Mexico, US, and while studying adolescents from medieval England, Lewis, Shapland and Watts (2016b) found that certain chronic conditions, particularly tuberculosis, were strongly related to delays on the age of PHV.

Previous bioarchaeological studies have stated that menarche occurs during the deceleration stage of puberty (Arthur, Gowland and Redfern, 2016; Lewis, Shapland and Watts, 2016b). As an indicator of a girl's physiological maturation, a normal age of menarche usually clinically correlates with a good general health status during puberty (Canelón and Boland, 2020). From a life history point of view, energy allocation across the life course results in trade-offs between biological processes of reproduction, growth and body homeostasis (i.e., somatic maintenance); early-life adversities would then favour an acceleration in pubertal maturity, particularly an earlier age of menarche, in an effort to ensure the birth of offspring before risk of death increases later in life (Sheppard, Pearce and Sear, 2016). However, this scenario is not seen in this sample; conversely, a delayed age of menarche is observed when compared to biomedical data of the period. Menarche timing alteration has been found to be associated with several environmental factors; early onset menarche has been linked to higher SES, higher parental education, intrauterine stress, low weight at birth, childhood obesity (Muzzo, 2007; Karapanou and Papadimitriou, 2010) and emotional and physical abuse (Klimek et al., 2023), while delay has been associated to poor nutritional status, conditions that reduce total body fat, low SES, migration and rural upbringing (Gluckman and Hanson, 2006). Earlier age of menarche when intrauterine stress

and low weight at birth are present is linked to a subsequent catch-up growth strategy, when childhood metabolism adapts to foetal-infant developmental constraints via insulin resistance and by retaining more visceral adipose tissue, which could lead to childhood obesity (Sheppard, Pearce and Sear, 2016). As higher body fat is linked to higher concentrations of sex hormones, and thus to earlier menarche age, a girl facing early-life stressors but "catching-up" during childhood would experience normal or earlier age of menarche (Klimek *et al.*, 2023). The findings of this study in combination with clinical data for the time showing lower body fat in girls of low SES (Burrows *et al.*, 1988), suggest that age of menarche in this population was delayed because chronic malnutrition might have prevented these individuals from accumulating body fat during the key stage of childhood. Coming from low SES communities in urban Santiago, these individuals experienced diminished living conditions, and endured times of socio-political change and severe economic crisis (Espinoza, 1988), which would support this explanation. And although skeletal evidence can only provide an estimate on when menarche has already occurred (Henderson and Padez, 2017), it serves to shed light on the health conditions these individuals might have experienced in the years prior to puberty.

Finally, Lewis, Shapland and Watts (2016b) suggested that transition (when PHV is attained) and completion stages were found to be heavily impacted by physical health. The results of this study contrast with this observation, as neither of these stages was affected by CA/SA discrepancies or showed disruptions when assessed through SFI or CoD.

Documented cause of death.

As one of the unique insights into pubertal development given by this skeletal collection, recorded CoD offers another lens to analyse the results of this study. Individuals dying in the first half of puberty (before deceleration) from chronic diseases are older for their respective pubertal stage than those without chronic diseases; this finding is more marked in females than males. Studies in modern populations have linked the presence of chronic health conditions to a delay in pubertal timing (Turkel and Pao, 2007; Umławska and Krzyzanowska, 2009; Lewis, Shapland and Watts, 2016b), as onset of puberty relies on the individual having acquired sufficient body mass to progress through this stage (Proos and Gustafsson, 2012). Particularly for females, less favourable conditions (such as malnutrition

and exposure to chronic illness) might delay the onset of puberty and sexual maturation, providing the body with extra time to catch up on the required energetic resources, in an effort to ensure later fertility and longer longevity (Hochberg and Belsky, 2013). It is possible, then, that the first half of skeletally defined pubertal stages might be more sensitive to detrimental environmental factors reflecting an overall delay in the onset of puberty until the body is biologically capable to progress successfully. Once PHV has been attained and the individual is experiencing puberty, chronic conditions were not associated with a pattern of delay as the progression through the later stages would have already begun.

Additionally, documented causes of death for individuals in the maturation and end of growth spurt stages do not provide evidence of chronic conditions but do imply high levels of interpersonal violence among older adolescents during the period; 68% of the individuals in these two pubertal stages suffered violent deaths (e.g., gunshot wounds, traumatic brain injuries, acute anaemia due to violent traumas). It is worth noting that 53% of the violent deaths happened in 1973, year in which a military coup d'état deposed the lawful Chilean government of President Salvador Allende; in the first months after the coup, the armed forces killed thousands of Chilean leftists, real or suspected, or forced their "disappearance", mainly targeting young adults (González, 2015). Although no direct associations can be made, it is however possible to state that older adolescent deaths for this sample were not related with chronic conditions, except in three cases: B0380, a 16 year old male in the maturation stage who died from testicular cancer; B0004, a 20 year old male in the maturation stage with cause of death recorded as “epilepsy”; and B0075, a 21 years of age female in the end of growth spurt stage who died from cachexia.

5.4.3 Methodological considerations

As “non-survivors” (Wood *et al.*, 1992), individuals in bioarchaeological studies are unlikely to be fully representative of their population, which makes the study of pubertal timing from skeletal remains a task with several limitations. The main issues with the study of pubertal development and timing are the small sample sizes of non-adults (and adolescents, by extension) within archaeological populations, complexity of assessing biological sex in immature skeletal remains, and availability of skeletal elements needed for age estimation and pubertal stage assessment (e.g., permanent lower canine and hamate) (Arthur, Gowland

and Redfern, 2016). Many of these limitations were identified and, to some extent, addressed at the initial application of the method (Shapland and Lewis, 2013, 2014; Lewis, Shapland and Watts, 2016b) and by subsequent studies (Arthur, Gowland and Redfern, 2016; Henderson and Padez, 2017; Blom *et al.*, 2021).

This study used individual's antemortem data (documented biological sex, CA, and cause of death) in addition to clinical data for the specific period and population, allowing aspects of the accuracy of the pubertal estimation method to be tested while also exploring the implications of pubertal delay. Despite the plentiful additional evidence that was accessible in the present study, some limitations remain. Direct comparison between clinical studies in living individuals and bioarchaeological skeletal prove challenging, not just because of the differential representativeness of the samples, but also due to the variety of features studied in living individuals to assess puberty, such as height, development of secondary sexual characteristics, and self-reported onset of puberty and menarche age. As Avery *et al.* (2022) note, longitudinal clinical studies usually report "average age-at-entry" for each pubertal stage, while osteological data use a cross-sectional approach, grouping together all the individuals in said stage, producing "average in-stage age". This study benefited from documented ages-at-death, which made it possible to study the sample in two levels: investigating the general trends (that behave as "average in-stage age") and individual experiences (that can be comparable to "average age-at-entry"), combining bioarchaeological data and historical records to better understand this unique period of human growth and development.

5.5 Conclusion

The aim of the present research was to examine pubertal timing and the potential impact physiological stress had on pubertal stages in an identified skeletal sample from urban Santiago, Chile during late-20th century. Being the first to explore pubertal timing in bioarchaeological remains from South America, these results have the potential to integrate with other studies conducted in different geographical areas and for different time periods, to understand progression and variation within pubertal timing in human populations.

The results indicate that this sample was experiencing onset of puberty at a similar age to that reported in living adolescents for the period, while the progression through puberty was highly disrupted and stretched farther, implying the overall duration of puberty was increased. One of the more significant findings to emerge from this study is the large delay in the acceleration towards PHV and subsequently in age of menarche was found in the females from this sample. This indicates that, although starting puberty slightly before than girls for the period, females in this sample experienced a longer pubertal period, with delayed PHV and menarche. This points toward underlying health problems that could be interfering with the biological processes leading to puberty, an assumption supported by epidemiological and historical records for the period and the high prevalence of skeletal stress markers present throughout the sample. Thus, the increase in age for the majority of pubertal stages robustly indicates that disease exposure and susceptibility was increased during puberty in individuals of low SES living in Santiago de Chile between 1960 and 1990.

Although limited, the study of adolescence within bioarchaeology is slowly increasing (Avery *et al.*, 2022). This study contributes to the understanding of a previously not studied key stage in growth and development in bioarchaeological remains from Chile. Reconstructing past pubertal timing at an individual and population level also reveals important information on the nature of adolescence, an "unruly and shaggy" period of physical and social transition.

5.6 References

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Chapter 6

Mortality risk and physiological stress in older adults of documented chronological age from mid-20th century Santiago, Chile

Abstract

Context: The effects of senescence on older adults have been understudied in bioarchaeology due to methodological challenges associated with age estimation.

Objectives: This study evaluates frailty and risk of death of older adults in relation to the presence of both early and later life physiological stressors.

Materials and Methods: 782 skeletal individuals with documented antemortem data (sex, age-at-death, and cause of death) were analysed. The sample were aged between 40 and 96 years at death and came from low socioeconomic status communities in late-20th century Santiago, Chile. *Chronological vs Skeletal age*. Effect size between mean chronological and skeletal ages was analysed between decade of life, and Kaplan-Meier survival analysis with a log-rank test was used to find the threshold age dividing the sample into early vs late adulthood. *Physiological stress*. Presence or absence of skeletal stress markers related to early life (cribra orbitalia, linear enamel hypoplasia, and porotic hyperostosis) and later life (bone loss, osteoarthritis, and periosteal new bone formation) physiological stress were recorded. Cox proportional hazard models were used to evaluate the effect of skeletal stress marker presence on survivorship for the sample.

Results: *Chronological vs Skeletal age*. Discrepancies of more than 50% from 50 years of age were found, being more significant from 70 years onward. Statistically significant differences ($p=0.017$) in the mean survival time between females and males were found using 60 years of age as a threshold. The sample was further analysed using two age groups: mature adults (40-59 years of age) and senile adults (60-96 years of age). *Physiological stress*. Survival analysis suggests that presence of later life stressors was significantly associated with greater mortality risk in older age (osteoarthritis in mature females, and bone loss in all senile individuals), while presence of cribra orbitalia was associated with poorer survivorship in older adulthood.

Conclusions: Individuals in this sample were resilient enough to survive childhood health assaults and went on to live into older adulthood, but early life stressors might have affected their immune response in later life. Older adults in this sample, particularly females, were more at risk of death when later life stressors such as osteoarthritis and bone loss were present.

Keywords: old age, physiological stress, DOHaD, osteological paradox

Riesgo de muerte y estrés fisiológico en adultos mayores de edad cronológica documentada de mediados del siglo XX de Santiago de Chile

Contexto: Los efectos de la senescencia en los adultos mayores han sido poco estudiados en bioarqueología debido a los desafíos metodológicos asociados con la estimación de la edad.

Objetivos: Este estudio evalúa la fragilidad y el riesgo de muerte de los adultos mayores en relación con la presencia de factores de estrés fisiológico durante la infancia y la vejez.

Materiales y Métodos: Se analizaron los restos óseos de 782 individuos con sexo, edad (entre 40 y 96 años) y causa de la muerte documentados, provenientes de comunidades de bajo nivel socioeconómico de finales del siglo XX de Santiago. *Edad cronológica vs esquelética*. Se analizó la discrepancia entre la edad media cronológica y esquelética por décadas de vida. Se utilizó el análisis de Kaplan-Meier para dividir la muestra en adultez temprana y tardía. *Estrés fisiológico*. Se registró la presencia o ausencia de marcadores esqueléticos relacionados con estrés fisiológico en la infancia (cribra orbitalia, hipoplasia del esmalte e hiperostosis porótica) y en la vejez (pérdida ósea, osteoartritis y parches periósticos). Se utilizaron modelos de riesgos proporcionales de Cox para evaluar el efecto de la presencia de estrés en la supervivencia de la muestra.

Resultados: *Edad cronológica vs esquelética*. Se encontraron diferencias de más del 50% a partir de los 50 años, siendo más significativas a partir de los 70. Diferencias significativas (p=0,017) en el tiempo medio de supervivencia entre mujeres y hombres se hicieron patentes utilizando como umbral los 60 años. *Estrés fisiológico*. La muestra se analizó a continuación utilizando dos grupos de edad: adultos maduros (40-59 años) y seniles (60-96 años). El análisis de supervivencia indicó que el estrés en la vejez se asoció significativamente a un mayor riesgo de mortalidad en mujeres (osteoartritis en mujeres maduras y pérdida ósea en todos los individuos seniles), mientras que la presencia de cribra orbitalia se asoció con una menor supervivencia en la edad adulta.

Conclusiones: Los individuos de esta muestra fueron lo suficientemente resilientes como para sobrevivir a los problemas de salud en la infancia y llegaron a vivir hasta la edad adulta, pero el estrés durante edades tempranas podría haber afectado su respuesta inmunológica en la vida adulta. Los adultos mayores de esta muestra, en particular las mujeres, presentaron un mayor riesgo de muerte cuando estaban presentes factores estresantes durante la vejez, como la osteoartritis y la pérdida ósea.

Palabras clave: vejez, estrés fisiológico, DOHaD, paradoja osteológica

6.1 Introduction

Biological ageing is the progressive decline of the body's physical condition that leads to higher risks of morbidity and mortality. A cumulative and variable process within and between populations, ageing is mediated by the complex interaction between genetic influences, hormonal levels, environmental factors, and epigenetics (Appleby, 2018). Even time period and cultural expectations of adulthood within a particular population can affect the way in which the physical body reflects the process of ageing, as the physical impairments of old age are heavily dependent on the experience of individual and communal health and wellbeing (Ljuslinder, Ellis and Vikström, 2020).

Few bioarchaeological studies explore ageing and older adult's lifeways and health experience, partly because of the methodological challenges associated with estimating age in older individuals. Once growth and development has ceased, age assessment methods rely on degenerative changes of the skeleton to estimate age-at-death; the problematic issues underlying these methods relate to the inexact correlation between physiological and chronological age, and the high variability that exists on the process of ageing itself: as bodies get older, skeletal indicators that bioarchaeologists use to estimate age become even less reliable and age ranges become larger and overlap (Milner *et al.*, 2021). While age estimation of non-adults is generally more precise, enabling the allocation of individuals to shorter age ranges, the tendency within bioarchaeology is to categorise adults very broadly into larger subgroups, to reflect these methodological issues (Buckberry, 2015). One approach to categorise older individuals into biologically meaningful categories was undertaken by Roksandic and Armstrong (2011). Their model outlines four stages of adulthood: young, full, mature, and senile adulthood, from which the latter two are of interest for this study. The onset of mature adulthood is defined by the complete fusion of all skeletal epiphyses and the start of degenerative changes, with the last stage, senile adulthood, defined by severe degenerative changes in the skeleton. Both late stages, mature and senile adulthood, overlap by sharing some skeletal and dental markers such as cranial suture obliteration and substantial antemortem tooth loss with resorption, with both stages correlating with the final stages of age estimation methods for the pubic symphysis, iliac auricular surface, and sternal rib end (İşcan, Loth and Wright, 1984; İşcan, Loth and Wright, 1984; Lovejoy *et al.*, 1985; İşcan, Loth and Wright, 1985; Brooks and Suchey, 1990; Buikstra and Ubelaker, 1994).

This division of older adulthood into "mature" and "senile" adults on the basis of the degenerative changes in the body will be tested within this study.

Assessing age-related changes of the body associated with physiological stress offers another source of challenges for bioarchaeologists. On the one hand, the Developmental Origins of Health and Disease (DOHaD) conceptual framework suggests a relationship between early life physiological stress and negative health outcomes in later adulthood (Gowland, 2015), linking stress assaults during childhood to greater morbidity and mortality in later life (DeWitte, 2014; Milner and Boldsen, 2017). However, the Osteological Paradox suggests that presence of skeletal stress markers might also be indicative of greater resilience, as individuals with skeletal indicators of physiological stress were able to survive enough time for their skeletons to respond to such stress events by forming pathological lesions (Wood *et al.*, 1992; DeWitte and Stojanowski, 2015). The paradox is especially relevant to individuals surviving into later life. Taking both theoretical frameworks into mind, bioarchaeological research on skeletal indicators of physiological stress in adults should be approached with caution, as presence of skeletal stress markers can be indicative of both frailty and resilience (DeWitte and Stojanowski, 2015; Milner and Boldsen, 2017; Kyle *et al.*, 2018). However, it is not impossible to better understand the impact of stress throughout the life course on older adults. To reconcile both perspectives, it has been argued that bioarchaeological research should determine the criteria by which frailty and resilience will be differentiated, taking full account of the biocultural and demographic context of the population under study (DeWitte and Stojanowski, 2015).

The ageing process is known to be correlated with a higher risk of morbidity and mortality in both females and males (Niccoli and Partridge, 2012). Current understanding of the process of ageing accepts that this complex process is the result of evolutionary constraints attributable to the hazard of survival and the metabolic costs of longevity assurance, while also considering that stress-induced DNA damage is an important part of the senescence puzzle (von Zglinicki, Bürkle and Kirkwood, 2001). Stress-induced changes in metabolism and DNA damage interact with the accumulation of mutations that lower fitness in older adults, and are experienced differently depending on biocultural contexts such as sex, gender, and socioeconomic status, among others (Gowland, 2007). Despite known gaps in sex equality, higher body fat percentages and lower physical activity levels in females,

there is a near-universal pattern in modern populations of longer life expectancy for women compared to males (Seifarth, McGowan and Milne, 2012). However, advancing age in women tends to be related to higher odds of frailty than men, particularly in Latin American countries (Alvarado *et al.*, 2008). Experiences of impoverished childhoods and low SES during adulthood are also related to higher likelihood of frailty in older adults (Alvarado *et al.*, 2008; Harttgen *et al.*, 2013), and it has been suggested that individuals from poverty-stricken communities tend to age faster than those in high socioeconomic areas, due to exposure to unhealthy environments during their life (Adams and White, 2004). In this context, bioarchaeology would benefit from any insights into the effects of deprivation in an ageing body, as this was most likely experienced by many past populations.

This study is in a privileged position to explore the intersection of old age, sex, and SES represented as risk of death related to physiological stress during the life course. Taking advantage of the uniqueness of this skeletal sample, with documented antemortem information such as age-at-death, sex, and cause of death, this study uses 40 years of age as the threshold for "older adult". The purpose of this study is to explore trends in survival and physiological stress in older individuals from mid-20th century Santiago, Chile. The experience of "old age" is then explored using physiological stress to analyse the risk of mortality associated with age-at-death and biological sex, assessing frailty throughout the life course using early and later life skeletal stress markers.

6.2 Materials and Methods

6.2.1 Skeletal sample

This study examined the skeletal remains of 782 individuals between 40 and 96 years of age-at-death from the COSS. From all the older adult individuals that comprise the COSS, the sample studied here was selected on the basis of the availability of documented antemortem data (biological sex, age-at-death, and cause of death).

Firstly, documented age-at-death and skeletal age estimation were analysed to test discrepancies between ages. The effect of physiological stress on survival was examined later.

6.2.2 Chronological vs Skeletal age

Age data.

Chronological age-at-death (CA), as well as all other antemortem data, was documented for all individuals in this sample from physical archives found at the original site, the Santiago General Cemetery. Skeletal age (SA) estimations were recorded by staff from the Department of Anthropology at the University of Chile, using bioarchaeological methods including cranial suture closure (Meindl and Lovejoy, 1985), pubic symphysis morphology (Brooks and Suchey, 1990), auricular surface of the ilium morphology (Meindl and Lovejoy, 1985; Osborne, Simmons and Nawrocki, 2004) and sternal end of the 4th rib morphology (İşcan, Loth and Wright, 1984, 1985).

Statistical analyses.

Taking advantage of the individuals' documented CA, the sample was divided by decade of life (e.g., 40-49 years of age, 50-59 years of age, and so on); however, as the use of age range categories is an abstract fabrication, this study also explored whether biologically meaningful categories are a better approach to any age-related study. To achieve this, two issues were tested: if discrepancies between CA and SA exist in this sample, and if differences in survival between decades of life could generate biologically meaningful age subgroups. Firstly, to test discrepancies between CA and SA per decade of life, effect size was calculated using Hedges' g; this statistical test was selected as it performs better in small not normally distributed sample sizes (Cohen, 1988). Common language effect size (CLES) is reported to give a representative percentage of each Hedges' g calculated value (Lakens, 2013). To more effectively interpret the magnitude of differences observed, all effect sizes are reported, even the not statistically significant ones, with results of $g>0.60$ (CLES>65%) found to be representative of a large effect size (Thompson, 2007). Secondly, to test if larger effect sizes correlate with differences in survivorship after a certain age-at-death and following the work of Roksandic and Armstrong (2011), the sample was tested using Kaplan-Meier survival curves to explore if two distinct subgroups within the older adult stage ("mature" and "senile") could be determined.

6.2.3 Physiological stress

Macroscopic analysis.

Presence or absence of six skeletal stress markers was recorded following recommended standard techniques in bioarchaeology (Roberts and Connell, 2004; Mitchell and Brickley, 2017). Stress markers were selected to explore early and later life physiological stress: early life stressors selected were cribra orbitalia (CO), linear enamel hypoplasia (LEH), and porotic hyperostosis (PH), while later life stressors corresponded to bone loss (BLOS), osteoarthritis (OA), and periosteal new bone formation (PNBF). Diagnostic features to diagnose and record each skeletal stress marker are summarised in Table 6.1 (see Chapter 2.2.4 for detailed descriptions).

Table 6.1 Skeletal stress markers recorded for the sample and their diagnostic features.

	Stress marker	**Abb.**	**Diagnostic features**	**References**
Early life stressors	Cribra orbitalia	CO	Porous lesions on one or both orbital roofs. Graded 1 to 4 from present to moderate/severe, according to Rivera and Mirazón Lahr (2017) (see Chapter 2)	Rivera and Mirazón Lahr (2017)
	Linear enamel hypoplasia	LEH	Transversal irregularities on the surface of the teeth. Seen as pits, grooves, or missing enamel of varying size.	Hillson, 2014
	Porotic hyperostosis	PH	Porous and hypertrophic lesions on the cranial vault, particularly on frontal and parietals	Brickley, 2018; Buikstra, 2019
Later life stressors	Bone loss	BLOS	Signs of osteopenia, such as spinal compression fractures, and loss of cortical bone and bone mass	Buikstra, 2019
	Osteoarthritis	OA	Presence of at least two of four of the traditional arthritic traits on synovial joints: lipping, porosity, eburnation, and surface osteophytes. Recorded present also when only eburnation was found (pathognomonic sign)	Rogers and Waldron, 1995; Calce *et al.*, 2018b
	Periosteal new bone formation	PNBF	Woven and/or lamellar new bone formation on the periosteal surface. Only asymmetrical bone formations were recorded as pathological	Ortner, 2003; Lewis, 2017b

Although the groups of early and later life markers recorded here are all indicators of non-specific stress, they have different formation processes, timing, and aetiologies. Both CO and PH are porous lesions reflecting marrow hypertrophy in the skull, and their presence has been associated with nutritional deficiencies, parasitic infection, scurvy, inflammation, respiratory infections, and anaemia (Brickley, 2018; Buikstra, 2019; Rinaldo *et al.*, 2019; O'Donnell *et al.*, 2020; Godde and Hens, 2021). Active porous lesions are only formed in childhood until 10 years of age in girls and 11.5 years of age in boys (Simonson and Kao, 1992), thus all healed lesions recorded as CO and PH in older adults would have had their origin during early life. LEH is associated with systemic physiological stress and metabolic disruption during pre- and postnatal development, and its causes span nutritional deficiency, disease and trauma (King, Humphrey and Hillson, 2005; Buikstra, 2019; Rj *et al.*, 2021).

Late life stressors, particularly BLOS and OA, are associated directly with the process of ageing (Waldron, 2009). OA is traditionally associated with processes of "wear and tear" when synovial joint surfaces experience pathological changes related to cumulative mechanical stress. However, the expression of OA is now believed to be highly multifactorial and the result of the interaction between numerous environmental and genetic factors (Weiss and Jurmain, 2007; Domett *et al.*, 2017; Calce *et al.*, 2018b). BLOS is recognised as a metabolic and degenerative disease that impacts the balance between bone formation and resorption, leading to decreased bone density and a higher propensity to fracture (Tomasevic-Todorovic et al., 2018; van Spelde *et al.*, 2021). Age-related BLOS has been observed clinically in both sexes, where it is associated with genetic influences, diet and nutrition, and physical activity. However, BLOS has been noted to be more prevalent in females, and has been related to pregnancy and breastfeeding, and to hormonal changes associated with menopause (Mays, 1996; Demontiero, Vidal and Duque, 2012; Whitmarsh *et al.*, 2019; Agarwal, 2021; van Spelde *et al.*, 2021). A third later life stress indicator, PNBF, is related to processes of inflammation, and can develop secondary to infection, trauma, or even be related to certain nutritional imbalances (Weston, 2008; Buikstra, 2019). PNBF can form at any age, and although periosteal response can be understood as cumulative, it is used here as a later life stress response indicator because bone remodels over time. Any new formation of either woven or lamellar bone seen on the periosteum in this sample of old adults would be indicative of an active inflammation process involving the bone around the time of death (Waldron, 2009; DeWitte, 2014; Buikstra, 2019).

Statistical analyses.

Prevalences of each skeletal indicator of stress were calculated to broadly explore the presence of these markers in this sample. Cox proportional hazard models were used to explore the impact of physiological stress on survivorship, analysing the interdependent effect of multiple factors or covariates (presence or absence of skeletal stress markers) on the time of death (chronological age-at-death) depending on age group (all, mature adults, senile adults) and biological sex (pooled, females, males). Each skeletal stress marker was recorded with a binary variable outcome of presence (coded 1) or absence (coded 2); censored data (when a skeletal element was not available to study) was included by coding it independently as 0, to prevent absence overestimation. A hazard ratio (HR) greater than 1.0 indicated an increased risk of death, while HR<1.0 indicated a reduced risk of death as the value of the covariate increases (Clark *et al.*, 2003). Results are discussed here in percentages (see Chapter 2.2.4). All statistical analyses were performed using RStudio 2021.09.0 Build 351.

6.3 Results

6.3.1 Demography

Documented age-at-death and biological sex distributions of the sample, segregated by decade of life, are shown in Figure 6.1. Males were slightly over-represented (58.18%, n= 455) compared to females (41.82%, n = 327) overall and in all age groups segregated by decade, except for the 80-89 years of age group, where there are almost double the number of females. A small number of individuals equally split between males and females lived into their 10th decade. Broadly speaking, females were underrepresented in the sample, but lived longer overall than males.

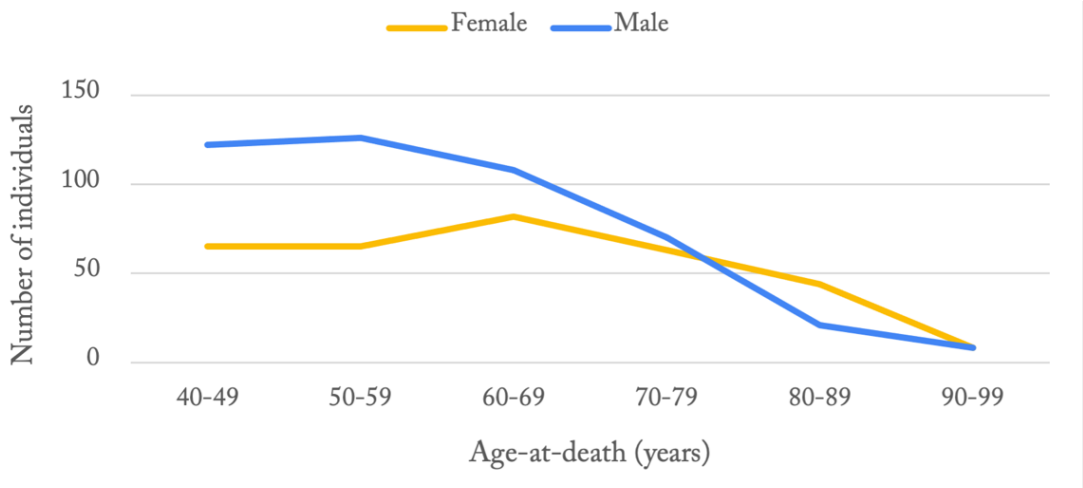

Figure 6.1 Distribution of individuals by biological sex and age-at-death, segregated by decade of life.

Documented year of death was available for 97.82% (n = 765) of the sample. The individuals in this study died between 1957 and 1986; to explore mean age-at-death in comparison with life expectancy reported by Chilean governmental records, three cohorts (1957-1967, 1968-1977, and 1978-1986) were artificially created (Figure 6.2). These cohorts pooled together all females/males in the sample depending on their year of death, thus age-at-death mean and standard deviation for each cohort includes both upper and lower age boundaries within older adults. This exercise was made to explore the representativeness of the COSS older adult sample when compared with Chilean historical records. Ages-at-death for males in the COSS sample broadly correlate with life expectancy for Chilean males during all periods. Contrary to the Chilean scenario, where females tend to live longer than males, mean age-at-death for females in the COSS sample was younger than their male counterparts and the Chilean average, across all periods. Mean age-at-death for females between 1978 and 1986 was considerably younger than the mean for Chilean females for the period. Average life expectancy in Chile for the period 1960-1990 was 66.74 years of age (63.68 years of age for males, 69.92 years of age for females); average age-at-death for the same period for COSS individuals was 61.65 years of age (63.19 years of age for males, 59.61 years of age for females).

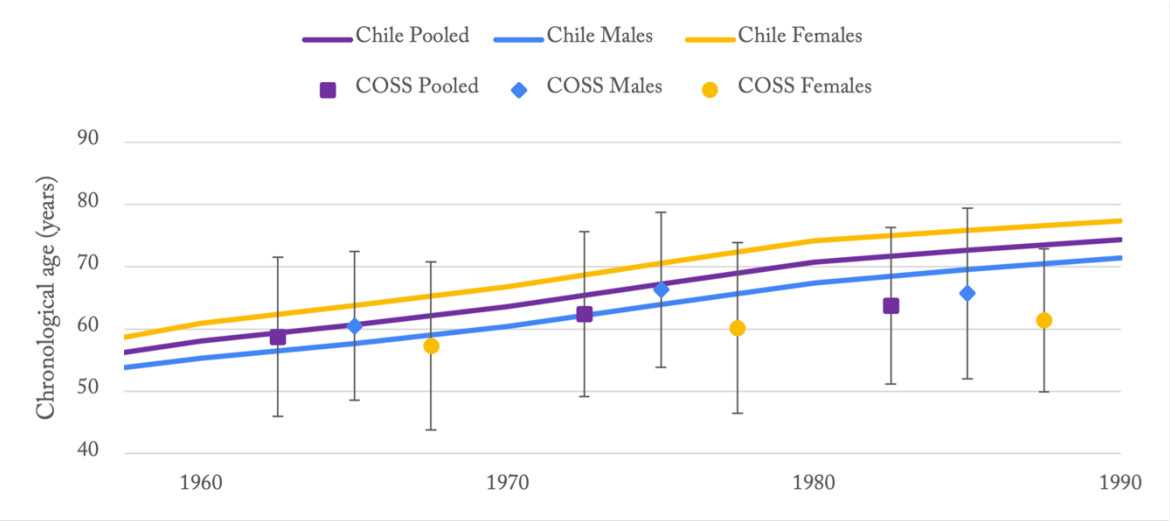

Figure 6.2 Life expectancy for Chilean population (lines) and age-at-death (points and brackets, representing mean age-at-death and standard deviation) for individuals in this sample. Chilean life expectancy data from Ministry of Health digital archives.

Cause of death (CoD) was documented for 618 of the individuals in the sample (79.03%). Prevalence of different CoD shows that cardiovascular conditions (e.g., heart failure, cardiac arrest, atherosclerosis, among others) and infectious diseases (e.g., tuberculosis, septic shock, gangrene, among others) dominate in all decades of older life (Figure 6.3). Metabolic diseases, particularly those associated with cachexia and malnutrition, are the third more prevalent CoD, with a very low prevalence of neoplastic conditions (e.g., brain tumour, cancer, myeloma, among others) and traumatic injuries (e.g., traumatic brain injury, gunshot wounds, physical asphyxia via hanging, among others).

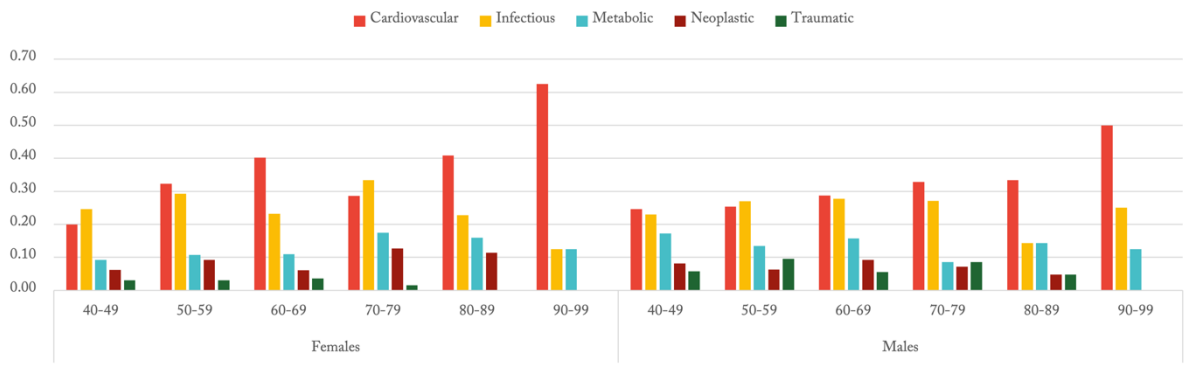

Figure 6.3 Prevalence of cause of death (CoD) type by biological sex and decade of life.

6.3.2 Chronological versus Skeletal age

Results from the effect size difference between documented CA and SA estimations show that skeletal age estimations tend to underestimate age for both females and males in all age categories (Table 6.2). The difference between CA and SA in individuals older than 60 years of age exceeds 50%, while after 70 years of age the difference between CA and SA can be considered a large effect size of more than 65% difference (Table 6.3). This strongly suggest that, for this population, there is an age threshold between what Roksandic and Armstrong (2011) define as "mature" and "senile" adults; this threshold lies between 60 and 70 years of age for this population. This assumption was tested using Kaplan Meier survival curves to test which age threshold (60 or 70 years of age) significantly impacted survival in this sample (Figure 6.4). Statistically significant differences (p=0.017) in the mean survival time between females and males were found using 60 years of age as threshold. Hence, this sample was further analysed using two age groups: mature adults (40-59 years of age) and senile adults (60-100 years of age). Because of this, new CoD prevalence charts were constructed to reflect prevalence of different causes of death within mature and senile adults (Figure 6.5).

Table 6.2 Descriptive statistics for chronological and skeletal ages per decade of life segregated by sex.

	Females					**Males**				
		CA		**SA**			**CA**		**SA**	
	n	MA	SD	MA	SD	*n*	MA	SD	MA	SD
40 – 49	65	44.38	2.91	45.12	10.30	122	44.57	2.76	46.97	11.54
50 – 59	65	55.31	2.89	54.80	13.31	126	54.22	2.93	53.71	12.96
60 – 69	82	64.56	2.74	62.53	14.68	108	64.31	2.88	57.05	14.72
70 – 79	63	73.94	2.79	64.34	13.05	70	73.81	2.68	62.21	13.85
80 – 89	44	83.93	3.13	69.56	12.00	21	83.10	2.79	67.43	14.74
90 – 99	8	91.38	2.07	69.06	15.47	8	93.00	2.33	73.44	10.26

CA: chronological age; SA: skeletal age; n: sample size; MA: mean age; SD: standard deviation.

Table 6.3 Effect size difference between documented chronological age and skeletal age estimations.

	Effect size Females (CA v. SA)			Effect size Males (CA v. SA)		
	g	CI	CLES	g	CI	CLES
40 – 49	-0.10	-0.44 – 0.25	47.18	-0.13	-1.32 – 1.06	46.34
50 – 59	0.05	-0.29 – 0.40	51.41	0.03	-1.13 – 1.18	50.85
60 – 69	0.19	-0.12 – 0.50	55.34	0.35	-0.82 – 1.53	59.77
70 – 79	1.01	0.64 – 1.38	76.24	0.66	-0.57 – 1.88	67.96
80 – 89	1.63	1.14 – 2.11	87.55	0.98	-0.30 – 2.26	75.58
90 – 99	1.91	0.73 – 3.09	91.16	1.78	0.13 – 3.43	89.59

g: Hedge's g; CI: confidence interval (95%); CLES: common language effect size (in %). Highlighted are Hedge's g > 0.60 (CLES > 65%).

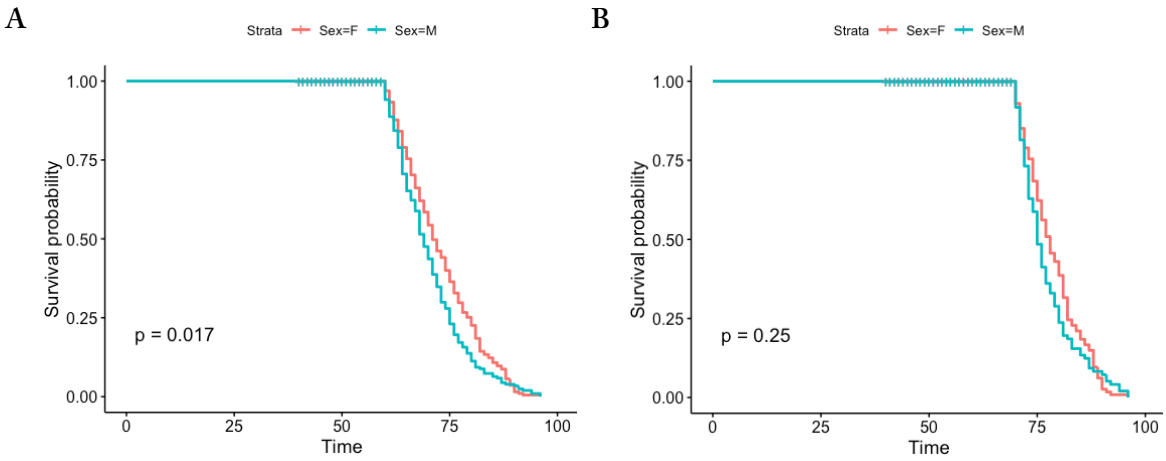

Figure 6.4 Kaplan-Meier survival curves for mature vs senile individuals in the older adult sample using 60 (A) and 70 (B) years of age as threshold.

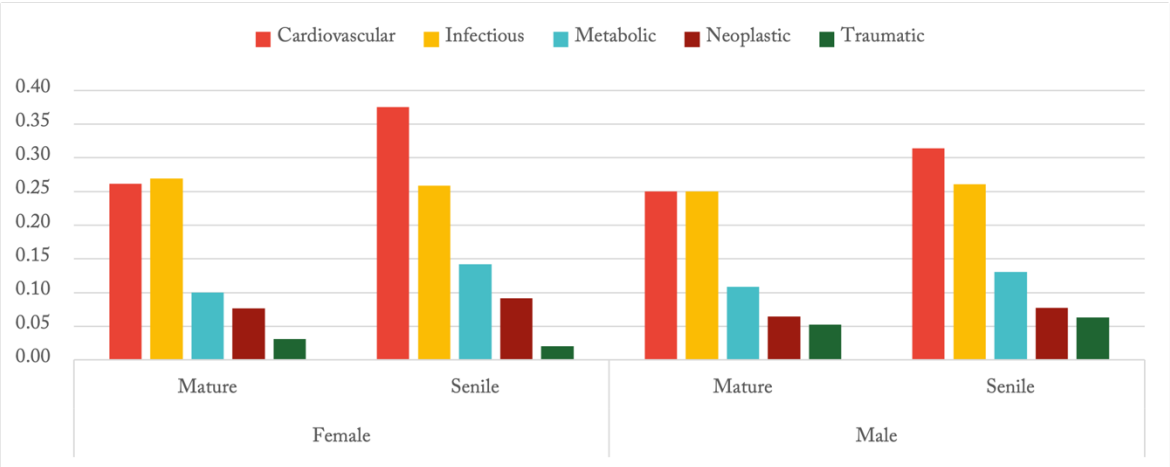

Figure 6.5 Prevalence of cause of death (CoD) type by biological sex and age group (mature and senile adults).

6.3.3 Physiological stress

Crude prevalence of each stress marker was calculated separately for the mature and senile groups (Table 6.4). Markers associated with morbidity in later life, especially OA, and PNBF, were the most prevalent across sexes and age groups. BLOS, PH, LEH and CO had prevalences of <10% across biological sex and age groups. Although clinical studies associate a higher prevalence of osteopenia and osteoporosis to females mainly due to hormonal differences (Leboime *et al.*, 2010), BLOS prevalence for this sample was slightly higher in males than females for both age groups.

Table 6.4 Crude prevalence of skeletal stress markers by biological sex and age group.

	Females						**Males**					
	Mature			**Senile**			**Mature**			**Senile**		
	N	**n**	**P**	**N**	**n**	**P**	**N**	**n**	**P**	**N**	**n**	**P**
CO	130	7	5.38	195	5	2.54	248	16	6.45	204	10	4.83
LEH	130	3	2.31	195	3	1.52	248	4	1.61	204	5	2.42
PH	130	2	1.54	195	3	1.52	248	4	1.61	204	1	0.48
BLOS	130	3	2.31	195	4	2.03	248	9	3.63	204	6	2.90
OA	130	22	16.92	195	28	14.21	248	40	16.13	204	35	16.91
PNBF	130	15	11.54	195	16	8.12	248	27	10.89	204	29	14.01

N: total number of individuals with the skeletal elements present for analysis; n: number of individuals exhibiting the skeletal stress marker; P: prevalence in percentage (%).

Statistical significance of the Cox proportional hazard models was analysed using likelihood ratio test (Xu, Shaw and Mehrotra, 2018). Four models were found to be significant for different sets of covariates: the complete sample (All: Pooled), all senile adults (Senile: Pooled), and mature and senile females (Mature: Females and Senile: Females) (Table 6.5). Informative covariates for each of the significant models display differential hazard ratios, pointing to differences in risk of death given the presence (positive B coefficient) or absence (negative B coefficient) of skeletal stress markers (Table 6.6). When holding all other covariates constant, there is a 58.57% (HR = 1.4137) increase in the risk of death when cribra orbitalia (CO) is present in all individuals in this sample, and a 64.38% (HR = 1.8076) increased risk of death when osteoarthritis (OA) is present in mature females. Bone loss (BLOS) presence is linked to an increased risk of death in all individuals and senile females by risk factors of 1.9746 and 9.8806 (66.38 and 90.81%, respectively). Conversely,

when all covariates are held constant, absence of periosteal new bone formation (PNBF) in all individuals and senile individuals reduces the hazard by risk factors of 0.7804 and 0.6692 (43.83 and 40.09%, respectively).

Table 6.5 Statistical significance of Cox proportional hazards models.

	Pooled		Females		Males	
	-2 Log	**C index**	**-2 Log**	**C index**	**-2 Log**	**C index**
All	0.043	0.526	0.130	0.533	0.089	0.524
Mature group	0.293	0.530	0.049	0.569	0.640	0.517
Senile group	0.024	0.527	0.015	0.549	0.113	0.541

-2 Log: likelihood ratio (p-value); C index: concordance index. Highlighted are models with p-values <0.05

Table 6.6 Results of significant covariates from significant Cox models.

Model	Covariates	B	SE B	HR/OR	CI	p-value
All: Pooled	CO	0.3462	0.1717	1.4137	1.0096 - 1.9797	0.044
	PNBF	-0.2479	0.1173	0.7804	0.6201 - 0.9822	0.035
Mature: Females	OA	0.5920	0.2449	1.8076	1.11846 - 2.921	0.016
Senile: Pooled	BLOS	0.6803	0.3227	1.9746	1.0490 - 3.7169	0.035
	PNBF	-0.4016	0.2047	0.6692	0.4480 - 0.9997	0.049
Senile: Females	BLOS	2.2906	0.5389	9.8806	3.4363 - 28.410	<0.001

B: beta coefficient; SE B: standard error of beta coefficient; HR/OR: hazard ratio/odds ratio; CI: confidence intervals; p-value: Pr(>|z|). Highlighted are covariates with p-value < 0.05 and HR > 1.0

6.4 Discussion

6.4.1 Ageing older adult skeletons

Discrepancy exists over what an "older adult" is; from a biocultural perspective, ageing is at the same time a biological process and a sociocultural construct that arises from the contextual cues a particular population considers to make an individual "old" (Gowland, 2007; Appleby, 2018). During mid-20th century Chile, period in which the individuals in this study lived, medical doctors considered women "senile"[3] once they reached 50 years of age, while men were though to retained their physical and mental capacities for a few more

[3] In its common meaning of "relating to or having diminished cognitive function, as when memory is impaired, because of old age", not related to the age category used by Roksandic and Armstrong (2011) and this study.

years, until they were 60 years of age (Correa Gómez, 2013). This more or less corresponds with the thresholds used in bioarchaeology to describe the age at which estimations in older adults cannot be precised. Bioarchaeological age estimation of older adults is impacted by the inadequacy of current ageing methods to accurately estimate age in individuals that have reached (and passed) skeletal maturity. Although the accuracy of ageing methods for younger individuals (<30 years) is widely accepted (Falys and Lewis, 2011), once the skeleton has ceased to growth and has started to display degeneration related changes, age estimation techniques result in ever expanding ranges, reducing the value of any given mean age and creating large standard deviations for each age range (Cox, 2000). Because of this combination of biological, social and methodological factors, the age from which an individual becomes "old" in bioarchaeology varies (Falys and Lewis, 2011). While Buikstra and Ubelaker (1994) proposed to classify as "older adults" all individuals over 50 years of age, other researchers lean towards ages such as 45+ or 60+ as the milestone for old age (Brothwell, 1981; Lovejoy *et al.*, 1985); some even assert that in many cases it might be impossible to distinguish between an older adult or an adult with a pathological condition (Roksandic and Armstrong, 2011). OA, for example, has been linked to impact age estimations using the os coxa, as arthritic traits affect the auricular surface, pubic symphyses, and acetabulum (Calce *et al.*, 2018b). The value of the present study to this debate lies in its integration of documented age-at-death with osteological data from a large cohort of older adult individuals.

Results from effect size and Kaplan-Meier survival curves in this study strongly support the model proposed by Roksandic and Armstrong (2011) to categorise adulthood. For the population in this study, there is a significant difference in survivorship of older adults when a certain age (60 years of age) has been reached, which serves to differentiate Roksandic and Armstrong's (2011) "mature" and "senile" adults as two biologically distinctive groups. Although some researchers criticise the use of ordinal systems to explore ageing in bioarcheology, arguing data about the individual might be potentially lost (Milner and Boldsen, 2012), categorisation into mature and senile adults allows to assess ageing in a broader sense than using age-at-death, focusing research on the relationship between processes of continuity and/or decline of physiological stress during later stages of life (Appleby, 2018). Of importance is to note that the age threshold to differentiate between mature and senile adults according to Roksandic and Armstrong's model might be

population specific, as ageing processes are highly variable between individuals, and are dependent on genetics, nutritional intake, and sociocultural practices, among others (Buckberry, 2015). Therefore, further analyses with other documented populations should be undertaken.

However, these findings may be somewhat limited by the age estimation methods themselves. Age estimation data from the UChile database were generated using traditional methods assessing cranial suture closure (Meindl and Lovejoy, 1985), morphological changes of the pubic symphysis (Brooks and Suchey, 1990) and auricular surface of the ilium (Meindl and Lovejoy, 1985; Osborne, Simmons and Nawrocki, 2004), and sternal end of the 4th rib degeneration (İşcan, Loth and Wright, 1984, 1985); efficacy of these methods should not be overestimated as they produce very large ranges for age estimations in older adulthood, therefore it is likely that the chronological age-at-death will fall within the estimated range, but will not correlate to a mean age within that age range. Some researchers have called for the use of other age estimation methods when dealing with older adults, such as using the degeneration of the sternal end of the clavicle (Falys and Prangle, 2015) and the composite method using pubic symphysis morphology (Truesdell, 2023). Further studies, which take these variables into account, would benefit the issue of assessing biologically meaningful age groups in older adulthood.

6.4.2 Early and late life stressors in older adults

Any bioarchaeological study regarding frailty and physiological stress in human skeletal remains presents with the dilemma outlined by the "osteological paradox" (Wood *et al.*, 1992): that presence of skeletal stress lesions might be representing either frailty or resilience in a population. The same skeletal lesions may be illustrating both paradoxical interpretations: their presence might indicate severe assaults to health that were insurmountable or might be a sign of resilience as the individual survived long enough for their skeleton to show signs of physiological stress. Considering this, exploring both early and late life stressors in older adults can help understand the pattern of how physiological stress might impact survivorship in a population. In non-adults, presence of early life stressors is associated with a higher risk of death (see Chapter 4); the same scenario, with presence of skeletal stress markers, in older adults show higher resilience during childhood

and survival into adulthood. Thus, although resilience during early years of life meant survival into adulthood, stress insults during childhood still might have an impact into later health outcomes (DeWitte and Stojanowski, 2015). Exploring early life stressors in older adult skeletons might help to understand whether the resilience these individuals showed was at a cost (earlier mortality later on) or a benefit (living longer overall). The association between adult health outcomes and physiological stress during childhood is well established and is the essential baseline of the DOHaD framework: periods of physiological stress and ill health are faced by involving higher energy costs towards survival, thus hindering growth and development, at the expense of their long-term health and longevity (Humphrey and King, 2000; Bateson *et al.*, 2004; Gluckman and Hanson, 2006; DeWitte and Wood, 2008; Gowland, 2015).

In that context, results showing presence of CO to be significantly associated with elevated risk of death in all older adults in this sample can be understood as baseline frailty carried from childhood to adulthood, as it would be expected by the DOHaD conceptual framework. In adults, investigations into CO presence and its association with survival are limited: porous lesions on the orbits due to iron-deficiency anaemia or nutritional deficiencies develop to the first years of life (Brickley, 2018; Godde and Hens, 2021); the individuals in this sample were resilient enough to survive childhood health assaults such as those linked to CO and went on to live into older adulthood while still displaying lesions (McFadden and Oxenham, 2020). Iron-deficiency anaemia during childhood might be related to diminished immunocompetence in adulthood for this sample. Iron plays an essential role in supporting the immune system response, as its metabolism promotes growth and induces differentiation of immune cells (Hassan *et al.*, 2016); iron involvement in immune cell proliferation, particularly lymphocytes, is associated with generating a specific response against infection (Beard, 2001). Moreover, malnutrition, including iron-deficiency anaemia, has been highlighted as one of the most illustrative factors of the DOHaD hypothesis (Gowland, 2015). The pattern of increased risk of death when CO was present can also be correlated with CoD prevalence in older adults in this sample: cardiovascular disease in adulthood has been found to have links with early life stressors (Osmond and Barker, 2000), while individuals with diminished immune systems are more prone to develop poorer health outcomes when presented with infectious diseases. Fisher's exact test results for the association between CO presence and cardiovascular CoD showed a p-value = 0.0067,

indicating that there is a significant association between these two variables, a scenario that is also consistent to the Chilean population of the time, where cardiovascular conditions and infectious diseases were the first and second CoD reported (Solimano and Mazzei, 2007).

A compromised immune response in older adulthood for this sample can also be linked to later life stressors. Results from significant Cox models found an increased risk of death when OA and BLOS were present in mature and senile females, respectively. It is interesting to note that BLOS prevalence was higher in males, but BLOS presence using a Cox model was found to be associated with an increased risk of death in all senile adults, and just in senile females. It is possible that the Cox model showing all senile individuals having an increased risk of death when BLOS was present might represent an artefact of pooling together females and males, due to significance also found only in senile females. The dissimilar results between prevalence and survival analysis might be also showing that prevalence alone is not a good estimator of the effect of physiological stress on survival, and other statistical techniques (such as survival analysis) must be used to explore this issue.

Both OA and BLOS are skeletal stress markers whose aetiologies are usually related to the process of senescence, however more recently their presence has been linked to a compromised immune system. Degenerative joint diseases, particularly OA, are characterised by progressive articular cartilage degradation due to biomechanical stress leading to porosity and destruction of subchondral bone, eburnation, sclerosis, and osteophyte formation (Buikstra, 2019). These has been traditionally seen as result of "wear and tear" biomechanical processes and thus usually linked to physical activity and ageing in bioarchaeology (Klaus, Larsen and Tam, 2009; Waldron, 2009; Larsen, 2015; Calce *et al.*, 2018a, 2018b), but clinical studies have shown that chronic inflammation has a direct role in OA pathogenesis (Liu-Bryan and Terkeltaub, 2015; Lopes *et al.*, 2017). Evidence suggests that low-grade inflammation is involved in the pathophysiology of OA, creating an interplay between mechanical damage and immune response (Orlowsky and Kraus, 2015; Woodell-May and Sommerfeld, 2020). In recent years, new mechanisms have been described through which immunology-related pathways assist with osteogenesis, supporting the idea that the immune system plays a central role in regulating bone homeostasis, regeneration, and resorption (Takayanagi, 2012, 2015; Ginaldi and De Martinis, 2016). In addition, shared immune mediators in arthritis and osteoporosis has been found in clinical

studies (Ginaldi and De Martinis, 2016). It can thus be suggested that OA and BLOS could be considered at least partially chronic immune diseases, and increased risk of death when present in this sample could be related not just to the traditional factors outlined before, but also to chronic inflammation and a diminished immune response in adult life. This relatively new conceptual field of "osteoimmunology", linking immune and skeletal systems and responses, might offer new insights to traditional paleopathological issues, such as the effects of OA and BLOS, and even fracture healing (Lončar, Halcrow and Swales, 2023).

Several animal and human studies suggest that sex differences in immune function are protective for females, as sex hormones play a critical role in human immunocompetence, with oestrogen considered an immuno-enhancer (Seifarth, McGowan and Milne, 2012). Clinical studies have shown that men are affected by OA more than women before 45 years of age, but the prevalence increases in women after 55 years of age; this shift is usually attributed to the protective effect of oestrogen in women before the menopause (O'Connor, 2007; Weiss and Jurmain, 2007). Differential immune response between sexes might be related to higher susceptibility to infection in males, while in general it is believed that lower androgen levels lead to a more robust immune system; thus, females seem to display an oestrogen protected and enhanced immune system against physiological stress. The seemingly contradicting findings of this study, where females were at a higher risk of death when OA and BLOS were present and the correlation of it with higher risk of death when CO was present in the entire sample, might be explained by the same protective immunocompetence. A more robust immune system in females is also linked to immune-pathogenic responses and a predisposition to autoimmunity disorders due to hyper immune responses (Ngo, Steyn and McCombe, 2014; Taneja, 2018). In addition, early environmental factors including nutritional status and microbiome composition, might also alter the immune response, with a diminished nutrition during early years linked to an altered immune response in later stages of life (Klein and Flanagan, 2016). As part of low SES communities in Santiago de Chile, it is highly likely that these individuals suffered detrimental living conditions during their life course, particularly during early life (Medina and Kaempffer, 2007). Income inequality has a great impact on health and survival (Contreras *et al.*, 2001; Subramanian *et al.*, 2003), which can be seen in the individuals in this sample, who were dying younger than the expected country average. This might also be reflected in the lower mean age-at-death for females compared to males in this sample, and

when compared to life expectancy of the Chilean population of the period. Females in this sample not only died at younger ages than their male counterparts, they also had a heightened risk of death when experiencing early life and later life assaults on their health.

Age-related bone loss is usually seen as the result of ontogenetic processes over the life cycle (Agarwal, 2021), with osteoporosis risk factors linked to genetics, ageing, mechanical loading, nutritional status, lifestyle and, in females, pregnancy, breastfeeding, and menopause (Agarwal, 2012; Buikstra, 2019; Brickley, Ives and Mays, 2020). Sex differentials in this study are consistent with clinical studies that link presence of BLOS in older females as an inevitable result of the costs of reproduction in adulthood and to the decrease in hormonal levels during senescence that protect and maintain bone density, while it has also been linked to behavioural changes linked to urbanisation (Agarwal, 2021). Clinical studies on age-related BLOS, particularly osteoporosis, recognise its relation to an increased risk of fracture (Leboime *et al.*, 2010; Demontiero, Vidal and Duque, 2012), a scenario that is also known in bioarchaeology (Brickley, Ives and Mays, 2020). Results of this study found no evidence for increased fragility related to mechanical failure and bone or fracture (FRA) in senile females; BLOS was more prevalent in males in all ages while traumatic CoD for senile females do not list fractures. Higher prevalence of FRA in males might also be an indicator of interpersonal violence and conflict, accident-related events, or activity related practices (Lovell, 1997; de la Cova, 2012). Another possible explanation for this might be that risk of death when BLOS is present might not be related to fractures in this sample, but to the previously outlined interplay between immune and skeletal systems, as clinical studies have found that low bone mass density is a risk factor for infections and sepsis (Zhang *et al.*, 2022). However, infectious diseases are not the leading CoD for senile females, and their prevalence is higher in all other age groups, while PNBF was not more prevalent nor found significant in the senile females group. Therefore, this interpretation needs to be taken with caution.

6.5 Conclusion

This combination of findings provides support for the conceptual premise of the DOHaD hypothesis and highlights the importance of exploring multiple skeletal indicators of physiological stress in combination, and not as isolated markers. The study also illustrates

how early life stressors may play a significant role in later life health outcomes, and how bioarchaeological research should interpret later life stress markers: both OA and BLOS are regarded as skeletal indicators of the process of senescence, from wear and tear to the product of health investment into reproduction, but their origins might be traced back to childhood. Although the individuals in this sample survived childhood and lived well into old age, their skeletons still show traces of stress episodes in early life, raising the question of how other factors, such as social inequalities, might have contributed to their later life survival (Gowland, 2015).

The results of this study suggest that frailty increased with age and women were more likely to be frail than males, as has been found in Western modern populations and particularly, Latin America (Alvarado *et al.*, 2008; Harttgen *et al.*, 2013). The findings also broaden our understanding of skeletal response to physiological stress, in line with the osteological paradox and DOHaD theoretical framework, linking the state of health and risk of death from disease in later adult life with the environmental conditions of early life. Genetics, early life physiological stress, reproductive behaviours, and levels of physical activity and nutrition throughout the life cycle, in addition to cultural related influences (such as sex/gender, socioeconomic status, or community lifestyle), all layer onto the pattern of survival in this population. These findings have important implications for bioarchaeological research, as it stresses how critical it is to adopt a biocultural approach in our studies, particularly when analysing older adults. Although these individuals are non-survivors, their skeletons tell the story of the interplay of frailty and resilience through the life course and can enrich our interpretations of the ageing process and survival.

6.6 References

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Chapter 7

General Discussion

7.1 Introduction

This research aimed to broaden our understanding of the impact of physiological stress on growth and survival of an understudied documented skeletal collection from Santiago de Chile. Each research chapter sought to capitalise on the potential to address bioarchaeological evidence in combination with the historical and social context of this sample, improving our understanding of the life experience of a Latin American population in the context of economic migration and urbanisation.

The main aims of each chapter can be summarised as follows:

- **Chapter 1.** To review the central themes necessary to understand the different chapters within this thesis, including a literature review of current approaches in bioarchaeology and palaeopathology, theoretical frameworks used throughout this research, and a brief summary of this population's historical context.
- **Chapter 2.** To present the COSS collection, its origin and current curatorial state. To describe the methods used across the different research chapters.
- **Chapter 3.** To explore growth disruption in foetal, perinatal, and infant individuals (<1 year of age) comparing documented chronological age to age estimations from skeletal and dental elements. To analyse the impact of physiological stress on growth disruption.
- **Chapter 4.** To examine the impact of physiological stress in non-adult survival. To explore if presence of skeletal stress markers is associated with higher risk of death during childhood.
- **Chapter 5.** To explore pubertal timing through osteological assessment of pubertal stages in this population. To analyse frailty in order to explore the impact of physiological stress during pubertal development.
- **Chapter 6.** To evaluate differential survival in biologically meaningful categories of older adulthood according to Roksandic and Armstrong (2011). To explore frailty and risk of death in adults older than 40 years of age in relation to the presence of both early and later life physiological stressors.

Key findings of each research chapter (i.e., 3-6) can be summarised as follows:

- **Chapter 3.** When compared to documented age-at-death, dental age estimations are the most accurate for this sample. Estimations using skeletal elements varied. Femoral length underestimated age-at-death, indicating the COSS population were largely smaller for their age that the modern and historical populations used in the development of age estimation methods. Estimates using pars basilaris dimensions were inconsistent, randomly over and underestimate age-at-death regardless of age group or sex. This contradicts findings based on samples from European descent, where the pars basilaris method has been found to be highly accurate. There is a positive association between presence of physiological stress and growth, particularly related to the presence of cribra orbitalia and periosteal new bone formation. In addition, growth disruption due to physiological stress is present both pre- and postnatally, suggesting elevated maternal physiological stress in this population.
- **Chapter 4.** The sample showed sex differentials in survival, with greater mortality risk associated to the presence of cribra orbitalia and porotic hyperostosis in foetal and infant males compared to females. During childhood, there is evidence of greater risk of death when skeletal indicators of rickets and dental pathologies of different aetiology (linear enamel hypoplasia in females and caries in males) were present. This suggests a decreased survival in this sample was associated with basal malnutrition across childhood, with a compromised immune response during early stages of life, which correlated with living in low socioeconomic status communities.
- **Chapter 5.** Although onset of puberty occurred at a similar age in this population when compared to clinical records of adolescence for the period, progression through pubertal development was slower and extended in time. There was a significant delay in the stages leading to the peak high velocity point and subsequent delay in age of menarche compared to the clinical data for the period. These results suggest that frailty indicators might link disease exposure and susceptibility to disruption in pubertal timing, which might impact osteological assessment of puberty. In terms of methodological accuracy, the method devised by Shapland and Lewis (2013, 2014) and Lewis, Shapland and Watts (2016) for skeletal assessment of pubertal stages was reliable in this population when comparing age-at-death and skeletal ages estimates, with the only exception being females in the early stages of puberty.

- **Chapter 6.** A statistically significant difference in survival time between females and males was found after 60 years of age, suggesting this as the age threshold for Roksandic and Armstrong's (2011) categorisation of older adulthood into mature and senile groups in this population. Results suggests that presence of both early and later life stressors impacted survival in older age: cribra orbitalia was associated with poorer survivorship in older adulthood, with osteoarthritis and bone loss associated with higher risk of death in mature females and all senile individuals, respectively. These findings are in line with DOHaD framework, suggesting that older adults in this population were resilient enough to survive childhood health insults, but these early life stressors might have affected their immune response in later life.

Each research chapter presented in this thesis is a discrete body of work, containing its own discussion on how that work fits into the broader context of palaeopathology and bioarchaeological research. Nevertheless, some key themes run through the entirety of this thesis, arising from the uniqueness of studying a documented skeletal collection. These will be discussed in depth here. This general discussion begins with reflections on the uniqueness of working with a documented skeletal collection, the strengths and weaknesses of combining historical records and paleopathological data, and the ethical issues that should be considered when working with modern cemetery populations. The following sections will bring together all the evidence gathered across research chapters, discussing the availability of documented antemortem data for this population and how it relates to bioarchaeological and historical evidence, and the insights that have arisen from bioarchaeological evidence of physiological stress and frailty. The chapter finishes with some future research possibilities and concluding remarks about the entire thesis.

7.2 The value of a documented skeletal collection

This thesis is the first comprehensive investigation on the *Colección Osteológica Subactual de Santiago*, one of the few known documented skeletal collections from Central and South America. Although several identified osteological collections exist around the world (Santos, 2020), some of them have been more intensively studied than others. These

often-used collections have been the basis for the development and testing of methodologies of osteological analysis, simultaneously serving in anthropological and medical training and research of normal human variation (Moorrees, Fanning and Hunt, 1963; Simon and Hubbe, 2021) and as comparative samples in palaeoanthropology and human evolutionary studies (Bekvalac, Cowal and Mikulski, 2006). The identified osteological collections that appear most frequently in research have their origins in the United States and Europe, and usually represent modern individuals of European and African descent. Collections from South America are rarely represented in this body of work, resulting in two fundamental problems. First, there are few methods specific to South American populations. Second, the accuracy of methods based on European and African populations when applied to South American populations is fairly limited.

It is widely recognised that population specificity can prove to be a limitation when applying methodologies to samples from different ecogeographic and time contexts (New and Algee-Hewitt, 2021). Population specificity has been long discussed and accepted within the field; normal anatomical variation between individuals and populations is an issue that must be considered when using reference collections to extrapolate methods and arrive to conclusions when researching different populations. Thus, increasing access to diverse reference samples is an important part of the bioarchaeology and forensic anthropology research agenda. For the latter, modern identified skeletal collections might convey greater population affinity to people living nowadays in a particular location. To adequately cater for population specificity in human skeletal remains and its implications for every area of biological anthropological research, access to identified skeletal collections from every part of the world is essential.

Central and South American identified skeletal collections are a necessity for further development of the field of bioarchaeology and forensic anthropology in the global south (Plens, Górka and López Quintero, 2022). Typically curated by higher education institutions, these collections range from a few dozen individuals to more than a thousand, with variable amounts of documented antemortem data associated to the individuals (Table 7.1). Unlike reference collections from the USA or Europe, collections from Central and South America are most often only accessed and studied nationally, developing local criteria for a particular population that are not usually widely used across the continent. Only a few

Table 7.1 Known modern identified osteological collections in Central and South America (adapted from the Forensic Anthropology Society of Europe (FASE) website. Original content can be found in http://forensicanthropology.eu/osteological-collections).

Skeletal collection	Location	No. of individuals	Known information
Reference collection from the Xoclán cemetery	Mérida, Mexico	84 (56 males, 28 females)	Sex, age-at-death, date of birth, death and inhumation, cause of death, citizenship, place of origin, and last residence
Chacarita Collection	Buenos Aires, Argentina	159	Age, date and cause of death, nationality
Northwestern Reference Osteological Collection	Río Negro, Argentina	13	Sex, age-at-death, date and cause of death
Osteological and Tomographic Collection of FOP/UNICAMP	São Paulo, Brazil	320	Sex, age-at-death, ancestry, and cause of death
Identified Skeletal Collection of Sergipanos of the University Tiradentes	Aracaju, Brazil	230	Sex and age-at-death
Collection of Identified Skeletons from UNIFAP Medical School	Araripinae, Brazil	400 (248 males, 152 females)	Not reported
The 21st Century Collection of the Center for Studies in Forensic Anthropology (CEAF/UPE)	Pernambuco, Brazil	239	Sex, age-at-death, date of burial, and date of exhumation
Universidad de Antioquía's human skeletal reference collection	Medellín, Colombia	+ 100	Sex and age-at-death
Human Skeletal Reference Collection of Modern Colombian Population	Bogotá, Colombia	600 (406 males, 194 females)	Sex, age-at-death, manner, cause and mechanism of death
Colección Osteológica Subactual de Santiago (COSS)	Santiago, Chile	1635	Sex, age-at-death, date of birth and death, cause of death

of osteological methods have been developed or evaluated using South American osteological samples. These methods have focused on estimation of stature (Genovés, 1967; Del Angel and Cisneros-Reyes, 2004; Menéndez and Gómez-Valdés, 2011), sex (Carvallo and Retamal, 2020), age-at-death (Rivera-Sandoval, Monsalve and Cattaneo, 2018), or a combination of them (Ross and Manneschi, 2011). Of these, only the methodology of Genovés (1967, modified by Del Angel and Cisneros-Reyes in 2004) has been tested on different populations

(Pelin and Duyar, 2003; Auerbach and Ruff, 2010) and used in forensic and archaeological contexts across Latin America, with variable success (Menéndez and Gómez-Valdés, 2011).

The COSS has been extensively studied by Chilean and Latin American researchers and used by hundreds of students as their primary source of practical knowledge, with the vast majority of the work done using the COSS as reference collection published in Spanish (Table 2.5). This fact contributes to the relatively low international profile of the COSS and has reduced its research potential by narrowing down the potential researchers who can work directly with the collection. The lack of knowledge of the Spanish language contributes to the unfamiliarity of international researchers towards not only this collection in particular, but also to some degree to the archaeological field outside English speaking countries. It is no secret that English is currently considered "the language of science", and that the vast majority of scientific knowledge is published and shared in that language. The extent to which Latin American archaeological and bioanthropological resources have been overlooked outside the continent hints towards what Ramírez-Castañeda (2020, p.6) identifies as the “negative hegemony of English in the scientific community”. Although the effort to create multilinguistic options for promoting inclusion and diversity in scientific research should not rest on researchers from English as a Foreign Language (EFL) countries alone, that is exactly what is usually necessary to bring to the fore such resources. For the particular case of identified skeletal collections in Central and South America, their inclusion on the Forensic Anthropology Society of Europe (FASE) online database for identified osteological collections (Petaros et al., 2021) was one step forward. However, until more research on these collections is published entirely in English (and not just including a translated title to be picked up by search engines radars), it is essential for the language barrier in the field of (bio)archaeology to be a topic to take into account when conducting research.

Beyond their significant value to the development and testing of osteological methods, skeletal reference collections can also help understanding sociocultural, economic, and political changes in their origin countries (Bekvalac, Cowal and Mikulski, 2006), as this thesis has proven. As these collections often comprise more modern individuals than other skeletal collections derived from archaeological excavations, they can be contextualised with historical events that add depth and nuance to the understanding of the lived experience of

the individuals and populations represented. A remarkable example of this issue is the Coimbra collection in Portugal, a skeletal collection comprising individuals with DAD (in addition to cause of death, local origin, among other data) that died between 1995 and 2008 (Ferreira et al., 2014). The documented biographical information and chronological continuity has provided a unique opportunity to document secular trends regarding growth and ageing, and the impact of physiological stress (Henderson and Padez, 2017; Arrieta, Ramos Gaspar and Santos, 2021). Besides studies focusing on sex and age-at-death, research using documented occupation (Alves Cardoso and Henderson, 2013) and cause of death (Santos, 1995; Santos and Roberts, 2006) have been of particular interest, expanding research by contrasting bioarchaeological evidence with clinical biomedical documentation.

A particular note of caution must be always taken when working with historical biomedical records, where data available responds to the knowledge used at the time. This, however, does not mean documented causes of death from cemetery records for this population cannot be studied in conjunction with palaeopathological changes to the skeleton. The issue has been addressed by what Mitchell (2011) calls "the Cunningham debate" (Cunningham, 2002): the idea that disease is a socially experienced entity, a combination of a biological and social phenomenon and thus, modern views will never be able to fully understand the true meaning of disease in the past, as our social context differs from that of past populations. Mitchell (2011) agrees to a point with this, but argues it is possible to use historical records to retrospectively diagnose or explore disease in the past, an argument that the findings of this thesis agree upon: when clear descriptions of signs and symptoms are made in historical sources, modern researchers will be able to determine biological aetiologies or possible biomedical explanations that can be, in turn, be seen on skeletal remains. Particularly for documented collections such as the COSS, causes of death are available for a large number of individuals from cemetery records, which in turn come from governmental documents issued by medical doctors. It is important to note that medical terminology used in Chile changed during the century the COSS population lived and died. Issues such as the use of similar terms to describe the same lethal symptoms responded to the medical knowledge of the time and the emergence of new medical treatments that managed to lower the lethality of diseases; such was the case in Chilean biomedical literature before the 1950's for the use of "tuberculosis" and "bronchopneumonia" indistinctly as causes of death related to the same chronic respiratory infection caused by the Mycobacterium tuberculosis bacteria.

This situation only changed after 1952 when the Ministry of Salubrity (predecessor of the current Ministry of Health) changed the guidelines and treatments for tuberculosis, greatly improving life expectancy for affected individuals (Paluzzi, 2004; Herrera and Farga, 2015; Lopez, 2016). For the purposes of this thesis, both tuberculosis and bronchopneumonia were grouped together under the broader concept of "infectious diseases", but caution should be taken if, for example, only the study of tuberculosis is in question. Other source of difficulties when working with historical records is the differences in medical knowledge during different time period, and the disparate concepts used throughout time to describe the same illness. Although the time period when the COSS individuals lived is not so far removed from more modern times, differences in the cultural idiosyncrasy particular to the time can prove problematic. The medical field during mid-20th century Chile saw "social faults" such as "promiscuity, immorality and vices" as an intrinsic part of disease's aetiology, management, and successful treatment (see Chapter 1.3). In the eyes of medical doctors of the time, poverty and overcrowding living conditions contributed to the spread of so-called "diseases of social importance" that were not effectively treated, not only because of the lack healthcare but mainly because of the "uncivilised" state of the population (Chávez Zúñiga, 2019). In that context, diffuse concepts such as "social misery" are listed as cause of death for COSS individuals, showing how important it is to have in mind that medical concepts might be outdated for modern times and may not correspond to current nosological classifications. Although this might present itself as a challenge for its use in conjunction with bioarchaeological evidence and palaeopathological analyses (e.g., what is the biological origin of the cause of death described as "social misery"?) and seem to support Cunningham's argument (2002), it also presents a valuable strength for the bioarchaeological approach, as the study of past populations is a composite of biological evidence and the complexity of sociocultural contexts.

Of equal importance to advocating for the value of documented collections, and to the fundamental understanding of all archaeological work using documented collections, is the need to be aware of their potential problems and biases. No matter the extent of their numbers, even if they are comprised of hundreds or thousands of individuals, documented skeletal collections do not represent their whole population due to two different phenomena: they represent the non-survivors among the living societies they come from (Wood et al., 1992; DeWitte and Stojanowski, 2015) and, particularly for cemetery-derived collections

such as the COSS, the origin of a collection was dictated by specific factors that answer sociocultural, political, and economic issues at the time of they were established, biasing which individuals were recovered and retained as part of skeletal assemblages (Cardoso, 2021).

It is particularly important to bear in mind the possible bias in cemetery-based skeletal collections which arises from the motivations for excavation at the time. In the case of the COSS, as extensively described in Chapter 2.1, its establishment responded to a very particular set of issues that combined to create the skeletal collection: the secular nature of the Santiago General Cemetery, the availability of cheap short-term graves that were to be exhumed, the fact that relatives from the diseased did not or could not pay for cemetery fees associated with an extension of the burial, the changes in laws and regulations that allowed for the skeletal remains to be relocated to a higher education institution, and the willingness of the University of Chile and its staff during the time to care for and house the remains. All these matters lead to the establishment of a skeletal collection that represented a very specific population of Santiago at the time, mainly impoverished individuals whose living relatives did not have the monetary means to change the final destination of the remains of their family and friends. In practice, this results in two issues of very different nature that nonetheless impact the way the COSS should be understood: the individuals that comprise the COSS are not representative of modern Chileans, and the ethical considerations that should be discussed when studying it. For years, the way the COSS has been positioned within bioanthropological scholarship shows it as a representative of the modern population of Santiago, and to some extent to certain researchers, as a representation of the modern Latin American population (O'Bright, Peckmann and Meek, 2018). However, we must take into consideration how these collections, and the COSS in particular as explored in this thesis, might be showing us intrinsically frail individuals, whose health and pathological conditions are associated with marginalisation and lower SES. By using the COSS as a model to understand modern individuals from Santiago, Chile, or even Latin America, researchers are actively erasing the socio-political background of this population, creating a narrative where all modern *santiaguinos* can be represented by the COSS individuals. The findings of this thesis show that when compared to clinical data from the period, the lifeways and frailty experience of the COSS individuals did not always reflect all of their living contemporaries. This issue affects not only research done using the COSS collection but

encompasses broader themes in bioanthropology regarding the representativeness of the original samples used to develop the methods we use in our daily work to assess age-at-death, sex, stature, pubertal timing, frailty experience, among others.

7.2.1 Ethical considerations

It has been only in recent years that archaeology as a discipline has been challenged with the notion of accountability involving ethical practices when working with human remains (Squires, Roberts and Márquez-Grant, 2022). While studying human skeletal remains, bioarchaeologists and forensic anthropologists should always seek to examine the ethical implications of our work. The lack of explicit ethical statements in published papers regarding archaeological materials is troublesome on different levels (Squires, Roberts and Márquez-Grant, 2022), but the one pertaining to this research refers to the study of the relatively modern (understood here as post-medieval in Europe, and post-Colonial in Latin America) human remains.

Conversely to the case of North America and some parts of Europe, where perpetual care of human remains is a standard burial practice (Sharman and Albanese, 2018), primary burial rites in Chile are agreed between the cemetery and the family in a short- or long-term basis. Extensively reviewed in Chapter 2.1, the circumstances surrounding the creation of the COSS can be summarised as follows. The construction of the skeletal collection complied with all legal requirements, and both the origin (SGC) and destination (UChile) institutions acted within their powers to ensure the custody and conservation of these human remains was maintained at the highest standards for the time. Currently, and during the time when COSS individuals were exhumed, it is mandatory for all cemeteries to inform the customer (i.e., who purchases the burial rights, in the majority of cases the deceased's next of kin) in advance to an exhumation, to give the opportunity to purchase a burial extension, purchase another grave plot and transfer the remains, or to arrange cremation (Ministerio de Salud (Chile), 1970). After mandatory communication from cemetery authorities is issued, and if it was impossible to contact the customer or if the customer did not want or could not pay for an extension of burial, the cemetery is allowed to dispose of the remains in any manner that is legally possible, including relocation to a communal grave, cremation, or entrusting the human remains to a higher education institution (Ministerio de Salud (Chile),

1970). The particular reasons why these individuals were exhumed and entrusted to the UChile might respond to several reasons, all of them rooted in a lack of monetary resources from the deceased's families.

Acknowledging the fact that the human remains that comprise the COSS only do so because of their low SES, is of extreme importance. From cemetery records and historical data, we have inferred that in life these individuals, and their next of kin in charge of burial arrangements, belonged to the lowest socioeconomic strata in Santiago at the time. This fact must be considered when discussing their inclusion in a skeletal collection, as their selection for exhumation was mediated by their lack of wealth, not consent. It has been recognised that modern skeletal collections typically represent individuals of low SES (Sharman and Albanese, 2018); particularly for this population, an impoverished life experience translated to lack of control or autonomy over their ultimate fate after death. Lack of consent, encompassing the individual and their directly related community, on how their remains would be treated after burial meant that these individuals continued to be marginalised even after death. This is not the scenario for every cemetery-derived skeletal collection, however; Vanderbyl, Albanese and Cardoso (2020) analysed a skeletal collection from Lisbon, Portugal, and found no association between low SES and inclusion into the collection, arguing that the poorer members of society would go directly to communal graves or cremation. This was not the case for SGC regulations; no communal grave or exempt of charge cremation was available for low SES individuals, being short-term graves in grave plots or niches the cheapest alternative within the cemetery.

In addition to the contextual origin of the COSS, the way in which the individuals within the collection have been treated after their entrusting to the UChile should also be revised. The perception of all archaeological materials, human remains included, as public heritage goes hand in hand with the idea of the bioarchaeology discipline as "stewards of the past" (Zimmermann, 1998), rooted deep in the archaeological ethos that denounces and works against the destruction of archaeological sites and data. Documented skeletal collections (and all anatomical collections, by extension) fall into this scope; by creating these collections, archaeologists and the institutions that house them safeguard the information that these human remains can deliver to science, in present and future times. However, this approach has not been without fault; in the past, storage and curation of skeletal collection

has partly erased the personal identity of the individuals, objectivising them without malice, for the sake of the scientific research value of the same human remains (Muller, Pearlstein and de la Cova, 2017). This approach affected the treatment in storage and curation of the COSS during the first decades of its creation, when individuals' remains were disaggregated into ossuaries of anatomical elements (i.e., skulls, long bones, pelvises, vertebrae, etc.), in line with teaching guidelines of the time (Meza-Escobar et al., 2023). It has only been recently, and mainly due to the efforts of the Bicentennial Project, that a large campaign for restitution of the individual's remains has been in place. In addition to mending past faults, achievements in documentation made by the Bicentennial Project and this thesis means that for 1104 individuals, a restitution of identity has been made. Documented identity means that COSS individuals can be studied as known individuals, giving them a renovated existence and making them social beings (Tarlow, 2006), but also makes it possible a potential reburial of the remains if their relatives wish to reclaim them.

It is imperative to recognise the role of structural violence and marginalisation in creating this particular skeletal collection, while striving to reclaim the forgotten identities of these individuals (de la Cova, 2019). It is also essential that bioarchaeology takes into account not only the historical background the studied population, but also the process by which the population came to be studied (Muller, 2020). In the case of cemetery-derived skeletal collections such as the COSS, acknowledging and incorporating this to the research, seems to be the only way for preventing a remarginalisation of already marginalised individuals. The bias towards individuals of low SES does not mean the collection lacks value; on the contrary, it gives the researcher an important opportunity to study the lower strata of society whose lives are comparatively less well documented and who experienced important social and economic transitions of direct relevance to this thesis – economic migration and adverse living conditions through life. In doing so, this thesis offered a platform for better understanding the lives of this understudied group, while also recognising the limits of representativeness, as bioarchaeological research will only go so far, never fully recovering, representing or recognising the complex historical "truth" this population experienced (Tarlow, 2001).

7.3 Bioarchaeological research in light of documented antemortem data

By analysing only individuals with documented antemortem data (DAD) from a larger sample of individuals within the osteological collection, each research chapter in this thesis has produced valuable data regarding several different topics. Cemetery-based bioarchaeological studies with access to individuals with DAD can provide an array of research opportunities but might also be impacted by potential limitations due to the nature of the documentation.

Estimations of biological sex and skeletal age-at-death are essential parameters for formulating and answering research questions in bioarchaeology and forensic anthropology. The availability of both sex and age-at-death antemortem data is the part of the uniqueness of the COSS, but it might not be without biases.

7.3.1 Documented biological sex

Many skeletal collections are affected by sex biases, comprising far more males than females (Sharman and Albanese, 2018). Although the COSS is also affected by this, the selected samples in this thesis strived to represent males and females somewhat equally: in the non-adult sample 40% was female (n=66) and 60% male (n=98), while the older adult sample was 42% females (n=327) and 58% males (n=455). However, on three smaller age groups used within this thesis, male/female ratio similarity fell short of the desired level.

In Chapter 3, male/female ratio in the foetal-infant group might have had some impact on the results (Table 7.2). When segregating by sex, there was no statistical difference between estimates for DA and SA-F, but SA-P showed a discrepancy of 1 week between male (advanced) and female individuals (deficit). This effect might be related to the differences in *intra utero* growth strategies between sexes (Eriksson et al., 2010), with male foetuses growing faster but investing less in placental growth (Roseboom et al., 2011); but it could also be a statistical artifact of the small sample size. As osteological sex estimation in non-adults is highly inaccurate, and not routinely undertaken as a result, the same study conducted using any other sample without DAD would not report this as an issue. Further studies with a larger sample and different population with DAD are required to test the role of biological sex on growth disruption in foetal-infant individuals.

Table 7.2 Sex and age-at-death distribution in the analysed foetal-infant sample.

Sex	Age group			Total
	Foetal (< 40 GWA)	Perinatal (40 GWA - 43 WA)	Infant (44 - 78 WA)	
Male	3	6	20	29
Female	3	2	9	14
Total	6	8	29	43

Disparities between male/female ratio are also seen in early stages of adolescence, where male individuals outnumbered females (see Figure 5.2). Overrepresentation of males in this sample seems to be in agreement with global findings that suggest a higher mortality among adolescent males compared to their female counterparts, mainly due to injuries and violence found in this age group (Ward et al., 2021). However, the disparity in male/female ratio might impact the study's findings on age at female pubertal onset, under- or overestimating the true CA at which initiation stage was attained. On the other hand, the reverse scenario is true for older adults over 80 years of age, where females surpassed males (see Figure 2.7). This was to be expected, as females tend to live longer than their male counterparts (Seifarth, McGowan and Milne, 2012). As before, further studies are required to explore the impact of sex disparities in this sample.

7.3.2 Documented age-at-death

A known issue with documented skeletal collections is the variable reliability of "known" ages (Sharman and Albanese, 2018), as it is not uncommon for individuals to not know or inexactly report their own ages. Inaccuracies in reported age at death would directly affect the outcomes of this thesis, as discrepancies between documented chronological ages were assumed to be accurate when compared to physiological age estimations derived from skeletal and dental elements. Results across age group subsamples (foetal-infant, childhood, adolescence, and older adulthood) showed that statistically significant discrepancies between CA and PA were only found in adults older than 70 years of age. Although not significant statistically, discrepancies between CA and PA also existed in the foetal-infant sample, where CA was not well predicted by femoral length (due to growth disruption and possible population specificity) and pars basilaris measurements (due to population specificity). The magnitude of the discrepancy was not significant, but its existence raised important questions

about the accuracy of age estimation methods in this population. Nevertheless, higher accuracy of osteological age assessment in immature individuals was to be expected, as age estimations in non-adults depend on growth and development, which is highly predictable and, particularly for dental development, greatly resistant to environmental changes (Cardoso, 2007, 2009; Lewis, 2007). In contrast, estimation of skeletal age in adults depends on interpreting morphological changes associated with bone degeneration rates, which results in increasingly larger age ranges and greater inaccuracy the longer an individual lives (Buckberry, 2015). While there are reasonably grounds to assume that inconsistency between CA and PA are due to issues with methods of calculating PA, inaccuracies in CA affecting older adults in this population might also be expected from a historical point of view: mandatory identification was enforced by the Chilean government from 1930, and by 1943 all new-borns were required to be enrolled with the Civil Registry and Identification Service of Chile (SRCeI) within three days of the birth (Palacios, 2013). Hence, younger individuals within the COSS would have had their births more accurately recorded than older adults, whose date of births were potentially less accurate. Particularly in the countryside and low SES urban communities, it was common for births not being documented in the parish or town council for months, and even years, with dates of birth usually being recorded from memory alone (Palacios, 2013). Thus, it is possible that some ages have been misreported in cemetery records, dependant from the burial authorisation certificate issued by the SRCeI. Nevertheless, the impact of this issue on the present study are expected to be minimal as errors in reported CA are likely to be comparatively small compared to errors in PA.

Although no statistically significant difference was found between CA and SA in non-adults, it is still important to point out that SA estimations are not without bias. Age estimation methods derived from clinical datasets, such as Maresh's (1970) dental development method, were devised using samples of modern, living non-adults, which might limit any comparison made with archaeological samples, where all individuals are by definition non-survivors (DeWitte and Stojanowski, 2015). To overcome this limitation when assessing growth disruption, this research employed the widest error ranges as the methods accounts for, producing age ranges where CA often fell within the lower and upper boundaries, thus predicting more accurately age-at-death (Hodson, 2018). However, other sources of bias arose when estimation age-at-death from skeletal elements, particularly when using measurements from the pars basilaris of the occipital (SA-P). Contrary to previous

studies that support the use of SA-P as a good estimator of age-at-death (Nagaoka, Kawakubo and Hirata, 2012; Thornton, Edkins and Hutchinson, 2020), SA-P results generated using methods by Scheuer and MacLaughlin-Black (1994), showed random over and under-estimation of age-at-death, suggesting that this skeletal element cannot be used to accurately predict CA in this South American sample. However, when analysing pars basilaris measurements using the regression formulae devised by Irurita and Alemán (2017) from a sample from Granada, Spain, results for this sample were highly accurate. This finding points out to the need of revise the population specificity of our methods to estimate the biological profile of an individual.

Ageing is a biological, chronological, sociological, psychological, and functional process (Gowland, 2007), its ordeal dependant on genetic factors and environmental influences that model the way in which each person experiences it. Age-related health outcomes are then linked to an individual's life history and responds to their sociocultural historical context: the way we experience ageing now is different to that in past populations. Estimating age accurately in older adults continues to be a difficult task in bioarchaeology and forensic anthropology, particularly because the areas used to estimate age-at-death are also anatomical areas that are prone to suffer pathological changes associated with ageing and physical activity. For example, Calce et al. (2018) investigated the association between osteoarthritis and age-at-death estimations, found that presence and severity of OA are correlated with less accurate age estimations using joints from the os coxa (i.e., pubic symphysis and auricular surface of the ilium) when analysing changes proposed in traditional literature (Lovejoy et al., 1985; Brooks and Suchey, 1990); instead, they propose the use of degenerative arthritic traits themselves to estimate age-at-death. However, as other studies have point out, OA is a pathological condition, not a normal part of the human aging process (Winburn and Stock, 2019). This is particularly important when studying palaeopathology, as age is a multifaceted phenomenon and skeletal age estimations should take into consideration contextual information about the individual and/or the population.

7.3.3 Expanding demographic questions

As outlined before, the COSS represents low SES individuals who lived in specific areas within Santiago during the late 19th and early 20th centuries. This assumption is

supported by information on cemetery documentation, where the address of the purchaser of the burial plot was recorded. Largely, this information points towards fairly socially homogeneous communities, historically associated with working class populations and economic migrants to the city. Thus, the definition of "local" people (Sharman and Albanese, 2018) in this thesis responds to a slightly wider region than just the city, as it encompasses economic migrants from Central Chile. Nevertheless, economic migrants were socially and ethnically akin to the urban working class, forming cohesive communities relatively soon after their arrival to the city (Cambiaso et al., 2001). Thus, the community where the COSS individuals lived was a fairly homogeneous one, providing an advantage when answering research questions about environmental challenges and frailty experience.

7.4 Understanding the life course through skeletal evidence of stress

Milner and Boldsen (2017, p.29) stated that "simple tallies of skeletal lesions are insufficient to gain an impression about the toll diseases and injuries took on human groups in the past. It is, nevertheless, essential to start with skeletal lesion frequencies. But by themselves, they tell us little about life experience". This research has actively tried not to rely on frequency and prevalence of skeletal stress markers, aiming to explore how their presence affected frailty experience in relation with pubertal timing, growth disruption, morbidity and mortality through other statistical techniques. In this endeavour, this thesis has found a complex pattern of pathological changes associated with a reduced health experience during life. The potential reasons behind this are discussed in the following sections.

7.4.1 Exploring the maternal-infant nexus in later life

Findings of growth disruption in foetal-infant individuals (Chapter 3) attest to the importance of understanding the unique biological connection between a mother and her child. Further, the findings on this thesis might extends our understanding of the maternal and infant stress experience presented by the findings in Chapter 3, by linking the consequences of early life stress on later life health experiences: findings in Chapter 5 suggest that a delay in pubertal timing was associated with frail females that died of chronic disease,

while findings in Chapters 4 and 6 show that cribra orbitalia (CO) was found to be associated with decreased survival for foetal-infant individuals (<1 year of age) and older adults (>60 years of age). Although it is impossible to assert the specific time in life when porous lesions seen in older adults were formed, it has been reported that CO can only be formed in early childhood if a non-adult individual was suffering from iron deficiency anaemia (Brickley, 2018). Thus, it could be proposed that greater mortality risk associated with presence of CO in this population could be showing two groups of non-survivors facing the same health assault: those who could not withstand it, and those resilient enough to survive. Because this scenario would mean nutritional deficiencies were occurring at the very early stages of life, older adulthood resilience in this population could be linked to the maternal input into immunity ontogeny.

Human immunity is a highly complex system, and outside the scope of the present thesis to present in detail. In brief, it is recognised that the human immune system is made up of innate (non-specific) and adaptive (specialised) immunities; these two fundamental lines of defence differ on how they approach an intruding pathogen: innate response is fast and non-specific but does not have immunologic memory, while adaptive immunity is antigen-dependant and antigen-specific, requiring environmental influences to develop and specialise (Marshall et al., 2018). Both innate and adaptive immunities work together in the adult immune response, but their roles vary during the life course. Although it has been proposed that variation in human immune systems is largely driven by non-heritable influences (Brodin et al., 2015), environmental exposure only starts to play a significant role in the immune response after 5 years of age, when thymic output increases, surpassing maternally derived immune mediators (antibodies), and the contribution of commensal microbiomes, environmental microbes, and new food antigens is fully incorporated into the immature immune system (Kollmann et al., 2017). As the immune system gradually matures during childhood, non-heritable influences replace the antibodies transferred from the mother *intra utero* (transplacentally) and during breastfeeding as functional units of immunity (Simon, Hollander and McMichael, 2015). Iron, in addition to several other micronutrients, plays an essential role in supporting this period of immune maturation and metabolism, promoting growth and inducing differentiation of immune cells (Erickson, Medina and Hubbard, 2000). Some studies have found that iron deficiency anaemia negatively influences non-specific (innate) immune response in otherwise healthy patients

(Ekiz et al., 2005; Hassan et al., 2016), linking pathological issues related to dietary deficiencies of iron to early childhood immune responses. This might explain the elevated risk of death found in foetal-infant individual with presence of CO. In addition, under this assumption, decreased survival in older adulthood could be highly linked to maternal buffering and the very earlier stages of immunity, where maternal antibodies are the key players in the immature immune system (Kollmann et al., 2017).

However impossible it is to ascribe a specific timing to CO lesions, this scenario would be in line with the increasing epidemiological and experimental evidence evidencing that environmental exposure influences health not only postnatally, but also during gestation (Santurtún, Riancho and Riancho, 2019). Maturation of the immune system is a highly energy draining task, as acquired (adaptive) immune responses must be learn by the body, gaining insights into infections and mutualistic parasites (McDade, 2003). For those at the margins of nutritional adequacy, as low SES communities often are, the energetic cost is even more considerable, while health conditions associated with infections might disrupt nutrient's digestion and absorption, increasing the cost of already depleted energy reserves (McDade, 2005). Maternally transmitted immunity, then, proves to be an evolutionary advantage, "borrowing" immunity for the foetus and infant from intrauterine life to the first months after birth through breastfeeding; transplacental transfer of antigens from mother to child occurs during the final trimester of pregnancy and offers "passive immunity" to babies for around 6 months after delivery (Miller, 2020). In this scenario, research has shown that undernutrition in mothers during pregnancy and breastfeeding has a severe impact on the immunity of their offspring, decreasing long-term immune investment that can last into adolescence and adulthood (McDade, 2005). More significantly for the findings in this thesis, intrauterine undernutrition is also associated with differential allocation of resources within the immune response, particularly during early life: in a situation of physiological stress, the immune system will reroute resources away from energetically costly processes (such as the specific immune defences that are being acquire), prioritising less costly nonspecific defences related to the innate immunity, such as inflammation and the acute phase response (McDade, 2003). These changes in resource allocation mean that at birth a child from an undernourished mother will see their immune response maturation impaired, and if undernutrition continues postnatally, even a "reinforced" innate immunity will not be able to cope with health assaults in an optimal way during childhood (Weyand and Goronzy,

2016). For certain health assaults, such as iron-deficiency anaemia in childhood, an impaired immune response can lead to skeletal changes visible even into adulthood. It is possible, therefore, to propose that the older adult sample in this research, where CO presence was associated with decreased survival and likely representing first generation economic migrants, saw their intrauterine life, infancy and childhood influenced by poverty, malnutrition, and migration, with their immune response forever altered by physiological constraints in those early stages of life. These findings and potential explanations are particularly valuable as they support the importance of exploring the maternal-infant nexus in bioarchaeological studies.

As growth and development is heterochronic (i.e., different anatomical elements develop at different rates), pathological changes related to physiological stress develop during specific times within the life course (Lewis, 2017). Growth and development rate also varies throughout childhood, with growth spurts happening at different times by different reasons (Bogin, 2020). Any disruption of somatic homeostasis brought by changes in environmental factors can affect the pattern of pathological lesions seen in skeletal remains: an improvement in environmental conditions can shield the individuals from stressors, while the opposite scenario might increase their exposure to physiological stress (Goodman and Armelagos, 1989). In this sample of foetal-infant individuals, macroscopically evident pathological changes might be related to their particular bioarchaeological status: as non-survivors, these individuals would have died either *intra utero* or in the year after birth as a consequence of their frailty. These individuals do not represent the "normal" population (Wood et al., 1992), and although most conditions do not leave a trace on the skeleton (Goodman and Armelagos, 1989), the findings in this thesis suggest that during their short lives their bodies were subjected to chronic levels of physiological stress, hence presence of skeletal lesions should not be unexpected (Roberts and Manchester, 2010). In cases of physiological constraints (i.e., limits placed on adaptive plasticity through differential modulation of energy to competing processes), the body will focus on survival by exercising trade-offs in energetic allocation that reduce investments in growth to maintain essential tissue function. However, this process must accommodate the fast and continuous growth experienced during early life, prioritising which anatomical areas should maintain growth: the most important organ for the allocation of such limited and precious nutritional resources is the brain, and with it the skull (Barker et al., 2012). Hence, bones from the cranial vault will continue to develop in their "normal" growth patterns even in the presence of physiological constraints. Early development of the

human skull starts within the first trimester *intra utero*, with ossification centres from the frontal bone appearing in membrane between the 6th and 7th week of prenatal life. The orbital plate, which will form the orbital roof, ossifies slower than the frontal plate (i.e., the forehead part of the frontal) but is present, although extremely slight and thin, by 13 gestational weeks (Scheuer and Black, 2004). All cranial bones are highly vascularised elements, harbouring hematopoietic (red) bone marrow until 6 to 10 years of age, when systematic conversion of red bone marrow to mixed and eventually yellow (fatty) bone marrow occurs in the skull (Brickley, 2018). The presence of red bone marrow in cranial bones is what relates the presence of porous lesions to anaemia, which presents as an expansion of red marrow to promote the production of red blood cells. Thus, if growth and development of cranial bones is prioritised even in the presence of physiological stress, the skull would be first skeletal area to be affected and macroscopically show pathological changes, such as porous lesions.

7.4.2 Bone and immune responses to physiological stress

Older adults, however, can be seen as "another kind" of non-survivors. They did die, but not before having lived longer lives than their contemporaries, suggesting greater resilience to early life insults. Greater mortality risk in older adulthood associated with presence of CO was discussed in light of the maternal-infant nexus in an earlier section, but how to interpret the additional association of osteoarthritis (OA) and bone loss (BLOS) with poorer survivorship in older adulthood, if these indicators respond to late life stressors? The answer might lie in the pathophysiology of molecular signalling mechanisms of bone response. Sex differentials were found associated with late life stressors, with OA negatively affecting survival in "mature" (i.e., between 40 and 60 years of age) females, and BLOS associated with increased risk of death in all senile individuals (i.e., older than 60 years of age), and the possible explanations for this difference were discussed in Chapter 6. However, in a broader sense, these findings might be suggesting that these individuals experienced differential responses from adaptive plasticity at some point during their lives.

Adaptive (developmental) plasticity "describes situations where a specific input during an individual's development produces a lasting alteration in phenotype" (Nettle and Bateson, 2015, p.1). As outlined before, both innate and acquired immunity can be

influenced by genetic and environmental inputs that produce differential responses, and the new field of osteoimmunology might offer understanding into how bone remodelling (which is the key function dysregulated in both OA and BLOS) might be affected by the immune response. In healthy individuals under normal homeostatic conditions, the skeletal system not only provides support, mobility, and protection, but it is also the mineral reservoir of calcium and phosphates, responding as an organic and active system in continuous remodelling (Currey, 2006). The dynamic balance between osteoblasts and osteoclasts is a strongly regulated equilibrium controlled by the communication through direct contact between cells or via secretory proteins between osteoblasts and osteoclasts; these communication routes are called signalling pathways, of which the RANK/RANKL/OPG pathways is a major one (Figure 7.1). Essentially, the integrity of bone is reliant upon the interplay between various molecular signalling factors within the paracrine system, which includes RANKL (i.e., receptor activator of nuclear factor κβ ligand), RANK (its receptor molecules on the surface of osteoclast precursor cells), and osteoprotegerin (OPG), a soluble inhibitor receptor protein for RANKL (Klaus, 2014). In this pathway, regulation of the bone remodelling cycle is given by mediators that induce either the protein-ligand RANKL or OPG, that acts as a decoy for RANKL; although RANKL can be produced by various types of cells such as osteoblasts, osteocytes and chondrocytes, only the osteocyte is capable of sensing changes in mechanical load and microdamage within the bone matrix, stimulating bone remodelling (Gosman, 2012). Then, when RANKL binds to its receptor RANK on an osteoclastic precursor cell, it signals further osteoclast differentiation, which ultimately results in an active osteoclast. Hence, the RANKL:OPG ratio is what actually determines the rate of osteoclastogenesis (osteoclast-mediated bone resorption) (Kenkre and Bassett, 2018).

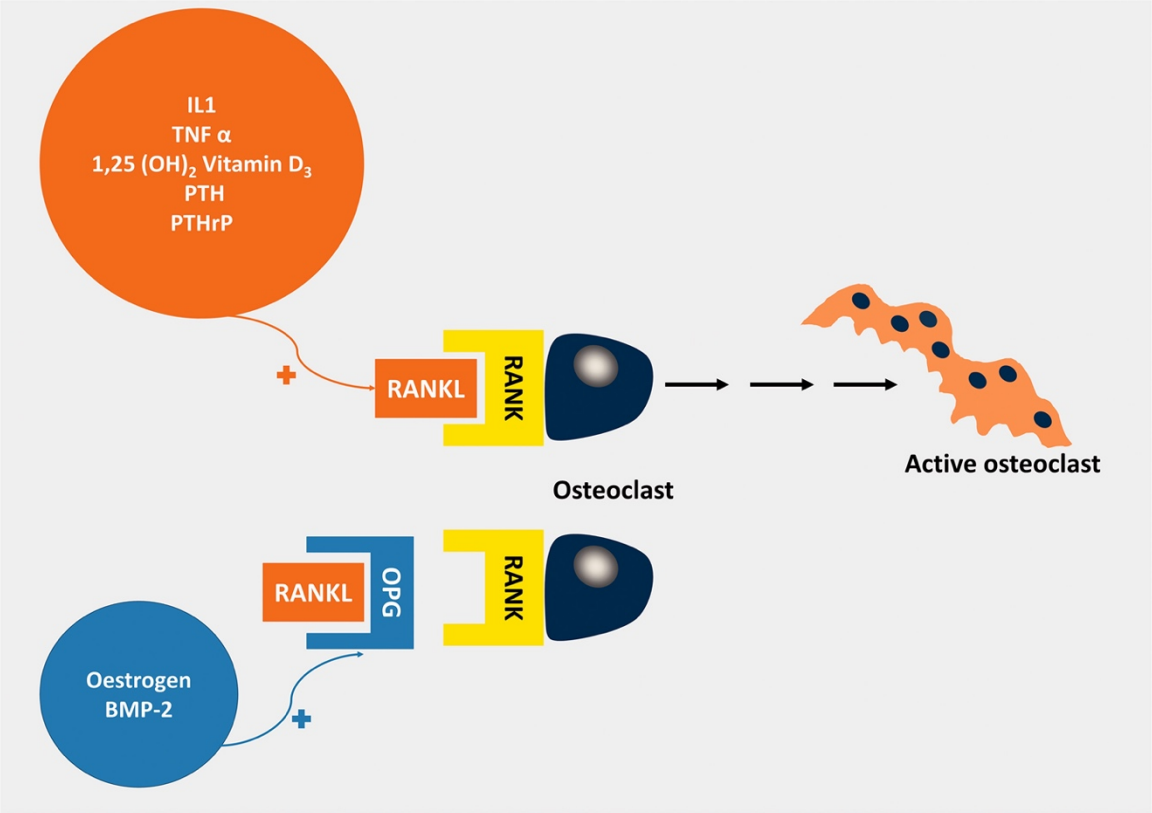

Figure 7.1 Factors that influence the RANK/RANKL/OPG signalling pathway of activation and suppression of bone remodelling. Osteoprotegerin (OPG) acts as a decoy receptor for RANKL, interfering with its binding to RANK (image from Kenkre and Bassett, 2018, p.315).

Some of the mediators of the remodelling bone cycle are also associated with the immune response. Interleukins (IL-s) are a group of cytokines protein made by leukocytes (white blood cells) and are highly involved in regulating the immune response; tumor necrosis factors (formerly called tumour necrosis factor alpha or TNFα) can act as cytokines and are involved in cell signalling within the immune system as part of the inflammatory response. BMP-2, a bone morphogenetic protein, is key in the development of bone and cartilage, but is also involved in cytokine-cytokine receptor interaction (Boyce and Xing, 2007). The involvement of RANK/RANKL/OPG pathway mediators in both osteoclastogenesis and immune response indicates there is extensive cross-talk between the immune and skeletal systems. Thus, dysregulation of the RANK/RANKL/OPG signalling pathway generates a mismanagement of the bone remodelling process that can cause pathological changes in the skeleton that can be observed macroscopically, such as BLOS and OA. Although having different aetiologies, both OA and BLOS are characterised by

abnormal bone resorption and have both seen to be linked to the immune system (Geusens and Lems, 2011; Jones, Glimcher and Aliprantis, 2011; Lopes et al., 2017).

In addition to OA and BLOS, other findings within this research might be related to the altered immune response and bone remodelling described. An increased mortality risk was found in non-adults during childhood (1 to 11 years of age) when presence of rickets was found in the skeleton. In addition to its key role in calcium homeostasis and bone metabolism, vitamin D is also involved in immunomodulation, having an immunoregulatory role in both innate and adaptive immune systems (O'Brien and Jackson, 2012). Vitamin D cholecalciferol (D3) is naturally synthesized in the skin and functions as a pro-hormone, being converted to calcitriol (1,25-dihydroxyvitamin D), an active metabolite that acts as a mediator to produce RANKL in the RANK/RANKL/OPG pathway (Boyce and Xing, 2007). Deficiency of vitamin D in non-adults can cause rickets, while also affecting the immune function. Interestingly, foetal intrauterine growth restriction and persistence of increased fragility and higher incidence of autoimmune disease in children has been linked to vitamin D deficiency during pregnancy (Zhang et al., 2022).

Consistent with this, findings in Chapter 5 points toward a frailty phenotype associated with chronic disease as the potential cause of pubertal delay in this sample. Adolescence is already a biologically and mentally demanding phase of the life course, with an increase in the demand for resources during the pubertal growth spurt triggering trade-off dynamics between the immune system and physiological development (McDade, 2003). In addition to that, chronic disease and frailty cause the activation of the immune system and increase the secretion of pro-inflammatory cytokines (Gerasimidis, McGrogan and Edwards, 2011), which might result in compromised skeletal growth and development.

The findings in this thesis suggests the relationship between skeletal and immune systems in this population can be potentially seen in the interplay between maternal passive immunity, childhood rickets, and altered bone resorption in older adulthood, but further research is needed. The complexity of the interaction between bone and immune responses is the focus of ongoing research, meaning there are still many unanswered questions. This overview of potential ties between both processes has shown a direction for future research, advances aiming to analyse the extent of the influence of physiological stress on the interplay between immunity and skeletal pathological changes.

7.4.3 Intergenerational impact of physiological constraints

This research is part of a larger trend in bioarchaeology that in the last decade has used life course approaches to explore the changes in bone morphology and development. Looking at both population and individual level data, the research chapters have used multiple indicators of early and later life stress to explore the life experience of this skeletal collection, with each research chapter focused on a life stage exploring the critical differences in the body's response to physiological stress depending on the age of the individual. Nonetheless, it is possible to explore how the factors associated with risk of death and frailty were affecting the population's mortality and morbidity as a whole. The fact that the majority of the individuals in this skeletal collection have documented year of death, and that those without documentation on the matter must have died some time closer to the rest of the population due to years of use for each cemetery burial area, gives us a very good idea on their historical background. In addition, the use of the cheapest (and in some cases free) short-term graves hints to their SES prior to their deaths. This allows us to circumscribe their life experience to a particular sociocultural context: that of the most impoverished communities of Santiago, living during or right after the rural-to-urban migration, facing social and economic changes brought by the industrialisation of the country and the rapid urbanisation of the capital. In combination with the bioarchaeological evidence gathered throughout this research, their deaths inform us much about their lives and their individual frailty, but also about the risk factors associated with living in low SES communities in a time of social and economic change.

Previous sections have discussed the bioarchaeological evidence presented in this thesis through the lens of the short and long-term impact of physiological stress in skeletal and immune responses. Following this premise, and incorporating the historical documentation available for this population, it is logical to question if the responses to the physiological constraints affecting these individuals through different stages of the life course can be rooted in previous generations' health experiences. Because of the epidemiological studies that originated the DOHaD framework, medical and biological research is now fully aware of the relationship between early life experiences and the factors that can lead to the development of diseases in adulthood (Barker, 2012). Currently, it is well established that pre- and perinatal exposure to detrimental environmental factors such as undernutrition,

physiological and psychological stress, lower socioeconomic status, among others, has been linked to several adult health outcomes, emphasising the interplay between environmental, genetics, and epigenetics causes of adult chronic non-communicable diseases such as cardiovascular disease, diabetes, and cancer (Thorsell and Nätt, 2016). With disease susceptibility in adulthood highly influenced by environmental exposures during early life, developmental plasticity reflects the individual's epigenetic reshaping of cells, tissues, organs and systems of the body during critical periods of growth and development (Bateson et al., 2004). Critical windows of development have been recognised at different stages of prenatal and infant life, and although physiological constraints during these stages can be compensated by improvements in nutrition later in development, such compensation comes with a cost later in life, as the body's energetic resources were diverted from growth and development to repair and survival (Gluckman et al., 2008). Miller (2020) identified the key association between breast milk and the immune function of mother and child, pointing out that while maternal constraints such as birth mode (vaginal or caesarean deliveries) and breastfeeding/weaning practices highly impact the foetal and perinatal adaptive responses, it also allows for passive immunity to protect the neonate during critical windows of immune development. Maternally transmitted antigens in breastmilk then act by "immunological imprinting" on the child, not only offering them an umbrella of immunity while innate and acquire immune systems mature, but also transmitting the mother's immune phenotype, reflecting her acquire environmental experience (Figure 7.2). In fact, the process of passive immunity transmission via breastmilk outlined by Miller (2020) proposes maternally-guided ecology-specific immune profiles that are shared intergenerationally via pregnancy and breastfeeding, that permeates the immune phenotype and response (including cytokine activity) of ancestors and descendants.

If Miller's (2020) model is correct, physiological constraints that influence the immune response and shape the immune phenotype can have an intergenerational effect, while at the same time maintaining immune flexibility to new encountered environmental pathogens. Hence, it could conceivably be hypothesised that ancestral changes in immune response throughout life will also affect the skeletal response to physiological stress in their descendants, as immune and skeletal responses are highly linked. Although intergenerational cycles are known in social sciences (e.g., intergenerational poverty), the impact of biologically mediated theoretical links between generations is yet to be more widely considered on

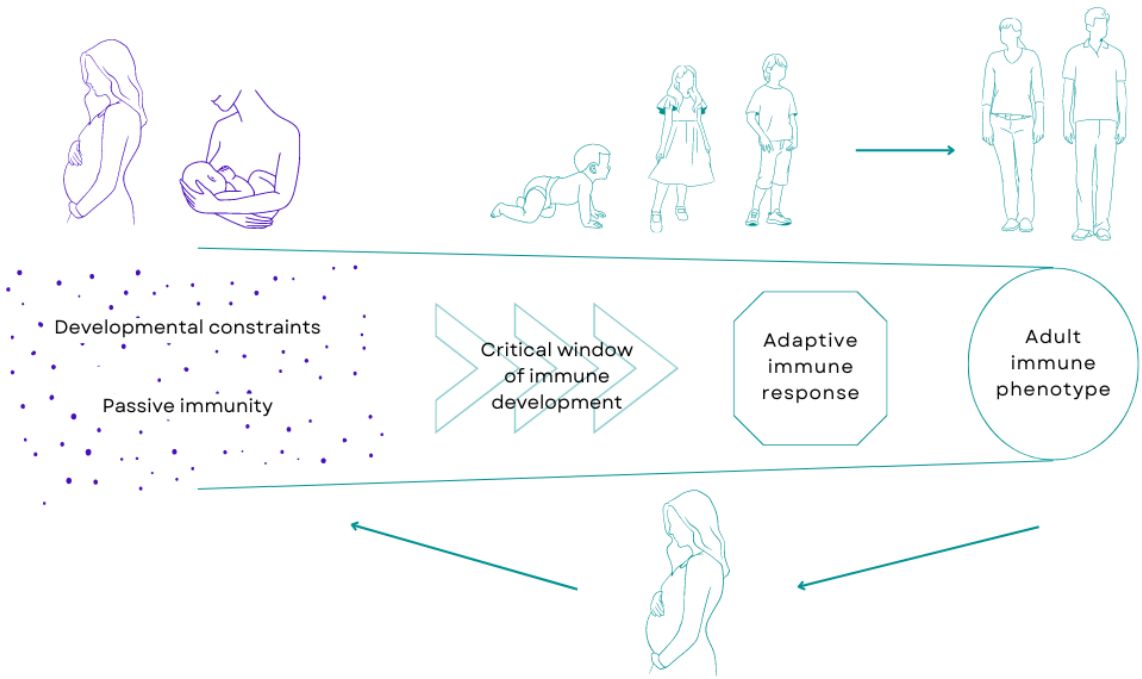

Figure 7.2 Intergenerationally transmitted adult immune phenotype via pregnancy and breast milk theoretical model. Maternal interplay of developmental constraints and passive immunity during critical windows of development results in adaptive immune responses in the child, which in turn shapes their adult immune phenotype. When the child reaches maturity and if she becomes pregnant, their immune phenotype is passed to the next generation (adapted from Miller, 2020, p.93).

bioarchaeological studies. Currently, palaeopathological research recognises the existence of skeletal indicators of physiological stress as part of the life history of an individual, acknowledging that early life assaults can affect adult health outcomes; it also identifies the need to understand skeletal changes as adaptable and non-stationary, with bone responses varying during the life course. To this approach, it might be necessary to incorporate an intergenerational approach to skeletal responses to physiological constraints. Such strong biocultural contextualisation is needed to analyse bioarchaeological evidence in an effort to reduce the uncertain issues of hidden heterogeneity and its effects on selective mortality (DeWitte and Stojanowski, 2015).

A "maternally-guided ecology-specific immune phenotype" that is transmitted intergenerationally via transplacental exchange and breastfeeding (Miller, 2020) supports the idea of physiological stress responses associated with environmental factors affecting the mother being transferred to the next generation through pregnancy and breastfeeding. Of such environmental factors, the most obvious one to be considered in detail for this

population is socioeconomic status; throughout epidemiological research, a persistent finding is that health outcomes are associated with social factors, and individuals who occupy lower SES positions within society tend to bear a greater burden of disease throughout their lives (McCrory et al., 2019). Research has also shown that there is a significant association between delays in bone growth and development and low SES (Alves-Cardoso and Campanacho, 2022); this correlates with findings in Chapters 3 and 5, where growth and pubertal delays were found. Further, an intergenerational immune phenotype associated with the endurance of poor living conditions and economic migration might be then related to the skeletal response to physiological stress of non-adults and older adults in this population. This might be reflecting the existence of structural inequalities that affect bone quality and development and impacted their health outcomes.

Recognising the coexistence of multiple intersectional dimensions within each individual (e.g., gender, age, SES) and the possibility of an intergenerational biological baggage that affects bone and immune responses in later life, challenges the bioarchaeological analysis to explore how response to physiological stress is mediated by more than just the health of an individual, but encompassing intergenerational health experiences. Finally, as Mant, de la Cova and Brickley (2021, p.583) so clearly state, "complicating, problematizing, and questioning what we think we know about past lives demonstrates our respect to the individuals whose remains we have the privilege of studying and our commitment as a field to social justice". It also expresses our respect and understanding for the living population where the individuals whose remains we study once lived and their descendants today; for the individuals in the COSS, this means a renewed appreciation of the lifeways and frailty experience of the population of Santiago de Chile during the 19th and 20th centuries.

7.5 Concluding remarks

Bioarchaeologists investigate the health and well-being of past populations by analysing skeletal and dental remains. Through these analyses, it is possible to reconstruct the physiological responses to various stressors, such as malnutrition, disease, and social inequality. However, to fully understand the complex interplay between biological stress and disease, it is necessary to incorporate cross-disciplinary perspectives that draw from multiple

fields of study. By integrating different methods and theories, this thesis has provided fresh insights into the social and environmental factors that contributed to health outcomes in the population of Santiago de Chile during the late 19th and early 20th centuries. By exploring the ways in which human skeletal variation responds to physiological stress and disease, this thesis has aimed to expand current understanding of this particular population, while also exploring broader themes in bioarchaeology. Specifically, two main lines of investigation have been incorporated into the discussion of this thesis findings: the pathophysiology of molecular signalling mechanisms and the new field of osteoimmunology. Through an examination of these concepts, a more precise understanding of pathological phenotypes, disease aetiology, and the significance of skeletal lesions has been outlined, enhancing our understanding of how pathological phenotypes in bone are formed with greater precision.

In the recent past, bioarchaeology has benefit from incorporating new disciplinary insights from across the natural and social sciences, involving a deeper understanding of the association and interplay of human biology, social theory, and identities intersections (Bauer, 2014; Klaus, 2014; Yaussy, 2019). The concept of biological (physiological) stress, firstly introduced by Selye (1959, 1976) more than sixty years ago within the biomedical field, has proven to be of great use within bioarchaeology, and a driving concept within this thesis. Understanding of biological dynamics of homeostasis, resource allocation and allostatic load, paired with the social aspects of behaviour and environmental influences, has allowed the study of past populations, the COSS included, through a richer lens. Moreover, the use of theoretical frameworks with influences from medicine, epidemiology, ecology, sociology, and psychology (such as LCT and DOHaD theoretical frameworks) has driven the interpretation of bioarchaeological evidence towards the understanding on how the cumulative burden of environmental challenges, from past generations health experiences, gestation, and life events, resonate on bone responses to physiological stress. By combining new approaches, bioarchaeologists have gained insights into the long-term health consequences of social and environmental stressors in the past, investigating the impact of historical events and biological life milestones on the health and well-being of marginalised populations (Marklein, Leahy and Crews, 2016; McCrory et al., 2019; Garland, 2020; Mathena-Allen and Zuckerman, 2020; Avery et al., 2023).

This thesis has emphasised the importance of considering the cumulative effects of chronic stress – since before birth and throughout life – on multiple physiological systems. Of particularly importance, the study of allostatic load of individuals from different social and economic backgrounds has allowed to investigate the health inequalities that existed in the past and the ways in which social and environmental factors contributed to these disparities (McCrory et al., 2019).

This thesis lays the groundwork for future research into long-term effects of chronic stress on human health and well-being in the COSS population, and for investigating the social and environmental factors that contributed to these health outcomes. Undoubtedly, more research is needed to expand the findings of this thesis and the proposed mechanisms by which physiological stress affects bone responses. The remainder section will address limitations of this study, some recommendations for future research work, and a final conclusion of the thesis.

7.5.1 Limitations of this study

The majority of the limitations faced by this research were a direct result of the worldwide response to the COVID-19 pandemic. Outlined briefly in Chapter 2, restrictions in international travel meant a significant delay and, eventually, a reduced period of data collection for this thesis, which reduced the amount and variety of information that was collected during fieldworks, after restrictions were partially lifted. Originally, the project hoped to examine all COSS individuals with DAD, using a wider array of methodologies, including age-at-death estimations using different methods, in depth palaeopathological analyses with recording of presence/absence and severity, and potential use of isotopic analyses to study migration patterns within the population. Changes due to the pandemic meant that to gain a deeper understanding of how this population lifeways were impacted by frailty experiences during the life course, only non-adults and older adults were studied directly by the researcher. This, however, allowed this research to gain a deeper and richer understanding of two less studied life stages. In addition, methods were drastically changed, with some analyses made relying on age estimations retrieved from the UChile database, particularly for older adults. Although this might introduce some restrictions to the results, it is important to note that effect size analyses on the discrepancies between CA and SA

were only found significant for adults older than 70 years of age, which is to be expected as a fault of the age estimations methods themselves, and not on the researchers who made said estimations.

7.5.2 Future research

Areas for future research can capitalise directly on the availability of DAD for this population, extending the value of this data for bioarchaeological as well as forensic studies. Three themes of further research were identified during the course of this research but were not pursued as they were outside the scope of this project and/or due to pandemic-related time restrictions. Future directions within these themes could benefit from analyses beyond macroscopic research, incorporating other areas of study to expand the findings of this thesis.

Examination of prenatal and birth experiences.

Further study of the foetal-infant individuals in the COSS could help elucidate how the first stages of life *intra utero* and right after birth are affected by maternal buffering and environmental challenges. Research questions and potential future research within this theme are as follows:

- Is it possible to study maternal physiology and diet during late foetal and early infant life using collagen from high-resolution dentine sections of foetal-infant teeth?

  Childhood dentine collagen $\delta^{13}C$ and $\delta^{15}N$ profiles have been used to study breastfeeding and weaning (Beaumont et al., 2015), and could be also used to study the relationship between maternal diet and physiology. Results might reflect ill or malnourished mother and/or breastfeeding signalling in dentine. This study, however, involves destructive analyses and might not be suitable for a small collection of foetal-infant individuals, such as the COSS.
- Can non-destructive histological assessment of micro-CT scans differentiate between stillborn and short-lived infants from this population?

  Analyses of bone micro-CT led by Booth, Redfern and Gowland (2016) suggest that it is possible to use bone diagenesis and bacterial bioerosion to discern between perinates (<1 month old) and older infants, due to stillborn and

perinates dying before the full development of osteolytic gut bacteria within their bodies. This study could be replicated and expanded analysing documented preterm and perinate individuals from the COSS.

- Is there evidence of sexually dimorphic growth strategies and/or growth disruption on osteometric measurements of long bones?

  Long bone measurements have been used to assess intra-limb growth prioritisation and arrest variation in relation with growth disruption (Hodson, 2021). A study on the differences in asymmetrical growth and variations in allometry between sexes, analysing all long bones (humerus, radius, ulna, femur, tibia, and fibula), could help identify disproportional growth within and between anatomical elements, helping with interpretations on stress exposure.

New explorations into older adulthood.

- Can non-traditional methods (besides changes on pubic symphysis and auricular surface of the ilium) generate more accurate age-at-death estimates for older adults?

  Estimations using the degeneration of sternal end of the clavicle have been found to better predict age-at-death in mature adults (Falys and Prangle, 2015), while regression models derived from histomorphometry analyses have found to be more accurate for individuals over 50 years old, although they seem to be highly population specific (Karydi et al., 2022). Taking advantage of this sample's DAD, these techniques could be used to test the accuracy of estimations of skeletal age in contrast with chronological age in older adults.

Rural versus urban origins.

- Is it possible to explore economic rural to urban migration in this sample using human $\delta^{18}O$ results? If so, how did the living experience of growing up in rural or urban communities in Central Chile during early 20th century affect frailty and mortality risk?

  Studies have found differences in patterns of physiological stress and survival between urban and rural populations (Redfern et al., 2015). Using oxygen isotopic analysis technique to identify migrants and, potentially, their origin

geographical location (Lightfoot and O'Connell, 2016), could help understand intra-sample variation and migration processes in this population. Frailty and mortality risk in the COSS could be compared to samples representing rural populations during the period, such as the Rinconada collection (Maipú, Región Metropolitana), housed at the National History Museum, Chile.

7.5.3 Conclusion

By addressing traditional bioarchaeological questions through cross-disciplinary approaches using individuals with DAD, this thesis has added to the expanding body of research focused on marginalised populations in the field of bioarchaeology. The late 19th and early 20th centuries marked a period of significant transformations in Chilean history, encompassing profound changes in both the physical landscape and the society itself. Although historical research has explored economic migration and poverty across various geographic and social contexts, bioarchaeological investigation of this subject remains relatively unexplored in the country.

By examining under-represented groups, this thesis has expanded our understanding of how frailty experiences shaped the lifeways of *santiaguinos* during this era. The flourishing development of the nation, with steady increments in population size and wealth opportunities, did not permeate all levels of society, leaving behind the lower classes. The COSS individuals reflect how those early life stressors, passed from mother to child and carried through life into old age, had a significant impact on both the immediate health and survival of children, and the health outcomes of adults. Historical records show low SES communities in Santiago struggled to thrive in such poor living conditions, with bioarchaeological evidence gathered by this thesis supporting this scenario: a population that faced environmental challenges beyond their social and biological ability to cope, with ensuing allostatic overloads that rendered their survival a highly difficult task. Given the current world situation, studies conducted on past populations can offer significant and vital insights into the long-term consequences of social inequality for future generations.

From differentials in biological factors (e.g., hormonal levels and developmental stages related to biological sex and chronological age) to behavioural aspects of an individual

within its society (e.g., adolescence and senescence experiences), several issues do not have to be estimated when working with documented skeletal collections. From a life course perspective, this has allowed the research to contextualise each individual of the COSS within their own potential life experience, with a particular scenario of biological and environmental challenges and problematics. By exploring childhood and older adulthood stages within the life course, this thesis also allowed to contrast the frailty experiences of two different subsets of non-survivors: those who died in childhood, unable to overcome health insults in early life, and those who died later in life, having been resilient enough to surmount assaults to their health and live on to die in old age. The different skeletal indicators of stress associated with growth disruption or mortality risk in each age group can serve as a window into their health experience, which is permeated by their identity within the society. Thereby, the analysis of this skeletal collection offered a way to further advance our knowledge of the life experience of those usually forgotten or underrepresented by history, as the working classes were and still are.

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Appendix

Supporting documents

This appendix includes all recording forms used during data collection, as well as copyright licenses needed for the use of images within this thesis.

For all recording forms, mandatory antemortem information includes UChile database's skeleton ID number, chronological age-at-death, and biological sex. Years of birth (YoB) and death (YoD) and cause of death (CoD) were recorded only when available. The three variances of the recording form each contain recording of pathological indicators and skeletal diagrams relevant to each age group.

Documents, in order of appearance, are as follows:

- **A.1 Recording forms**
    - **Recording form · Foetal-infant** – To be used in all individuals up to 1 year of age. It includes a section on age estimation using pars basilaris measurements and dental development, and a section on postcranial measurements.
    - **Recording form · Non-adult** – To be used in all individuals between 1 and 21 years of age. It includes a section on assessment of pubertal stages, to be used in conjunction with the visual aid chart developed for this study. Said section was only filled in when individuals were between 8 and 21 years of age.
    - **Recording form · Adult** – To be used in all individuals older than 40 years of age.
- **A.2 Copyright licenses**

A.1 Recording forms

The University Of Sheffield.

PhD research project · Ofelia Meza-Escobar
Supervisor: Dr Elizabeth Craig-Atkins

RECORDING FORM · FOETAL-INFANT

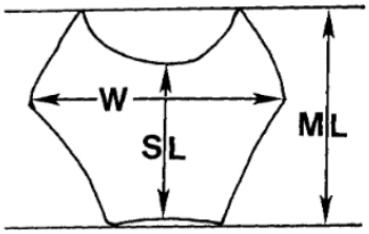
INFORMATION			
ID UChile		ID	
Sex		Age	
YoB/YoD		CoD	

AGE ESTIMATION

Mark measurements taken from lightly eroded or reconstructed bone with an asterisk (*).
Measurements should be undertaken twice to determine intra-observer error.

SKELETAL - Pars basilaris (mm)
(Scheuer and MacLaughlin-Black, 1994)

Maximum length (ML)		
Sagittal length (SL)		
Maximum width (MW)		



Skeletal age

RIGHT tooth is preferred, any exception will be marked 'L'.

DENTAL – Tooth development and eruption
(AlQahtani et al., 2010)

Tooth	Tooth formation stage		Tooth	Tooth formation stage	
i^1			i_1		
i^2			i_2		
$c^{'}$			$c_,$		
m^1			m_1		
m^2			m_2		
I^1			I_1		
I^2			I_2		
$C^{'}$			$C_,$		
M^1			M_1		

Dental age

POSTCRANIAL MEASUREMENTS

LEFT bone is preferred, any exception will be marked 'R'.
Mark measurements taken from lightly eroded or reconstructed bone with an asterisk (*).
Measurements should be undertaken twice to determine intra-observer error.

Humerus	**mm**		**Ulna**	**mm**	
Maximum length			Maximum length		
Head – vertical diameter			Physiological length		
Shaft – min circumference			Minimum circumference		

Femur	**mm**		**Tibia**	**mm**	
Maximum length			Maximum length		
Bicondylar length			Circumference at nutrient foramen		
Midshaft circumference			Length		

PATHOLOGY

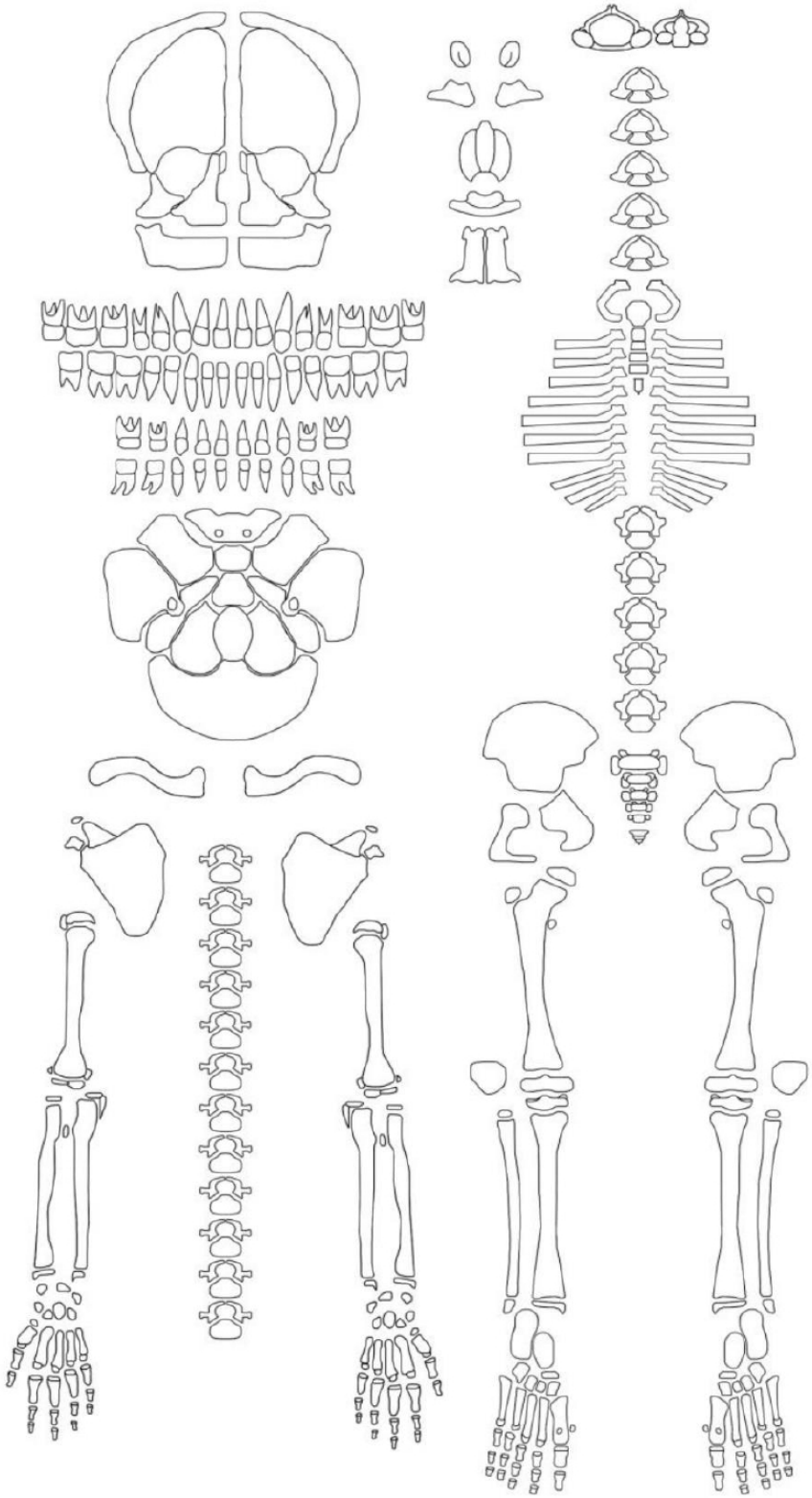
Record presence/absence with ✓; if present, record location and severity (severe, moderate, mild).

Frailty/stress markers				
Cribra orbitalia	Cribra femora	Periosteal reaction	Linear enamel hypoplasia	New bone formation

Cranium					
Porosity/NBF on sphenoid	Porosity/NBF on maxillae	Porosity/NBF on mandible	Porosity/NBF in orbits	Porosity/NBF on cranial vault	Medial angulation of mandibular ramus

Ribs			**Dentition**		**Scapulae**
Alteration of neck angle	Lateral straightening	Flaring/swelling of rib ends	LEH (general)	Caries	Porosity/NBF on infra- and supra-spinous regions

Long bones					
Flaring/swelling of metaphyses	Porosity of growth plate	Cupping deformities	Bowing	Thickening	Coxa vara



COMMENTS

RECORDING FORM · NON-ADULT

INFORMATION			
ID UChile		ID	
Sex		Age	
YoB/YoD		CoD	

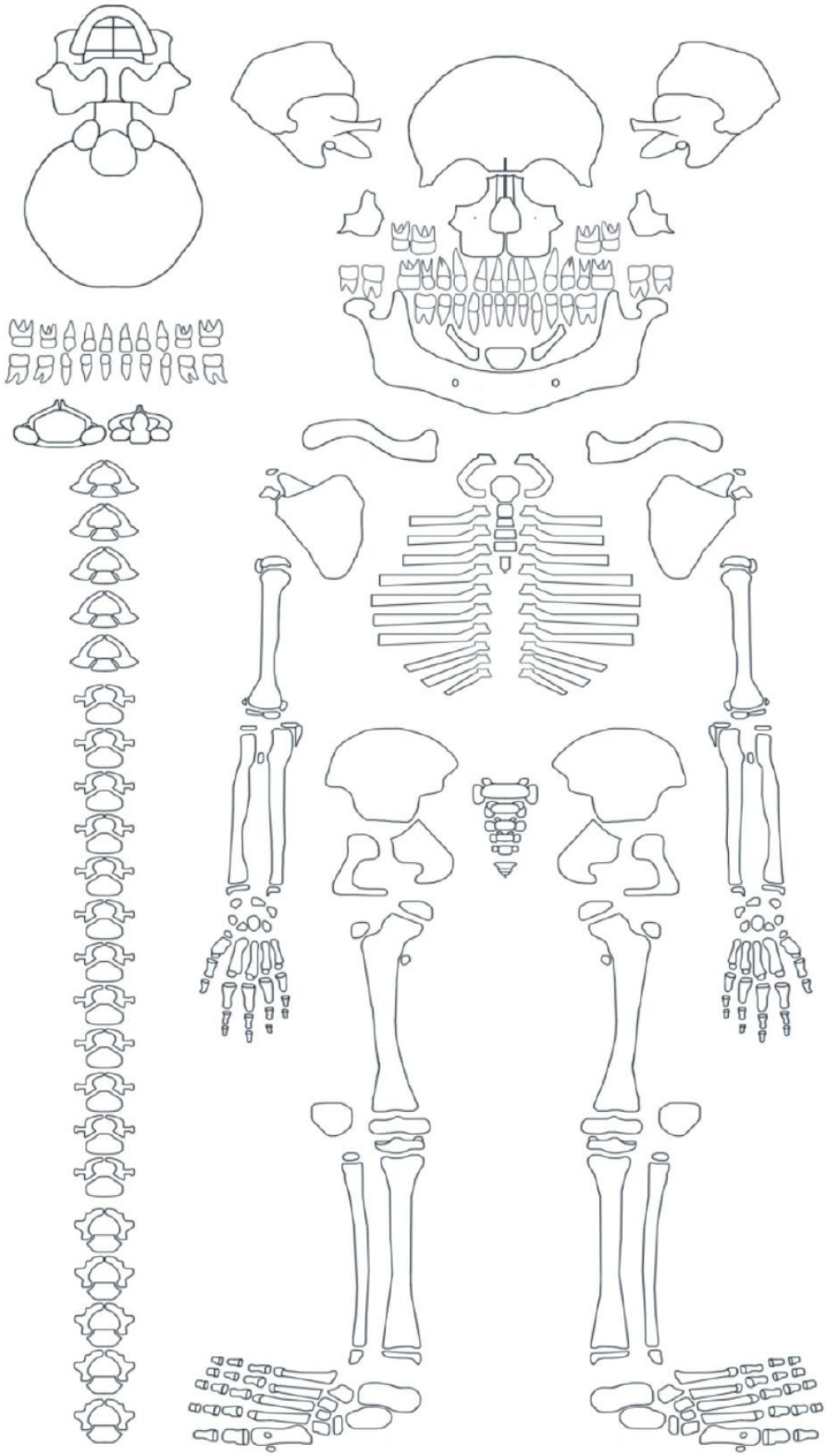
PUBERTY ASSESSMENT Assign pubertal stage when 3 or more features are in accordance (Shapland and Lewis 2013, 2014; Lewis, Shapland and Watts, 2016)			
Circle development stage	Hamate development G H H.5 I nr		
	Canine root development E F G H nr		
1: Unfused *2: Fusing* *3: Complete* *nr: not recorded* ** e: equal width* ** c: capping*	Proximal phalanges*	Middle phalanges*	Distal phalanges*
	MC1*	MC 2-5*	Distal radius
	Proximal ulna	Capitulum of humerus	Iliac crest
Yes/No	Iliac crest ossified but unfused epiphysis?		
Stage 1-6	C3	C4	C5
Pubertal stage			

PATHOLOGY Record presence/absence with ✓; if present, record location and severity (severe, moderate, mild).				
Frailty/stress markers				
Cribra orbitalia	Cribra femora	Periosteal reaction	Linear enamel hypoplasia	New bone formation

Cranium					
Porosity/NBF onsphenoid	Porosity/NBF onmaxillae	Porosity/NBF on mandible	Porosity/NBF inorbits	Porosity/NBF oncranial vault	Medial angulationof mandibular ramus

Ribs			Dentition		Scapulae
Alteration of neckangle	Lateral straightening	Flaring/swelling of rib ends	LEH (general)	Caries	Porosity/NBF oninfra- and supra-spinous regions

Long bones					
Flaring/swellingof metaphyses	Porosity of growth plate	Cupping deformities	Bowing	Thickening	Coxa vara



COMMENTS

RECORDING FORM · ADULT

INFORMATION			
ID UChile		ID	
Sex		Age	
YoB/YoD		CoD	

PATHOLOGY
Record presence/absence with ✓; if present, record location and severity (severe, moderate, mild).

Frailty/stress markers				
Cribra orbitalia	Cribra femora	Periosteal reaction	Linear enamel hypoplasia	New bone formation

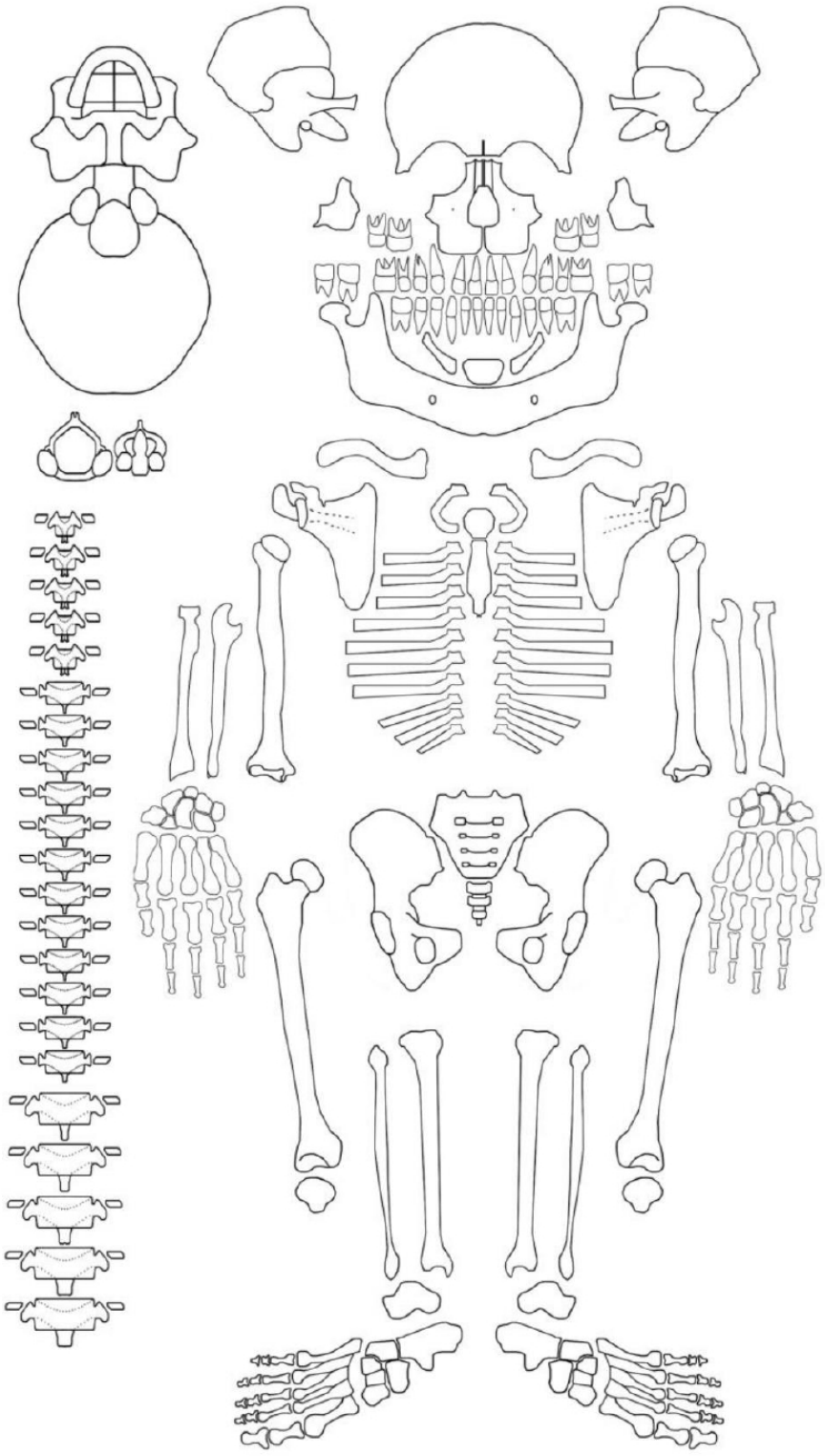
Cranium					
Porosity/NBF on sphenoid	Porosity/NBF on maxillae	Porosity/NBF on mandible	Porosity/NBF in orbits	Porosity/NBF on cranial vault	Thickened vault (particularly frontal)

Ribs					
Alteration of neck angle	Lateral straightening	Flaring/swelling of rib ends	Ankylosis costovertebral and costosternal	Sternum protrusion and rib angulation	Transverse fractures costosternal end

Pelvis					Vertebrae
Lateral narrowing	Pubic symphysis bulging	Acetabulae pushed dorsally into pelvic cavity	Angulation/bending sacrum	Curvature/abnormal shape of ilia	Kyphosis or scoliosis (T9-L3)

Long bones					
Flaring/swelling of metaphyses	Porosity of growth plate	Bending (*genu varum*), legs over arms	Bowing	Thickening	More angulation of femoral neck (*coxa vara*)
NBF (common towards ends)	Pumice-stone-like quality to surface	Pathological fractures	Angulation of knees (knock-knees)	Medio-lateral widening proximal femora (sub-trochanteric)	Cupping deformities

COMMENTS
Particularly TRAUMA (aetiology, location, healing stage, etc)



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