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Skeletal Indicators of Early Life Stress:
Insights into cribra orbitalia and porotic hyperostosis in a living subsistence population

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in Anthropology

by

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"…ἃ γὰρ δεῖ μαθόντας ποιεῖν, ταῦτα ποιοῦντες μανθάνομεν…"

- Aristotle: Nicomachean Ethics, Book II, 1103a

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Abstract

Skeletal indicators of early life stress:

Insights into cribra orbitalia and porotic hyperostosis in a living subsistence population

by

Amy Anderson

Porous cranial lesions (PCLs) of the orbital roofs (cribra orbitalia) and cranial vault (porotic hyperostosis) are among the most commonly observed pathological findings in archaeological skeletal remains. These lesions develop in childhood but can remain visible throughout adulthood. Though widely used by bioarchaeologists to infer compromised health in past populations, they are largely ignored in clinical practice and have never been studied in a population-representative sample. Consequently, the causes of PCLs and their consequences for health are a subject of on-going bioarchaeological debate. This dissertation takes an ethnobioarchaeological approach to the relationship between PCLs and health in a contemporary subsistence population, using biomedical data from the Tsimane Health and Life History Project to address the adult health correlates of PCLs and the childhood skeletal response to anemia, one known cause of PCLs.

Investigating PCLs in living individuals required that I first validate their visibility on clinical computed tomography (CT) criteria, which I did with an archaeological reference sample (n = 22) of individuals from sites of Pachacamac and Chicama, Peru. I found that porosity in the outer table of the cranial vault (porotic hyperostosis) was best observed on CT using volume-rendered reconstructions, and only porosity with pore diameters larger than the image resolution of the scan could be reliably identified. Porosity in the occipital squama is thus more likely to be visible on CT than porosity in other bones of the cranial vault. In the orbital roofs, porous

lesions (cribra orbitalia) were consistently identifiable only when they presented with enlarged diploic spaces.

I then used the resulting CT criteria to identify PCLs on existing CT scans of living adults. Because childhood anemia is one known cause of PCLs, I expected PCLs to be common among the Tsimane, a contemporary population of Amazonian forager-horticulturalists with high prevalence of childhood anemia. Given the established links between childhood stress and adult morbidity and the archaeological evidence that PCLs are associated with younger age at death, I expected the identification of PCLs to be associated with a distinct health signature in Tsimane adults. Using cranial CT scans and longitudinal biomedical data on a population-representative sample of 375 adults (45% female; aged 40+ years), I compared health outcomes for individuals with and without visible PCLs, including immune cell counts, biomarkers of inflammation, clinical diagnoses, and functional disability assessments.

I observed PCLs in 17.2% of the overall sample, with cranial vault lesions in 12.3% and orbital roof porosity in 6.1%. PCLs of the orbital roofs (cribra orbitalia) were associated with 3.8 (95% CI: 1.3, 11.2) times the risk of developing symptomatic tuberculosis and a lower CD4+/CD8+ T cell ratio for age (β = -0.78, (-1.52, -0.06)), an indicator of immunosenescence. However, orbital lesions were not associated with higher incidence of other respiratory infections, other markers of cell-mediated immunity, or adult hemoglobin levels. Vault lesions (porotic hyperostosis) were not associated with meaningful differences in measured health outcomes. These findings suggest that lesion-causing processes in early life can result in heightened lifetime susceptibility to some infections. It is also clear that, even if childhood anemia causes Tsimane PCLs, lesions do not indicate continued risk of anemia in adults.

As a first look at whether Tsimane PCLs could feasibly be caused by specific forms of childhood anemia, I investigated whether anemia was associated with differences in childhood

skeletal metabolism—as measured in blood by the bone turnover marker osteocalcin—and whether the skeletal effects of iron-deficiency anemia differed from those of anemia of inflammation. I found that among 362 observations of Tsimane children aged 4 months to 8 years (49% female), iron-deficiency anemia was associated with lower osteocalcin-for-age whereas high inflammation (C-reactive protein and erythrocyte sedimentation rate), with or without anemia, was not. However, because iron-deficient anemia cases (serum transferrin receptor >5 mg/L) tended to have lower hemoglobin than iron-replete cases, it is unclear whether the mechanism behind lower bone turnover is iron deficiency per se or the degree of anemia.

Taken together, this research strengthens the empirical foundations necessary for the study of health in past populations by linking skeletal stress markers to measures of individual health. In the process of doing so, it narrows the gap between skeletal biology research in living and past populations by using health-representative samples of a contemporary population and developing methods for creating comparable PCL data from living and long-deceased individuals.

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I. Introduction

1.1 Statement of the problem

Much of what we know—or think we know—about health and the human cost of societal change in prehistory depends on our ability to interpret evidence of health or disease from the skeleton. It is no straightforward task to determine from a prehistoric cemetery whether areas of unusual bone formation or destruction (skeletal lesions) are in fact associated with differences in mortality risk (DeWitte & Stojanowski, 2015a; Usher, 2000; J. W. Wood et al., 1992; Wright & Yoder, 2003)—let alone differences in disease experience. Cemetery samples can tell us about the relationship between skeletal lesions and age at death, but we can learn much more about the relationship between skeletal lesions and the experiences that precede death by directly investigating the associations between skeletal lesions and health in living people.

The overarching goal of this research is to strengthen the empirical foundation necessary for the study of health in past populations by linking skeletal indicators of childhood stress to measures of health in living individuals. I have focused on the knowledge gap surrounding porous cranial lesions (PCLs), a group of skeletal lesions notable for the stark contrast between their centrality in bioarchaeological studies of population health and their obscurity in contemporary medical practice (DeWitte & Stojanowski, 2015b). Because PCLs are rarely reported in clinical contexts, knowledge of the causes, consequences, and basic epidemiology of these lesions has come primarily from bioarchaeological studies and is hampered by the biases and limitations of archaeological skeletal samples. PCLs, often reported under the terms porotic hyperostosis and cribra orbitalia, are commonly noted in archaeological skeletal remains of children and adults, often associated with younger ages at death, and often interpreted as a consequence of childhood anemia (Steckel, Larsen, Sciulli, & Walker, 2005). Yet, their

relationship to health has never been investigated in a population-representative sample of living people.

To address this gap, this dissertation investigates the radiological evidence for PCLs and their associations with adult health in a contemporary subsistence population. It uses cranial computed tomography (CT) scans of adults aged 40+ years and mixed longitudinal health data from the Tsimane Health and Life History Project (THLHP). To assess the visibility of PCLs on THLHP cranial scans, it employs CT scans of an archaeological reference sample with directly observable lesion morphology before investigating PCL frequency and associations with adult health among living Tsimane horticulturalists. Finally, it evaluates the potential of anemia—the most commonly invoked causal explanation for PCLs—for driving changes in skeletal metabolism among Tsimane children.

1.1.1 A brief roadmap for what follows

The introductory chapter that follows begins with an outline of the interdisciplinary research gap that motivates this dissertation (1.2). The reader is then introduced in more detail to the subject of this interdisciplinary case study with an overview of PCLs, their possible causes, and their possible consequences (1.3). The Developmental Origins of Health and Disease (DOHaD) paradigm provides the major theoretical framework for this study, given that PCLs; (1) develop in childhood, (2) can still be observed in adults, and (3) appear to be associated with lasting impacts on physiological frailty (1.4). With the basic phenomena of interest defined, section 1.5 introduces the study population, the Tsimane of lowland Bolivia. There follows an overview of the skeletal biology of anemia and the clinical evidence that childhood anemia is a primary cause of PCLs (1.6). Finally, the introduction presents a theoretical consideration of

competing physiological demands for energy during skeletal growth and development, which sets the stage for investigating anemia and inflammation as drivers of childhood skeletal metabolism in the study population. We then close the introduction with an outline of subsequent chapters.

1.2 The osteological paradox and other problems

Broadly speaking there are two major challenges to inferring health from skeletal remains, both of which could be meaningfully addressed by investigating associations between skeletal lesions and physiological outcomes in population-representative samples of living people.

The first challenge comes from the confluence of sampling bias and undefined latent variables in skeletal data from archaeological cemeteries. By their very nature, cemeteries are never representative samples of the populations from which they are drawn (Ortner, 1991; J. W. Wood et al., 1992). Demographic estimates from cemetery samples are averages from the decades or centuries of a cemetery's use by a population experiencing shifting rates of growth and migration (*demographic nonstationarity*). Moreover, death itself creates systematically biased samples of living populations (*selective mortality*). That is to say, the childhood experiences represented by children in a cemetery sample are those of the sick, frail, or unlucky children who did not survive childhood.

Critically, the patterns of apparent illness or stress that are visible on human skeletons owe much to unobserved variation (*hidden heterogeneity*) in individual risks, and the sources of hidden heterogeneity are multiplied in archaeological mortality samples—not everyone is exposed to the same environmental stressors, not everyone exposed to the same stressor will develop a skeletal response, and not everyone in a birth cohort faces the same risk of dying in

the immediate future. The interactions between the risk of encountering a stress, the probability of a skeletal response to that stress, and an individual's immediate risk of death renders any single interpretation of 'health' from skeletal stress indicators in a mortality sample fraught with uncertainty, an identification problem referred to as the osteological paradox (J. W. Wood et al., 1992). This problem is magnified when we do not have a strong basis of knowledge about the disease processes that produce observable skeletal lesions. Osteoarthritis, for example, is increasingly common in older individuals because it is an age-progressive condition. It could naively be interpreted as an indicator of physiological robusticity in the archaeological record, but epidemiological evidence demonstrates that osteoarthritis is associated with higher age-specific mortality risk (Wilkie et al., 2019).

The second major challenge stems from the relationship between paleopathology and the clinical sciences. Here too, our understanding of the relationship between health/stress/disease and the evidence it leaves on the skeleton is constrained by sampling bias. Much of what we know about how disease affects skeletal tissues comes from pathological museum collections aggregated largely in the 19th and early 20th centuries and from published medical case studies with radiological imaging, both of which are inherently biased toward more fantastic cases (S. Mays, 2012). Medical museum and clinical samples are neither population-representative, being comprised solely of ill individuals, nor disease-representative, since both tend to emphasize extreme or unusual cases that do not typify the standard course of a given disease. Many diseases that leave their mark on the skeleton are chronic, progressive conditions (e.g., syphilis, osteomalacia, osteoarthritis), and most cases encountered in the archaeological record are likely to be less progressed than the cases from which skeletal disease profiles are constructed.

This research gap remains because, though the skeleton is the archaeologist's primary source of information on health, it is neither the most sensitive nor specific source of diagnostic information for the physician. Many conditions can be diagnosed without the expense or radiation exposure of clinical radiology. Critically, this does not mean that such conditions do not affect the skeleton, but rather that we may systematically lack medical documentation of their skeletal effects. This is yet another source of hidden heterogeneity that obscures interpretations of health from skeletal evidence, though it is not so much hidden as overlooked, a consequence of the unidirectional flow of knowledge from the clinical sciences to paleopathology. This thesis instead takes a paleopathological phenomenon (PCLs) as its starting point for investigating the developmental origins of health and disease in a contemporary population.

1.3 The curious case of porous cranial lesions: archaeologically common and clinically rare

Porous cranial lesions (PCLs)—characterized by areas of pitting or porosity on the outer surface of the normally smooth bones of the cranial vault and orbital roofs—are commonly reported in large-scale archaeological studies of human skeletal remains as a proxy indicator of population health (Marciniak et al., 2022; Obertova & Thurzo, 2004; Steckel, Larsen, Roberts, & Baten, 2019; Vercellotti et al., 2014). They are one of the most frequently recorded pathological findings in ancient skeletal remains (Ortner, 2003; Ortner & Putschar, 1981); among almost 10,000 prehistoric skeletons from North America and Europe examined as part of the Global History of Health Project, 24% exhibited porous lesions of the orbital roofs (Mcfadden & Oxenham, 2020).

Though they are observed in the skeletal remains of both children and adults, PCLs form largely in the first decade of life (Blom et al., 2005; Mittler & van Gerven, 1994; Stuart-Macadam,

1985). Lesions in adult remains are almost invariably in a stage of healing, with individual foramina (pores) exhibiting smooth, rounded margins; lesions with sharp-edged, unremodeled foramina that indicate active lesion formation are found almost exclusively in the remains of children (Mensforth, Lovejoy, Lallo, & Armelagos, 1978; J. W. Wood et al., 1992). This age distribution of unremodeled lesions strongly suggests that PCLs are markers of conditions experienced in childhood, even when they are observed in the skeletal remains of adults (Stuart-Macadam, 1985).

Bioarchaeologists interpret PCLs as skeletal indicators of nonspecific childhood stress (see below for an overview of known and potential causes of PCLs). Other phenomena in this category include dental enamel defects (Goodman & Armelagos, 1988) and indicators of growth stunting such as short limb bones (Kemkes-Grottenthaler, 2005). In contemporary populations, height and dental health are routinely recorded at regular preventative care visits to medical professionals or as part of largescale global health initiatives (Peres et al., 2019; WHO, 2014, 2020). However, collection of comparable population-representative data on PCLs would require clinical imaging procedures such as magnetic resonance imaging (MRI), radiography (x-ray), or computed tomography (CT) scanning. Such procedures are costly, and most involve patient exposure to radiation. Clinical imaging is therefore typically used to aid in diagnosing existing patient complaints or monitor recovery after patient treatment. As a result, evidence of PCLs in living individuals comes exclusively from a biased sample of the population—clinical settings where patients are unwell, often with multiple comorbidities. The current project leverages an existing population-representative sample of CT scans—obtained to study ageing and brain health at the population level—to produce the first study investigating the relationship between PCLs and health in a population-representative sample of living people.

1.3.1 A note on terminology

Among those who study pathological phenomena in dry bone, the terminology in use for porous cranial lesions has been regrettably imprecise. The terms *cribra orbitalia* and *porotic hyperostosis* have both been used to refer to abnormal porosity in the cortical surface of the orbital roofs, while *porotic hyperostosis*, *osteoporosis symmetrica*, and *cribra cranii* have all been used to denote abnormal porosity in the cortical surface of the bones of the cranial vault—most commonly the occipital squama and parietal bosses, less commonly the frontal squama, and occasionally the greater wings of the sphenoid bone.

Diagnostic interpretation is additionally hampered by the expanded application of the term '*porotic hyperostosis*' to include all porous lesions of the cranial vault. Where the term *porotic hyperostosis* has been applied to porous lesions of the cranial vault, expansion of the cranial marrow space (the hyperostosis side of the equation) was emphasized in the first half of the twentieth century, but later researchers often used *porotic hyperostosis* to describe surface porosity in the absence of attendant expansion of the adjacent marrow space (Ortner, 2003). This definition drift is understandable, given the immediate observability of surface porosity for the osteologist; subsurface changes are more visible using radiography or computed tomography. However, multiple disease processes can result in the appearance of surface porosity, and only a subset of these—most notably anemia—are capable of causing expansion of the marrow space.

Among contemporary paleopathologists the most common practice is to use '*porotic hyperostosi*s' to describe any area of pitting, porosity—or indeed even raised trabeculated patterns that are interpreted as healed porosity—on the cranial vault (Buikstra & Ubelaker, 1994). Likewise, any evidence of porosity or even vascular impressions in the orbital roofs are categorized as '*cribra orbitalia*.' I deviate from this popular use in order to acknowledge the centrality of surface porosity as the defining factor of these lesions in the current study and to

avoid implying expansive changes where none are observed. Following the example of Brickley (Brickley, 2018), I refer instead to *porous cranial lesions* (PCLs) and further distinguish lesion locality as either *orbital roof porosity* or *cranial vault porosity*.

1.3.2 What causes porous cranial lesions?

While researchers in the mid-twentieth century considered orbital and vault porosity both to be manifestations of anemia (Blom et al., 2005; Lallo, Armelagos, & Mensforth, 1977; Moseley, 1965b), evidence has accumulated in recent decades that porous lesions of the orbital roofs and cranial vault share a range of overlapping but not identical causes that are likely not yet fully itemized (Koontz Scaffidi, 2020; Richman, 2009; Rivera & Mirazon Lahr, 2017). Current bioarchaeological consensus considers PCLs to be nonspecific skeletal indicators of physiological stress because their known or suspected causes include a range of nutritional deficiencies, infections, and trauma.

Multiple processes can create a porous appearance of cortical bone, from new bone formation to poor bone formation to erosion of existing bone. Newly formed bone adjacent to the outer cortex is initially porous, and localized inflammation, neoplasms, and subperiosteal hemorrhage can all incite areas of porous new bone formation across the skeleton, including the cranium (Ortner, 2003). Documented causes of orbital hemorrhage—and thus possible causes of orbital roof lesions—include infantile scurvy, lacrimal gland infections, trauma, whooping cough, and childbirth (Cole & Waldron, 2019; Klaus, 2017; Ortner & Ericksen, 1997). Surface porosity can also result from disruptions in bone mineralization due to vitamin D deficiency or the proliferation of capillaries in response to localized inflammation (Brickley, Ives, & Mays, 2020). Orbital roof porosity has also been experimentally induced in juvenile Wistar rats through

a combination of early weaning, bloodletting, and a magnesium-deficient diet, though the small sample size (n = 10) and simultaneous manipulation of multiple variables makes identifying a specific cause from the results of this experiment untenable (Polo-Cerdá, Miquel-Feucht, & Villalaín-Blanco, 2000). Finally, it has also been suggested that cases of 'pinprick' porosity in the orbital roof may in fact be non-pathological developmental variants resulting from a combination of the formation of the supraorbital angle and the blood supply of the periorbita (Cole and Waldron, 2019).

1.3.3 What do porous cranial lesions predict?

PCLs, orbital roof lesions in particular, are often associated with younger ages at death in mortality samples from archaeological cemetery excavations. This pattern cannot be entirely attributed to the higher frequency of PCLs in the skeletal remains of children compared to adults. A number of survival analyses from disparate sites (medieval Nubia, medieval Slovakia, Ancestral Pueblo Southwest) report that orbital roof lesions are associated with higher mortality risk for children but no difference in mortality risk for individuals older than 20 years (Mittler & van Gerven, 1994; O'Donnell, 2019; Obertova & Thurzo, 2004). Additionally, analyses of the largescale data from the Global History of Health Project's North American and European modules show that PCLs are associated with reduced probability of young adult survival (Roberts & Steckel, 2019; Steckel, 2005), though not universally across all sites (Mcfadden & Oxenham, 2020). Lesions of the orbital roofs and cranial vaults also appear to differ in their associations with age at death, further suggesting differences in the underlying disease processes or differences in the ages and thus the developmental sensitivity of children forming orbital and vault lesions. Hazard models of mortality among individuals with PCLs have found lower life

expectancies for individuals with orbital roof porosity, but often find higher life expectancy for individuals with healing cranial vault porosity compared to individuals without lesions (DeWitte & Wood, 2008; O'Donnell, 2019; Usher, 2000). In aggregate, these studies suggest that associations between PCLs and mortality risk decline with age and vary within and between populations.

Most studies do not distinguish between lesions that do or do not show evidence of healing at time of death, which conflates mortality risks associated with active lesion-causing stress and the subsequent mortality risks faced by those who survive such stress. Where the two are examined separately, unremodeled lesions are associated with higher mortality hazards compared to absence of lesions, while individuals with healing orbital roof lesions have similar mortality hazards to those without lesions, and healing cranial porosity is associated with reduced mortality hazards (Mittler & van Gerven, 1994; O'Donnell, 2019; Speal, 2017). To the extent that associations with age at death do reflect mortality risk, analyses of adults are primarily investigating the mortality risk associated with healed lesions, while children will express a combination of unremodeled and remodeling lesions (Mcfadden & Oxenham, 2020).

The current study is unique in that it provides a more sensitive test of the long-term health costs of PCLs by evaluating their relationship to health outcomes in living individuals. It is unclear to what extent the patterns observed in archaeological data can truly be attributed to lasting physiological frailty in the wake of lesion-causing stress. In the first place, declining lesion frequency with age is precisely what we would expect to see for a skeletal lesion that develops in childhood and has a lifetime to heal—even if PCLs are entirely unrelated to an individual's risk of dying. More importantly, though the physiological cost of lesion-causing stress may have an absolute value, observable patterns of lesions in mortality samples are sensitive to the relative risks of mortality from different causes, and the archaeologist is generally blind to this source of

heterogeneity. To wit, in an archaeological mortality sample the inclusion of otherwise healthy individuals who died from violence or accidents could mask the signal of any elevated susceptibility to death from infectious causes experienced by individuals with skeletal lesions. However, PCLs may be measurably associated with morbidity in samples of living people even where they are not significantly associated with hazards of all-cause mortality. Elucidating the specific health correlates of PCLs provides empirical grounds for inferring experiences of physiological stress in the archaeological record even when the consequences for mortality risk are unclear.

1.4 Theoretical Framework

1.4.1 Developmental Origins of Health and Disease

The *Developmental Origins of Health and Disease* (DOHaD) framework posits that early life environment has lasting impacts on health and the risk of developing diseases over the life course (Barker, 1995; Barker et al., 1991). Empirical studies of early life environment as a predictor of later life disease risk (Boyce & Ellis, 2005; Gluckman, Hanson, Spencer, & Bateson, 2005; Suzuki, 2018) have reinvigorated biomedical interest in the long shadow of early life stress. Because many skeletal indicators of nonspecific stress develop in early life and are observable in adults, studies of the skeleton as an embodied archive of health and disease are often inherently—and increasingly, explicitly—oriented within the framework of the DOHaD paradigm (Amoroso & Garcia, 2018; Cheverko, 2018; Garland, 2020; Goodman & Armelagos, 1988; Roberts & Steckel, 2019; Sabazali & Cheverko, 2022; Steckel, 2005; Temple, 2018). However, with a handful of exceptions looking at dental enamel defects in living populations (e.g., Cooper et al., 2002; Dunn et al., 2022; Masterson et al., 2018), most papers applying a DOHaD framework to skeletal indicators of early life stress are archaeological studies assessing

whether skeletal indicators of early life stress predict adult lifespan. Archaeological studies use age at death as a proxy for adult health primarily because mortality is the main observable health outcome in archaeological settings.

There are three distinct pathways through which early life stress may shape health outcomes later in life. In the *predictive-adaptive model*, early life experiences calibrate individual physiology and set developmental trajectories in response to environmental cues. This calibration is adaptive when adult environments are similar to early life environments but can lead to physiological dysregulation and disease when early life cues are not predictive of later environments (Gluckman, Hanson, & Spencer, 2005). Low birth weight, an indicator of reduced intra-uterine growth, has been associated in later life with a range of poor health outcomes and risk indicators including higher blood pressure, cholesterol, insulin-resistance, infectious mortality, and lower cell-mediated immunity. This has often been interpreted as a consequence of environmental mismatch between intra-uterine cues of low resource availability and subsequent experience of excess resource availability over the life course (Kuzawa, 2005).

However, the health outcomes associated with low birth weight are also consistent with a *plasticity-constraint model*, which predicts that physiological responses designed to weather adversity in early life are achieved at a cost to health in later life (Stearns, 1992). Early life tradeoffs in energy allocation can maximize chances of immediate survival, but due to constraints on physiological plasticity such trade-offs result in compromised long-term health (Worthman & Kuzara, 2005) and a decreased capacity to weather future challenges, an outcome also referred to as *physiological scarring* (Hayward, Rigby, & Lummaa, 2016; Zheng, 2014). A third category of distorted morphology in early life simply results from encountering constraint beyond the possibility of adaptive physiological plasticity— for instance, porosity that results from impaired bone mineralization due to vitamin D deficiency. While adaptive mechanisms

may minimize an individual's overall costs of vitamin D deficiency by preferentially protecting vitamin D-dependent physiological processes in other organ systems, skeletal porosity here is an undeniable expression of the physiological cost of a nutrient deficit.

1.4.2 A DOHaD perspective on porous cranial lesions

Among the skeletal indicators of childhood stress that remain visible in adults, porous cranial lesions are unusual in that they result from something other than the direct effects of growth disruption, though the full range of PCL causes is very likely still unknown. Because the known or putative causes of porous cranial lesions to date include anemias, other micronutrient deficiencies, and infections, the health sequelae for individuals who develop PCLs are very likely to be mixed consequences of all three scenarios of early life stress outlined above. If PCLs result from childhood infections, they may be proxy indicators of adaptive plasticity in energy allocation. If PCLs result from dietary micronutrient deficiencies unrelated to anemia (e.g., vitamins C and D), they mark individuals who experienced specific resource shortfalls without the potential for adaptive plasticity. It is also theoretically possible that some PCLs result from a predictive-adaptive response to anemia risk, specifically forms of anemia that are accompanied by increased red blood cell production.

In the case of lesions that result from expansion of erythropoietic marrow in response to anemia, the extended investment in erythropoietic tissue may protect against subsequent bouts of anemia. In the context of endemic hookworm, chronic infection with bacteria, viruses, and other pathogens, and occasional food insecurity faced by individuals in the Tsimane population, early life cues of these anemia risks are likely to be predictive of future experiences.

In this first exploration of PCLs' associations with adult health—rather than adult mortality—I ask if PCLs are associated with any facet of morbidity in later life; the causal processes behind any observed pattern remain inaccessible. In archaeological cemetery samples associations between PCLs and younger age at death have often been interpreted as evidence of physiological scarring from childhood stress. This interpretation implies that individuals with PCLs face an increased susceptibility to illness great enough to produce elevated mortality risks for years after experiencing lesion-causing stress, but a relationship between PCLs and adult morbidity has never been directly investigated.

It has also been suggested that PCLs are of only limited relevance to DOHaD conceptions of health over the life course, given the lack of clarity on the developmental window for PCL formation and the possibility that it extends past the first decade of life (Mcfadden & Oxenham, 2020). Initial proponents of DOHaD focused on the fetal origins of adult health and later extended this to include the first 1,000 days of post-natal life as a critical developmental period for setting lifelong health trajectories (Gluckman, Hanson, Spencer, et al., 2005). However, recent studies have found that porous lesions of the orbital roofs are significantly more common in children and adolescents with fatal respiratory infections (Gomes, Petit, Dutour, & Santos, 2022; O'Donnell, Hill, Anderson, & Edgar, 2020). Susceptibility to chronic or recurrent respiratory infections presents a plausible pathway between lesion-causing stress and sustained mortality risk. Respiratory infections are consistently a leading cause of death in historic records (Bartlett & Mundy, 1995; Mulholland, 2007) and in low-income populations (Mathers, Boerma, & Ma Fat, 2009), and initial infection appears to have far-reaching effects on subsequent respiratory morbidity. The current study is well positioned to test whether PCLs are associated with higher susceptibility to respiratory infections, since respiratory infection is the

most common category of illness among Tsimane of all ages (M. Gurven, Kaplan, & Supa, 2007).

1.5 Study population: The Tsimane of lowland Bolivia

The Tsimane are an indigenous population of ≈17,000 people who live a subsistence lifestyle in close to 100 villages in the Beni department of Amazonian Bolivia. Most Tsimane villages have little public infrastructure, no running water, and minimal access to electricity and clinical medicine (M. Gurven et al., 2017). Their diet is the result of a mixed subsistence strategy: horticultural staples include plantains, manioc, and rice (62% of calories); hunting yields 6% of all calories; fishing brings in 16%. Market foods, currently 8% of average daily calories per person, make up a growing proportion of the diet (Kraft et al., 2018). The Tsimane language is one of two members in the linguistically isolated Mosetenan language family, without apparent ties to other indigenous languages spoken in Bolivia (Sakel, 2011). The lack of navigable roads in the Tsimane territory limited regional travel and maintained traditionally subsistence lifestyles through much of the twentieth century, but logging and motorized vehicle access is increasingly removing barriers to nearby market centers.

Tsimane health and lifeways have been extensively documented by two longitudinal health and anthropology projects. The Tsimane' Amazonian Panel Study (TAPS), which collected longitudinal data from 2002 to 2010 on Tsimane in 13 villages along the Maniqui River, focused on the health and wellbeing impacts of increasing engagement with the market economy (Godoy & Leonard, 2020). The Tsimane Health and Life History Project is an ongoing longitudinal study of health and aging among Tsimane communities started in 2002 (Gurven et al., 2017). THLHP data collection initially included 18 villages but has since expanded to cover

90, with data on demography, physical activity, resource transfers, and physical and mental health across the life course.

The Tsimane environment is high-pathogen, energy-limited, and hookworm-endemic. Chronic parasitic infection is common. 57% of study participants in past reports were infected with at least one helminth species, with hookworm (undetermined, but likely *Necator americanus*) (45.3%) and roundworm (*Ascaris lumbricoides*, 19.9%) the most prevalent (Aaron D. Blackwell et al., 2011). Levels of immunoglobulin-E, a class of antibody produced in response to parasitic worm infections, are 150-200 times higher among Tsimane individuals than in age-matched Americans. Elevated inflammation is prevalent. Total white blood cell counts among the Tsimane are roughly 1.5 times higher than US National Health and Nutrition Examination Survey (NHANES) values, and 89% of Tsimane have eosinophil counts higher than NHANES 95th percentile (Aaron D Blackwell et al., 2016). Roughly one third of Tsimane men and women and roughly a third of children are anemic (DeLouize et al., 2022) despite high estimated levels of dietary iron (Kraft et al., 2018).

The population experiences high mortality, with death rates across the life course similar to European mortality from the 1800s (M. Gurven, Kaplan, Winking, Finch, & Crimmins, 2008). As of 2002, life expectancy at birth is 53 years, a substantial increase from the pre-1990 figure of 43 years (M. Gurven et al., 2007). This increase in life expectancy appears to be driven by decreased adult mortality rather than improved survival of infants and children—approximately a quarter of the population does not survive past age 14. Respiratory infection is the most commonly documented cause of death for Tsimane individuals of all ages except for early adulthood, where it is tied by deaths from accidents or violence (M. Gurven et al., 2007), demonstrating the critical importance for immunological robusticity at every stage of life in this pathogen-rich environment. Tradeoffs between investments in linear growth and adaptive

immunity have been documented in Tsimane preadolescents (Garcia et al., 2020), providing additional evidence of the ways in which requirements of immunity shape Tsimane life histories.

Their subsistence lifestyle and high prevalence of childhood anemia (39% of children ages 0.5-15 years) make them a relevant case for comparison to prehistoric populations among whom PCLs are commonly observed. The relatively egalitarian Tsimane social structure limits the confounding effect that social inequality has on differential health and mortality risk, a natural control that the authors of the osteological paradox encouraged researchers to leverage in order to avoid the heterogeneity in disease exposure and individual frailty caused by social inequality (J. W. Wood et al., 1992). The current study benefits from the existing infrastructure of the Tsimane Health and Life History Project.

1.6 Evidence for childhood anemia as a cause of porous cranial lesions

Because the current study is focused on PCLs in the Tsimane, a neotropical Amerindian population living a subsistence lifestyle, some potential causes of PCLs can be heavily discounted. Childhood deficiencies in vitamin D (rickets) and C (infantile scurvy) are unlikely in a non-industrialized tropical population because there is sufficient sun exposure in the tropics as well as year-round fruit consumption. Hereditary anemias like thalassemia and sickle cell, the most substantively documented cause of porous cranial lesions, are found in African, south Asian, and Mediterranean populations because they provide a degree of resistance to malaria but are absent from populations indigenous to the Americas, where malaria arrived only in the 1800s (Angel, 1966). Acquired childhood anemia, which is present in roughly a third of Tsimane children (Alami et al., 2020), deserves serious consideration.

1.6.1 Epidemiology of anemia in childhood

Anemia is clinically defined as inappropriately low hemoglobin for body size and physiologically defined as a shortfall in red blood cells' capacity to deliver oxygen to the body's tissues. This insufficiency can be caused by a wide range of conditions, including: congenital malformations of the red blood cells such as those in sickle cell or spherocytic anemia; prolonged inflammation due to infection or chronic disease; and dietary deficiencies of folate, B-12, or even copper (Hermiston & Mentzer, 2002; Myint, Oo, Thein, Tun, & Saeed, 2018). Globally the most common forms of anemia are acquired iron-deficiency anemia and anemia of inflammation (Camaschella, 2015). Iron-deficiency anemia typically results from iron-poor diets or slow, persistent blood loss from gastrointestinal parasites like hookworm. Anemia of inflammation is a consequence of immune responses to inflammatory stimuli and can cause functional iron deficiency even in the presence of adequate iron stores (Marks, 2012).

Young children face high risks of developing iron-deficiency anemia because iron is critical to the rapid cellular replication that constitutes growth (Ryan, 1997). The weaning period brings particularly high anemia risk. Neonatal iron stores typically meet iron needs during the first four to six months of life (Oski, 1993), but solid foods considered appropriate for infant consumption are often poor dietary sources of iron (Domellof et al., 2014). Moreover, the introduction of solid foods also introduces potentially pathogenic bacteria, heightening the risk for anemia of inflammation—a risk that may be partially mitigated by low-iron weaning foods (Ryan, 1997).

1.6.2 Clinical evidence of PCLs: Hair-on-end sign

Childhood anemia has long been considered the most promising single explanation for the high frequency of porous cranial lesions in the archaeological record (Hengen, 1971;

Moseley, 1965a; Papathanasiou, Meinzer, Williams, & Larsen, 2018), though clinical cases of anemia almost never reports changes to the orbital roof. Clinical radiologists sometimes report cases of anemia with reduced bone density and expansive changes in the cranial vault (under the term 'hair-on-end' sign), and a similar radiological appearance has been documented in archaeological cases of extreme cranial vault porosity with enlarged trabecular spacing and expansion of the cranial marrow space (Angel, 1964; Moore, 1929). The visibility of PCLs on dry skeletons curated from clinically diagnosed thalassemia patients confirms the match between archaeological and clinical lesions with hair-on-end expansion of the calvarial diploë (Chaichun et al 2021). Radiological findings broadly consistent with cranial vault lesions have also been observed in children with iron-deficiency anemia from dietary or parasitic causes, though the extent of cranial changes does not appear related to the severity of anemia, and the prevalence of cranial changes in response to non-hereditary anemias remains unknown (N. Agarwal, M, Dhar, & Bhardwaj, 1970; Aksoy, Muzaffer; Camli, Necdet; Erdem, 1966; Britton, Howard A.; Canby, John P.; Kohler, 1960; Burko, Henry; Mellins, harry Z.; Watson, 1960; Eng, 1958; Lanzkowsky, 1968; Shahidi, Nasrollah T.; Diamond, 1960). Reports of non-hereditary anemias presenting with cranial vault changes are often twins or premature births, who are born with lower iron stores and are thus at higher risk of developing iron deficiency in the first few months of life, when cortical bone provides less resistance to expanding marrow (Britton, Howard A.; Canby, John P.; Kohler, 1960).

While hair-on-end sign is the most specific skeletal indicator of anemia, it is not the most common skeletal change associated with anemia. Only 5–20% of cases of thalassemia, itself one of the more severe but relatively rare and regional hereditary anemias, show development of hair-on-end sign (Tayles, 1996). Radiographic examination of 147 children (most younger than three years) with acquired iron-deficiency anemia found that only two displayed expansion of the

marrow space, but 95 exhibited thinning of the cranial outer table without visible marrow expansion (N. Agarwal et al., 1970). The authors attribute these changes to the erosive effects of pressure exerted by the build-up of erythroid precursors in the underlying marrow, but it is unclear whether radiologically visible outer table thinning is synonymous with the cranial vault porosity observed directly in archaeological remains.

There is less clinical evidence linking orbital roof lesions to anemia, but this may reflect clinical priorities and technological limitations of clinical radiology rather than solely a divergence in the processes that lead to porosity of the orbital roofs and cranial vault. Though thickening and porosity of the orbital roofs can occasionally be observed in the radiographic images accompanying published clinical cases of anemia with onset in infancy (Procianoy, Brandão Filho, Cruz, & Alencar, 2008; Stuart-Macadam, 1987), these orbital changes are generally unmentioned in the case report and have not been emphasized as a radiological feature of anemias. These orbital changes may be disregarded in clinical practice because the thin, curved bone of the orbital roof is not easily visualized by radiologists using most standard cranial imaging protocols, and orbital roof changes are of limited diagnostic value in the context of other patient symptoms (Exner, Bogusch, & Sokiranski, 2004; Steyn, Voeller, Botha, & Ross, 2016a).

1.6.3 How anemia affects the skull

Because the cranial lesions linked to anemia are caused by expansion of the red blood cell producing marrow and subsequent pressure erosion of the surrounding cortical bone, there are two critical determinants of cranial involvement in anemia: 1) increased erythropoiesis (red blood cell production) and 2) age of anemia onset. Congenital hemolytic anemias and B-vitamin-deficiency anemias are both accompanied by increased erythropoiesis. Acquired iron-deficiency

anemia has been connected to both increases and decreases in erythropoiesis, though this may reflect differences between physiological response to blood loss (increased erythropoiesis) and dietary iron deficiency (decreased erythropoiesis) (Marks, 2012; Oxenham & Cavill, 2010; Walker, Bathurst, Richman, Gjerdrum, & Andrushko, 2009). Anemia of inflammation is accompanied by reduced erythropoiesis, making it an implausible cause of expansive cranial lesions (Cavill, 2002; Oxenham & Cavill, 2010). Anemia of inflammation has been suggested as a possible cause for cranial porosity accompanied by thinning of the cranial vault (Rivera & Mirazon Lahr, 2017), but the mechanistic link between hypocellular marrow and outer table porosity needed to underwrite this hypothesis has not been established. Critically, many cases of acquired anemia have multiple contributing factors, and the implications of such complex cases on the potential for skeletal lesions is unclear (Mcilvaine, 2015).

Age of anemia onset determines the skeletal consequences of anemia because of age-associated changes in the composition of bone marrow (Gurevitch, Slavin, & Feldman, 2007). At birth all bone marrow is involved in red blood cell production but with age this red marrow is systematically converted to fat-storing yellow marrow (Kricun, 1985). As a result, erythropoiesis can be increased after the first few years of life by reconverting yellow marrow back to red. However, in infancy additional red marrow is gained at the expense of the cortical bone surrounding it.

It is unclear how prolonged or severe anemia need be to incite marrow expansion, nor at what age these erosive skeletal changes become a highly improbable consequence of anemia, though it has been suggested that the potential for marrow expansion is negligible after age twelve (Brickley, 2018). Cranial changes are documented more frequently in congenital anemias such as thalassemia and sickle cell anemia, which may be in part because congenital anemias tend to manifest earlier in infancy than acquired anemias.

Finally, it is important to note that while the strongest clinical evidence for porous cranial lesions comes from chronic anemias with onset in infancy, cranial changes are an exceedingly rare clinical finding for acquired anemia. Additionally, many PCLs do not exhibit evidence of marrow expansion (Galea, 2015; Morgan, 2014; Robertson, 2017; Wapler, Crubézy, & Schultz, 2004). Such lesions are thus likely to be missed in clinical settings and may be more common consequences of childhood anemia than currently thought, but these lesions also lack evidence of the mechanism by which anemia is known to cause cranial lesions. Anemia may therefore ultimately explain only a minority of PCLs in the archaeological record (M. B. Brickley, 2018; Wapler et al., 2004), and the predominant cause of these common skeletal lesions remains, in this author's mind, an open question.

1.7 Skeletal responses to acquired childhood anemias in an energy-limited environment: A life history perspective

The on-going debate about whether PCLs can be commonly ascribed to effects of acquired iron-deficiency anemia or anemia of inflammation can be broadened to investigate whether these distinct causes of anemia have distinct effects on general processes of skeletal growth and maintenance in childhood. One might expect differences in the skeletal effects of childhood anemia from different causes, given the potential for different erythropoietic responses to anemia of inflammation and iron-deficiency anemia.

Childhood, especially infancy, is a time of rapid bone formation. Contributions of bone formation to height are cumulative; long bone diaphyses lengthen but never shorten. However, other aspects of skeletal morphology—trabecular structures, cortical thickness—remain responsive to inputs from mechanical loading, nutritional status, hormones, and signals from the immune system. Bone mass can be lost as well as gained, though the overall trajectory of bone mass in childhood is positive.

Individual energy budgets during growth and development are divided between growth of new somatic tissue and maintenance of existing somatic tissue (Stearns, 1992). When energy is limited, competing physiological demands may result in energetic tradeoffs that maximize the probability of immediate survival. For example, immune activation can draw resources away from growth, skeletal and otherwise.

Immune activation, itself an investment in somatic maintenance, may also draw resources away from the maintenance of skeletal tissue, a hypothesis consistent with the decreases in bone mass widely observed in the presence of chronic inflammation (Hardy & Cooper, 2009). However, the skeletal and immune systems are intimately interdependent and expecting an energetic tradeoff between them may be underestimating the extent to which actions of bone and immune cells are coordinated. All immune cells are produced within the bone marrow (Mercier, Ragu, & Scadden, 2012), and bone and immune cells display an intricate web of regulatory relationships within and between both systems (Berger, Griffin, & Dent, 2020). In light of all this, the skeletal effects of inflammation-driven anemia may be markedly different from the skeletal effects of anemia caused by either iron-deficient diet or by occult blood loss from chronic infection by gastrointestinal parasites.

1.8 Dissertation Objectives

Chapter II lays the foundations for observing PCLs in living individuals using clinical computed tomography (CT). It develops CT-specific identification criteria based on archaeologically defined lesion characteristics using an archaeological reference sample of 22 dry crania with varying degrees of PCL expression. By comparing PCL evaluations of the same crania by four observers across multiple viewing modes, this chapter makes recommended best practices for identifying PCLs using CT and identifies the minimum threshold of lesion

expression that can be reliably identified in clinical scans. Scanner settings tested in this chapter are selected to approximate those used for brain CTs obtained as part of the larger Tsimane Health and Life History Project.

Chapter III applies the lessons learned in chapter two to identify PCLs in a sample of living Tsimane adults (aged 40+ years) and then explores associations between CT-based assessment of these skeletal indicators of childhood stress and subsequent health outcomes in later life. As a non-industrialized population with high prevalence of childhood anemia, the Tsimane of lowland Bolivia are an excellent contemporary population for testing the lasting consequences of PCLs on individual frailty. Based on archaeological reports of younger age at death for individuals with PCLs and given the well-established links between childhood stress and adult health, this chapter investigates whether PCLs in older Tsimane adults are associated with elevated mortality risk or worse health outcomes as measured by multiple markers of immune competence, functional disability, and physician diagnoses.

To test anemia's influence on skeletal tissue in childhood **Chapter IV** explores the relationship between anemia, inflammation, and skeletal metabolic activity among Tsimane children (aged < 9 years). It tests whether childhood anemia, with and without iron deficiency, is associated with differences in skeletal metabolism that might indirectly support anemia as a feasible cause of PCLs in this population, and whether anemias with different proximate causes differ in their effects on the skeleton. It also examines whether the high levels of immune activity common to disease experience during Tsimane childhood might reduce the energy available for the growth and maintenance of mineralized tissues in this high-pathogen, energy-limited environment. This chapter uses epidemiological data from biomedical rounds, including biomarkers from dried blood spots and clinical diagnoses.

Finally, **Chapter V** synthesizes and summarizes findings from this research and lays out the implications of its results for our understanding of health and disease experience as embodied in the skeleton. It ends by outlining future directions for interdisciplinary research in human skeletal biology that will (1) expand our understanding of the skeleton as a dynamic organ system, (2) broaden the empirical base of knowledge that grounds bioarchaeological reconstructions of past population health, and (3) center the skeleton as a bridge between our understanding of variation in human health in the past and present.

1.9 References

Aksoy, Muzaffer; Camli, Necdet; Erdem, S. (1966). Roentgenographic Bone Changes in Chronic Iron Deficiency Anemia: a study in twelve patients. *Blood*, *27*(5).

Alami, S., von Rueden, C., Seabright, E., Kraft, T. S., Blackwell, A. D., Stieglitz, J., … Gurven, M. (2020). Mother's social status is associated with child health in a horticulturalist population. *Proceedings of the Royal Society B: Biological Sciences*, *287*(1922). https://doi.org/10.1098/rspb.2019.2783

Amoroso, A., & Garcia, S. J. (2018). Can early-life growth disruptions predict longevity? Testing the association between vertebral neural canal (VNC) size and age-at-death. *International Journal of Paleopathology*, *22*(April), 8–17. https://doi.org/10.1016/j.ijpp.2018.03.007

Angel, J. L. (1964). Osteoporosis: Thalassemia? *American Journal of Physical Anthropology*, *22*(3), 369–373.

Angel, J. L. (1966). Porotic hyperostosis, anemias, malarias, and marshes in the prehistoric eastern Mediterranean. *Science*, *153*(3737), 760–763.

Barker, D. J. P. (1995). Fetal origins of coronary heart disease. *British Medical Journal*, *311*(171). Retrieved from doi:10.1136/bmj.311.6998.171

Barker, D. J. P., Godfrey, K. M., Fall, C., Osmond, C., Winter, P. D., & Shaheen, S. O. (1991). Relation of birth weight and childhood respiratory infection to adult lung function and death from chronic obstructive airways disease. *British Medical Journal*, *303*(6804), 671–675. https://doi.org/10.1136/bmj.303.6804.671

Bartlett, J. G., & Mundy, L. M. (1995). Community-acquired pneumonia. *The New England Journal of Medicine*, *333*(24), 1618–1624.

Berger, S. M., Griffin, J. S., & Dent, S. C. (2020). Phenotypes and pathways: Working toward an integrated skeletal biology in biological anthropology. *American Journal of Human Biology*, (April), 1–19. https://doi.org/10.1002/ajhb.23450

Blackwell, A. D., Gurven, M. D., Sugiyama, L. S., Madimenos, F. C., Liebert, M. A., Martin, M. A., … Snodgrass, J. J. (2011). Evidence for a peak shift in a humoral response to helminths: Age profiles of IgE in the shuar of Ecuador, the Tsimane of Bolivia, and the U.S. NHANES. *PLoS Neglected Tropical Diseases*, *5*(6). https://doi.org/10.1371/journal.pntd.0001218

Blackwell, A. D., Trumble, B. C., Suarez, I. M., Beheim, B., Snodgrass, J. J., Kaplan, H., & Gurven, M. (2016). Immune function in Amazonian horticulturalists. *Annals of Human Biology*, (May), 1464–5033. https://doi.org/10.1080/03014460.2016.1189963

Blom, D. E., Buikstra, J. E., Keng, L., Tomczak, P. D., Shoreman, E., & Stevens-Tuttle, D. (2005). Anemia and childhood mortality: Latitudinal patterning along the coast of pre-Columbian Peru. *American Journal of Physical Anthropology*, *127*(2), 152–169. https://doi.org/10.1002/ajpa.10431

Boyce, W. T., & Ellis, B. J. (2005). *Biological sensitivity to context : I . An evolutionary – developmental theory of the origins and functions of stress reactivity*. 271–301.

Brickley, M. B. (2018). Cribra orbitalia and porotic hyperostosis: A biological approach to

diagnosis. *American Journal of Physical Anthropology*, *167*(4), 896–902. https://doi.org/10.1002/ajpa.23701

Brickley, M., Ives, R., & Mays, S. (2020). Vitamin D deficiency. In *The Bioarchaeology of Metabolic Bone Disease* (2nd ed., pp. 75–128). https://doi.org/10.1016/B978-0-08-101020-4.00005-7

Britton, Howard A.; Canby, John P.; Kohler, C. M. (1960). Iron deficiency anemia producing evidence of marrow hyperplasia in the calvarium. *Pediatrics*, *25*(April 1960), 621–627.

Buikstra, J. E., & Ubelaker, D. H. (1994). *Standards for data collection from human skeletal remains: Proceedings of a seminar at the Field Museum of Natural History*. Fayetteville, AR: Arkansas Archeological Survey.

Burko, Henry; Mellins, harry Z.; Watson, J. (1960). Skull changes in iron deficiency anemia simulating congenital hemolytic anemia. *The American Journal of Roentgenology, Radium Therapy, and Nuclear Medicine*, *86*, 447–452.

Camaschella, C. (2015). Iron-deficiency anemia. *The New England Journal of Medicine*, *372*(19), 1832–1843. https://doi.org/10.1056/NEJMra1401038

Cavill, I. (2002). Erythropoiesis and iron. *Best Practice & Research Clinical Haematology*, *15*(2), 399–409. https://doi.org/10.1053/beha.2002.0004

Cheverko, C. M. (2018). *The Biological Impact of Development Stress in the Past: Correlations between Growth Disruptions and Mortality Risk in Bioarchaeology*. The Ohio State University.

Cole, G., & Waldron, T. (2019). cribra orbitalia: Dissecting an ill-defined phenomenon. *International Journal of Osteoarchaeology*, *29*(4), 613–621. https://doi.org/10.1002/oa.2757

Cooper, C., Javaid, M. K., Taylor, P., Walker-Bone, K., Dennison, E., & Arden, N. (2002). The fetal origins of osteoporotic fracture. *Calcified Tissue International*, *70*(5), 391–394. https://doi.org/10.1007/s00223-001-0044-z

DeLouize, A. M., Liebert, M. A., Madimenos, F. C., Urlacher, S. S., Schrock, J. M., Cepon-

Robins, T. J., … Sugiyama, L. S. (2022). Low prevalence of anemia among Shuar communities of Amazonian Ecuador. *American Journal of Human Biology*, *34*(1), e23590. https://doi.org/10.1002/ajhb.23590

DeWitte, S. N., & Stojanowski, C. M. (2015a). The Osteological Paradox 20 years later: Past perspectives, future directions. *Journal of Archaeological Research*, *23*(4), 397–450. https://doi.org/10.1007/s10814-015-9084-1

DeWitte, S. N., & Stojanowski, C. M. (2015b). The Osteological Paradox 20 Years Later: Past Perspectives, Future Directions. *Journal of Archaeological Research*, *23*(4), 397–450. https://doi.org/10.1007/s10814-015-9084-1

DeWitte, S. N., & Wood, J. W. (2008). Selectivity of Black Death mortality with respect to preexisting health. *Proceedings of the National Academy of Sciences*, *105*(5), 1436–1441.

Domellof, M., Braegger, C., Campoy, C., Colomb, V., Decsi, T., Fewtrell, M., … van Goudoever, J. (2014). Iron requirements of infants and toddlers. *Journal of Pediatric Gastroenterology and Nutrition*, *58*(1), 119–129. https://doi.org/10.1097/MPG.0000000000000206

Dunn, E. C., Mountain, R. V, Davis, K. A., Shaffer, I., Smith, A. D. A. C., Roubinov, D. S., … Boyce, W. T. (2022). Association Between Measures Derived From Children's Primary Exfoliated Teeth and Psychopathology Symptoms: Results From a Community-Based Study . *Frontiers in Dental Medicine* , Vol. 3. Retrieved from https://www.frontiersin.org/articles/10.3389/fdmed.2022.803364

Eng, L.-I. L. (1958). Chronic Iron Deficiency Anaemia with Bone Changes Resembling Cooley's Anaemia. *Acta Haematologica*, *19*, 263–268.

Exner, S., Bogusch, G., & Sokiranski, R. (2004). Cribra orbitalia visualized in computed tomography. *Annals of Anatomy*, *186*(2), 169–172. https://doi.org/10.1016/S0940-

9602(04)80035-9

Galea, J. (2015). *Analysing the microarchitecture of cribra orbitalia via micro-computed tomography in post-medieval remains from the Bristol Royal Infirmary Jessica Christine Galea.* (January 2013). https://doi.org/10.13140/2.1.1243.6803

Garcia, A. R., Blackwell, A. D., Trumble, B. C., Stieglitz, J., Kaplan, H., & Gurven, M. D. (2020). Evidence for height and immune function tradeoffs among preadolescents in a high pathogen population. *Evolution, Medicine, and Public Health and Public Health*, *2020*(1), 86–99.

Garland, C. J. (2020). Implications of accumulative stress burdens during critical periods of early postnatal life for mortality risk among Guale interred in a colonial era cemetery in Spanish Florida (ca. AD 1605–1680). *American Journal of Physical Anthropology*, (January), 1–17. https://doi.org/10.1002/ajpa.24020

Gluckman, P. D., Hanson, M. A., & Spencer, H. G. (2005). Predictive adaptive responses and human evolution. *Trends in Ecology and Evolution*, *20*(10), 527–533. https://doi.org/10.1016/j.tree.2005.08.001

Gluckman, P. D., Hanson, M. A., Spencer, H. G., & Bateson, P. (2005). Environmental influences during development and their later consequences for health and disease: implications for the interpretation of empirical studies. *Proceedings of the Royal Society B*, *272*, 671–677. https://doi.org/10.1098/rspb.2004.3001

Godoy, R. A., & Leonard, W. R. (2020). *Tsimane' Amazonian Panel Study, El Beni, Bolivia, 2002-2010*. https://doi.org/10.3886/ICPSR37671.v1

Gomes, R. A. M. P., Petit, J., Dutour, O., & Santos, A. L. (2022). Frequency and co-occurrence of porous skeletal lesions in identified non-adults from Portugal (19th to 20th centuries) and its association with respiratory infections as cause of death. *International Journal of Osteoarchaeology*, (January), 1–12. https://doi.org/10.1002/oa.3132

Goodman, A. H., & Armelagos, G. J. (1988). Childhood Stress and Decreased Longevity in a Prehistoric Population. *American Anthropologist*, *90*(4), 936–944. https://doi.org/10.1525/aa.1988.90.4.02a00120

Gurevitch, O., Slavin, S., & Feldman, A. G. (2007). *Conversion of red bone marrow into yellow – Cause and mechanisms*. 531–536. https://doi.org/10.1016/j.mehy.2007.01.052

Gurven, M., Kaplan, H., & Supa, A. Z. (2007). *Mortality Experience of Tsimane Amerindians of Bolivia : Regional Variation and Temporal Trends*. *398*(September 2006), 376–398. https://doi.org/10.1002/ajhb

Gurven, M., Kaplan, H., Winking, J., Finch, C., & Crimmins, E. M. (2008). Aging and inflammation in two epidemiological worlds. *The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences*, *63*(2), 196–199. https://doi.org/63/2/196 [pii]

Gurven, M., Stieglitz, J., Trumble, B., Blackwell, A. D., Beheim, B., Davis, H., … Kaplan, H. (2017). The Tsimane Health and Life History Project: Integrating anthropology and biomedicine. *Evolutionary Anthropology*, *26*(2), 54–73. https://doi.org/10.1002/evan.21515

Hardy, R., & Cooper, M. S. (2009). Bone loss in inflammatory disorders. *Journal of Endocrinology*, *201*(3), 309–320. https://doi.org/10.1677/JOE-08-0568

Hayward, A. D., Rigby, F. L., & Lummaa, V. (2016). Early-life disease exposure and associations with adult survival, cause of death, and reproductive success in preindustrial humans. *Proceedings of the National Academy of Sciences of the United States of America*, *113*(32), 8951–8956. https://doi.org/10.1073/pnas.1519820113

Hengen, O. P. (1971). Cribra orbitalia pathogenesis and probable etiology-annotated. *HOMO*, *22*, 57–75.

Hermiston, M. L., & Mentzer, W. C. (2002). *A practical approach to the evaluation of the anemic child*. *49*, 877–891.

Kemkes-Grottenthaler, A. (2005). The short die young: The interrelationship between stature and longevity—evidence from skeletal remains. *American Journal of Physical Anthropology*, *128*(2), 340–347. https://doi.org/https://doi.org/10.1002/ajpa.20146

Klaus, H. D. (2017). Paleopathological rigor and differential diagnosis : Case studies involving terminology , description , and diagnostic frameworks for scurvy in skeletal remains. *International Journal of Paleopathology*, *19*, 96–110. https://doi.org/10.1016/j.ijpp.2015.10.002

Koontz Scaffidi, B. (2020). Spatial paleopathology: A geographic approach to the etiology of cribrotic lesions in the prehistoric Andes. *International Journal of Paleopathology*, *29*, 102–116. https://doi.org/10.1016/j.ijpp.2019.07.002

Kraft, T. S., Stieglitz, J., Trumble, B. C., Martin, M., Kaplan, H., & Gurven, M. (2018). Nutrition transition in two lowland Bolivian subsistence populations. *The American Journal of Clinical Nutrition*, *108*(6), 1183–1195. https://doi.org/10.1093/ajcn/nqy250

Kricun, M. E. (1985). Red-yellow marrow conversion: its effect on the location of some solitary bone lesions. *Skeletal Radiology*, *14*(1), 10–19.

Kuzawa, C. W. (2005). Fetal origins of developmental plasticity: Are fetal cues reliable predictors of future nutritional environments ? *American Journal of Human Biology*, *17*, 5–21. https://doi.org/10.1002/ajhb.20091

Lallo, J. W., Armelagos, G. J., & Mensforth, R. P. (1977). The Role of Diet , Disease , and Physiology in the Origin of Porotic Hyperostosis. *Human Biology*, *49*(3), 471–483.

Lanzkowsky, P. (1968). Radiologic features of iron deficiency anemia. *American Journal of Diseases in Childhood*, *116*, 16–29.

Marciniak, S., Bergey, C. M., Silva, A. M., Hałuszko, A., Furmanek, M., Veselka, B., … Perry, G. H. (2022). An integrative skeletal and paleogenomic analysis of prehistoric stature variation suggests relatively reduced health for early European farmers. *Proceedings of the National*

Academy of Sciences, *119*(15), e2106743119. https://doi.org/10.1073/pnas.2106743119/-/DCSupplemental.Published

Marks, P. W. (2012). Anemia : Clinical Approach. In L. M. Schmaier, Alvin H; Hillard (Ed.), *Concise guide to hematology* (First, pp. 24–34). Oxford: Blackwell Publishing Ltd.

Masterson, E. E., Fitzpatrick, A. L., Enquobahrie, D. A., Mancl, L. A., Eisenberg, D. T. A., Conde, E., & Hujoel, P. P. (2018). Dental enamel defects predict adolescent health indicators: A cohort study among the Tsimane' of Bolivia. *American Journal of Human Biology*, *30*(3), 1–10. https://doi.org/10.1002/ajhb.23107

Mathers, C. D., Boerma, T., & Ma Fat, D. (2009). Global and regional causes of death. *British Medical Bulletin*, *92*(1), 7–32. https://doi.org/10.1093/bmb/ldp028

Mays, S. (2012). The relationship between paleopathology and the clinical sciences. In A. L. Grauer (Ed.), *A Companion to Paleopathology* (pp. 285–309). https://doi.org/10.1002/9781444345940.ch16

Mcfadden, C., & Oxenham, M. F. (2020). A paleoepidemiological approach to the osteological paradox : Investigating stress , frailty and resilience through cribra orbitalia. *American Journal of Physical Anthropology*, *173*(2), 205–217. https://doi.org/10.1002/ajpa.24091

Mcilvaine, B. K. (2015). Implications of Reappraising the Iron-Deficiency Anemia Hypothesis. *International Journal of Osteoarchaeology*, *25*(6), 997–1000. https://doi.org/10.1002/oa.2383

Mensforth, R. P., Lovejoy, C. O., Lallo, J. W., & Armelagos, G. J. (1978). Part Two: The role of constitutional factors, diet, and infectious disease in the etiology of porotic hyperostosis and periosteal reactions in prehistoric infants and children. *Medical Anthropology*, *2*(April 2016), 1–59. https://doi.org/10.1080/01459740.1978.9986939

Mercier, F. E., Ragu, C., & Scadden, D. T. (2012). The bone marrow at the crossroads of blood and immunity. *Nature Reviews Immunology*, *12*(1), 49–60. https://doi.org/10.1038/nri3132

Mittler, D. M., & van Gerven, D. P. (1994). Developmental, diachronic, and demographic analysis of cribra orbitalia in the medieval christian populations of Kulubnarti. *American Journal of Physical Anthropology*, *93*(3), 287–297. https://doi.org/10.1002/ajpa.1330930302

Moore, S. (1929). The bone changes in sickle cell anemia with note on similar changes observed in skulls of ancient Mayan Indians. *Journal of Missouri Medical Association*, *26*, 561.

Morgan, J. (2014). *The Methodological and Diagnostic Applications of Micro-CT to Palaeopathology : A Quantitative Study of Porotic Hyperostosis* (The University of Western Ontario). Retrieved from https://ir.lib.uwo.ca/etd/2056/

Moseley, J. E. (1965). The Paleopathologic riddle of "symmetrical osteoporosis." *American Journal of Roentgenology*, *95*(1), 135–142. https://doi.org/10.2214/ajr.95.1.135

Mulholland, K. (2007). Perspectives on the burden of pneumonia in children. *Vaccine*, *25*(13), 2394–2397.

Myint, Z. W., Oo, T. H., Thein, K. Z., Tun, A. M., & Saeed, H. (2018). Copper deficiency anemia: review article. *Annals of Hematology*, *97*(9), 1527–1534. https://doi.org/10.1007/s00277-018-3407-5

O'Donnell, L. (2019). Indicators of stress and their association with frailty in the precontact Southwestern United States. *American Journal of Physical Anthropology*, (May), 1–14. https://doi.org/10.1002/ajpa.23902

O'Donnell, L., Hill, E. C., Anderson, A. S., & Edgar, H. J. H. (2020). Cribra orbitalia and porotic hyperostosis are associated with respiratory infections in a contemporary mortality sample from New Mexico. *American Journal of Physical Anthropology*, *173*(4), 721–733. https://doi.org/10.1002/ajpa.24131

Obertova, Z., & Thurzo, M. (2004). Cribra orbitalia as an indicator of stress in the early medieval Slavic population from Borovce (Slovakia). In *Anthropologie* (Vol. 42).

Ortner, D. J. (1991). Theoretical and Methodological Issues in Paleopathology. In J. O. Donald & A. C. Aufderheide (Eds.), *Human paleopathology: current syntheses and future options* (pp. 5–11). Washington and London: Smithsonian Institution Press.

Ortner, D. J. (2003). *Identification of Pathological Conditions in Human Skeletal Remains* (2nd ed.). San Diego: Academic Press.

Ortner, D. J., & Ericksen, M. F. (1997). Bone changes in the human skull probably resulting from scurvy in infancy and childhood. *International Journal of Osteoarchaeology*, *7*, 212–220.

Ortner, D. J., & Putschar, W. G. J. (1981). *Identification of Pathological Conditions in Human Skeletal Remains*. https://doi.org/10.1017/CBO9781107415324.004

Oski, F. A. (1993). Iron Deficiency in Infancy and Childhood. *New England Journal of Medicine*, *329*(3), 190–193. https://doi.org/10.1056/NEJM199307153290308

Oxenham, M. F., & Cavill, I. (2010). Porotic hyperostosis and cribra orbitalia : the erythropoietic response to iron-deficiency anaemia. *Anthropological Science*, *118*(May), 199–200. https://doi.org/10.1537/ase.100302

Papathanasiou, A., Meinzer, N. J., Williams, K. D., & Larsen, C. S. (2018). History of anemia and related nutritional deficiencies: Evidence from cranial porosities. In *The Backbone of Europe: Health, Diet, Work and Violence over Two Millennia*.

Peres, M. A., Macpherson, L. M. D., Weyant, R. J., Daly, B., Venturelli, R., Mathur, M. R., … Watt, R. G. (2019). Oral diseases: a global public health challenge. *The Lancet*, *394*(10194), 249–260. https://doi.org/10.1016/S0140-6736(19)31146-8

Polo-Cerdá, M., Miquel-Feucht, M., & Villalaín-Blanco, J. D. (2000). Experimental cribra orbitalia in Wistar rats: an etiopathogenic model of porotic hyperostosis and other porotic phenomena. *Proceedings of XIIIth European Meeting of the Palepathology Association*, (January 2001), 253–259.

Procianoy, F., Brandão Filho, M., Cruz, A. A. V. e, & Alencar, V. M. (2008). Subperiosteal hematoma and orbital compression syndrome following minor frontal trauma in sickle cell anemia: case report. *Arquivos Brasileiros de Oftalmologia*, *71*(2), 262–264. https://doi.org/10.1590/s0004-27492008000200024

Richman, R. (2009). *Spatial analysis of porotic hyperostosis and cribra orbitalia: Do they share an etiology?* University of California Santa Barbara.

Rivera, F., & Mirazon Lahr, M. (2017). New evidence suggesting a dissociated etiology for cribra orbitalia and porotic hyperostosis. *Am J Phys Anthropol*, *164*(1), 76–96. https://doi.org/10.1002/ajpa.23258

Roberts, C. A., & Steckel, R. H. (2019). The Developmental Origins of Health and Disease: Early Life Health Conditions and Adult Age at Death in Europe. In R. H. Steckel, C. S. Larsen, C. A. Roberts, & J. Baten (Eds.), *The Backbone of Europe: Health, diet, work and violence over two millennia* (1st ed., pp. 325–351). Cambridge: Cambridge University Press.

Robertson, S. K. (2017). *Cribra orbitalia in an archaeological context : new insights from micro-CT analysis of the Christchurch Spitalfields collection*. (August).

Ryan, A. S. (1997). Iron-Deficiency Anemia in Infant Development: Implications for Growth, Cognitive Development, Resistance to Infection, and Iron Supplementation. *Yearbook of Physical Anthropology*, *40*, 25–62.

Sabazali, C., & Cheverko, C. M. (2022). Exploring the inclusion of developmental stress trajectories on past adult mortality patterns: Quantifying skeletal plasticity and adult mortality risk in post-medieval London using a probability tree model. *International Journal of Osteoarchaeology*, *32*(3), 706–717. https://doi.org/10.1002/oa.3097

Sakel, J. (2011). Mosetén and Chimane Argument Coding: A Layered System. *International Journal of American Linguistics*, *77*(4), 537–557. https://doi.org/10.1086/662159

Shahidi, nasrollah T.; Diamond, L. K. (1960). Skull changes in infants with chronic iron-deficiency anemia. *New E*, *262*(3), 137–139.

Speal, C. S. (2017). *Mortality -- The Ultimate stress marker: Skeletal lesions and survival analysis at Roman Viminacium*. London.

Stearns, S. C. (1992). *The evolution of life histories*. Oxford: Oxford University Press.

Steckel, R. H. (2005). Young adult mortality following severe physiological stress in childhood: Skeletal evidence. *Economics and Human Biology*, *3*(2), 314–328. https://doi.org/10.1016/j.ehb.2005.05.006

Steckel, R. H., Larsen, C. S., Roberts, C. A., & Baten, J. (Eds.). (2019). *The Backbone of Europe: Health, Diet, Work and Violence over Two Millennia*. Cambridge: Cambridge University Press.

Steckel, R. H., Larsen, C. S., Sciulli, P. W., & Walker, P. L. (2005). The Global History of Health Project: data collection codebook. *Ohio State University, Columbus, OH*, 40. Retrieved from http://global.sbs.ohio-state.edu

Steyn, M., Voeller, S., Botha, D., & Ross, A. H. (2016). Cribra orbitalia: Prevalence in contemporary populations. *Clinical Anatomy*. https://doi.org/10.1002/ca.22734

Stuart-Macadam, P. (1985). Porotic hyperostosis: representative of a childhood condition. *American Journal of Physical Anthropology*, *66*, 391–398. https://doi.org/10.1002/ajpa.1330660407

Stuart-Macadam, P. (1987). A radiographic study of porotic hyperostosis. *American Journal of Physical Anthropology*, *74*(4), 511–520. https://doi.org/10.1002/ajpa.1330740409

Suzuki, K. (2018). The developing world of DOHaD. *Journal of Developmental Origins of Health and Disease*, *9*(3), 266–269. https://doi.org/10.1017/S2040174417000691

Tayles, N. (1996). Anemia, genetic diseases, and malaria in prehistoric Mainland Southeast Asia. *American Journal of Physical Anthropology*, *101*(1), 11–27. https://doi.org/10.1002/(SICI)1096-

8644(199609)101:1<11::AID-AJPA2>3.0.CO;2-G

Temple, D. H. (2018). Bioarchaeological evidence for adaptive plasticity and constraint : Exploring life-history trade-offs in the human past. *Evolutionary Anthropology*, *28*(September), 34–46. https://doi.org/10.1002/evan.21754

Usher, B. M. (2000). *A Multistate model of health and mortality for paleodemography: Tirup Cemetery*. The Pennsylvania State University.

Vercellotti, G., Piperata, B. A., Agnew, A. M., Wilson, W. M., Dufour, D. L., Reina, J. C., … Sciulli, P. W. (2014). Exploring the multidimensionality of stature variation in the past through comparisons of archaeological and living populations. *American Journal of Physical Anthropology*, *155*(2), 229–242. https://doi.org/10.1002/ajpa.22552

Walker, P. L., Bathurst, R. R., Richman, R., Gjerdrum, T., & Andrushko, V. A. (2009). The causes of porotic hyperostosis and cribra orbitalia: A reappraisal of the iron-deficiency-anemia hypothesis. *American Journal Of Physical Anthropology*, *139*(2), 109–125. https://doi.org/10.1002/ajpa.21031

Wapler, U., Crubézy, E., & Schultz, M. (2004). Is cribra orbitalia synonymous with anemia? Analysis and interpretation of cranial pathology in Sudan. *American Journal of Physical Anthropology*, *123*(4), 333–339. https://doi.org/10.1002/ajpa.10321

WHO. (2014). *Global nutrition targets 2025: stunting policy brief (WHO/NMH/NHD/14.3)*. https://doi.org/10.7591/cornell/9781501758898.003.0006

WHO. (2020). *Improving early childhood development: WHO guideline*. Retrieved from https://apps.who.int/iris/handle/10665/331306

Wilkie, R., Parmar, S. S., Blagojevic-Bucknall, M., Smith, D., Thomas, M. J., Seale, B. J., … Peat, G. (2019). Reasons why osteoarthritis predicts mortality: path analysis within a Cox proportional hazards model. *RMD Open*, *5*(2), e001048. https://doi.org/10.1136/rmdopen-

2019-001048

Wood, J. W., Milner, G. R., Harpending, H. C., Weiss, K. M., Cohen, M. N., Eisenberg, L. E., … Weiss, K. M. (1992). The osteological paradox : Problems of inferring prehistoric health from skeletal samples. *Current Anthropology*, *33*(4), 343–370.

Worthman, C. M., & Kuzara, J. (2005). Life history and the early origins of health differentials. *American Journal of Human Biology*, *17*(1), 95–112. https://doi.org/10.1002/ajhb.20096

Wright, L. E., & Yoder, C. J. (2003). Recent progress in bioarchaeology: Approaches to the Osteological Paradox. *Journal of Archaeological Research*. https://doi.org/10.1023/A:1021200925063

Zheng, H. (2014). Aging in the Context of Cohort Evolution and Mortality Selection. *Demography*, *51*(4), 1295–1317. https://doi.org/10.1007/s13524-014-0306-9

IISetting the stage for investigating an archaeologically defined skeletal phenomenon in a living population: Do computed tomography findings agree with traditional osteological examination?

2.1 Introduction

2.1.1 Medical imaging and osteological inference

When paleopathologists diagnose a disease in human skeletal remains, they do so using operational definitions of the potential diagnoses, or 'skeletal disease scripts' (S. A. Mays, 2020). These operational definitions consist primarily of bone changes observed in clinical cases with diagnoses made independent of skeletal observations (Waldron, 2009) and sometimes of bone changes inferred from a biological approach to pathophysiology (Mays, 2012; Ortner & Ericksen, 1997). Such a diagnosis-centered approach is indispensable to paleopathological practice and an inextricable part of the traditional relationship between paleopathology and the clinical sciences (Mays, 2012). However, advances in medical imaging, particularly in computed tomography (CT), have made it possible to create 3-D reconstructions of the skeletal surface that parallel the archaeologist's direct view of human skeletal remains. This parallelism opens the door to a logical reversal—rather than asking which skeletal changes are associated with a given diagnosis, a lesion-centered approach asks which aspects of disease are associated with a given lesion.

Mapping the relationship between skeletal manifestations of disease and the lived experience of individuals remains a critical endeavor for paleopathology (DeWitte & Stojanowski, 2015; Mays, 2012; J. W. Wood et al., 1992), one that can be productively addressed

with examinations of skeletal lesions in contemporary cases. Existing clinical CT scans with accompanying data on symptoms and diagnoses stand to clarify lesion formation processes and the relationship between skeletal plasticity and individual experiences of disease. Not only will these investigations uncover the clinical implications of specific skeletal lesions but also extricate how severity of stress, age of onset, and comorbidities contribute to differences in lesion expression.

In order to bring osteological perspectives to clinical contexts, we first need to ascertain how well paleopathologically-defined features can be detected with medical imaging and how their expression in medical imaging might differ from that directly observed in skeletal materials. This ensures that clinical and archaeological investigation are not miscommunicating due to 1) differing definitions of skeletal phenomena or 2) constraints on the sensitivity of diagnostic imaging. The current study validates—and delineates the limitations of—a CT-based approach to evaluating porous cranial lesions on clinical CT scans with bone-optimized reconstructions.

2.1.2 Porous cranial lesions: A case study

Porous cranial lesions in the orbits and cranial vault (cribra orbitalia and porotic hyperostosis, broadly defined) are often used by bioarchaeologists as skeletal indicators of childhood physiological stress (O'Donnell, 2019; Obertova & Thurzo, 2004; Steckel, Rose, Larsen, & Walker, 2002). They present a good validation case because they are primarily archaeological phenomena, both in definition and in representation. Their frequency in the archaeological record far exceeds reports of their presence in clinical literature (Józsa & Pap, 1990; Stuart-Macadam, 1987). This disparity between the archaeological record and clinical literature is surprising since the social and environmental factors that bioarchaeologists link to

these skeletal lesions include chronic microbial and parasitic infection, food insecurity, and inadequate dietary diversity, all of which are still common on a global scale (Ortner, 2003; Rivera & Mirazon Lahr, 2017; Stuart-Macadam, 1987b; Walker et al., 2009). The higher prevalence of porous cranial lesions reported in anthropological studies of modern mortality samples suggests that the absence of these lesions reported in clinical contexts may not reflect their absence in contemporary populations (Beatrice & Soler, 2016; David, 2018; O'Donnell et al., 2020; Steyn, Voeller, Botha, & Ross, 2016; Wright & Chew, 1998). Here we evaluate the feasibility of applying paleopathological criteria to CT scans for porous cranial lesion identification—an approach that could lead to different conclusions about the prevalence of these skeletal lesions in contemporary populations and to new insights on their causes and consequences.

2.1.3 Archaeological and radiological approaches to porous cranial lesions

The different methods by which osteologists and radiologists examine the skeleton can emphasize different aspects of lesion-related morphology (S. Mays, 2012). Because osteologists largely define and identify porous cranial lesions based on direct macroscopic examination of the ectocranial surface they tend to emphasize surface porosity, while radiologists have historically viewed the cranium on radiographs in the sagittal or coronal plane and focused instead on expansion of the bone layers in the cranial vault. The main clinical radiological finding equated with porous cranial lesions is a radially striated 'hair-on-end' appearance of the diploic trabeculae with abnormal thickness of the diploë. This finding is noted in radiography of severe childhood anemia cases, most commonly thalassemia and sickle-cell anemia (Angel, 1964; Resnick and Niwayama, 1988). These anemia-related skeletal changes are initiated in the diploic space; cranial

surface porosity that results from anemia should thus always be accompanied by underlying diploic changes.

The cranial porosity encountered in archaeological cases is caused by multiple lesion formation processes, marrow hyperplasia being just one. Radiological and histological examination of archaeological skeletal remains reveals that cranial surface porosity is not invariably accompanied by expansion of the underlying diploic bone (M. B. Brickley, 2018; Ortner, 2003). Histological cross sections of archaeological skeletal remains with porous cranial lesions confirm differences in underlying morphology that speak to the range of processes capable of producing a porous appearance of the ectocranial surface (M. B. Brickley, Ives, & Mays, 2020; Ortner, 2003; Wapler et al., 2004). It is also clear, given the age distribution of unremodeled lesions in the archaeological record, that these lesions form almost exclusively in childhood, and that lesions in adults are largely evidence of individual medical history (Blom et al., 2005; Stuart-Macadam, 1985). In the case of new bone formation in response to localized inflammation, lesion formation need not be confined to childhood, though the higher rate of bone turnover during growth and development makes osseous responses to stimuli more likely at younger ages.

Stuart-Macadam's (1987) radiological study identified a suite of radiological features that are more commonly seen in radiographs of crania with porous vault and orbital lesions. Taken in aggregate, these lesion-associated traits suggest that marrow hyperplasia is a cause of porous cranial lesions but likely not the cause of all such lesions in the study. The only feature common to the majority of lesion cases—an abnormal texture of the diploë described as 'diploic granularity'—was also the least specific and deemed the most difficult trait to evaluate. The hair-on-end feature was pathognomonic for porous cranial lesions but rare, only found in 8% of cases examined by Stuart-Macadam.

We propose that the causes and consequences of porous cranial lesions in the living be investigated by applying paleopathological criteria for their identification to clinical cranial imaging. Rather than identifying these lesions in living individuals based primarily on anemia-related changes in the diploic space, cranial surface porosity might be investigated as a phenomenon in its own right. Its associations with underlying osseous abnormality, clinical diagnoses, and individual disease experience can then be examined. Advances in medical imaging technology, particularly computed tomography, make it possible to realize this approach (Exner et al., 2004; Naveed, Abed, Davagnanam, Uddin, & Adds, 2012; Rivera & Mirazon Lahr, 2017).

Cranial computed tomography (CT) provides more detailed images than traditional radiographs. Rather than the single superimposed image produced by radiography, CT affords both three-dimensional views of the skull's ectocranial surface and discrete cross sections in axial, coronal, and sagittal planes (Beckett, 2014). Cranial surface porosity, underlying osseous features, and their relationship both to each other and to disease can thus be investigated using CT reconstructions. Paleopathologists have already used CT to differentiate causes of cranial vault lesions (Zuckerman, Garofalo, Frohlich, & Ortner, 2014) and test the relationship between causes of orbital and vault lesions (Rivera & Mirazon Lahr, 2017). Applying a lesion-centered approach to CT scans of living individuals is a natural extension of this research and opens new possibilities for exploring the connection between porous cranial lesions and individual disease experience. A ready pool of data for such studies exists in scans obtained during medical treatment.

As a first step toward investigating these lesions in contemporary populations, we assess the comparability of cranial lesion data produced using traditional in-person and photo-based osteological evaluations of crania and evaluations of the same crania using multiple CT viewing scenarios. Following in-person evaluation by lead author (AA) of 22 archaeological crania for

lesion presence and lesion surface morphology, four observers (including AA) conducted independent evaluations of porous cranial lesions using the same crania based on photographs, 3-D CT reconstructions of the cranial surface, and cross-sectional 2-D CT views. In cross section, observers primarily recorded pitting and porosity at the ectocranial surface to evaluate the utility of 2-D cross-sectional views for capturing the surface features that define porous cranial lesions in archaeological settings.

2.2 Materials and Methods

2.2.1 Subjects

This study utilized crania housed at the Smithsonian National Museum of Natural History (NMNH) biological anthropology collection. Because this study was intended to establish a baseline for an analysis of porous cranial lesions from existing CT scans of living adults, we focused solely on adult crania. Adult status was determined by the presence of fully erupted third molars, evidential wear on the dentition and closure of late adolescent apophyses. Eligible crania selected were >90% complete. Individuals with extreme cranial modification or excessive taphonomic degradation were excluded from analysis. Crania were chosen to represent the range of bilateral porous cranial lesions expressed in adults (Fig. S1).

The study sample consisted of 22 adult crania (15 estimated male) collected by Aleš Hrdlička in 1913 (Hrdlička, 1914). All but one came from the site of Pachacamac (A.D. 200-1533) on the southern Peruvian coast. The final individual, selected for their extreme cranial vault lesions, was from the Chicama valley, 685 km north of Pachacamac. Broad age categories were estimated based on dental wear and ectocranial suture closure (Meindl & Lovejoy, 1985), and sex was estimated from cranial nonmetric features (Buikstra & Ubelaker, 1994).

2.2.2 CT Scanning

Scan parameters and subject positioning were selected to approximate those used for brain CT scans of living patients. Scanning was implemented using the NMNH Siemens SOMATOM Emotion 6 computed tomography (CT) scanner using 0.63 mm slice thickness, 130 kVp, 140 mAs (Table 1). Scans were obtained with a pitch of 0.65. Image resolution in the axial plane was determined by the scan's field of view (199 mm) and the 512 x 512 scan matrix, producing pixels approximately 0.39 mm^2. Slice thickness constrained image resolution in the Z plane, creating coronal and sagittal images with pixels 0.39 mm x 0.63 mm. Each cranium was positioned in Frankfort plane (to replicate living patient positioning), the midline centered on nasion. Siemens ultra-sharp U90 bone reconstruction algorithm (kernel) was applied to the raw data to optimize visualization of skeletal structures. In clinical contexts, such an algorithm would typically be used to assess skull trauma as well as a standard soft-tissue algorithm to identify intracranial hemorrhage (Maetani et al., 2016).

Table 2. 1 A selected lexicon of CT terminology

	Term	**Definition**	**Synonyms**	**Importance**
Setting scan parameters	Gantry	The circular frame housing the x-ray tube, collimators, and detectors	--	Positioning of the gantry relative to the patient determines the angle of scan acquisition.
	Pitch	Distance traveled by the scanner table in one 360° gantry rotation divided by beam width	Increment; table feed	Pitch determines how much interpolation is done to construct slices from helical CT. Lower pitch = higher image resolution. Higher pitch = lower resolution.
	Slice thickness	Depth in the Z axis (usually axial) of each constructed CT image	--	Slice thickness determines image resolution in coronal and sagittal reconstructions.
	Field of View (FOV)	Diameter of the area being scanned	--	Field of view (mm) divided by the size of the scan matrix (often 512) yields the image resolution (mm/pixel) in the axial plane. FOV can often be set prior to scanning, and smaller FOV results in higher resolution images.

	kilovoltage peak (kVp)	Maximum voltage of x-ray tube	--	Higher voltage can return higher image quality, but also results in higher radiation exposure to the patient.
	Milliamperes (mA)	Measure of the current in the x-ray tube	--	Like kVp, higher mA can return higher quality images, but this must be balanced against the need to minimize patient radiation exposure.
	Voxel	a cubic unit equivalent to a pixel for 3-D graphics	--	Voxel size is determined by axial resolution and slice thickness (0.39 x 0.39 x 0.625, in the case of this study). When slice thickness = axial resolution, voxel dimensions are cubic (isotropic) and image resolution in axial, coronal, and sagittal planes is identical.
	Reconstruction algorithm	A filter that modifies CT projection data in order to reduce image blurring of backprojected 2-D images	reconstruction kernel; filter; convolution algorithm	Algorithms are chosen at the time of scanning based on the purpose of the scan. Those optimized for viewing bone (hard/sharp algorithms) produce sharper images with higher spatial resolution but can create higher density values at borders where density contrasts are high (edge hardening).
Viewing scan reconstructions	Window Width	The range of CT density values mapped onto the shade palette of the CT image display	--	Setting the window width and level to the appropriate values can maximize visual differentiation for the tissue of interest while minimizing visibility of other tissue types.
	Window Level	The CT density value mapped to the middle tone of the CT display palette	--	

2.2.3 Cranial lesion evaluation

2.2.3.1 In-person lesion classification

Lesion presence and morphology were classified in person by ASA using the Stuart-Macadam (1985) classification schema: 0 – absence of lesion; 1 – scattered fine foramina; 2 – large and small isolated foramina; 3 – foramina coalescing in a trabecular structure; 4 – outgrowth in trabecular structure from the normal contour of the outer bone table (Figure S2.1). When morphology varied within a single lesion, the highest score was recorded. Of the six cases of ectocranial vault lesions classified as 3, four presented with well-healed superficial impressions

(Fig. S2.1g), while in the other two cases trabeculated foramina perforated the outer table (Fig. S2.1h).

2.2.3.2 Photograph and CT-based lesion classification

Four observers—three osteologists familiar with porous cranial lesions (ASA, LO, EH), and an experienced clinical radiologist (MLS)—scored the crania for porous lesions of the orbital roofs and cranial vault using three separate viewing scenarios: photographs, 2-D orthogonal CT multi-planar reconstructions (MPR), and 3-D CT volume renderings. Order of crania was randomized for each observer at each observation session. Photographs were assigned anonymized identifiers to limit recognition of individual specimens across modalities, and CT observation sessions for 2-D MPR and 3-D volume rendering were conducted on separate days.

CT scans were evaluated using the freeware DICOM viewer Horos version 4.0.0 (Horos, 2019). In 2-D MPR the viewing window was set to Horos' bone-optimized preset, and observers were able to scroll through cross-sectional slices of the scan in coronal, sagittal, or axial planes. For 3-D volume rendering, observers were able to rotate volume-rendered images, and each observer chose settings within Horos that they deemed best to maximize detail visibility (Fig. S2.2; Fig. S2.3). While this introduces extra variables to evaluations from the 3-D renderings, it is also likely to capture a realistic source of variation in the way this viewing modality is used by different individuals and on different display screens. All observers chose to view renderings at the finest level of detail and best possible resolution and set the rendering colors to either 'VR muscle and bone' or 'VR bone.' Observers differed in their choices of preset window width and level settings ('Default,' 'Full Dynamic,' 'Bone CT'), voxel opacity function (logarithmic or linear), and individually customized shading settings (see Figs. S2.2 and S2.3).

In both photographs and CT 3-D volume rendering, observers scored lesion surface morphology using Stuart-Macadam's (1985) scoring classification. Observers did not score degree of lesion healing; no individual in the sample (all adults) appeared to have unremodeled lesions at time of death. On 2-D MPR images observers determined the presence or absence of three lesion-related traits (Figure 2.1): radial trabecular orientation (radiologic 'hair-on-end' feature), superficial pitting of the outer table (ectocranial pitting), and porosity of the outer table. For orbital roofs, observers scored only cortical porosity.

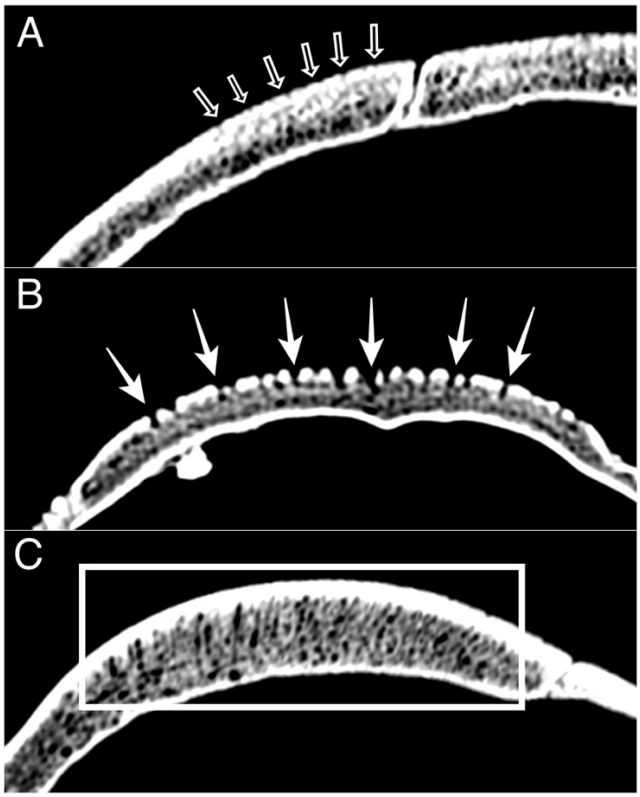

Figure 2. 1 Lesion-related traits as seen on 2-D CT MPR. A) Open arrows indicate ectocranial pitting: Dimples or divots disrupt the contour of the outer table's ectocranial surface (sagittal view, frontal and parietal bones). B) Solid arrows indicate porosity: porous channels run from the ectocranial surface to the diploic bone beneath the outer table (axial view, occipital bone). C) Hair-on-end sign: Bone in the diploic space has a radial orientation with trabeculae lying perpendicular to the outer table. Note ectocranial pitting also visible in C (sagittal view, frontal and parietal bones).

2.3 Results and Discussion

Lesion morphology scores were correlated among in-person, photographic, and 3-D CT assessments (Figure 2.2), though each viewing modality has its own strengths and weaknesses for evaluating lesions, and the effect of viewing modality differed for vault lesions and orbital roof lesions (Table 2.2). The performance of each viewing modality was impacted by differences in image resolution and the depth or superficiality of lesion-related changes. For example, the high resolution of photographs relative to CT reconstructions resulted in higher correlation between photo-based and in-person evaluations of lesion morphology (categories 0-4) (Table 2.3). The relatively low resolution of 3-D volume-rendered CT, on the other hand, removed the visibility of pseudopathological features and returned better agreement with direct observations of vault lesion presence/absence as a result (Table 2.4). Evidence of vault lesions was clearest in CT viewing modalities when true porosity penetrated the full depth of the outer cortex (Figure 2.1A). However, 3-D volume-rendered CT was more sensitive than 2-D MPR for detecting the shallower imprints of ectocranial pitting, and superficial osseous changes were visible only in photographs.

The range of lesion morphology visible on CT is delineated below. Because the effect of CT on evaluations of cranial vault lesions differs from its effect on orbital roof lesions, the effects of viewing modality on lesion visibility are discussed separately for vault and orbital lesions.

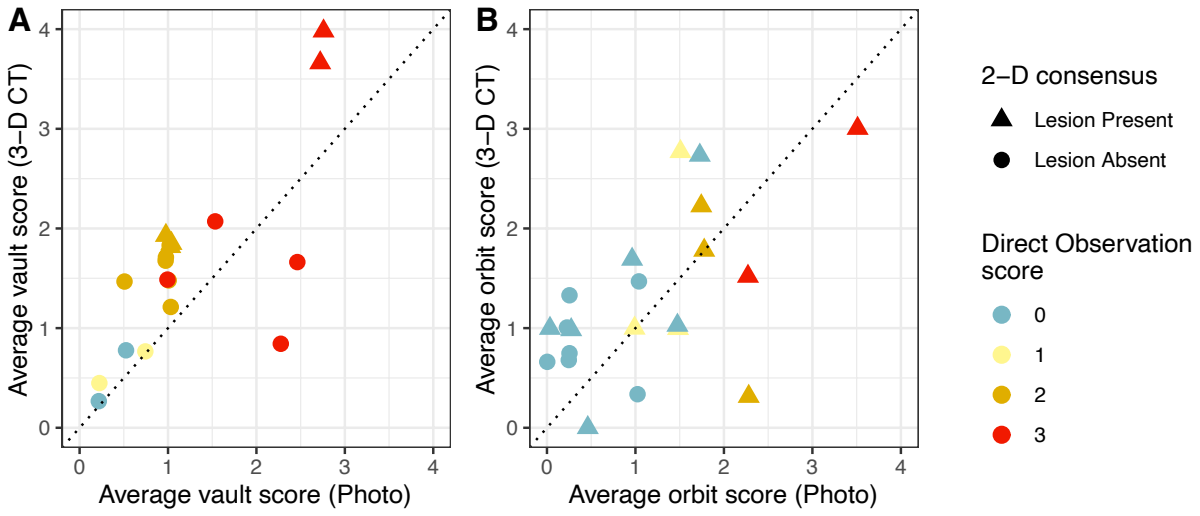

Figure 2. 2 Comparison of lesion evaluations for each cranium across all viewing modalities (n = 22): in-person cranial lesion classification (color scale), average cranial lesion scores from four observers based on evaluation of photographs (x-axis) and based on 3-D volume rendering (y-axis), and majority opinion on lesion presence/absence from 2-D MPR (shape scale).

Table 2. 2 Ordered logistic regression of the effect of viewing modality on lesion morphology. Reporting differs for orbital and vault lesions, particularly for CT.

	Odds Ratio	lower 95% CI	upper 95% CI
Modality Photo	1.17	0.74	1.87
Modality3-D CT	0.49	0.30	0.78
Lesiontype_orbit	0.12	0.07	0.20
modalityphoto:lesiontype_orbit	2.02	1.01	4.07
Modality3-D CT:lesiontype_orbit	6.61	3.23	13.61

Reference case is direct observation of vault lesions. Vault lesions are likely to be given lower scores from 3-D CT than from direct observation, while orbital lesions are likely to be given higher scores. Lesion classification categories were treated as a heuristic ordinal scale for this model. The model was estimated in R v. 3.6.1 (http://cran. r-project.org/) using the *polr* command in the *MASS* package.

Table 2. 3 Relative performance of photographs and volume-rendered (3-D) CT images for determining lesion morphology category(0-4).

	Vaults		Orbits	
	Photo	**3-D**	**Photo**	**3-D**
Mean score difference (relative to Direct Obs.)	1.30	-0.22	1.44	0.63
Pearson's R (correlation with Direct Obs.)	0.78 (0.68, 0.85)	0.59 (0.43, 0.71)	0.67 (0.53, 0.77)	0.32 (0.11, 0.51)
Intraclass Correlation Coefficient (95% Confidence Interval)	0.80 (0.66, 0.90)	0.66 (0.46, 0.82)	0.58 (0.37, 0.77)	0.27 (0.04, 0.61)

Intraclass Correlation Coefficients show interobserver concordance within each viewing modality.

Table 2. 4 Relative performance of different viewing modalities for detecting lesion presence/absence.

Vault	Photo	2-D MPR	3-D
Sensitivity*	1	0.63	0.93
Specificity†	0.10	0.75	0.5
Positive Predictive Value **	0.79	0.9	0.86
Negative Predictive Value††	1	0.38	0.67
% Agreement with Direct Obs.	79.5	52.3	83.0
Orbits	**Photo**	**2-D MPR**	**3-D**
Sensitivity	0.94	0.97	0.69
Specificity	0.52	0.39	0.29
Positive Predictive Value	0.53	0.48	0.35
Negative Predictive Value	0.94	0.96	0.62
% Agreement with Direct Obs.	67.0	44.3	48.7

$* \frac{\text{number of lesions reported present}}{number\ of\ lesions\ truly\ present}$

$\dagger \frac{\boldsymbol{number\ of\ lesions\ reported\ absent}}{\boldsymbol{number\ of\ lesions\ truly\ absent}}$

**the probability a lesion identified as present was truly present (i.e., reported during direct observation): $\frac{lesion\ frequency * sensitivity}{((lesion\ frequency * sensitivity) + (1 - lesion\ frequency) * (1 - specificity)}$

$\dagger\dagger$probability that a case identified as absent of lesions was truly absent (i.e., similarly reported during direct observation): $\frac{(1 - \text{disease frequency}) * \text{specificity}}{(\text{frequency} * (1 - \text{sensitivity}) + (1 - \text{frequency}) * \text{specificity})}$

Sensitivity and specificity, used clinically to measure the accuracy of a diagnostic test in detecting a disease, are used here to describe the detection rates of cranial lesions from different viewing modalities. Values are calculated using the directly observed lesion frequencies as 'true' prevalence.

2.3.1 Cranial vault lesions

2. 3.1.1 3-D volume-rendered CT

Evaluations from 3-D volume-rendered CT out-performed 2-D MPR and photographs in detecting the presence of cranial vault lesions; observer consensus on 3-D volume-rendered CT images provided the closest match to in-person evaluation of cranial vault lesion presence (83% agreement) (Table 2.4). The cranial surface reconstruction of 3-D volume-rendered CT also has the advantage over 2-D MPR of allowing observers to evaluate lesion morphology using the same criteria as direct observation of skeletal remains, though 3-D volume-rendered morphology scores tend to be influenced by the same factors that affect lesion visibility.

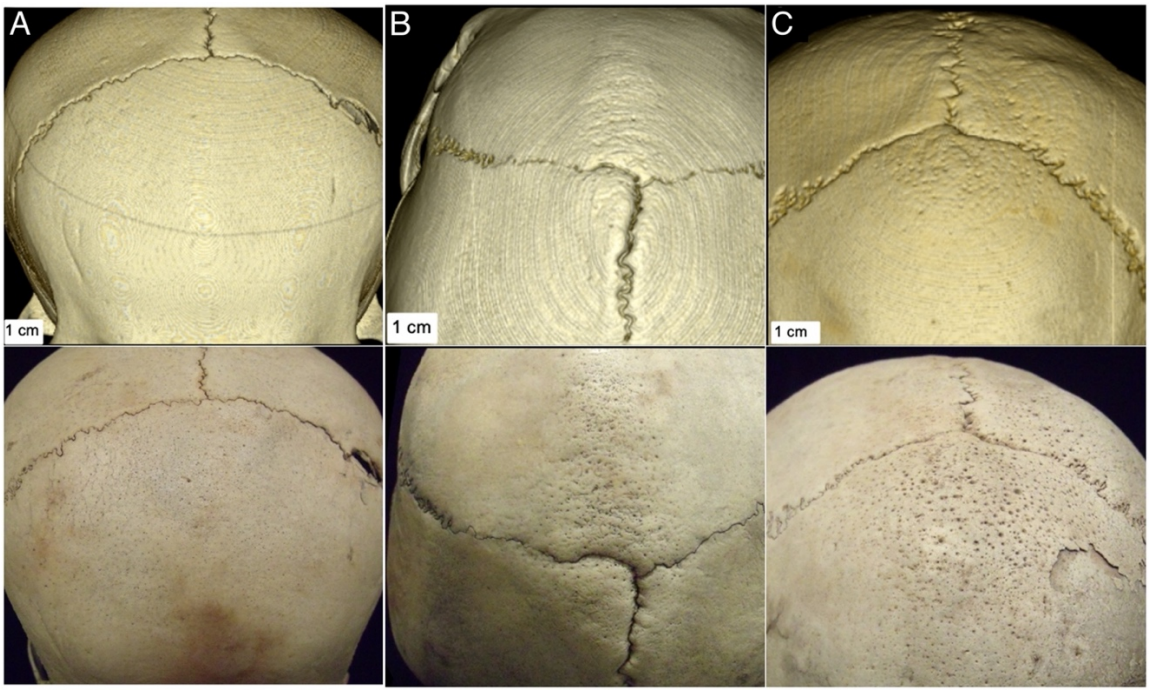

Figure 2. 3 Side-by-side comparison of ectocranial lesion visibility for three crania, photos (bottom) and 3-D CT volume renderings (top).A) Lesions classified from photographs as 1, 'scattered fine foramina,' were not well translated into 3-D CT renderings. B) Superficial ectocranial impressions created disagreement about lesion classification from photographs, and these superficial structures were not visible in 3-D CT renderings. C) Lesions scored from photographs as 2, 'large and small isolated foramina,' were consistently identified from 3-D CT

renderings, though small foramina were often not visualized unless they occurred near bregma. The concentric rings visible on 3-D renderings are minor stair-step artifacts in the scans that result from mapping objects that lie obliquely across the axial plane of acquisition. Image reconstruction with smoothing algorithms will minimize these artifacts but also obscures fine cranial porosity. 'Fine scattered foramina' is also described by Buikstra and Ubelaker (1994) as 'barely discernible porosity,' (p.151) while 'large foramina' is alternately described as 'true porosity' (p.126), a distinction which seems appropriate to their appearance in volume-rendered CT images.

Lesion visibility appeared to be determined by two features: the extent of true porosity and the size of cortical defects (pitting or porosity). The majority of observers agreed that cranial vault lesions were present on 3-D volume-rendered CT images when lesions manifest either as coalescing porosity with trabecular patterning (score: 3 or 4) or included foramina with a diameter larger than the scan resolution (Fig. 2.3). Foramina above this size threshold (> 0.5 mm) were identified as 'large foramina' (score: 2) from photographs and in-person observation (Fig. 2.4A) but often identified on 3-D CT images as 'fine scattered foramina' (score: 1). Of the 15 crania with in-person vault lesion scores >1, observers unanimously identified lesions in 13. Observer disagreement over the remaining two crania was likely due to the advanced state of lesion remodeling; superficial trabeculated impressions lacking open porosity created ambiguous lesion expression on CT images. Altogether, these results suggest that vault lesions with more than superficial involvement and porosity surpassing the scan resolution can be identified on CT scans of living individuals.

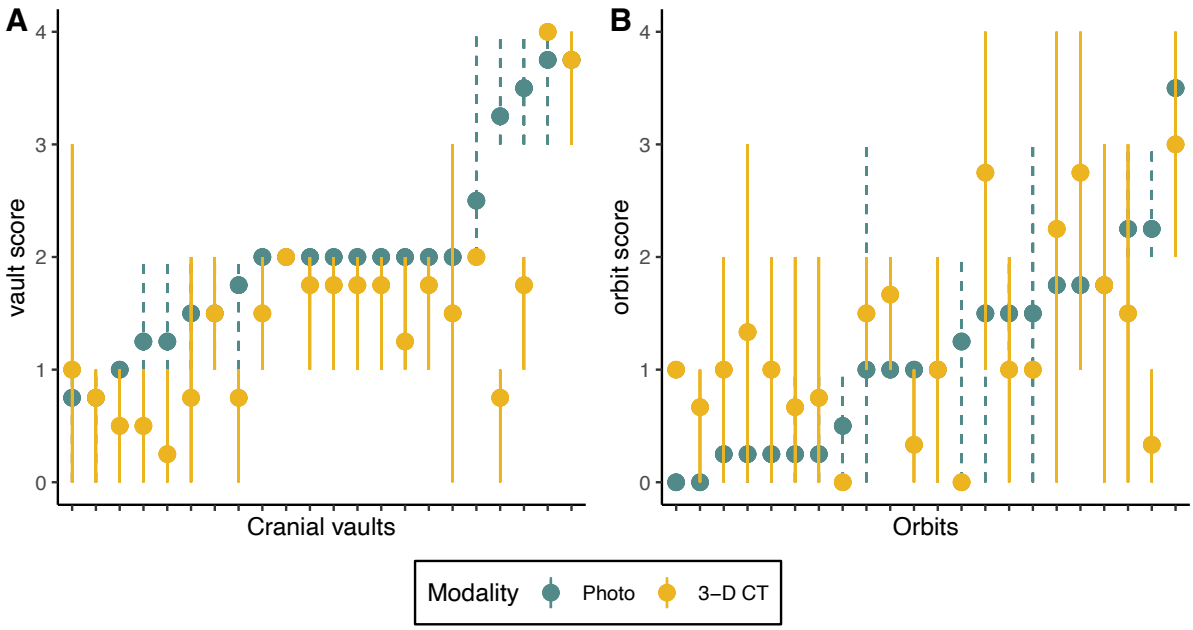

Figure 2. 4 Comparison of observer agreement within and between viewing modalities for a) vault lesions and b) orbital roof lesions.Each point is the average lesion score (n = 4 observers) for an individual cranium; vertical lines show the range of lesion morphology scores assigned to each cranium. Observations in each panel are ordered according to the mean photo-based score for the lesion of interest.

2.3.1.2 CT 2-D MPR

2-D MPR evaluations had the lowest sensitivity of all viewing modalities (0.63) for detecting cranial vault lesions but the highest specificity (0.75). Accordingly, one may be fairly confident that lesions identified as present from MPR are indeed present but expect a high frequency of false negatives. In light of the relatively low sensitivity of MPR for identifying lesion presence, it seems likely that the traditional planar view of crania in radiological examinations has contributed to the under-identification of porous cranial lesions in clinical settings, as ectocranial pitting is often the primary feature of remodeled lesions. Observer evaluation of 3-D volume-rendered CT appears to be more sensitive than 2-D MPR for detecting surface features of the outer table such as ectocranial pitting. Despite 2-D MPR's low sensitivity for identifying lesions based on surface features, it provides valuable views of the extent and nature of lesion-related

changes below the ectocranial surface and is thus indispensable to CT-based evaluations of porous cranial lesions.

Differences in observer agreement across lesion-related traits illuminate 2-D MPR's strengths and weaknesses. Observer agreement on presence/absence of true porosity was higher than agreement on ectocranial pitting. All agreed that true porosity was present in two crania and absent in 13 cases. This low frequency of true porosity demonstrates the extent of lesion healing in the study sample (see Mensforth et al., 1978 for a description of stages of healing in porous cranial lesions), and the higher observer agreement for this trait suggests that 2-D MPR is better at capturing lesions with unremodeled porosity.

Observers also visually evaluated crania for the presence of radially oriented trabeculae in the diploic space, indicating the presence of hair-on-end feature (Fig. 2.1C). True to previous archaeological reports of hair-on-end, unambiguous hair-on-end was only found in cases with other pronounced lesion-related changes (n = 3). 2-D MPR's greatest advantage remains that, unlike other viewing modalities, it provides a window into lesion-associated changes below the cortical surface, making it a critical component of a CT-based evaluation even if 3-D volume-rendered images provide a more sensitive tool for lesion detection.

2.3.1.3 Photographs

Despite yielding higher interobserver agreement on lesion morphology (ICC = 0.80; CI 0.66-0.90) than 3-D volume-rendered CT (ICC = 0.66; CI 0.46-0.82), photo-based evaluations had, unexpectedly, worse agreement with in-person assessment of lesion presence/absence (sensitivity = 1, specificity = 0.10). Compared to in-person evaluations, photo-based evaluations over-reported lesion presence (Fig. 5A). While AA reported absence of vault porosity in 27.3% of crania during initial in-person evaluation, there was no case in which all observers agreed from photographs that vault porosity was absent. False positive cases from photo-based

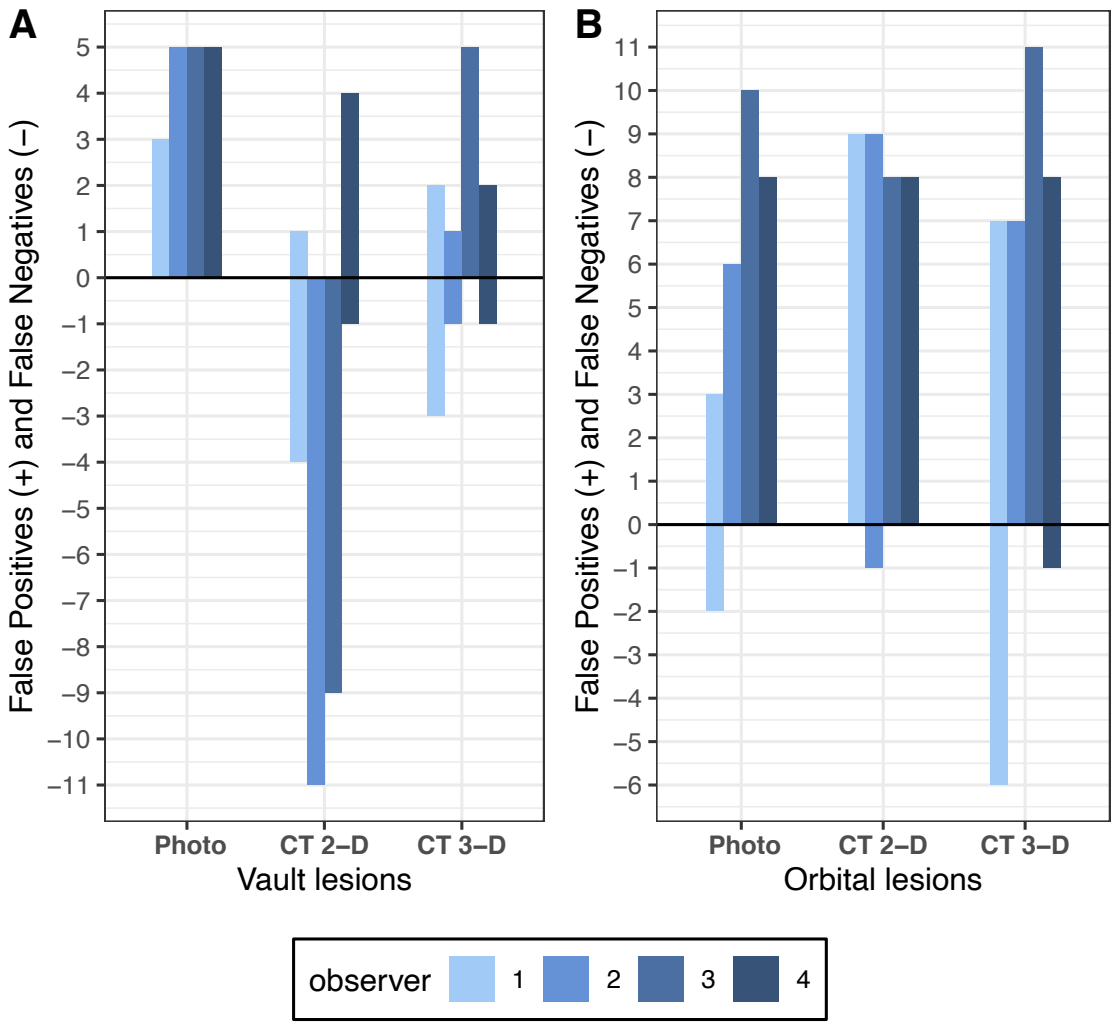

Figure 2. 5 Comparison of false positive and false negative lesion identification rates across viewing modalities, assuming that initial in-person evaluations of lesion presence/absence from direct observation are true. The positive values for each bar indicate the number of crania scored as absent of pathological condition during initial in-person evaluation that were thought to show evidence of lesions in subsequent observation sessions using different viewing modalities. The negative values indicate the number of crania identified as having lesions during in-person evaluation that were subsequently scored as absent of pathological condition. The length of each bar indicates the amount of deviation from in-person presence/absence evaluations, and the position of the bar indicates the direction of deviation. Note: Possible number of 'false' identifications is constrained by the lesion frequencies from in-person evaluations; vault and orbital values here should thus not be directly compared.

evaluations were primarily reported as scattered fine foramina (score: 1). We attribute this discrepancy to the fact that in-person lesion evaluations by AA were conducted after selecting the study sample from a broader collection of skeletal material, providing an opportunity to calibrate expectations for postmortem damage in the Pachacamac remains. Photo-based evaluations are therefore more likely to misclassify postmortem damage as pathological.

Volume-rendered 3-D CT images, on the other hand, have lower resolution than photographs, smoothing over surface features smaller than the scan resolution. The limitations of CT resolution render CT-based evaluations less prone to misidentifying postmortem surface erosion as pathological but also reduce the likelihood of recognizing subtler pathological features that are visible during traditional osteological examinations of dry bone. Nevertheless, even for in-person evaluations of skeletal remains, disagreement over the line between pathological and non-pathological expression is a recognized source of noise in osteological data (Ubelaker, 2003). Archaeologists occasionally avoid dealing with ambiguous cases by increasing the porosity threshold for considering pathological changes to be present (Lewis, 2017; Watts, 2013), a strategy that may create more comparable data on lesions in past and present populations (though lesion frequencies should never be compared directly – see Wood et al (1992)).

2.3.2 Orbital roof lesions

Across all viewing modalities, only orbital roof lesions with coalescing, trabeculated foramina (in-person score: 3) were easily visualized. In these cases (n = 2), widening of the inter-trabecular spaces in the spongy bone of the orbital roof was clearly visible in 2-D MPR (Fig. 2.6), and the lesions were easily and consistently identified as present in all viewing modalities. In contrast, observers were unable to agree, in any viewing modality, whether lesions were present when

orbital roofs displayed only isolated foramina or vascular impressions. As a whole, orbital roof lesions had high observer disagreement in all viewing modalities (Table 2.4; Table S2.1), but there is a clear threshold of lesion visibility for examining these lesions in living individuals: orbital roof lesions with widened inter-trabecular spaces can be identified on standard cranial CT scans.

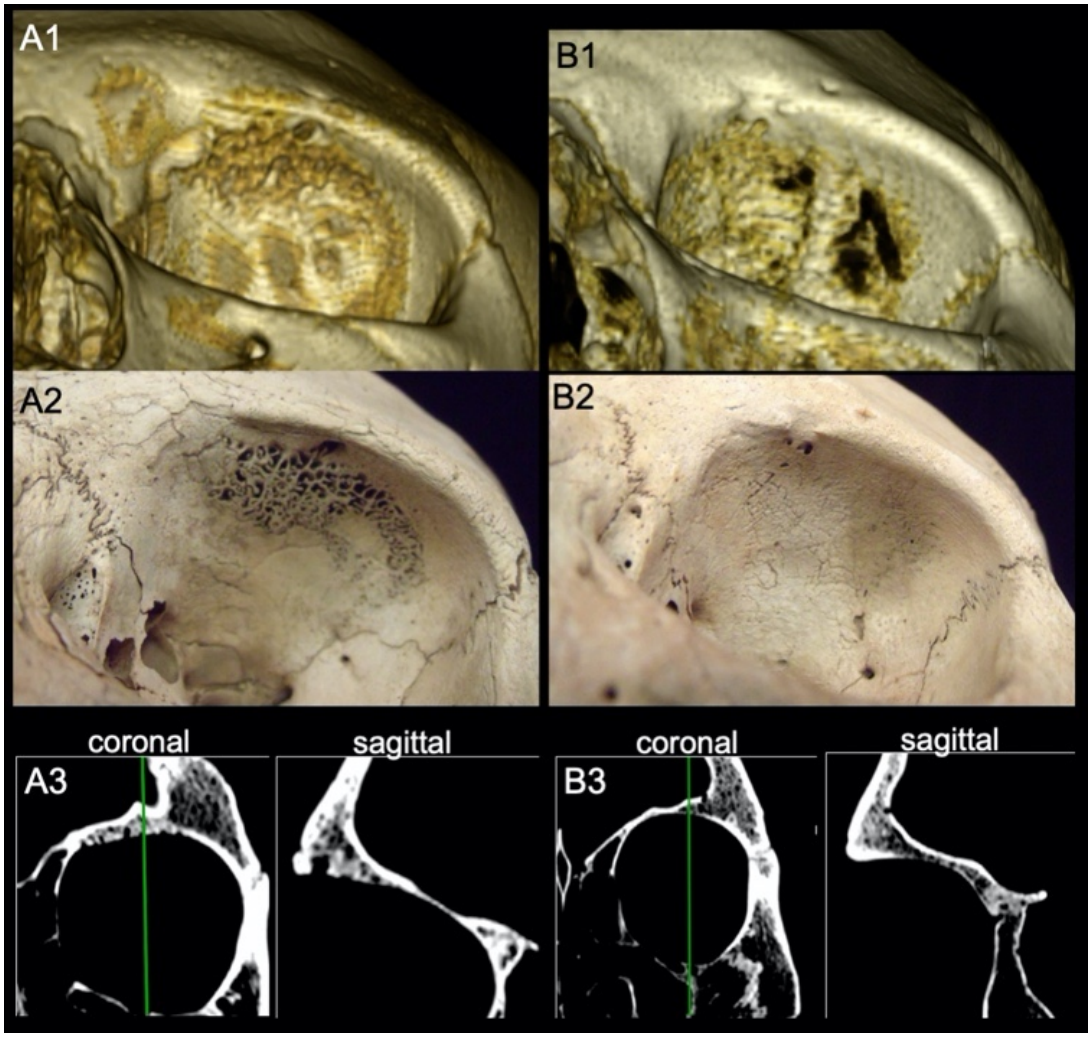

Figure 2. 6 Orbital roof lesions with skeletal changes indicative of marrow hyperplasia(A) are easily visible in all viewing modalities, but orbital roof porosity is often exaggerated in CT reconstructions (B).

2.3.2.1 CT imaging

In the absence of substantial trabecular bone in the orbital roof, CT reconstructions often introduce limitations on orbital roof visibility caused by CT artifacts that render gaps in the region of interest (Figure 2.6B). These artifacts primarily result from the scan's failure to render the orbital roof due to the thinness of the horizontal plate of the frontal bone and its oblique orientation relative to the axial acquisition of the CT scan. The failure to capture thin areas of the orbital roof led to over-identification of orbital roof porosity in CT viewing modalities. When the orbital roof *was* visibly rendered, the axial scan acquisition caused a stairstep effect with visible slices in the volume-rendered orbit resulting in observer uncertainty over subtler features such as isolated foramina and vascular impressions.

2.3.2.2 Photographs

Though photo-based evaluations yielded better interobserver agreement than CT and provided the closest match to in-person evaluations, observers disagreed on whether orbital lesions were present in almost half of photographic cases. A number of factors contribute to disagreement in identifying lesion presence or absence using traditional osteological methods and seem to pose particular difficulty in evaluating orbital roof lesions. For example, well-healed lesions can be difficult to identify in photographs, especially when there is variegated discoloration of the orbit (Figures S4a, S4b). Plant roots can create postmortem discoloration in a vascular pattern (Figures S4c, S4g). The appearance of non-pathological porosity varies with age, and the same sensitivity to porosity may not be appropriate for individuals of all ages (Figures S4d, S4e, S4f). Dirt inclusions can make porosity difficult to assess, and lamellar bone deposition is hard to determine without in-person observation (Figure S4h). Finally, the depth and curvature of the orbit itself poses a challenge for producing photographs with adequate clarity for evaluation of pathological conditions, though techniques like focus stacking can help address this issue (Clini, Frapiccini, Mengoni, Nespeca, & Ruggeri, 2016). CT obviates most of

these challenges, though CT-specific issues (see *3.2.1* above) take their place. Ultimately, minor deviations in orbital roof morphology are sources of observer uncertainty, regardless of viewing modality.

2.3.3 Technical considerations: CT scanning parameters

Phenomena of osteological interest are most feasibly investigated in living individuals using existing CT scans and accompanying medical data. Accordingly, it is important to define the limitations of skeletal lesion detection imposed by the scan parameters of routine cranial CTs. In clinical practice, CT acquisition parameters for standard head CT differ depending on the purpose of the scan. Scans obtained for assessing cranial fracture subsequent to head trauma are the most likely to have submillimeter slices appropriate for visualizing skeletal lesions, and these scans will include a reconstruction using a bone-optimized algorithm. The names and precise parameters of bone algorithms vary across scan manufacturers, but comparable settings do exist (see McCollough, 2011 for a guide). The caveat remains though, that all bone-optimized algorithms contain corrections for the artificially high density values that can appear at the edges of dense materials such as bone (beam hardening artifacts). Algorithms from different scanners vary in the weight of their beam hardening correction, and the extent to which this impacts the visibility of porous skeletal lesions is uncertain.

Previous investigation into the CT visibility of orbital roof lesions has recommended positioning crania "at a slice angle of 90° between the slice plane and the orbital roof" (Exner et al., 2004, p.170). Though this patient positioning optimizes visibility by avoiding scanning artifacts and providing higher-resolution imaging of features in the orbital roof, it is rarely used in clinical CT protocols except in targeted investigations of orbital roof anomalies such as orbital

tumors (Rosel, 2015). Standard head CTs, obtained with the patient in a supine position, are more common and are indicated for patients with a wider range of potential diagnoses. It may be prudent for a future study to assess the effect of variations in skull positioning on the perceptibility of lesions. Positioning has not been found to affect cranial measurements from CT reconstructions (Hassan, Van Der Stelt, & Sanderink, 2009), but the visibility of submillimeter porosity may be more sensitive to minor changes in orientation of the skull.

CT slice thickness in the axial plane is a critical determinant of lesion visibility because it limits image resolution in the reconstructed sagittal and coronal planes. A slice thickness equal to the axial resolution (roughly 0.4 mm in a standard head scan) is likely to be optimal for visualizing porous lesions. Such a slice thickness is achievable with 32- or 64-slice scanners, though 16-slice scanners get close (0.5-0.75 mm minimum slice thickness, depending on the scanner model) (Goldman, 2008). Six-slice scanners such as the Siemens Emotion 6 used here can also achieve submillimeter slices but are rarely used with such thin slices in clinical settings due to the longer scanning time required – a less important consideration for scanning skeletal remains.

With the thin slices available in newer scanners, the true limit on image resolution will be the scan's field of view (FOV). In all CT scanners, pixel resolution of axial slices is determined primarily by the scanner's beam width and the FOV, and FOV is determined by the size of the area to be scanned; all else equal, juvenile head CTs will have higher resolution—and better lesion visibility—due to the smaller FOV for smaller crania. The visibility of fine porosity on the superior aspect of the calvarium is thus unlikely to be much improved by advances in scanning technology.

Pitch also influences the clarity of details in scan reconstructions, partly by influencing the minimum thickness of slices that can be constructed from helical scans. A pitch setting close to 1, which creates contiguous slices, has shown good results for viewing orbital roof lesions

(Naveed et al., 2012), but scans obtained for viewing the intracranial space often have lower pitch settings designed to minimize the likelihood of scanning artifacts and maximize image resolution. The low pitch (0.65) setting for scans in this study, chosen to match parameters for existing brain CT, results in some smoothing of density values, particularly in the Z plane. This smoothing of the data likely obscures some finer porosity, and detection of surface porosity is probably less sensitive on the lateral portions of the calvarium due to the combination of smoothing and lower resolution in the Z plane (Maetani et al., 2016). Fortunately, porous lesions of the occipital bone, which might be most obscured by the resolution constraints set by slice width, tend to have larger foramina than lesions on other areas of the cranial vault. Using the scan parameters of the current study, porosity is most likely to be visible on cranial CT if surface diameter of individual foramina exceeds 0.4 mm or remodeling has not begun to obscure any underlying pathway from the diploë to the ectocranial surface.

2.3.4 Technical considerations: CT viewing settings

Image reconstruction settings in 2-D MPR and 3-D rendering may also influence lesion visualization. Differences in the rendering algorithms used by different viewing software may affect the visibility of surface features (Khan, Khan, Yasin, Shafi, & Abid, 2020). Horos' volume rendering, for instance, uses a ray tracing algorithm, while some viewers render 3-D images with ray marching. The specific effects of different algorithms on visualizing cranial porosity are unclear but worth considering.

The difference between slice thickness and axial resolution has counterintuitive effects on the visualization of cranial surface porosity. For the current study, resolution in axial images is approximately 0.39 mm, while resolution in the other planes is limited by the slice thickness of

0.63 mm. Given the finer axial resolution, one might conclude that the axial plane should provide the best visualization of porous lesions, but in 2-D reconstructions, surface porosity of the cranium's outer table is best visualized in a slice plane orthogonal to the layers of the cranial vault. Porosity in the superior cranial vault is therefore hard to identify in axial slices that run parallel/oblique to the layers of the calvarium, however, axial images are best suited to capture porosity in the occipital squama. In 3-D renderings though, higher resolution in the axial plane helps to produce higher resolution reconstructions of the superior cranial vault's outer table.

Of course, viewing settings chosen by the observer will also impact lesion visibility, particularly in 3-D renderings, where the observer has more opportunities to customize settings. Multiple preset options exist within Horos and other DICOM viewers for window width and level, the color palette of rendered images, the algorithm used for calculating relative opacity of different density values, and the strength and diffusion of simulated light reflection from the tissue surface — and options also exist for entirely customized configurations. Figures S2.2 and S2.3 demonstrate some effects of altering these parameters to suit the preferences of individual observers. A single optimal setting configuration for viewing 3-D renderings of porous lesions has not been determined, but for moderate to severe lesions the range of settings used in this study do not appear to be a notable source of observer disagreement.

It is also worth noting that radiologists and osteologists may pick up on different skeletal features from the same images. In this study the radiologist's 2-D MPR assessments of lesion presence in the cranial vault are notably different from those of the osteologists, with far fewer false negatives (observer 4, Fig. 2.5A). It may be useful for a future study to compare scoring of multiple radiologists and osteologists to determine the effect of observer specialization on radiological assessments of paleopathological conditions, but it is likely that the majority of researchers interested in pursuing the evidence for porous cranial lesions in contemporary

populations will continue to be osteologists. This result also suggests that osteologists might increase accuracy of assessments on 2-D MPR with radiological training.

2.3.5 A case for quantitative approaches

Despite general correlation between methods, there is considerable observer disagreement in lesion evaluations within viewing modalities. Across photographs, 2-D MPR, and 3-D CT, there was no case in which all observers agreed that vault lesions were absent. This may indicate an over-sensitivity on the part of observers due to the focus of the current study — availability bias or confirmation bias — though such sensitivity is likely a common variable in much research that relies on subjective evaluations of phenomena of interest.

Investigations of porous cranial lesions in living individuals will benefit from employing quantitative methods that minimize the impact of subjective evaluations. Since the raw data of a CT scan comprise a matrix of density values, such quantification is a natural approach to CT. For instance, hair-on-end feature might be quantified as an entropy score of trabecular organization. Likewise, diploic granularity and porosity of the outer table, if they are visible to the trained eye, will also have signatures in the matrix of density data, and existing micro-CT work suggests that a range of disease processes might be identified based on quantitative differences in underlying trabecular architecture (Morgan, 2014). However, even quantitative analyses of porous cranial lesions using micro-CT found that, while differences in trabecular architecture could discriminate between unaffected orbits and those with moderate to severe lesions, subtler surface porosity was not associated with significant differences in underlying trabeculae (Morgan, 2014). Quantitative analysis of CT findings at multiple resolutions can

produce an integrated understanding of shared patterns of pathophysiology across clinical and archaeological samples.

Some researchers have approached cranial thickness as a meaningful metric in evaluating lesions in archaeological crania, though the conclusions of these studies are limited either by their use of radiographs or by small sample sizes. Stuart-Macadam (1987) found consistent differences between crania with and without lesions in the ratio of cortical to diploic bone thickness. Likewise, Zuckerman noted that among subadults with cranial lesions, individuals with lesions suggestive of scurvy had significantly thicker cranial vaults (Zuckerman et al., 2014), though as scorbutic hemorrhage frequently leads to anemia it is difficult to draw a clear distinction between the skeletal manifestations of these conditions (Brickley et al., 2020: 241). Using a particularly sophisticated approach, River and Mirazon Lahr (2017) documented a complex relationship between orbital roof porosity and individual patterns of cranial vault thickness. These studies suggest that a more comprehensive quantitative approach using CT scans is likely to yield productive and nuanced results about the covariation of osseous changes across the skull and the aspects of osseous change with most clinical significance.

2.4 Conclusion

Assessing the equivalence of evaluations of pathological skeletal lesions based on direct observation, photographs, and CT, we find that paleopathological criteria can be applied to standard cranial CTs to identify the presence of moderate to severe porous lesions of the cranial vault and to differentiate lesions with coalescing porosity from those with isolated foramina. Orbital roofs, however, are poorly visualized in standard head CT, and only extreme cases of cribra orbitalia are readily visible. Volume-rendered 3-D images are preferable for identifying

lesion presence, but evaluation of lesion depth and morphology should be supported with cross-sectional views from 2-D MPR.

Based on these results, paleopathologists and radiologists can identify cranial vault lesions in living individuals on cranial CT scans, provided scans are obtained with a bone algorithm and submillimeter slice thickness and lesions a) present with more than pinprick porosity and b) lesion healing is not too advanced. Best practices might involve consensus by multiple observers in order to mitigate individual observer biases (Mays, 2020). As scanning technologies improve and hospitals continue to adopt 64-slice CT scanners in their trauma centers, cranial scans with submillimeter slice thickness is becoming routine for cases of head trauma (Mutch, Talbott, & Gean, 2016; Orman et al., 2015). The resolution of routine clinical imaging will only improve, closing the distance between findings from CT and direct observation of skeletal materials and expanding the opportunities for integrating paleopathological and clinical perspectives.

The application of comparable methods of data collection in clinical and archaeological cases has two major benefits for paleopathology. First, it serves to broaden the range of reference material for paleopathological diagnosis and thus mitigate some of the biases in skeletal profiles of disease that result from the unrepresentative nature of available reference cases (Mays, 2018). Second, it facilitates a lesion-centered approach to existing medical imaging from contemporary clinical cases, a program of research that can be undertaken by osteologists without requiring radiologists to reify and document skeletal findings that may be of minimal diagnostic relevance to identifying and treating a patient's condition. Such investigation of skeletal lesions in living individuals will simultaneously serve to test the diagnostic accuracy of paleopathological inference and explore the connection, beyond diagnosis, between skeletal manifestations of disease and individual disease experience.

I have provided here a case study of the groundwork needed to join paleopathological insights and existing clinical data by mapping out the strengths and limitations of clinical CT for examining an archaeologically defined pathological lesion. But there are still many open questions about porous cranial lesions that cannot be answered from archaeological samples, including their rate of remodeling, how commonly they are retained into adulthood, and the factors that determine both of these. Studying these lesions in living individuals as well as cases of lesion-associated ailments that present without cranial lesions will elucidate the conditions necessary to produce osseous changes and the aspects of disease experience that differ between those who develop skeletal lesions and those who do not. An integrated approach to skeletal pathology, enabled by the methods demonstrated here, will generate more meaningful analyses of disease in past populations and an unprecedented understanding of the relationship between health and the skeleton.

2.5 References

Angel, J. L. (1964). Osteoporosis: Thalassemia? *American Journal of Physical Anthropology*, *22*(3), 369–373.

Beatrice, J. S., & Soler, A. (2016). Skeletal indicators of stress: A component of the biocultural profile of undocumented migrants in southern Arizona. *Journal of Forensic Sciences*, *61*(5), 1164–1172. https://doi.org/10.1111/1556-4029.13131

Beckett, R. G. (2014). Paleoimaging: A review of applications and challenges. *Forensic Science, Medicine, and Pathology*, *10*(3), 423–436. https://doi.org/10.1007/s12024-014-9541-z

Blom, D. E., Buikstra, J. E., Keng, L., Tomczak, P. D., Shoreman, E., & Stevens-Tuttle, D. (2005). Anemia and childhood mortality: Latitudinal patterning along the coast of pre-Columbian Peru. *American Journal of Physical Anthropology*, *127*(2), 152–169.

https://doi.org/10.1002/ajpa.10431

Brickley, M. B. (2018). Cribra orbitalia and porotic hyperostosis: A biological approach to diagnosis. *American Journal of Physical Anthropology*, *167*(4), 896–902. https://doi.org/10.1002/ajpa.23701

Brickley, M. B., Ives, R., & Mays, S. (2020). Biology and metabolism of mineralised tissues. In *The Bioarchaeology of Metabolic Bone Disease* (2nd editio, pp. 23–41). https://doi.org/10.1016/b978-0-08-101020-4.00003-3

Buikstra, J. E., & Ubelaker, D. H. (1994). *Standards for data collection from human skeletal remains: Proceedings of a seminar at the Field Museum of Natural History*. Fayetteville, AR: Arkansas Archeological Survey.

Clini, P., Frapiccini, N., Mengoni, M., Nespeca, R., & Ruggeri, L. (2016). SFM technique and focus stacking for digital documentation of archaeological artifacts. *International Archives of the Photogrammetry, Remote Sensing and Spatial Information Sciences - ISPRS Archives*, *41*(July), 229–236. https://doi.org/10.5194/isprsarchives-XLI-B5-229-2016

David, M. (2018). *Traumatic predictors of femicide: A forensic anthropological approach to domestic violence* (Florida Gulf Coast University). Retrieved from https://fgcu.digital.flvc.org/islandora/object/fgcu%3A32589

DeWitte, S. N., & Stojanowski, C. M. (2015). The Osteological Paradox 20 Years Later: Past Perspectives, Future Directions. *Journal of Archaeological Research*, *23*(4), 397–450. https://doi.org/10.1007/s10814-015-9084-1

Exner, S., Bogusch, G., & Sokiranski, R. (2004). Cribra orbitalia visualized in computed tomography. *Annals of Anatomy*, *186*(2), 169–172. https://doi.org/10.1016/S0940-9602(04)80035-9

Goldman, L. W. (2008). Principles of CT: Multislice CT. *Journal of Nuclear Medicine Technology*,

36(2), 57–68. https://doi.org/10.2967/jnmt.107.044826

Hassan, B., Van Der Stelt, P., & Sanderink, G. (2009). Accuracy of three-dimensional measurements obtained from cone beam computed tomography surface-rendered images for cephalometric analysis: Influence of patient scanning position. *European Journal of Orthodontics*, *31*(2), 129–134. https://doi.org/10.1093/ejo/cjn088

Horos. (2019). *Horos*. Retrieved from https://horosproject.org/faqs/

Hrdlička, A. (1914). Anthropological work in Peru in 1913, with notes on the pathology of the ancient Peruvians. *Smithsonian Miscellaneous Collections*, *61*(18), 1–69.

Józsa, L., & Pap, I. (1990). Morphology and differential diagnosis of porotic hyperostosis on historical anthropological material. *Anthropologia Hungarica*, *21*(1930), 69–80.

Khan, U., Khan, U., Yasin, A., Shafi, I., & Abid, M. (2020). CPU-GPU Rendering of CT Scan Images for Vertebra Reconstruction from CT Scan Images with a Calibration Policy. *Medical Imaging and Radiation Sciences*, 1–5. https://doi.org/10.31487/j.mirs.2020.01.02

Lewis, M. E. (2017). *Paleopathology of Children: Identification of Pathological Conditions in the Human Skeletal Remains of Non-Adults*. London: Elsevier Inc.

Maetani, K., Namiki, J., Matsumoto, S., Matsunami, K., Narumi, A., Tsuneyoshi, T., & Kishikawa, M. (2016). Routine Head Computed Tomography for Patients in the Emergency Room with Trauma Requires Both Thick- and Thin-Slice Images. *Emergency Medicine International*, *2016*(5781790). https://doi.org/10.1155/2016/5781790

Mays, S. (2012). The relationship between paleopathology and the clinical sciences. In A. L. Grauer (Ed.), *A Companion to Paleopathology* (pp. 285–309). https://doi.org/10.1002/9781444345940.ch16

Mays, S. (2018). How should we diagnose disease in palaeopathology? Some epistemological considerations. *International Journal of Paleopathology*, *20*.

https://doi.org/10.1016/j.ijpp.2017.10.006

Mays, S. A. (2020). A dual process model for paleopathological diagnosis. *International Journal of Paleopathology*, *31*(August), 89–96. https://doi.org/10.1016/j.ijpp.2020.10.001

McCollough, C. H. (2011). Translating Protocols Between Scanner Manufacturer and Model. In *Technology Assessment Initiative: Summit on CT Dose*. Retrieved from https://www.aapm.org/meetings/2010CTS/documents/1530_McCollough_Converting_Across_Scanner_Models_04-29-2010.pdf

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

Mensforth, R. P., Lovejoy, C. O., Lallo, J. W., & Armelagos, G. J. (1978). Part Two: The role of constitutional factors, diet, and infectious disease in the etiology of porotic hyperostosis and periosteal reactions in prehistoric infants and children. *Medical Anthropology*, *2*(April 2016), 1–59. https://doi.org/10.1080/01459740.1978.9986939

Morgan, J. (2014). *The Methodological and Diagnostic Applications of Micro-CT to Palaeopathology : A Quantitative Study of Porotic Hyperostosis* (The University of Western Ontario). Retrieved from https://ir.lib.uwo.ca/etd/2056/

Mutch, C. A., Talbott, J. F., & Gean, A. (2016). Imaging Evaluation of Acute Traumatic Brain Injury Christopher. *Physiology & Behavior*, *176*(1), 139–148. https://doi.org/10.1016/j.physbeh.2017.03.040

Naveed, H., Abed, S. F., Davagnanam, I., Uddin, J. M., & Adds, P. J. (2012). Lessons from the past: Cribra orbitalia, an orbital roof pathology. *Orbit*, *31*(6), 394–399. https://doi.org/10.3109/01676830.2012.723785

O'Donnell, L. (2019). Indicators of stress and their association with frailty in the precontact

Southwestern United States. *American Journal of Physical Anthropology*, (May), 1–14. https://doi.org/10.1002/ajpa.23902

O'Donnell, L., Hill, E. C., Anderson, A. S., & Edgar, H. J. H. (2020). Cribra orbitalia and porotic hyperostosis are associated with respiratory infections in a contemporary mortality sample from New Mexico. *American Journal of Physical Anthropology*, *173*(4), 721–733. https://doi.org/10.1002/ajpa.24131

Obertova, Z., & Thurzo, M. (2004). Cribra orbitalia as an indicator of stress in the early medieval Slavic population from Borovce (Slovakia). In *Anthropologie* (Vol. 42).

Orman, G., Wagner, M. W., Seeburg, D., Zamora, C. A., Oshmyansky, A., Tekes, A., … Bosemani, T. (2015). Pediatric skull fracture diagnosis: Should 3D CT reconstructions be added as routine imaging? *Journal of Neurosurgery: Pediatrics*, *16*(4), 426–431. https://doi.org/10.3171/2015.3.PEDS1553

Ortner, D. J. (2003). *Identification of Pathological Conditions in Human Skeletal Remains* (2nd ed.). San Diego: Academic Press.

Ortner, D. J., & Ericksen, M. F. (1997). Bone changes in the human skull probably resulting from scurvy in infancy and childhood. *International Journal of Osteoarchaeology*, *7*, 212–220.

Rivera, F., & Mirazon Lahr, M. (2017). New evidence suggesting a dissociated etiology for cribra orbitalia and porotic hyperostosis. *Am J Phys Anthropol*, *164*(1), 76–96. https://doi.org/10.1002/ajpa.23258

Rosel, P. (2015). The basics of orbital imaging. In Z. A. Karcioglu (Ed.), *Orbital Tumors: Diagnosis and Treatment* (2nd ed., pp. 83–95). https://doi.org/10.1007/978-1-4939-1510-1_9

Steckel, R. H., Rose, J. C., Larsen, C. S., & Walker, P. L. (2002). Skeletal health in the western hemisphere from 4000 B.C. to the present. *Evolutionary Anthropology*, *11*(4), 142–155. https://doi.org/10.1002/evan.10030

Steyn, M., Voeller, S., Botha, D., & Ross, A. H. (2016). Cribra orbitalia: Prevalence in contemporary populations. *Clinical Anatomy*, *830*(April), 823–830. https://doi.org/10.1002/ca.22734

Stuart-Macadam, P. (1985). Porotic hyperostosis: representative of a childhood condition. *American Journal of Physical Anthropology*, *66*, 391–398. https://doi.org/10.1002/ajpa.1330660407

Stuart-Macadam, P. (1987). Porotic hypertostosis: New evidence to support the anemia theory. *American Journal of Physical Anthropology*, *74*, 521–526.

Stuart-Macadam, P. (1987). A radiographic study of porotic hyperostosis. *American Journal of Physical Anthropology*, *74*(4), 511–520. https://doi.org/10.1002/ajpa.1330740409

Ubelaker, D. H. (2003). Anthropological Perspectives on the Study of Ancient Disease. In C. Greenblatt & M. Spigelman (Eds.), *Emerging Pathogens, Archaeology, Ecology and Evolution of Infectious Disease* (pp. 93–102). Oxford: Oxford University Press.

Waldron, T. (2009). *Paleopathology*. Cambridge: Cambridge University Press.

Walker, P. L., Bathurst, R. R., Richman, R., Gjerdrum, T., & Andrushko, V. A. (2009). The causes of porotic hyperostosis and cribra orbitalia: A reappraisal of the iron-deficiency-anemia hypothesis. *American Journal Of Physical Anthropology*, *139*(2), 109–125. https://doi.org/10.1002/ajpa.21031

Wapler, U., Crubézy, E., & Schultz, M. (2004). Is cribra orbitalia synonymous with anemia? Analysis and interpretation of cranial pathology in Sudan. *American Journal of Physical Anthropology*, *123*(4), 333–339. https://doi.org/10.1002/ajpa.10321

Watts, R. (2013). Childhood development and adult longevity in archaeological populations from Barton-upon-Humber, Lincolnshire, England. *International Journal of Paleopathology*, *3*, 95–104. Retrieved from

https://ezp.lib.unimelb.edu.au/login?url=https://search.ebscohost.com/login.aspx?direct=true&db=edsble&AN=edsble.631678&site=eds-live&scope=site

Wood, J. W., Milner, G. R., Harpending, H. C., Weiss, K. M., Cohen, M. N., Eisenberg, L. E., … Weiss, K. M. (1992). The osteological paradox : Problems of inferring prehistoric health from skeletal samples. *Current Anthropology*, *33*(4), 343–370.

Wright, L. E., & Chew, F. (1998). Porotic hyperostosis and paleoepidemiology: A forensic perspective on anemia among the ancient Maya. *American Anthropologist*, *100*(4), 924–939. https://doi.org/10.1525/aa.1998.100.4.924

Zuckerman, M. K., Garofalo, E. M., Frohlich, B., & Ortner, D. J. (2014). Anemia or scurvy : A pilot study on differential diagnosis of porous and hyperostotic lesions using differential cranial vault thickness in subadult humans. *International Journal of Paleopathology*, *5*, 27–33. https://doi.org/10.1016/j.ijpp.2014.02.001

III. A skeletal indicator of childhood stress commonly reported in archaeological studies is associated with immunosenescence among adults in a living subsistence population

3.1 Introduction

Studies of population health in prehistory—and thus the health impacts of shifts in subsistence and social structure such as those accompanying the agricultural revolution—rely largely on evidence from human skeletal remains. The pathological skeletal features most often reported in these studies are common, nonspecific indicators of physiological stress that develop early in life and can remain visible throughout adulthood: namely, dental enamel defects, stunted growth, and porous lesions of the cranial bones (Bocquet-Appel, Naji, & Bandy, 2008; Marciniak et al., 2022; Steckel et al., 2019; Steckel & Rose, 2002). All three have been interpreted as evidence of poor health in past populations from chronic systemic infections, heavy parasite loads, food insecurity, or inadequate dietary diversity (Mensforth et al., 1978; Rivera & Mirazon Lahr, 2017; Stuart-Macadam, 1987a; Walker et al., 2009). However, whereas enamel defects and growth stunting are routinely screened for in contemporary healthcare settings (Salanitri & Seow, 2013; WHO, 2014), porous cranial lesions are rarely reported outside of archaeological contexts. In fact, despite their prominent role in building narratives of past population health, the relationship between porous cranial lesions and health has never been studied in a population-representative sample of living people.

In dry bone, porous cranial lesions are characterized by the presence of numerous small holes, typically 1mm or less in diameter, that penetrate the normally smooth outer surface of the bones of the orbital roofs (termed cribra orbitalia, Figure. 3.1d) or cranial vault (termed porotic hyperostosis or cribra cranii, Figure. 3.1a-c), where porosity is typically found symmetrically distributed adjacent to the lambdoid or sagittal cranial sutures (Henschen, 1961; Walker et al., 2009). The surface porosity that defines these lesions for the paleopathologist has not been

readily visible to the radiologist until recently, with the development of multi-slice computed tomography scanners that enable volume-rendered images of surface features with submillimeter resolution. However, a subset of cranial vault lesions have long been observable in clinical radiographs (x-rays) (Angel, 1964; Chaichun et al., 2021; Moore, 1929; Moseley, 1965b). These are cases where outer surface porosity is accompanied by notable expansion of the underlying marrow space, and the most identifiable cases also present with a radial orientation of the spongy bone within the cranial vault, a radiological feature known as 'hair-on-end sign' (Figure 3.1a).

Radiological findings are most commonly reported in cases of hereditary anemias such as thalassemia (Caffey, 1951; Madani et al., 2007; Sebes & Diggs, 1979; Tyler, Madani, Chaudhuri, Wilson, & Dick, 2006), but cases of severe iron-deficiency anemia early in infancy can also lead to cranial marrow expansion, typically among premature or twin births, who are born with lower iron stores and thus face higher risk of developing anemia in the first few months of life (Burko, Henry; Mellins, harry Z.; Watson, 1960; Shahidi, Nasrollah T.; Diamond, 1960). More commonly, young children with iron-deficiency anemia from non-hereditary causes exhibit thinning of the skull's outer table without expansion of the marrow space (N. Agarwal et al., 1970), a feature that appears to be associated—but not synonymous—with the surface porosity observed in archaeological skeletal remains (Stuart-Macadam, 1987).

While anemia is the most common condition invoked to explain porous cranial lesions, cranial porosity has also been clinically documented in cases of infantile rickets (Schuller, 1950), where it results from impaired mineralization during rapid bone growth. Likewise, subperiosteal hemorrhage incurred by traumatic injury or infantile scurvy can stimulate the formation of porous new bone during healing (Damini, Dixit, Khullar, & Rajeshwari, 2021; Hunt Ingalls, 1936; Verma et al., 2007), and scurvy has become a common contender in paleopathological

differential diagnoses of these lesions (Ortner & Ericksen, 1997), though new bone formation following orbital hemorrhage is an exceedingly rare clinical finding (McNab, 2014). Other causes of cranial vault expansion such as neoplastic changes, Paget's disease, and hydrocephaly, are qualitatively distinct from the cranial porosity that is predominantly reported in archaeological contexts under the term porotic hyperostosis (Schuller, 1950). Regardless of their cause, it is clear from the archaeological evidence that these lesions form primarily in childhood because lesions in adults almost invariably show signs of healing at time of death (Blom et al., 2005; Stuart-Macadam, 1985). Lesions observed in adults are thus predominantly indicators of childhood medical history.

Archaeological studies report that orbital roof porosity in particular is often associated with younger age at death, even among young adults (Jatautis, Mitokaite, & Jankauskas, 2011; Mcfadden & Oxenham, 2020; Mittler & van Gerven, 1994; Obertova & Thurzo, 2004; Papathanasiou et al., 2018; Roberts & Steckel, 2019; Steckel, 2005). Since PCLs result mainly from events in early childhood (Stuart-Macadam, 1985), this pattern may be due in part to complete healing of lesions in some survivors, but the presence of partially remodeled lesions in older adults suggests that the pace of such healing is either highly variable or very slow. If porous cranial lesions are indeed associated with higher frailty in adolescents and adults, this implies a pathway to mortality through long-term heightened susceptibility to morbidity, and particularly to illnesses that were common causes of death for children and young adults in past populations. However, because (1) cemeteries do not provide population-representative data on health, (2) many aspects of individual exposure and experience are unknown, and (3) most illnesses do not affect the skeleton, bioarchaeological studies are poorly positioned to investigate the relationship between skeletal lesions and experiences of morbidity.

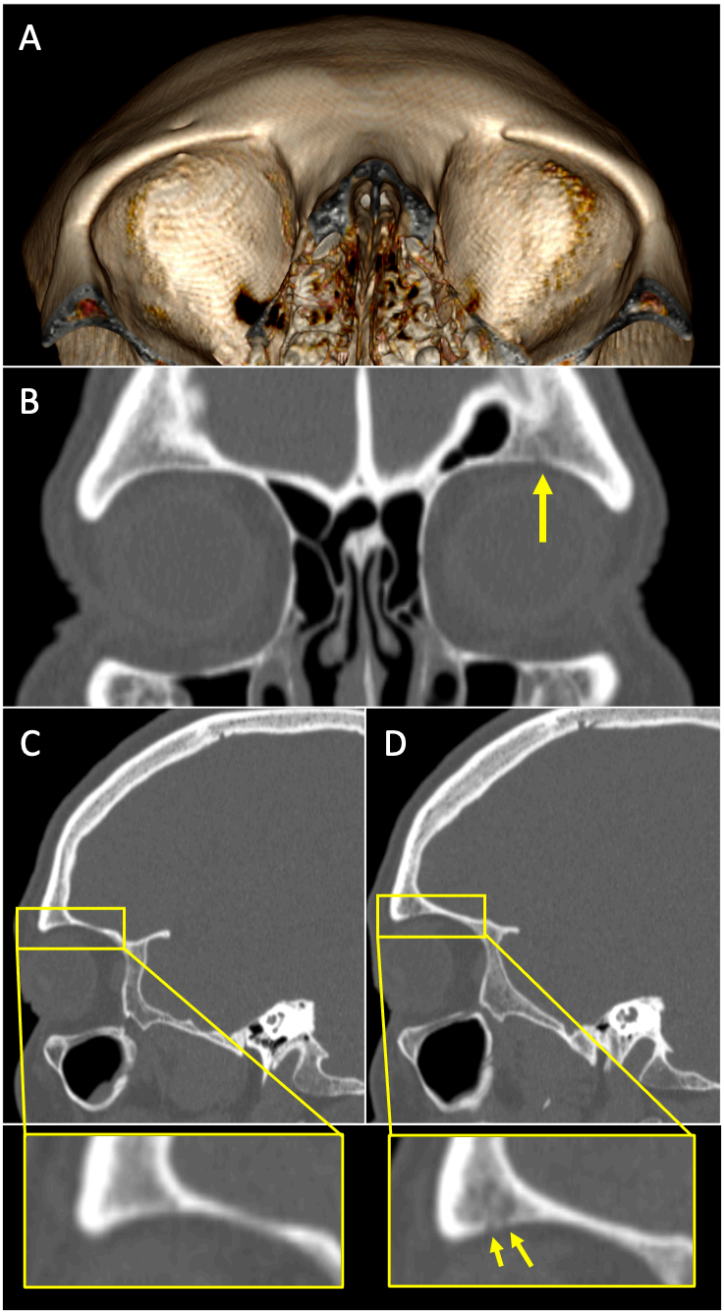

Figure 3. 1 CT appearance of orbital roof lesion presence and absence in study participant . A) Volume-rendered CT reconstruction showing an individual with orbital roof porosity present in the left orbit and absent in the right orbit. B) Coronal slice of CT MPR showing the same individual. C) sagittal slice of right orbit showing intact orbital roof. D) sagittal slice of left orbit with visible porosity in the anterior orbital roof. Arrows indicate individual foramina.

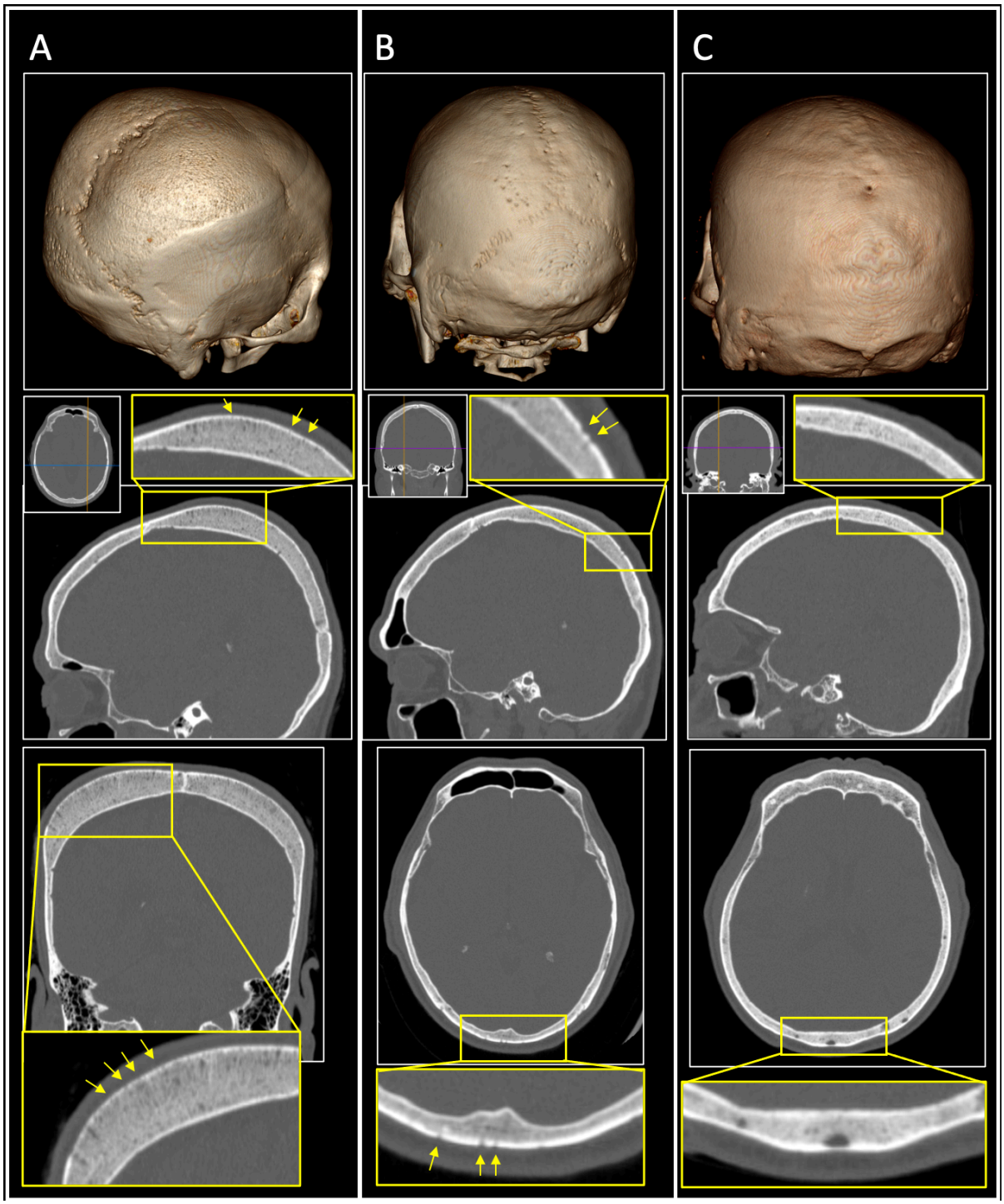

Figure 3. 2 CT appearance of cranial vault lesion presence and absence in study participants.A) cranial vault porosity with accompanying marrow expansion and hair-on-end appearance of cranial diploe. B) cranial vault porosity on the posterior parietal bones and occipital squama. C) absence of vault porosity. Arrows indicate individual foramina.

There is also non-archaeological evidence to suggest that porous cranial lesions are associated with elevated mortality risk in childhood. A recent study using post-mortem CT scans of contemporary New Mexican children found that, controlling for age, lesions were significantly more common among children who died of infectious or congenital conditions ('natural deaths') than those who died of traumatic causes. Porous cranial lesions were also associated with fatal respiratory infections in this sample, as reported by autopsy records, most commonly pneumonia (O'Donnell et al., 2020). The lasting implications of PCLs for adult health, however, have not been addressed.

To begin to test whether these apparent skeletal indicators of childhood stress are indeed associated with greater subsequent morbidity or signs of immune activation in adulthood, we use cranial computed tomography (CT) scans and longitudinal health data from a range of biomarkers, clinical diagnoses, and functional fitness tests. We first identify porous cranial lesions on CT scans from a representative sample of 375 living adults aged 40+ years from a contemporary subsistence population in lowland Bolivia and then explore whether these lesions are associated with differences in a wide range of health outcomes (Fig. 3.2). The CT scans were obtained as part of a Tsimane Health and Life History Project (THLHP) study on dementia and cognitive aging in the Tsimane population (Irimia et al., 2021). These data provide an unprecedented opportunity to test whether individuals with porous cranial lesions (PCLs) experience compromised health as adults.

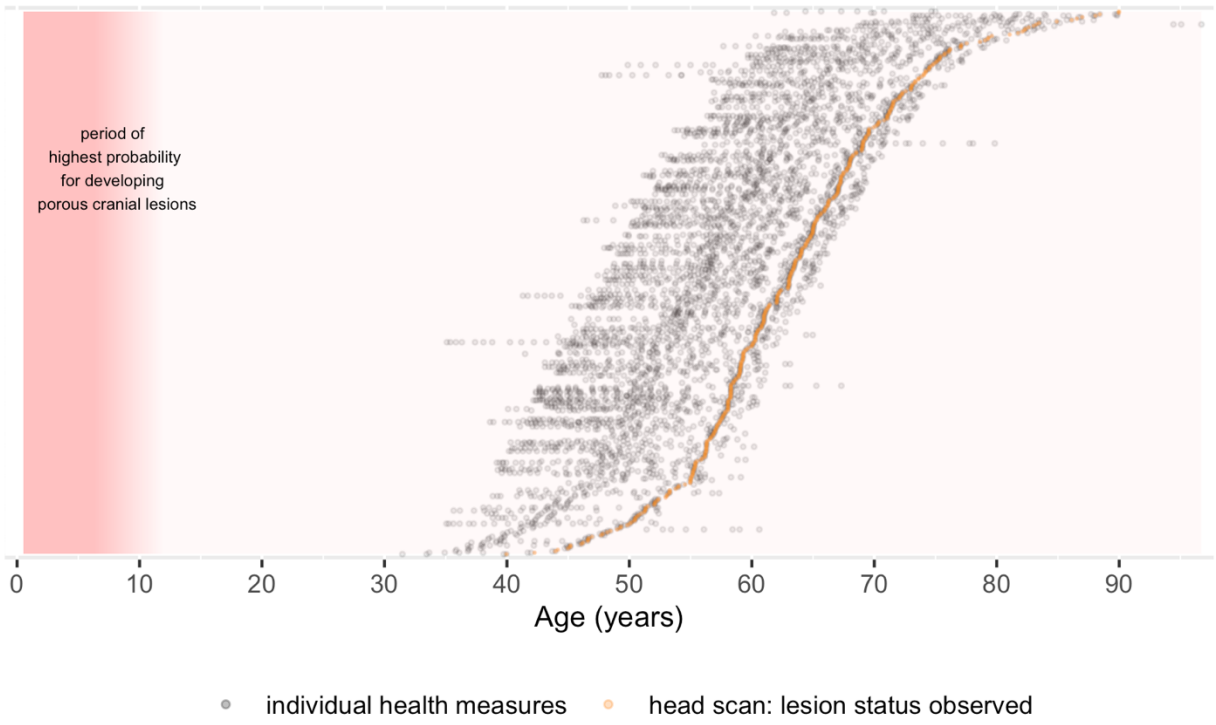

Figure 3. 3 Plot of the timing and frequency of available health data for each of the 375 individuals in the study(grey points) relative to the inferred age range of potential PCL development (pink area) and the age at which each person's PCL status is observed from their cranial CT scan (orange points). The background continues to be faintly pink for ages above twelve to represent the potential of developing isolated PCLs in response to localized infections or trauma to the eyes and head.

3.1.1 Why the Tsimane?

The Tsimane are an indigenous population of ≈17,000 people in the Beni department of Amazonian Bolivia. Most Tsimane villages have little public infrastructure, minimal access to electricity, and limited access to modern medical resources. Their subsistence lifestyle and tropical disease ecology are precisely the setting in which the archaeological literature suggests that we should encounter high prevalence of porous cranial lesions and find evidence of associated long-term health sequelae.

Childhood anemia, particularly due to blood loss from intestinal parasites, is often invoked as a probable cause of PCLs (Walker et al., 2009); 39% of Tsimane children ages 0.5-15

years are anemic despite high estimated dietary iron (DeLouize et al., 2022; Kraft et al., 2018), and one study reports a 76% prevalence of hookworm infection among children ages 2-10.9 years (Tanner et al., 2009). In light of recent reports that find an association between fatal respiratory infections and porous lesions of the orbital roofs (Gomes et al., 2022; O'Donnell et al., 2020), it is also notable that respiratory infection is the most commonly documented category of illness and the most common cause of death for Tsimane individuals of all ages (though young adults are equally likely to die from accidents or violence) (M. Gurven et al., 2007). It also highlights the critical importance for immunological robusticity at every stage of life in this pathogen-rich environment; if PCLs are associated with diminished capacity to weather subsequent health challenges, respiratory infections may be more common among individuals with PCLs. The THLHP thus provides a unique opportunity to examine the prevalence of PCLs and their correlated health conditions in a representative sample of adults in a subsistence society, and to link population-level trends in skeletal pathology to the known disease ecology of the population.

3.1.2 Physiological scarring

The *physiological scarring hypothesis* posits that the archaeologically documented association between PCLs and higher mortality risk stems from long-term health consequences of the childhood physiological stress indicated by these skeletal lesions. If lesion-causing processes have long-term costs to physiological function—or are more likely to form in individuals born with greater vulnerability to environmental stressors—the development of PCLs might be a harbinger of chronic vulnerability to morbidity and mortality. Where the force of mortality is weaker (Hamilton, 1966), this long-term vulnerability may manifest among older adults as a greater degree of geriatric frailty. Geriatric frailty, a holistic and inconsistently operationalized

measure of age-related risk of disease, disability, and death, is characterized by instability across multiple physiological systems (Rockwood, Hogan, & MacKnight, 2000). The current study assesses associations between porous cranial lesions and medical diagnoses, immune markers, functional ability, bone mineral density, and overall physiological dysregulation among Tsimane adults aged 40+ years.

3.2 RESULTS

3.2.1 PCL descriptives

Direct comparison of lesion frequencies in living populations and cemetery samples—or of lesion frequencies among cemetery samples—is inadvisable, given the different risk sets of each context, but the prevalence of porous cranial lesions among Tsimane adults is within the range of archaeological findings. Of the 372 cranial scans that could be evaluated for both cranial vault and orbital roof porosity, PCLs were observed on 64 (17.2%). 46 (12.3%) of the 373 observable cranial vaults had visible pitting or porosity of the cranial vault consistent with porous cranial lesions (Anderson et al., 2021), most frequently on the occipital squama. Marrow expansion of the cranial vault was present in only a single case of vault porosity, which also exhibited hair-on-end sign (Fig. 3.1a).

Orbital roof porosity was visible in 23 of 374 observable scans (6.1%). Orbital and vault porosity were not significantly associated with each other (chi-squared = 1.173, p = 0.278); only five individuals had concurrent orbital and vault porosity. Due to the visibility limits imposed by the scans' resolution (approximately 0.4 mm^2) and our conservative approach to identifying lesions (see Methods), the vault and orbital lesion frequencies reported here should be interpreted as the minimum prevalence of porous cranial lesions in older Tsimane adults.

3.2.2 Porous lesions of the orbital roofs are negatively associated with age.

The probability of orbital roof porosity (cribra orbitalia) is lower among the older adults in the study sample, while vault porosity showed no clear relationship with age (Fig. 3.3). This result is robust and remains whether ambiguous orbital lesion cases are considered present, absent, or excluded entirely from the models, and the pattern was similar for lesions in one or both orbits. Neither lesion frequency nor the relationship between lesion frequency and age differed by sex.

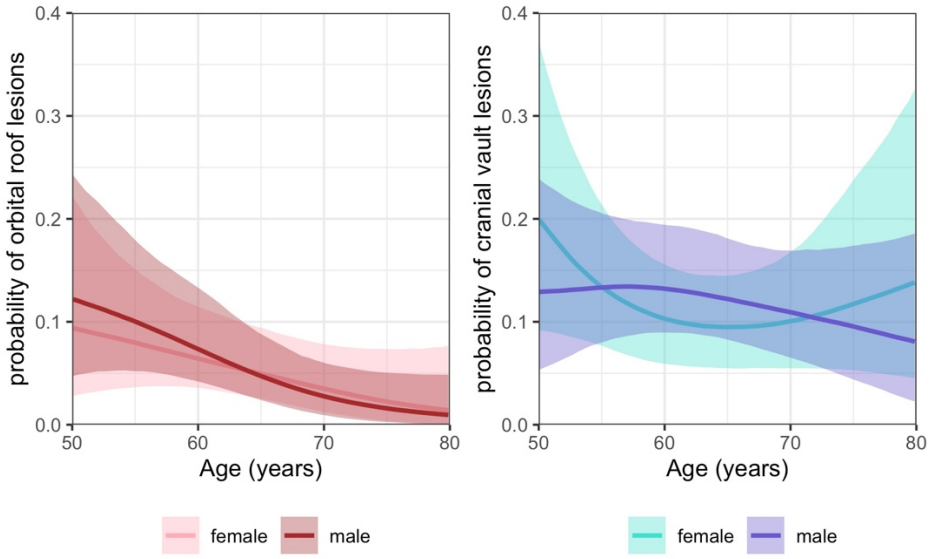

Figure 3. 4 Probability of having orbital roof porosity (left) but not vault porosity (right) is negatively associated with age. Plots show predicted probabilities from non-linear logistic regressions for lesion presence with a smoothing spline for age, grouped by sex.

3.2.3 Orbital roof porosity predicts higher risk of active tuberculosis but not other respiratory conditions.

We focus on pulmonary tuberculosis and other respiratory infections here not only because of their association with PCLs in recent publications (Gomes et al., 2022; O'Donnell et al., 2020) but also because of their high prevalence, high morbidity burden, and notable contribution to mortality across the life course in the Tsimane and other populations (M.

Gurven et al., 2007, 2017). Orbital roof porosity is associated with 3.8 (95% CI = (1.3, 11.2)) times the age-specific hazard of developing post-primary tuberculosis after controlling for sex, though with wide uncertainty (Fig. 3.4). Given that 7.8% of individuals in the study sample were diagnosed with symptomatic tuberculosis by physicians during the study period and that orbital roof porosity could be confirmed in only 6.1% of the study sample, this effect size is striking. In contrast, tuberculosis hazard does not differ when vault porosity is present (beta = 0.5, 95% CI = (0.1, 2.3)) and neither orbital nor vault porosity has a strong association with overall incidence of other respiratory infections ((beta = 0.7, 95% CI = (0.3, 1.53) and b = 1.8, 95% CI = (0.7, 1.93), respectively).

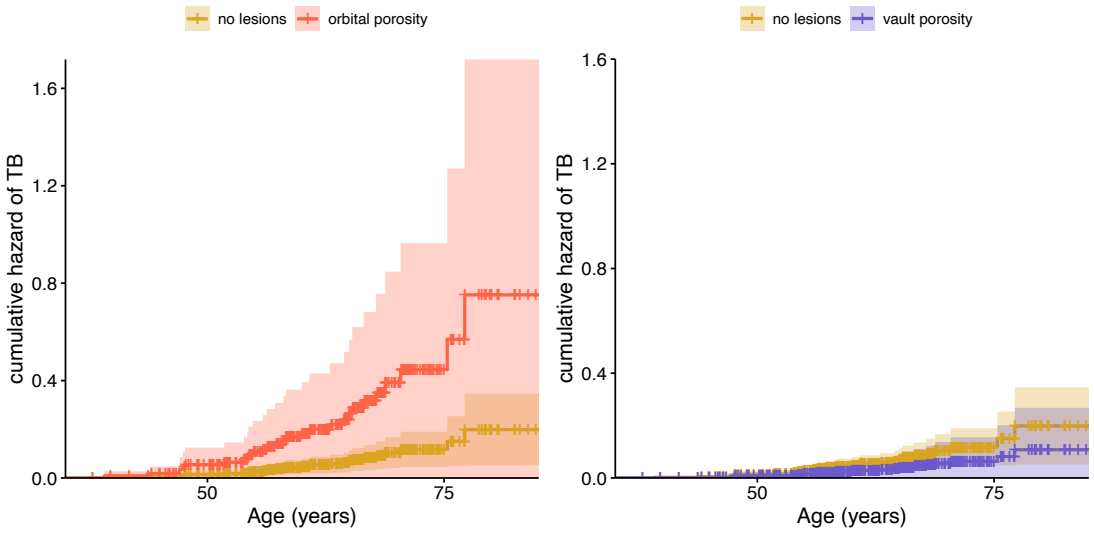

Figure 3. 5 Orbital roof porosity, but not cranial vault porosity, is associated with higher age-specific hazards of developing symptomatic tuberculosis, but with wide uncertainty on the effect size. Models control for sex and assume that all individuals were exposed prior to observation and carry latent or active infections.

3.2.4 Porous cranial lesions are not linked to anemia in adults.

Neither orbital roof porosity nor cranial vault porosity are associated with any differences in hemoglobin for the adults in this study (Table 3.1). Even the one individual with

marrow expansion and hair-on-end appearance of the cranial vault—considered pathognomonic for severe childhood anemia—had normal adult hemoglobin values (n = 7 observations over 10 years). Whether or not childhood anemia is the primary driver of porous cranial lesion formation in this population, PCL presence is not informative about adult anemia status.

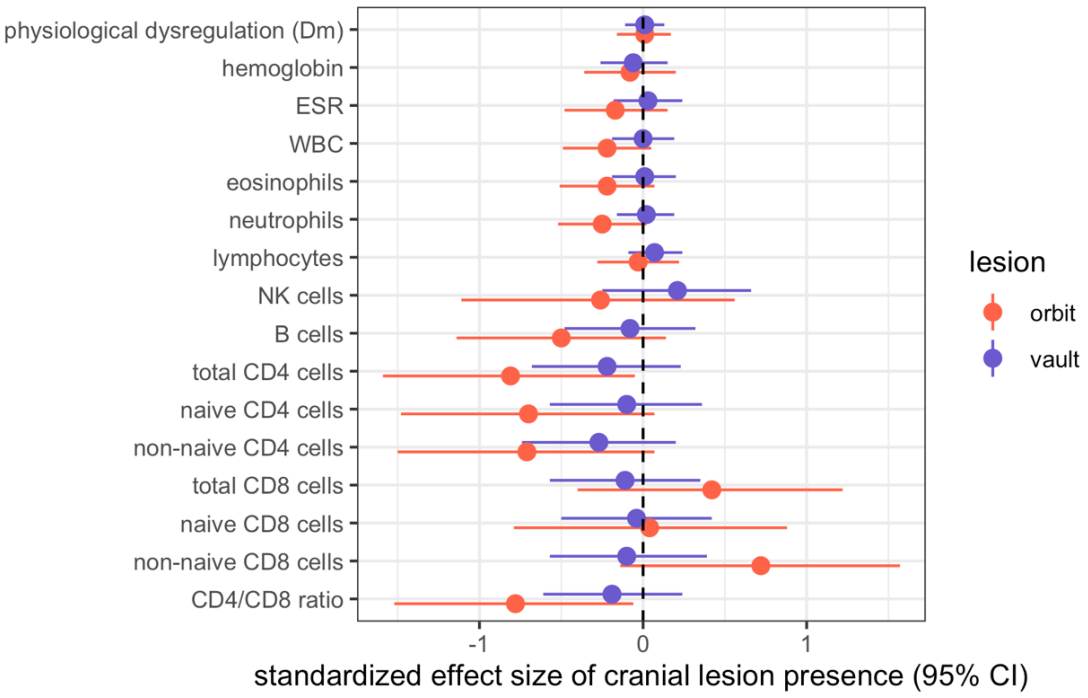

Figure 3. 6 A range of biomarkers show little association with cranial vault porosity, but cribra orbitalia predicts fewer CD4+ T cells and more non-naïve CD8+ T cells, a T cell profile associated with chronic helminth infections and higher susceptibility to active tuberculosis. Model results are presented in Table 3.1.

3.2.5 Orbital roof porosity is associated with lower ratio of CD4+/CD8+ T cells, a measure of immune senescence.

After controlling for age and sex, cranial vault porosity is not associated with any systematic differences in inflammation or white blood cell subtypes (Table 3.1), but orbital roof porosity appears to be accompanied by a lower neutrophil count and a lower CD4/CD8 ratio for chronological age, indicating a greater degree of immunosenescence (Figure 3.5). Controlling for current infection did not affect these results.

Among the subset of individuals with flow cytometry measures (n = 195; 253 obs), orbital roof porosity (n = 7; 10 obs) is associated with a lower total CD4+ T cell count and higher non-naïve CD8+ T cell count, which combine to produce a markedly lower CD4/CD8 ratio (standardized beta= -0.78, 95% CI = (-1.52, -0.06)). The CD4/CD8 ratio is a measure of immune competence that has been validated as an integrative marker of biological age in the general population (Garrido-Rodríguez et al., 2021; McBride & Striker, 2017). A low CD4/CD8 ratio is considered an immune risk phenotype and an indicator of immune senescence (McBride & Striker, 2017). Values below 1 have been associated with higher mortality risk and higher measures of oxidative stress among older adults (Muller et al., 2015; Wikby, Maxson, Olsson, Johansson, & Ferguson, 1998).

Table 3. 1 Standardized betas and 95% CI for models of physiological dysregulation (Dm), hemoglobin, erythrocyte sedimentation rate (ESR), and white blood cells (as absolute cell counts and as percent of total WBCs). All models control for age and sex. The model of D_m additionally controlled for age^2 and number of biomarkers measured for each observation of D_m.

	Orbital roof porosity		*Cranial vault porosity*	
	beta	95%CI	beta	95%CI
Dm	0.01	(-0.16, 0.17)	0.01	(-0.11, 0.13)
hemoglobin	-0.08	(-0.36, 0.2)	-0.06	(-0.26, 0.15)
ESR	-0.17	(-0.48, 0.15)	0.03	(-0.18, 0.24)
WBC	-0.22	(-0.49, 0.05)	0	(-0.19, 0.19)
eosinophils	-0.22	(-0.51, 0.07)	0.01	(-0.19, 0.2)
neutrophils	-0.25	(-0.52, 0.02)	0.02	(-0.16, 0.19)
lymphocytes	-0.03	(-0.28, 0.22)	0.07	(-0.09, 0.24)
NK cells	-0.26	(-1.11, 0.56)	0.21	(-0.25, 0.66)
B cells	-0.5	(-1.14, 0.14)	-0.08	(-0.48, 0.32)
total CD4 cells	-0.81	(-1.59, -0.05)	-0.22	(-0.68, 0.23)
naive CD4 cells	-0.7	(-1.48, 0.07)	-0.1	(-0.57, 0.36)
non-naive CD4 cells	-0.71	(-1.5, 0.07)	-0.27	(-0.74, 0.2)
total CD8 cells	0.42	(-0.4, 1.22)	-0.11	(-0.57, 0.35)
naive CD8 cells	0.04	(-0.79, 0.88)	-0.04	(-0.5, 0.42)
non-naive CD8 cells	0.72	(-0.14, 1.57)	-0.1	(-0.57, 0.39)
CD4/CD8 ratio	-0.78	(-1.52, -0.06)	-0.19	(-0.61, 0.24)

3.2.6 Porous cranial lesions do not predict greater physiological dysregulation or functional disability.

To measure allostatic load we used D_m, a composite metric of physiological dysregulation across multiple physiological systems (Cohen et al., 2013). Individual values of D_m are calculated using Mahalanobis distance, measuring the relationship of multiple biomarkers to each other compared to a 'healthy' baseline centroid. Both greater distances from baseline means and unusual combinations of biomarker values suggest greater physiological dysregulation and contribute to high values of D_m (Milot et al., 2014). While D_m is calculated using a range of biomarkers across multiple organ systems, it is not highly correlated with any individual biomarker. D_m predicts individual illness and mortality in multiple populations and appears to be robust to the choice of specific biomarkers (Milot et al., 2014), suggesting that D_m reflects organism-level breakdowns in the coordinated multi-system physiological regulation that is critical to maintaining health (Kraft et al., 2020). Neither orbital nor cranial vault porosity predict higher values of D_m.

Functional mobility provides yet another domain for assessing the long-term health consequences of childhood physiological stress. Functional disability is a central component of many assessments of geriatric frailty (Rockwood et al., 2000) and is itself a predictor of mortality risk for older adults (Kusumastuti et al., 2022). Childhood stress has been found to predict geriatric frailty, measured primarily as poor functional mobility, among older adults in other populations (Haapanen et al., 2018). Nevertheless, in the current study, presence of PCLs was not an independent predictor of functional disability as measured by task performance (Fig. 3.6) on a modified battery of mild exercises originally used in the MacArthur Studies of Successful Aging (Berkman et al., 1993; Stieglitz, Schniter, von Rueden, Kaplan, & Gurven, 2015).

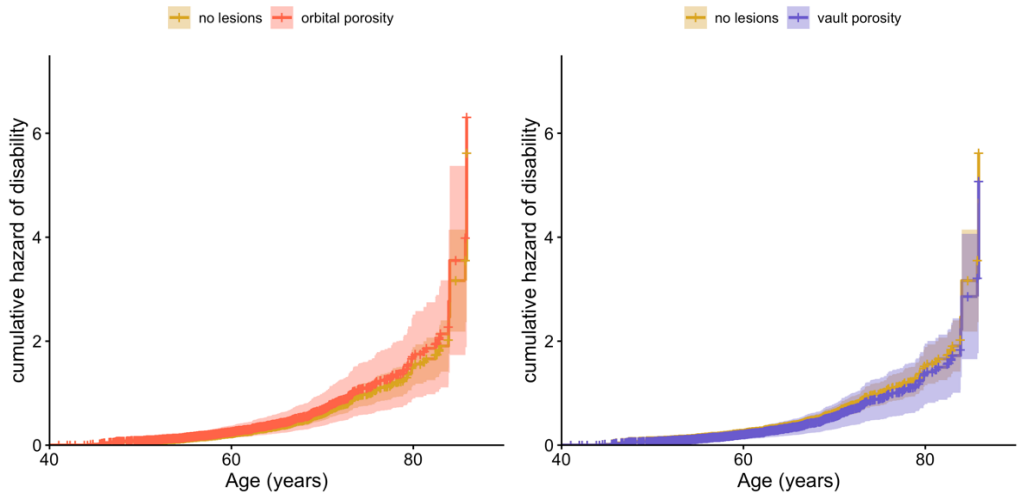

Figure 3. 7 Time to disability is not related to the presence of porous cranial lesions.The Cox proportional hazard model predicts age-specific cumulative hazard of difficulty performing basic tasks, controlling for sex. Tasks are included in the model as levels in a categorical fixed effect. Plots show predicted cumulative hazard of difficulty in one of the functional mobility assessment tasks (Table S3.7)—standing from a chair without using one's arms.

3.2.7 Cranial vault porosity is associated with low vertebral BMD.

A subset of individuals evaluated for PCLs (n = 94) also had existing measures for thoracic vertebral bone mineral density (BMD). Only three had clear cases of orbital roof porosity, rendering meaningful statistical associations untenable. Cranial vault porosity, which was better represented (n = 14), was associated with markedly lower thoracic BMD (beta = -0.56, 95% CI = (-1.02, -0.09)), controlling for age and sex. In fact, thoracic BMD among individuals with vault porosity was consistently below the sample average (Figure 3.7), regardless of the individual cranial bones involved. The low thoracic BMD associated with the cranial vault lesion identified in this study raises the question of whether some cases of cranial vault pitting or porosity are manifestations of systemic bone loss, or whether individuals who develop porous cranial lesions in childhood are at risk of low BMD in later life.

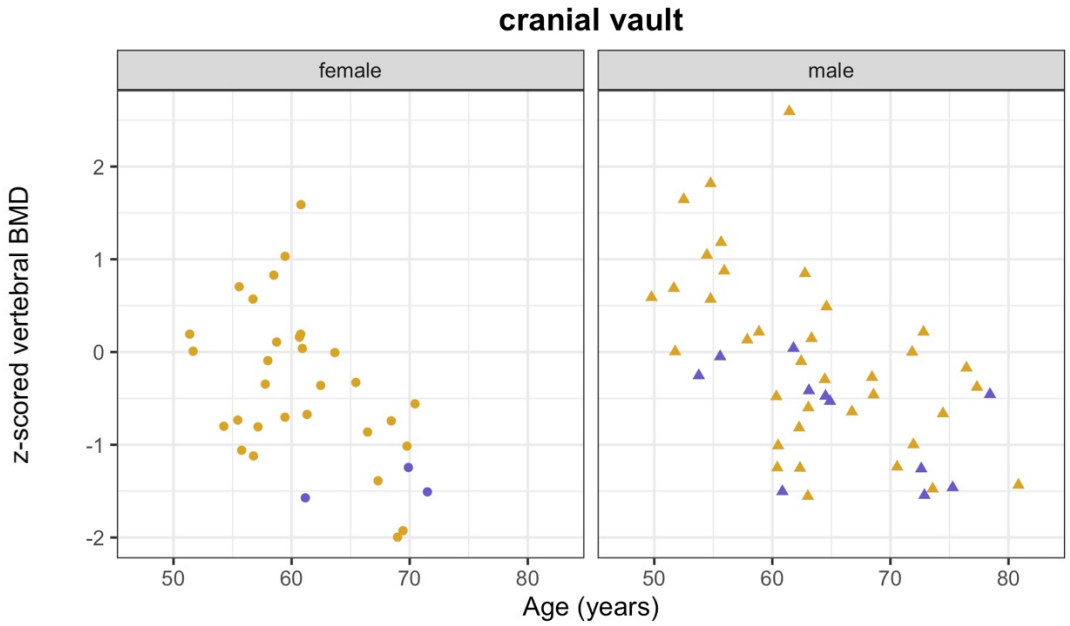

Figure 3. 8 Scatter plot of thoracic BMD in individuals with and without cranial vault porosity.

3.3 Discussion

Porous cranial lesions are widely interpreted by bioarchaeologists as skeletal indicators of nonspecific childhood physiological stress, and they are associated with shorter lifespans in many archaeological contexts. This is the first population-based study to explore the relationship between porous cranial lesions and the subsequent vulnerability to illness implied by their apparent association with elevated mortality risk. We leveraged existing cranial CT scans and longitudinal health data on medical diagnoses, immune biomarkers, functional mobility, and bone mineral density to investigate associations between porous cranial lesions of the orbits (cribra orbitalia) and bones of the cranial vault (cribra cranii/porotic hyperostosis) and measures of health in older adults (40+ years) from the Tsimane population of lowland Bolivia.

We find that porous lesions of the orbital roofs are associated with a greater degree of immune senescence for age and higher hazards of developing symptomatic tuberculosis among older adults. This apparent immunocompromise does not translate into higher incidence of other respiratory infections or greater levels of physiological dysregulation. Though associated

with immune senescence, orbital roof porosity is not an indicator of age-associated frailty more broadly, which is characterized by dysregulation and vulnerability across multiple systems.

The health correlates of orbital roof porosity—lower CD4/CD8 T cell ratio and higher hazard of tuberculosis—provide evidence for higher risks of immune senescence and age-related opportunistic infection in individuals with skeletal indicators of childhood physiological stress (Figure 3.8). However, these group-level associations should not be used to extrapolate specific individual experiences of chronic morbidity from archaeological skeletal remains. While risk of tuberculosis was higher for study participants with porous lesions of the orbital roofs, 82.6% of individuals with orbital roof lesions did not develop active tuberculosis during the study period.

Low CD4/CD8 ratio is an immune risk phenotype that has been shown to develop as a consequence of chronic immune activation, supporting the idea that individuals who experience lesion-causing stress in early childhood also experience elevated morbidity across the life course (McBride & Striker, 2017; Wikby et al., 1998; Wolday, Ndungu, Gómez-Pérez, & de Wit, 2021). Though chronic immune activation is a well described and widespread aspect of Tsimane life experience at all ages (Gurven et al., 2016), the previously reported distribution of CD4/CD8 ratios in Tsimane individuals is similar to German and English references in all but the 50+ age group, where Tsimane values are significantly lower (Blackwell et al., 2016; Bofill et al., 1992; Jentsch-Ullrich, Koenigsmann, Mohren, & Franke, 2005). The emergence of marked CD4 depletion among older Tsimane adults demonstrates the long-ranging costs of chronic immune activation, and its association with orbital roof lesions points to the early life influence on trajectories of disease experience. Here, skeletal lesions may be neither a cause nor a consequence of chronic immune activation, but merely an indicator of unobserved early life stress that serves to predict subsequent disease susceptibility (Figure 3.8).

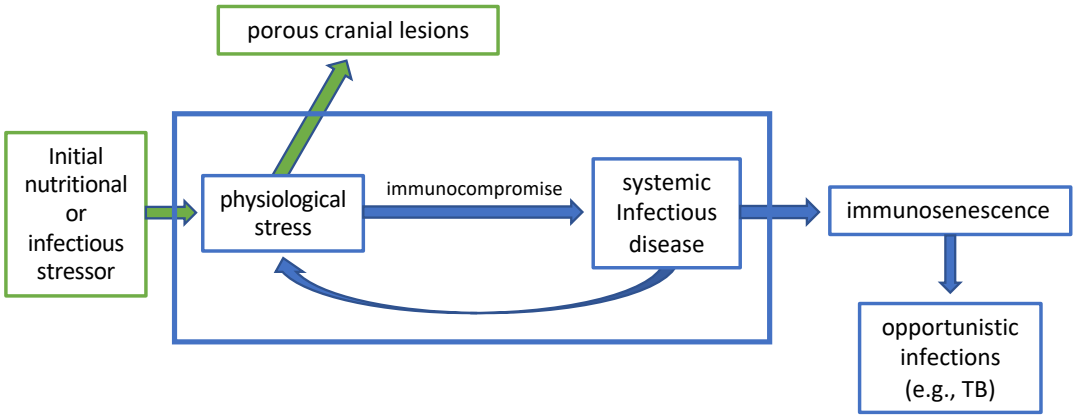

Figure 3. 9 Conceptual frameworkshowing pathways through which porous cranial lesions may be associated with health outcomes among older adults

Lower CD4/CD8 ratio and greater susceptibility to symptomatic tuberculosis are also documented sequelae of chronic hookworm infection (Chatterjee & Nutman, 2015; Guzzetta & Kirschner, 2013; Wolday et al., 2020), which presents a plausible but speculative candidate for the primary cause of porous cranial lesions in this population. Hookworm-driven blood loss anemia in early childhood has been proposed as a causal mechanism of porous cranial lesions (Reinhard, 1990; Walker et al., 2009), and chronic hookworm and other helminth infections are endemic in Tsimane communities (Anderson et al., 2019; Blackwell et al., 2016; Tanner et al., 2009; Vasunilashorn et al., 2011). Helminth infections appear to explain low CD4/CD8 ratios in a number of chronically exposed populations (Kalinkovich et al., 1998) and prompt a shift towards Th2 T cell-regulated immune responses that can increase susceptibility to tuberculosis by dampening the responsiveness of Th1-mediated immune responses to mycobacteria (Chatterjee & Nutman, 2015; Elias, Mengistu, Akuffo, & Britton, 2006). As well as being a consequence of untreated tuberculosis (Guzzetta & Kirschner, 2013), lower CD4 counts are a risk factor for developing symptomatic post-primary tuberculosis (Wolday et al., 2020). Without

better longitudinal coverage of flow cytometry data it is not possible to determine a causal relationship between these two variables.

Porosity of the cranial vault showed little relationship to any of the health outcomes measured here, with the exception of low thoracic bone mineral density. Bioarchaeological studies often report stronger apparent mortality risks associated with orbital roof porosity than with cranial vault porosity, and the results of the current study are consistent with this pattern. Alternately, the association between vault porosity and low vertebral BMD may indicate that apparent vault porosity as identified from volume-rendered cranial CTs is not indicative of childhood experience and is instead a manifestation of age-related systemic bone loss. If so, cranial vault porosity itself would serve as an additional indicator of geriatric frailty. This alternate explanation is not entirely satisfying, however, given that frequency of cranial vault porosity is uniform across all ages.

3.3.1 Study Limitations

Though the current study was able to examine the relationship between porous cranial lesions and later life morbidity in a variety of health domains, our study has several limitations. We investigated incidence of respiratory infection but did not have measures of severity or duration for individual bouts of infection, which are also salient contributors to overall morbidity burden and may be more relevant measures of morbidity-related mortality risk. Additionally, though population-representative, our sample is composed of adults aged 40+, and the cranial lesion status of non-survivors in these cohorts is unknown. Since the mortality risk associated with cranial lesions in archaeological settings is concentrated among children and young adults, the current study likely underestimates the lifetime impact of lesion-causing processes in the study population. A sample of older adults may comprise a robust subset of

survivors for whom lesion-causing stress has little measurable impact on life-long health experience, while PCLs might still be associated with higher group-level morbidity and mortality at younger ages. Likewise, while we can assess group-level associations, we cannot determine whether individual health consequences of cranial lesions diminish, remain constant, or accumulate over the life course. Future data (five-year CT follow-up and on-going collection of Tsimane census data) will shed light on the potential role of lesion-related mortality risk in producing the negative relationship observed between orbital roof lesions and age.

3.3 Conclusion

Cribra orbitalia, a skeletal lesion developed in early childhood that is among the most common pathological indicators in archaeological studies of human skeletal remains but largely ignored in clinical practice, is associated with a measure of chronic immune activation among adults in a contemporary subsistence population. Previous work interpreting these skeletal lesions in archaeological contexts has not been sufficiently grounded in population-based studies of their relationship to population health. While this is a promising first demonstration of a skeletal indicator of childhood stress *in vivo* that may provide a window into the developmental origins of health and disease in prehistory, the current study should be regarded as an initial step towards an integrated study of health in populations past and present. Further study is needed to determine whether these skeletal lesions are associated with elevated mortality risk among adults in this population and whether PCLs are consistently associated with morbidity in children and adults across populations.

3.4 Materials and Methods

3.4.1 Study population

The Tsimane are an indigenous population of ≈17,000 people who live a subsistence lifestyle in villages near the Maniqui River, a tributary of the Amazon. Starchy horticultural staples (62% of calories), wild game (6% of all calories), and fish (16%) make up the majority of the Tsimane diet, though consumption of market foods (recently estimated at 8% of average daily calories per person) is increasing (Kraft et al 2020).

Chronic parasitic infection is common in Tsimane villages. 57% of study participants in past reports were infected with at least one helminth species, with hookworm (undetermined, but likely *Necator americanus*) (45.3%) and roundworm (*Ascaris lumbricoides*, 19.9%) the most prevalent (Aaron D. Blackwell et al., 2011). Levels of immunoglobulin-E, a class of antibody produced in response to parasitic worm infections, are 150-200 times higher among Tsimane individuals than in age-matched Americans. Chronic inflammation is prevalent. Total white blood cell counts among the Tsimane are roughly 1.5 times higher than US National Health and Nutrition Examination Survey (NHANES) values, and 89% of Tsimane have eosinophil counts higher than NHANES 95th percentile (Aaron D Blackwell et al., 2016). Anemia is common despite high estimated levels of dietary iron (Anderson et al., 2019; Kraft et al., 2018; Vasunilashorn et al., 2010).

The population experiences high mortality, with death rates across the life course similar to European mortality from the 1800s (M. Gurven et al., 2008). As of 2002, life expectancy at birth is 53 years, a substantial increase from the pre-1990 figure of 43 years (M. Gurven et al., 2007). This increase in life expectancy appears to be driven by decreased adult mortality rather than improved survival of infants and children—approximately a quarter of the population does not survive past age 14.

Data for this study come from the Tsimane Health and Life History Project (THLHP, http:// www.unm.edu/~tsimane), a longitudinal study of health and aging among Tsimane

communities that started in 2002 (Gurven et al., 2017). For all Tsimane individuals with a cranial CT scan, there also exist assessments of functional ability, medical exams, and blood samples (Table S1). Clinical and health history data were collected by the THLHP mobile medical team during annual visits following routine medical examinations (patient history, symptom investigation and clinical diagnoses, blood pressure and temperature, height and weight). THLHP collection protocols for biomedical data are outlined in detail in numerous publications (Vasunilashorn et al., 2010; Blackwell et al., 2011; Gurven et al., 2012; Blackwell et al., 2016a; Gurven et al., 2016; Trumble et al., 2016; Gurven et al., 2017b).

3.4.2 Ethics approval

The study was reviewed and approved by the Institutional Review Board (IRB) of the University of California-Santa Barbara. The Gran Consejo Tsimane, the governing body overseeing Tsimane affairs and research projects, and the IRBs of the University of California-Santa Barbara and the University of New Mexico additionally reviewed and approved the studies from which existing data on Tsimane health is drawn. Informed consent was obtained at both the community and individual participant levels. During a community meeting open to all residents, communities determined collectively whether the study would be conducted. To date, all communities that have been approached have approved the study. Individuals gave informed consent before each medical visit, procedure, and biospecimen collection.

3.4.3 Biomarker Collection

Venous blood samples were collected by certified Bolivian biochemists into a heparin-coated vacutainer. Erythrocyte sedimentation rate (ESR), a marker of generalized inflammation

(Erikssen et al., 2000), was calculated using the Westergren (1957) method. Total white blood cell count and hemoglobin were measured using a QBC Autoread Plus dry hematology system (QBC Diagnostics) directly after blood draw, and leukocyte subtypes were counted manually using microscopy and a hemocytometer (Blackwell et al. 2016).

A subset of individuals (obs = 252; n = 195) also had lymphocyte subtypes quantified using flow cytometry. Flow cytometry was conducted on an Accuri C6 Flow Cytometer (BD Accuri Cytometers) within six hours of the blood draw in the THLHP clinic in San Borja on fresh heparinized blood. Lymphocytes were labeled with appropriate fluorescently tagged antibodies and categorized as helper T cell (CD4+CD8-), cytotoxic T cells (CD8+CD4-), natural killer cells (CD56+CD8-CD4-), and B cells (CD19+). Helper and cytotoxic T cells were further classified as naïve (CD45RA+) or non-naïve (CD45RA-). Absolute counts of each subset were calculated by multiplying the relative percentages determined by flow cytometer by the total lymphocyte count obtained from the QBC Autoread Plus (Aaron D Blackwell et al., 2016).

D_m, a composite measure of physiological dysregulation based on the Mahalanobis distance, incorporates intra-individual changes in the absolute levels of multiple biomarkers and their correlated structure relative to a healthy baseline as indicators of departure from an integrated physiological system. Overall D_m was calculated from up to 39 biomarkers across multiple physiological systems (median = 17). See Kraft et al. (2020)for a detailed discussion of how to calculate D_m.

As a measure of functional disability, we coded whether participants experienced any difficulty (yes = 1, no = 0) standing from a chair without using their arms, rapidly walking 3 m and returning to their starting position, picking up a pencil from the ground, and balancing in

the tandem position, and on each leg, without using their arms or body (Stieglitz, Schniter, et al., 2015).

3.4.4 Computed tomography data collection

The CT scans used in the current study were obtained between 2017 and 2018. Individuals who consented and were able to travel were transported to the German Busch Hospital in Trinidad, Bolivia, for chest and brain CT scans. CT scans were performed by a licensed radiological technician using a 16-detector row multi-slice CT (GE Brightspeed, Milwaukee, WI, USA) under the supervision of project clinicians.

ECG-gated non-contrast thoracic scans were obtained with 2.5 mm slice thickness (250 ms exposure, 0.5 s rotation speed, 120 kVp, and 40 mA with prospective triggering). Vertebral bone mineral density (BMD) was measured manually at the Los Angeles Biomedical Research Institute by a radiologist with 20+ years of experience. Each participant's reported BMD is the mean of three consecutive thoracic vertebrae, starting at the level of the section containing the left main coronary artery (LMCA) caudally (beginning at either T7 or T8, depending on the origin of the LMCA). The radiologist manually placed a circular region of interest at the center of each vertebra, with a 2–3 mm distance from the cortical shell to ensure that only trabecular bone was included. To the extent possible, areas with large vessels, bone island fractures and calcified herniated disks were excluded from the region of interest (Stieglitz et al., 2019).

Cranial scans were obtained using a slice thickness of 0.625mm (130 kvp, 140 mAs, pitch 0.65), and the raw data was processed using a bone-optimized reconstruction algorithm. The first author (AA) assessed porous cranial lesion status on each scan using the freeware DICOM viewer Horos (Horos, 2019) following the protocol laid out in Anderson et al. (2021). Briefly, AA first observed all cross-sectional slices of the scan in coronal, sagittal, or axial planes using

multiplanar reconstruction (MPR) with window width and level set to Horos' bone-optimized preset. MPR images were scored for the presence or absence of three lesion-related traits: radial trabecular orientation (radiologic 'hair-on-end' sign), superficial pitting of the outer table (ectocranial pitting), and porosity of the outer table for the frontal, parietal, and occipital bones. For orbital roofs, only cortical porosity was noted. MPR observations of each skull were then corroborated by observing surface features of a rotatable 3-D volume rendering, with color lookup table set to 'VR muscle and bone' and the generated light source set to 'diffuse.' Surface pitting or porosity were scored as 'fine,' 'distinct,' or 'trabeculated' for each bone of the cranial vault and each orbital roof, along with degree of observer certainty. Ambiguous cases were re-scored two weeks later.

Cranial vault porosity was considered present if pitting or porosity composed of more than five distinct foramina was observed on at least one bone of the cranial vault, though porosity comprised of distinct individual foramina was only observed on the posterior parietal bones and occipital squama. Because the data for this study were collected by a single observer and the results of Anderson et al. emphasize that lesions with foramina close to or below the scale of the scan's resolution ('pinprick' porosity) are difficult for observers to identify accurately, only lesions with larger, distinct foramina were identified as unambiguously present, though any indications of porous changes were noted during data collection in order to check the robusticity of results when marginal lesion cases are included/excluded.

Cribra orbitalia was considered present if it was clearly visible in at least one orbit in both coronal and sagittal planes of multiplanar reconstruction and using 3-D volume rendering. The majority of cribra orbitalia cases identified in the present study fall under Nathan and Haas' 'trabecular type' classification (Nathan & Haas, 1966). Orbital roof porosity with the appearance of the 'porotic' and 'cribrotic' types could not be reliably identified by observers in initial tests

(Anderson et al., 2021), and false positive cases often took on the appearance of porotic or cribrotic lesions. This study is therefore conservative, limited to observing orbital lesions with more extensive skeletal changes, and it is likely that our data contain a substantial number of false negatives due to these limitations.

3.4.5 Statistical analysis:

All analyses were conducted in R version 4.1.2 (https://cran.r-project.org/, (11 January 2022, date last accessed)) using the brms (Burkner, 2017)and survival packages (Therneau, 2022). Models were run with default non-informative brms priors unless otherwise specified.

3.4.5.1 What predicts lesions?

Because none of the processes that might contribute to age-related patterns in lesion frequency—selective mortality, lesion remodeling—are expected to have constant rates, demographic patterning of lesion presence and morphology was assessed using non-linear logistic regressions for lesion presence with a smoothing spline for age, grouped by sex.

3.4.5.2 What do lesions predict?

We assessed the effect of orbital roof porosity and cranial vault porosity/pitting on a range of outcome measures, with population-level effects controlling for age and sex and a group-level effect for participant identity to control for repeated measures per person in the mixed longitudinal THLHP sample, when appropriate. We report the effect of orbital roof porosity and cranial vault porosity using standardized betas and 95% CIs for continuous outcome variables and odds ratios with 95% CIs for binary outcomes. Models for orbital porosity were run twice – first with ambiguous cases (n = 37) coded as lesion absence and then with ambiguous cases coded as lesion presence. Results were considered robust when both models returned consistent results, and only results of the first models (more conservative coding for lesion presence) are reported.

Raw values for all continuous outcome measures were z-scored prior to analysis to produce comparable estimates of standardized effect sizes in all models. For models of cell counts from flow cytometry, for which fewer individuals had repeat measures, the group-level effect of individual identity was estimated only for the subset of individuals with flow cytometry data from multiple dates. The potential influence of current infection on hematological measures was tested by re-running each model with an additional population-level term for neutrophil count as a marker of current immune activation.

In models evaluating the effect of PCLs on incidence of recurrent health conditions over the study period, the outcome variable was a binary indicator of the diagnosis of interest at the time of medical clinic visit. Because respiratory infection is a broader diagnostic category than the other diagnoses investigated, we ran a series of models with respiratory infection categorized in several ways. Results were similar regardless of respiratory grouping: all ICD-10 respiratory diagnoses; upper or lower respiratory infections; respiratory diagnoses of infectious origin, including and excluding pulmonary tuberculosis. We report results for the incidence of respiratory diagnoses of infectious origin (pneumonia, bronchitis, whooping cough, tracheitis, pharyngitis, laryngitis, and unspecified upper and lower respiratory infections). We excluded tuberculosis diagnoses from this analysis of the incidence of infectious respiratory conditions due to the chronic nature of tuberculosis infection, although some of the 'unspecified' respiratory infections may be unidentified pulmonary tuberculosis. Sinusitis and other conditions likely directly related to chronic smoke exposure (e.g., pulmonary fibrosis) were also excluded.

We modeled the relationship between lesions and onset of symptomatic tuberculosis using a Cox proportional hazard model with sex, age, orbital roof porosity, and cranial vault porosity as covariates. The time-to-event begins at an individual's first medical visit, and the event of interest is an individual's first diagnosis of suspected tuberculosis. The association

between PCLs and functional disability was also evaluated using a cox model, with sex, orbital roof porosity and cranial vault porosity as predictors of time (age) to disability. Because functional disability was assessed using binary evaluations of performance on a range of tasks on a given date and most individuals completed the disability assessment multiple times, the model included task as a fixed effect and individual identity as a cluster term to account for non-independence of within-individual measures.

Models of the effect of porous cranial lesions on D_m included population-level controls for number of biomarkers per D_m calculation, sex, age, age^2, and a random intercept for individual identity to account for people with multiple observations. The quadratic age term improved model fit substantially, based on Bayesian model comparison using leave-one-out cross validation. Models were weighted by the number of biomarkers measured for each observation and run with a weakly informative gaussian prior for population-level effects.

3.5 References

Agarwal, N., M, S., Dhar, N., & Bhardwaj, P. (1970). Roentgenologic changes in iron deficiency anemia. *American Journal of Roentgenology*, *110*(3), 635–637.

Anderson, A. S., Trumble, B. C., Hové, C., Kraft, T. S., Kaplan, H., Gurven, M., & Blackwell, A. D. (2019). Old friends and friendly fire: Pregnancy, hookworm infection, and anemia among tropical horticulturalists. *American Journal of Human Biology*, *32*(2), e23337. https://doi.org/10.1002/ajhb.23337

Angel, J. L. (1964). Osteoporosis: Thalassemia? *American Journal of Physical Anthropology*, *22*(3), 369–373.

Berkman, L. F., Seeman, T. E., Albert, M., Blazer, D., Kahn, R., Mohs, R., … Rowe, J. (1993). High, usual and impaired functioning in community-dwelling older men and women:

Findings from the MacArthur Foundation Research Network on successful aging. *Journal of Clinical Epidemiology*, *46*(10), 1129–1140. https://doi.org/https://doi.org/10.1016/0895-4356(93)90112-E

Blackwell, A. D., Gurven, M. D., Sugiyama, L. S., Madimenos, F. C., Liebert, M. A., Martin, M. A., … Snodgrass, J. J. (2011). Evidence for a peak shift in a humoral response to helminths: Age profiles of IgE in the shuar of Ecuador, the Tsimane of Bolivia, and the U.S. NHANES. *PLoS Neglected Tropical Diseases*, *5*(6). https://doi.org/10.1371/journal.pntd.0001218

Blackwell, A. D., Trumble, B. C., Suarez, I. M., Stieglitz, J., Beheim, B., Snodgrass, J. J., … Gurven, M. (2016). Immune function in Amazonian horticulturalists. *Annals of Human Biology*, *43*(4), 382–396.

Blom, D. E., Buikstra, J. E., Keng, L., Tomczak, P. D., Shoreman, E., & Stevens-Tuttle, D. (2005). Anemia and childhood mortality: Latitudinal patterning along the coast of pre-Columbian Peru. *American Journal of Physical Anthropology*, *127*(2), 152–169. https://doi.org/10.1002/ajpa.10431

Bocquet-Appel, J. P., Naji, S., & Bandy, M. (2008). Demographic and health changes during the transition to agriculture in North America. In J. P. Bocquet-Appel (Ed.), *Recent Advances in Palaeodemography: Data, Techniques, Patterns* (pp. 277–292). https://doi.org/10.1007/978-1-4020-6424-1_10

Bofill, M., Janossy, G., Lee, C. A., MacDonald-Burns, D., Phillips, A. N., Sabin, C., … Kernoff, P. B. (1992). Laboratory control values for CD4 and CD8 T lymphocytes. Implications for HIV-1 diagnosis. *Clinical and Experimental Immunology*, *88*(2), 243–252. https://doi.org/10.1111/j.1365-2249.1992.tb03068.x

Burkner, P.-C. (2017). *brms : An R Package for Bayesian Generalized Linear Mixed Models using Stan.*

(Plummer 2013).

Burko, Henry; Mellins, harry Z.; Watson, J. (1960). Skull changes in iron deficiency anemia simulating congenital hemolytic anemia. *The American Journal of Roentgenology, Radium Therapy, and Nuclear Medicine*, *86*, 447–452.

Caffey, J. (1951). Cooley's erythroblastic anemia: Some skeletal findings in adolescents and young adults. *The American Journal of Roentgenology and Radium Therapy*, *65*(4).

Chaichun, A., Yurasakpong, L., Suwannakhan, A., Iamsaard, S., Arun, S., & Chaiyamoon, A. (2021). Gross and radiographic appearance of porotic hyperostosis and cribra orbitalia in thalassemia affected skulls. *Anatomy & Cell Biology*, *54*(2), 280–284. https://doi.org/10.5115/acb.20.323

Chatterjee, S., & Nutman, T. B. (2015). Helminth-Induced Immune Regulation: Implications for Immune Responses to Tuberculosis. *PLoS Pathogens*, *11*(1), 1–6. https://doi.org/10.1371/journal.ppat.1004582

Cohen, A. A., Milot, E., Yong, J., Seplaki, C. L., Fülöp, T., Bandeen-Roche, K., & Fried, L. P. (2013). A novel statistical approach shows evidence for multi-system physiological dysregulation during aging. *Mechanisms of Ageing and Development*, *134*(3–4), 110–117. https://doi.org/10.1016/j.mad.2013.01.004

Damini, S., Dixit, R., Khullar, T., & Rajeshwari, K. (2021). Bilateral proptosis: an unusual primary presentation of scurvy—a case report. *Egyptian Journal of Radiology and Nuclear Medicine*, *52*(1). https://doi.org/10.1186/s43055-021-00553-7

DeLouize, A. M., Liebert, M. A., Madimenos, F. C., Urlacher, S. S., Schrock, J. M., Cepon-Robins, T. J., … Sugiyama, L. S. (2022). Low prevalence of anemia among Shuar communities of Amazonian Ecuador. *American Journal of Human Biology*, *34*(1), e23590. https://doi.org/10.1002/ajhb.23590

Elias, D., Mengistu, G., Akuffo, H., & Britton, S. (2006). Are intestinal helminths risk factors for developing active tuberculosis? *Tropical Medicine & International Health*, *11*(4), 551–558. https://doi.org/https://doi.org/10.1111/j.1365-3156.2006.01578.x

Garrido-Rodríguez, V., Herrero-Fernández, I., Castro, M. J., Castillo, A., Rosado-Sánchez, I., Galvá, M. I., … Pacheco, Y. M. (2021). Immunological Features Beyond CD4/CD8 Ratio Values in Older Individuals. *Aging*, *13*(10), 13443–13459. https://doi.org/10.18632/aging.203109

Gomes, R. A. M. P., Petit, J., Dutour, O., & Santos, A. L. (2022). Frequency and co-occurrence of porous skeletal lesions in identified non-adults from Portugal (19th to 20th centuries) and its association with respiratory infections as cause of death. *International Journal of Osteoarchaeology*, (January), 1–12. https://doi.org/10.1002/oa.3132

Gurven, M. D., Trumble, B. C., Stieglitz, J., Yetish, G., Cummings, D., Blackwell, A. D., … Pontzer, H. (2016). High resting metabolic rate among Amazonian forager- horticulturalists experiencing high pathogen burden. *American Journal of Physical Anthropology*, *161*(3), 414–425. https://doi.org/10.1002/ajpa.23040

Gurven, M., Kaplan, H., & Supa, A. Z. (2007). *Mortality Experience of Tsimane Amerindians of Bolivia : Regional Variation and Temporal Trends*. *398*(September 2006), 376–398. https://doi.org/10.1002/ajhb

Gurven, M., Kaplan, H., Winking, J., Finch, C., & Crimmins, E. M. (2008). Aging and inflammation in two epidemiological worlds. *The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences*, *63*(2), 196–199. https://doi.org/63/2/196 [pii]

Gurven, M., Stieglitz, J., Trumble, B., Blackwell, A. D., Beheim, B., Davis, H., … Kaplan, H. (2017). The Tsimane Health and Life History Project: Integrating anthropology and biomedicine. *Evolutionary Anthropology*, *26*(2), 54–73. https://doi.org/10.1002/evan.21515

Guzzetta, G., & Kirschner, D. (2013). The Roles of Immune Memory and Aging in Protective Immunity and Endogenous Reactivation of Tuberculosis. *PLoS ONE*, *8*(4). https://doi.org/10.1371/journal.pone.0060425

Haapanen, M. J., Perälä, M. M., Salonen, M. K., Kajantie, E., Simonen, M., Pohjolainen, P., … Von Bonsdorff, M. B. (2018). Early life stress and frailty in old age: The Helsinki birth cohort study. *BMC Geriatrics*, *18*(1), 1–8. https://doi.org/10.1186/s12877-018-0873-5

Hamilton, W. D. (1966). The moulding of senescence by natural selection. *Journal of Theoretical Biology*, *12*(1), 12–45. https://doi.org/https://doi.org/10.1016/0022-5193(66)90184-6

Henschen, F. (1961). Cribra Cranii, a Skull Condition Said to be of Racial or Geographical Nature. *Pathologie et Microbiologie*, *729*(1961), 724–729.

Horos. (2019). *Horos*. Retrieved from https://horosproject.org/faqs/

Hunt Ingalls, T. (1936). The role of scurvy in the etiology of chronic subdurla hematoma. *New England Journal of Medicine*, *215*(27), 1279–1281.

Jatautis, Š., Mitokaite, I., & Jankauskas, R. (2011). Analysis of cribra orbitalia in the earliest inhabitants of medieval Vilnius. *Anthropological Review*, *74*(1), 57–68. https://doi.org/10.2478/v10044-010-0006-z

Jentsch-Ullrich, K., Koenigsmann, M., Mohren, M., & Franke, A. (2005). Lymphocyte subsets' reference ranges in an age- and gender-balanced population of 100 healthy adults—A monocentric German study. *Clinical Immunology*, *116*(2), 192–197. https://doi.org/https://doi.org/10.1016/j.clim.2005.03.020

Kalinkovich, A., Weisman, Z., Greenberg, Z., Nahmias, J., Eitan, S., Stein, M., & Bentwich R., Z. (1998). Decreased CD4 and increased CD8 counts with T cell activation is associated with chronic helminth infection. *Clinical and Experimental Immunology*, *114*(3), 414–421. https://doi.org/10.1046/j.1365-2249.1998.00736.x

Kraft, T. S., Stieglitz, J., Trumble, B. C., Garcia, A. R., Kaplan, H., & Gurven, M. (2020). Multi-system physiological dysregulation and ageing in a subsistence population: Physiological aging in perspective. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *375*(1811). https://doi.org/10.1098/rstb.2019.0610rstb20190610

Kraft, T. S., Stieglitz, J., Trumble, B. C., Martin, M., Kaplan, H., & Gurven, M. (2018). Nutrition transition in two lowland Bolivian subsistence populations. *The American Journal of Clinical Nutrition*, *108*(6), 1183–1195. https://doi.org/10.1093/ajcn/nqy250

Kusumastuti, S., Hoogendijk, E. O., Gerds, T. A., Lund, R., Mortensen, E. L., Huisman, M., & Westendorp, R. G. J. (2022). Do changes in frailty, physical functioning, and cognitive functioning predict mortality in old age? Results from the Longitudinal Aging Study Amsterdam. *BMC Geriatrics*, *22*(1), 193. https://doi.org/10.1186/s12877-022-02876-0

Madani, G., Papadopoulou, A. M., Holloway, B., Robins, A., Davis, J., & Murray, D. (2007). *The radiological manifestations of sickle cell disease*. 528–538. https://doi.org/10.1016/j.crad.2007.01.006

Marciniak, S., Bergey, C. M., Silva, A. M., Hałuszko, A., Furmanek, M., Veselka, B., … Perry, G. H. (2022). An integrative skeletal and paleogenomic analysis of prehistoric stature variation suggests relatively reduced health for early European farmers. *Proceedings of the National Academy of Sciences*, *119*(15), e2106743119. https://doi.org/10.1073/pnas.2106743119/-/DCSupplemental.Published

McBride, J. A., & Striker, R. (2017). Imbalance in the game of T cells: What can the CD4/CD8 T-cell ratio tell us about HIV and health? *PLoS Pathogens*, *13*(11), 1–7. https://doi.org/10.1371/journal.ppat.1006624

Mcfadden, C., & Oxenham, M. F. (2020). A paleoepidemiological approach to the osteological paradox : Investigating stress , frailty and resilience through cribra orbitalia. *American Journal*

of Physical Anthropology, *173*(2), 205–217. https://doi.org/10.1002/ajpa.24091

McNab, A. A. (2014). Nontraumatic orbital hemorrhage. *Survey of Ophthalmology*, *59*(2), 166–184. https://doi.org/10.1016/j.survophthal.2013.07.002

Mensforth, R. P., Lovejoy, C. O., Lallo, J. W., & Armelagos, G. J. (1978). Part Two: The role of constitutional factors, diet, and infectious disease in the etiology of porotic hyperostosis and periosteal reactions in prehistoric infants and children. *Medical Anthropology*, *2*(April 2016), 1–59. https://doi.org/10.1080/01459740.1978.9986939

Milot, E., Morissette-Thomas, V., Li, Q., Fried, L. P., Ferrucci, L., & Cohen, A. A. (2014). Trajectories of physiological dysregulation predicts mortality and health outcomes in a consistent manner across three populations. *Mechanisms of Ageing and Development*, *141–142*, 56–63. https://doi.org/10.1016/j.mad.2014.10.001

Mittler, D. M., & van Gerven, D. P. (1994). Developmental, diachronic, and demographic analysis of cribra orbitalia in the medieval christian populations of Kulubnarti. *American Journal of Physical Anthropology*, *93*(3), 287–297. https://doi.org/10.1002/ajpa.1330930302

Moore, S. (1929). The bone changes in sickle cell anemia with note on similar changes observed in skulls of ancient Mayan Indians. *Journal of Missouri Medical Association*, *26*, 561.

Moseley, J. E. (1965). The Paleopathologic riddle of "symmetrical osteoporosis." *American Journal of Roentgenology*, *95*(1), 135–142. https://doi.org/10.2214/ajr.95.1.135

Muller, G. C., Gottlieb, M. G. V., Luz Correa, B., Filho, I. G., Moresco, R. N., & Bauer, M. E. (2015). The inverted CD4: CD8 ratio is associated with gender-related changes in oxidative stress during aging. *Cellular Immunology*, *296*(2), 149–154. https://doi.org/10.1016/j.cellimm.2015.05.006

Nathan, H., & Haas, N. (1966). "Cribra orbitalia." A bone condition of the orbit of unknown nature: Anatomical study with etiological considerations. *Israel Journal of Medical Science*, *2*(2),

171–191.

O'Donnell, L., Hill, E. C., Anderson, A. S., & Edgar, H. J. H. (2020). Cribra orbitalia and porotic hyperostosis are associated with respiratory infections in a contemporary mortality sample from New Mexico. *American Journal of Physical Anthropology*, *173*(4), 721–733. https://doi.org/10.1002/ajpa.24131

Obertova, Z., & Thurzo, M. (2004). Cribra orbitalia as an indicator of stress in the early medieval Slavic population from Borovce (Slovakia). In *Anthropologie* (Vol. 42).

Ortner, D. J., & Ericksen, M. F. (1997). Bone changes in the human skull probably resulting from scurvy in infancy and childhood. *International Journal of Osteoarchaeology*, *7*, 212–220.

Papathanasiou, A., Meinzer, N. J., Williams, K. D., & Larsen, C. S. (2018). History of anemia and related nutritional deficiencies: Evidence from cranial porosities. In *The Backbone of Europe: Health, Diet, Work and Violence over Two Millennia*.

Reinhard, K. J. (1990). Archaeoparasitology in north America. *American Journal of Physical Anthropology*, *82*(2), 145–163.

Rivera, F., & Mirazon Lahr, M. (2017). New evidence suggesting a dissociated etiology for cribra orbitalia and porotic hyperostosis. *Am J Phys Anthropol*, *164*(1), 76–96. https://doi.org/10.1002/ajpa.23258

Roberts, C. A., & Steckel, R. H. (2019). The Developmental Origins of Health and Disease: Early Life Health Conditions and Adult Age at Death in Europe. In R. H. Steckel, C. S. Larsen, C. A. Roberts, & J. Baten (Eds.), *The Backbone of Europe: Health, diet, work and violence over two millennia* (1st ed., pp. 325–351). Cambridge: Cambridge University Press.

Rockwood, K., Hogan, D. B., & MacKnight, C. (2000). Conceptualisation and measurement of frailty in elderly people. *Drugs and Aging*, *17*(4), 295–302. https://doi.org/10.2165/00002512-200017040-00005

Salanitri, S., & Seow, W. K. (2013). Developmental enamel defects in the primary dentition: Aetiology and clinical management. *Australian Dental Journal*, *58*(2), 133–140. https://doi.org/10.1111/adj.12039

Schuller, A. (1950). A short review of cranial hyperostoses. *Acta Radiologica*, *34*(4–5), 361–373. https://doi.org/10.3109/00016925009135283

Sebes, I., & Diggs, L. W. (1979). Radiographic Changes of the Skull in Sickle Cell Anemia. *American Journal of Roentgenology*, *132*(March), 373–377.

Shahidi, Nasrollah T.; Diamond, L. K. (1960). Skull changes in infants with chronic iron-deficiency anemia. *New E*, *262*(3), 137–139.

Steckel, R. H. (2005). Young adult mortality following severe physiological stress in childhood: Skeletal evidence. *Economics and Human Biology*, *3*(2), 314–328. https://doi.org/10.1016/j.ehb.2005.05.006

Steckel, R. H., Larsen, C. S., Roberts, C. A., & Baten, J. (Eds.). (2019). *The Backbone of Europe: Health, Diet, Work and Violence over Two Millennia*. Cambridge: Cambridge University Press.

Steckel, R. H., & Rose, J. C. (Eds.). (2002). *The Backbone of History: Health and Nutrition in the Western Hemisphere*. Cambridge: Cambridge University Press.

Stieglitz, J., Schniter, E., von Rueden, C., Kaplan, H., & Gurven, M. (2015). Functional disability and social conflict increase risk of depression in older adulthood among Bolivian forager-farmers. *The Journals of Gerontology Series B: Psychological Sciences and Social Sciences*, *70*(6), 948–956. https://doi.org/10.1093/geronb/gbu080

Stieglitz, J., Trumble, B. C., Finch, C. E., Li, D., Budoff, M. J., Kaplan, H., & Gurven, M. D. (2019). Computed tomography shows high fracture prevalence among physically active forager-horticulturalists with high fertility. *ELife*, *8*, e48607. https://doi.org/10.7554/elife.48607

Stuart-Macadam, P. (1985). Porotic hyperostosis: representative of a childhood condition. *American Journal of Physical Anthropology*, *66*, 391–398. https://doi.org/10.1002/ajpa.1330660407

Stuart-Macadam, P. (1987). Porotic hyperostosis: New evidence to support the anemia theory. *American Journal of Physical Anthropology*, *74*, 521–526.

Stuart-Macadam, P. (1987). A radiographic study of porotic hyperostosis. *American Journal of Physical Anthropology*, *74*(4), 511–520. https://doi.org/10.1002/ajpa.1330740409

Tanner, S., Leonard, W. R., McDade, T. W., Reyes-Garcia, V., Godoy, R., & Huanca, T. (2009). Influence of helminth infections on childhood nutritional status in lowland Bolivia. *American Journal of Human Biology*, *21*(5), 651–656. https://doi.org/10.1002/ajhb.20944

Therneau, T. M. (2022). *A Package for survival analysis in R*. Retrieved from https://cran.r-project.org/package=survival

Tyler, P. A., Madani, G., Chaudhuri, R., Wilson, L. F., & Dick, E. A. (2006). *The radiological appearances of thalassaemia*. 40–52. https://doi.org/10.1016/j.crad.2005.07.006

Vasunilashorn, S., Crimmins, E. M., Kim, J. K., Winking, J., Gurven, M., Kaplan, H., & Finch, C. E. (2010). Blood lipids, infection, and inflammatory markers in the Tsimane of Bolivia. *American Journal of Human Biology*, *22*(6), 731–740. https://doi.org/10.1002/ajhb.21074

Vasunilashorn, S., Finch, C. E., Crimmins, E. M., Suvi, A., Stieglitz, J., Gurven, M., … Allayee, H. (2011). *Biodemography and Social Biology Inflammatory Gene Variants in the Tsimane , an Indigenous Bolivian Population with a High Infectious Load Inflammatory Gene Variants in the Tsimane , an Indigenous Bolivian Population with a High Infectious Load. 5565*(April 2016). https://doi.org/10.1080/19485565.2011.564475

Verma, S., Sivanandan, S., Aneesh, M. K., Gupta, V., Seth, R., & Kabra, S. (2007). Unilateral proptosis and extradural hematoma in a child with scurvy. *Pediatric Radiology*, *37*(9), 937–

939. https://doi.org/10.1007/s00247-007-0543-9

Walker, P. L., Bathurst, R. R., Richman, R., Gjerdrum, T., & Andrushko, V. A. (2009). The causes of porotic hyperostosis and cribra orbitalia: A reappraisal of the iron-deficiency-anemia hypothesis. *American Journal Of Physical Anthropology*, *139*(2), 109–125. https://doi.org/10.1002/ajpa.21031

WHO. (2014). *Global nutrition targets 2025: stunting policy brief (WHO/NMH/NHD/14.3).* https://doi.org/10.7591/cornell/9781501758898.003.0006

Wikby, A., Maxson, P., Olsson, J., Johansson, B., & Ferguson, F. G. (1998). Changes in CD8 and CD4 lymphocyte subsets, T cell proliferation responses and non-survival in the very old: The Swedish longitudinal OCTO-immune study. *Mechanisms of Ageing and Development*, *102*(2–3), 187–198. https://doi.org/10.1016/S0047-6374(97)00151-6

Wolday, D., Kebede, Y., Legesse, D., Siraj, D. S., McBride, J. A., Kirsch, M. J., & Striker, R. (2020). Role of CD4/CD8 ratio on the incidence of tuberculosis in HIV-infected patients on antiretroviral therapy followed up for more than a decade. *PLoS ONE*, *15*(5), 1–15. https://doi.org/10.1371/journal.pone.0233049

Wolday, D., Ndungu, F. M., Gómez-Pérez, G. P., & de Wit, T. F. R. (2021). Chronic Immune Activation and CD4+ T Cell Lymphopenia in Healthy African Individuals: Perspectives for SARS-CoV-2 Vaccine Efficacy . *Frontiers in Immunology* , Vol. 12. Retrieved from https://www.frontiersin.org/article/10.3389/fimmu.2021.693269

IV. Iron deficiency, but not inflammation, is associated with reduced bone turnover among children in a high-pathogen and energy-limited population

4.1. Background

4.1.1 Building bones: energy, metabolism, and the skeleton

During childhood, growth and somatic maintenance are major components of a finite energy budget. If one demand, for instance immune function, has greater consequences for a child's immediate survival it may be prioritized over less immediate demands, such as somatic growth, especially in high pathogen environments. Tradeoffs between linear growth and immune activation have been documented in children from multiple populations in energy-limited, high-pathogen environments (Blackwell et al., 2010; Garcia et al., 2020; McDade et al., 2008; Shattuck-Heidorn et al., 2017; Urlacher et al., 2018). Bone formation is an important component of linear growth, but development and maintenance of skeletal tissues more generally may tradeoff against immune activation in childhood.

Aside from linear growth, bone formation also contributes to bone mass and strength, and existing bone is continually renewed at the cellular level via remodeling, a tightly coupled process of sequential bone resorption and formation, all of which takes energy. Bone remodeling is important for mobilizing resources for immune and other physiological responses, as the skeleton provides a mineral reservoir for endocrine function, and recent studies suggest that it plays a mediating role in glucose metabolism (Allen & Burr, 2014; Chowdhury et al., 2020; Fulzele et al., 2010; Zhang, Riddle, & Clemens, 2015). Overall energetic expenditure by the skeleton is therefore best measured using markers of bone turnover, a term that encompasses the combined metabolic activity of all bone cells contributing to skeletal plasticity and homeostasis (see Table 4.1).

The balance of bone turnover in childhood is likely to have long term consequences for bone mass and bone mineral density throughout life. Bone mineral acquisition in the first three decades of life determines peak bone mass and predicts subsequent osteoporosis risk. In light of their high-pathogen, energy-limited environment, effects of immune activation on bone formation and resorption during childhood could be a critical early life determinant of Tsimane bone health trajectories. The current study investigates associations between anemia, inflammation, and bone turnover in Tsimane children to explore the relationship between energy expenditure by the skeletal and immune systems during childhood in a high-pathogen environment.

Table 4. 1 Glossary of terms for skeletal plasticity

Glossary	
bone modeling	the process of changing the shape of a skeletal structure either through bone resorption or formation, including the bone formation of linear growth
bone remodeling	the process of renewing existing skeletal structures at the cellular level through sequential resorption and formation, without changing the structural characteristics of the bone. The coupling of formation and resorption tends to weaken during senescence, particularly for post-menopausal women, resulting in age-associated bone loss.
bone turnover	the sum of metabolic activity by bone cells in the skeleton, resulting from the joint actions of formation and resorption and incorporating the metabolic load of both skeletal modeling and remodeling. Whereas modeling and remodeling are structural concepts that refer to the end result of actions by bone cells, bone turnover is a metabolic concept that indicates the total amount of energy spent on the actions of bone cells.

4.1.2 Identifying immune-skeletal tradeoffs using biomarkers of bone turnover

Bone turnover markers in blood are ideal for investigating tradeoffs between the immune and skeletal systems. These molecular byproducts are released into circulation at each stage of bone formation and resorption and reveal skeletal processes operating within hours to

weeks, a time frame similar to many biomarkers of immune activation. By contrast, height-for-age, a widely used metric of investment in growth, measures the outcome of cumulative lifetime growth and thus may be better suited to identifying energetic tradeoffs with chronic conditions.

We use osteocalcin as our indicator of skeletal metabolic activity. Osteocalcin is the second most common protein in bone's extracellular matrix, after collagen (Hauschka, Lian, & Gallop, 1975). It is synthesized by osteoblasts during bone formation but also released into circulation from demineralized extracellular matrix during bone resorption (Ivaska et al., 2004). Total serum osteocalcin is thus a marker of bone turnover. Several studies find that total serum osteocalcin is proportional to osteoblast activity, and consequently it is widely used as a marker of bone formation (Zoch, Clemens, & Riddle, 2016), though the extent to which osteocalcin reflects rates of bone mineralization depends on nutritional status, particularly vitamin K levels, and potentially insulin sensitivity (Ferron et al., 2010; Hauschka, Lian, Cole, & Gundberg, 1989). In addition to its role in building skeletal tissue, some research suggests that the uncarboxylated form of osteocalcin released during bone resorption can function as an endocrine hormone, coordinating energy metabolism across a range of organ systems (Ferron et al., 2010; Karsenty, 2003; Oury et al., 2011; Wei & Karsenty, 2015).

In children, circulating osteocalcin concentrations result from the combined actions of bone modeling—primarily growth—and the on-going remodeling involved in maintaining existing skeletal structures. Low osteocalcin concentrations indicate reduced rates of bone turnover, including both growth and remodeling. High osteocalcin concentrations denote high rates of bone turnover—though this could reflect either bone formation during rapid growth or rapid remodeling of existing bone. Rates of remodeling are affected by hormonal and nutritional status, as well as mechanical strain on the skeleton (Lanyon, 1984). Osteocalcin has been recently validated for use with dried blood spots, making it well-suited to research with remote

populations (Eick, Devlin, Paul, Sugiyama, & Snodgrass, 2019; Eick, Kowal, Devlin, & Sugiyama, 2020).

Among Tsimane children, high levels of C-reactive protein (CRP), a component of the acute-phase inflammatory response of innate immunity, have been found to predict lower gains in height at three-month follow-up but show no association with total height (Garcia, 2018; McDade et al., 2008). Similarly, children in the Shuar population of Ecuador with high CRP have lower gains in height during the following week but not the subsequent three months or twenty months (Urlacher et al., 2018). These results highlight the narrow timeframe in which energetic tradeoffs often operate.

We therefore expect that a possible tradeoff between simultaneous metabolic expenditure by the skeletal and immune systems may manifest among children in the current study as an inverse correlation between CRP and osteocalcin (**P1**). Since osteocalcin effectively measures energy allocated to the actions of bone cells, it should capture skeletal processes that affect acquisition and maintenance of bone mass as well as gains in stature. We also expect that osteocalcin, which can respond to stimuli within hours, will correlate more strongly with CRP than with the generalized inflammation marker erythrocyte sedimentation rate (ESR), which shifts more slowly (**P2**). If skeletal-immune tradeoffs are present, the probability and magnitude of such a tradeoff should be greater when individual energy budgets are smaller. In this case, we expect the inverse relationship between CRP/ESR and osteocalcin to be more noticeable when body energy stores (proxied by weight-for-height) are low (**P3**).

4.1.3 Considering anemia's influence on bone turnover in childhood

In addition to elevated immune activation, anemia is common among Tsimane children (Alami et al., 2020; Blackwell et al., 2016). These are related facts, given that the iron content of the Tsimane diet is more than twice the recommended daily intake to meet nutritional needs (Kraft et al., 2018). Rather than dietary deficiency, childhood anemia is most likely caused by the combined effects of chronic gastrointestinal blood loss from hookworm infection, and chronic inflammation and disrupted nutrient absorption due to innate immune responses in the face of infectious illnesses (Anderson et al., 2019). Anemia has been consistently associated with lower bone mineral density (Korkmaz et al., 2012; Moreau et al., 2012; Wong, Fuller, Gillespie, & Milat, 2016) and stunted growth (Angeles, Schultink, Matulessi, Gross, & Sastroamidjojo, 1993; Ryan, 1997) in other contexts. It merits consideration as a significant influence on Tsimane bone health and may independently contribute to energetic tradeoffs by further constraining individual energy budgets.

Studies of tradeoffs between immune investment and growth have often focused on calories as the critical resource that limits individual energy budgets. However, anemia of inflammation (AI) presents an underexplored pathway through which immune activity may additionally decrease the energy available for growth. AI limits the replication of many bacterial and some viral pathogens by restricting their access to iron. In response to inflammatory cytokines much of the body's iron is removed from circulation and sequestered in the liver, effectively creating functional iron deficiency despite adequate iron stores (Weiss, 2009; Zarychanski & Houston, 2008). Here the reallocation of iron privileges immunity over other physiological demands, and rather than calories the availability of oxygen constrains cellular respiration. Body energy stores (proxied by weight-for-height) are therefore not expected to moderate the effects of anemia.

Iron-deficiency anemia (IDA) on the other hand, like other nutrient deficiencies, results from an externally imposed shortfall rather than an endogenous adaptive response. If the constrained optimum of AI persists for too long, such as in chronic inflammatory conditions, it too becomes IDA (Cappellini et al., 2017). As a manifestation of true physiological deficit rather than a tactic of the immune system, IDA is more likely to be associated with dysfunction in other organ systems and may be more detrimental than AI to bone formation. We predict that iron-deficiency anemia in Tsimane children will be associated with lower osteocalcin concentrations (**P4**), in keeping with the results of other observational and experimental studies and consistent with a relationship between anemia and slower growth velocity (Katsumata, Tsuboi, Uehara, & Suzuki, 2006; S. Katsumata et al., 2009; Parelman, Stoecker, Baker, & Medeiros, 2006).

Since anemia of any cause reduces an individual's energy budget, anemia of inflammation is also expected to be accompanied by lower osteocalcin (**P5**), but since AI is a coordinated part of the innate immune response its effects on bone turnover may be bounded in ways that IDA's effects are not. On the other hand, anemia aside, chronic inflammation is known to decrease bone density by encouraging bone resorption and suppressing bone formation (De Benedetti, 2009; Hardy & Cooper, 2009). The Tsimane immune profile is characterized by elevated inflammation at all ages, and this immune activation may act in concert with the functional iron deficiency of AI to magnify reductions in bone formation (Blackwell et al., 2016; Gurven et al., 2016).

4.2 Materials and Methods

4.2.1 Study population

The Tsimane are a population of ~17,000 Amerindian forager-horticulturalists who live in dispersed villages in the basin of the Maniqui River, a tributary of the Amazon (Gurven et al., 2017). In general, Tsimane villages have little to no public health infrastructure, no running water, and limited access to modern medicine. The bulk of their diet is comprised of several starchy horticultural staples and wild fish and game. Infectious disease burden is high (M. Gurven et al., 2007) and chronic parasitic infection is common (Blackwell et al., 2011). The most commonly diagnosed childhood afflictions are respiratory infections (33%), gastrointestinal diseases (31%), and anemia (39%) (Alami et al., 2020; DeLouize et al., 2022).

By clinical standards based on reference samples from industrialized populations, Tsimane experience elevated levels of inflammation. 94% of Tsimane under age 18 have CRP levels above the median (0.4 mg/L) for an age-matched US-representative sample from the National Health and Nutrition Examination Survey (NHANES). Median ESR for Tsimane individuals under age 18 is ~30 mm/h (Blackwell et al., 2016); the threshold for elevated ESR in US populations is 15-20 mm/h (Wetteland, Roger, Solberg, & Iversen, 1996). White blood cell counts are also elevated compared to US references, with the most pronounced difference in eosinophils, which are involved in parasite defense. 89% of Tsimane eosinophil counts are above the NHANES 95th percentile.

While food insecurity is not uncommon among Tsimane (Stieglitz et al., 2014) and obesity is rare, most adults meet their caloric needs on most days (Kraft et al., 2018). In children, wasting is uncommon but stunting is widespread (Aaron D. Blackwell et al., 2017). Together these results suggest that macronutrient needs are largely met, but micronutrient requirements may not be. Indeed, daily per capita intake of calcium and vitamin K, both of which are critical

for bone formation, is low among Tsimane compared to recommended intakes based on US dietary references (Kraft et al 2018)—Tsimane dietary calcium is roughly a quarter and vitamin K less than a tenth of recommended daily intake. A broad range of systemic infections may additionally compromise nutrient uptake (Scrimshaw N. S., 1992).

Nutrition estimates are calculated using dietary recall data from adults, but childhood diets and complementary foods given to infants are not qualitatively different from adult diets. Most meals consist of a starch-based stew, containing either rice, plantains or less frequently store-bought pasta, mixed with meat or fish. Mothers do not prepare any special foods for infants, but often pre-masticate solids considered too tough or dry for infant consumption (Martin et al., 2016).

Tsimane prevalence of hookworm infection is ~50% (Blackwell et al. , 2013; Blackwell et al., 2011; Martin et al., 2013). Infection risk begins when children start walking, and prevalence rises across childhood as a function of cumulative exposure risk (Blackwell et al., 2011; Martin et al., 2013).

4.2.2 Data Collection

Blood samples and anthropometrics were collected between 2010 and 2014 during medical exams in individuals' village of residence. The THLHP mobile medical team visited each Tsimane village on an approximately annual basis and administered appropriate medications following on-site evaluation of individual exam results. Routine physical exams included collection of medical history and vital signs, assessment of current symptoms, and anthropometrics. Hemoglobin, hematocrit, and five-part blood count were measured on-site using a QBC hematometer. Individuals wore light clothing and no shoes during measurements

of weight with a Tanita BF-572 scale and standing height using a portable Seca 213 stadiometer (Blackwell et al., 2017).

Blood spot collection followed standard procedures for collecting whole capillary blood on filter paper (McDade, Williams, & Snodgrass, 2007), and samples were stored in the vapor phase of a liquid nitrogen tank for up to two months before being transported on dry ice to -80°C freezers at the UCSB Biobehavioral Health Laboratory until being transported on dry ice to Arizona State University for analysis (Blackwell et al., 2010).

4.2.3 Biomarkers of bone turnover, anemia, and inflammation

Osteocalcin, transferrin receptor, and C-Reactive protein were determined by enzyme-linked immunosorbent assay (ELISA). Each of the three assays required a 3.2-mm diameter punch of whole blood to provide sufficient signal and volume to run each sample in duplicate, for a total of three punches per blood spot card. Reported values are analyte concentrations calculated assuming a serum volume of 1.525uL in a 3.2-mm punch (Mei, Alexander, Adam, & Hannon, 2001). Samples with within-plate coefficients of variation (CVs) >15% were re-run where the remaining volume of sample allowed or were excluded from analyses.

Osteocalcin

Total intact serum osteocalcin was measured from dried blood spots using Abcam's Osteocalcin Human SimpleStep ELISA kit, following the protocol detailed by Eick and colleagues (Eick et al., 2020). A single 3.2-mm diameter punch was eluted in 100µL of kit-provided sample diluent overnight at 4 °C. The following morning 60µL of each elution was diluted with a further 60µL of sample diluent. 120uL of this 1:131.1 dilution provided sufficient signal and volume to run each sample in duplicate. The within and between assay CVs for

osteocalcin (n = 10 plates) were 7.4% and 13.5% for the high (209 pg/mL) and 3.1% and 5.7% for the low (103.5 pg/mL) controls, respectively.

Serum transferrin receptor

sTfR was measured using the Ramco human transferrin receptor ELISA kit (TF-94, Ramco Laboratories, Stafford, TX) following the DBS protocol by McDade and Duncan (Thomas W McDade & Shell-Duncan, 2002) but using the liquid sTfR standards and controls included with the kit. A 3.2-mm diameter punch from each sample spot was eluted overnight at 4°C in 250µL of PBS (pH 7.2) plus 0.05% Tween-20 to produce a 1:164 dilution. CVs for within and between assay CVs (n = 10 plates) were 4.6% and 16.3% for the normal (51.0 ng/mL) and 3.9% and 12.4% for the high (127.8 ng/mL) controls).

C-Reactive Protein

C-reactive protein (CRP) was assayed using an in-house ELISA protocol for dried blood spots based on the protocol by Brindle and colleagues (Brindle, Fujita, Shofer, & O'Connor, 2010). Dried blood spot CRP standards were created by combining predetermined quantities of a purified CRP stock solution with washed erythrocytes and pipetting the solution onto filter cards. For standards a single 3.2-mm punch was eluted in 2mL of PBS (0.01M phosphate buffer, 0.5M NaCl, 0.1% Tween 20, pH 7.2, ±0.3) plus 0.1% Tween-20 overnight at 4 °C. For samples and controls, a 3.2-mm punch was eluted in 100uL of PBS overnight at 4 °C. The following day 62.5µL of each eluted sample and control were diluted with 187.5µL of assay buffer to produce 250µL of a 1:1,312 dilution. The within and between assay CVs (n = 9 plates) were 3.8% and 14.6% respectively for the control run at a 1:328 dilution and 1.9% and 16.1% for the control run at 1:656 dilution.

Anemia was defined using World Health Organization age-specific hemoglobin thresholds of <11 g/dL for children under five and < 11.5 g/dL for older children (here up

to/including 8 years old) (WHO, 2011). We identified iron deficiency using serum transferrin receptor (sTfR), an indicator of iron status unaffected by the presence of inflammation (Beguin, Clemons, Pootrakul, & Fillet, 1993). Following the cutoff for iron deficiency in dried blood spots established by Wander and colleagues, samples were considered iron deficient if sTfR values exceeded 5 mg/L (Wander, Shell-duncan, & McDade, 2009). Given that most Tsimane present with clinically elevated markers of inflammation, it is difficult to identify cases in which inflammation may be the primary cause of anemia, and it is likely that chronic inflammation is a contributor to most anemia cases. Taking a conservative approach, we considered anemia of inflammation to be present if low hemoglobin and adequate iron stores were accompanied by ESR values in the fourth quartile of the sample range.

4.2.4 Statistical Analysis

Continuous biomarkers were log-transformed for normality where appropriate and z-scored prior to analysis. Weight-for-height and height-for-age z scores were calculated from population-specific growth references using the localgrowth package in R (*sensu* Blackwell et al., 2017; localgrowth R package: https://github.com/adblackwell/localgrowth). For analyses of body energy stores as a moderator of the relationship between inflammation and osteocalcin, weight-for-height was classified as low ($z < -1$), medium, or high ($z > 1$).

Relationships among variables were assessed using general additive models with thin plate splines for continuous variables using the mgcv package (version 1.8-40; Wood, 2011). We initially ran models with a random effect for individual identity to control for repeat observations on 41 children, suppressing calculation of random effect for single observation cases. Individual identity accounted for minimal variation in osteocalcin-for-age and final models

were run without a random effect term. Age-adjusted osteocalcin was calculated by standardizing the residuals from a univariate gam of log-transformed osteocalcin values. Standardized osteocalcin-for-age then served as the dependent variable for all subsequent models. We did not include sex as a covariate, as it showed no relationship to osteocalcin concentrations in prepubertal children (Fig. S3). If effective degrees of freedom for smoothed estimates of continuous covariates were close to 1, indicating a linear effect, we report results from equivalent generalized linear models. All analyses were conducted using R version 4.1.2.

4.2.5 Ethics approval

Informed consent was collected at multiple levels. The participant, the community, and the Tsimane governing body (Tsimane Gran Consejo) gave independent consent, and the procedures were approved by the institutional review boards at the University of California, Santa Barbara (protocol # 28-21-0788), and University of New Mexico (study #'s 07-157, and 17-262).

4.3 Results

4.3.1 Sample Descriptives

The study sample (Table 4.2) comprises 362 observations on 321 Tsimane children (49.4% female), aged 4 months to 8 years (mean = 4.6 years, sd = 2.1). Of the 260 children with measures of height, 51.5% met WHO standards for stunting (height-for-age < 2 standard deviations below mean values in WHO growth reference data), while only 3.7% with concurrent measures of weight met WHO criteria for wasting (weight-for-height z-score > 2). All individuals had ESR >10 mm/hr, the clinical threshold for elevated inflammation, though only 6% had CRP levels suggestive of active infection (> 5 mg/L, following McDade et al. (2005) for

dried blood spots). Osteocalcin concentrations do not vary by sex (Figure S4.3). Osteocalcin concentrations are highest in infants, and age is negatively correlated with osteocalcin until approximately 2.5 years, after which mean osteocalcin is stable across age (Figure 4.1).

Table 4. 2 Descriptive characteristics of sample

	Median	***(5th, 95th) percentile***	***n (obs)***	***n (people)***	***dates***
age (years)	4.5	(0.9, 8.3)	363	322	2010 - 2014
osteocalcin (ng/mL)	20.5	(8.8, 49.5)	362	321	2010 - 2014
serum transferrin receptor (mg/L)	3.4	(1.9, 5.2)	363	322	2010 - 2014
hemoglobin (g/dL)	11.2	(8.9, 12.9)	363	322	2010 - 2014
hematocrit (%)	35.1	(28.1, 40.2)	361	320	2010 - 2014
C-reactive protein (mg/L)	4.8	(2.4, 5.7)	307	292	2010 - 2014
ESR (mm/h)	35	(20, 55)	245	230	2010 - 2014
total leukocytes (cells/uL)	12000	(7000, 17255)	359	319	2010 - 2014
neutrophils (cells/uL)	4890	(1392.1, 9761.2)	300	270	2010 - 2014
eosinophils (cells/uL)	1563.2	(373.2, 4131.6)	300	270	2010 - 2014
lymphocytes (cells/uL)	4760	(2455, 7752)	285	255	2010 - 2014
height (cm)	97.2	(74, 120.8)	260	235	2011 - 2014
weight (kg)	15.2	(9, 24.2)	257	233	2011 - 2014
variable	***period prevalence***		***n (obs)***	***n (people)***	***Dates (period years)***
hookworm spp. (yes/no)	17.20%		325	290	2010 - 2014
A. lumbricoides (yes/no)	1.50%		325	290	2010 - 2014
G. lamblia (yes/no)	6.80%		325	290	2010 - 2014

Anemia prevalence among study participants was 47.4%, though most of these cases (86%) are mild and cannot be clearly attributed to a single cause. Among the 362 observations of children in the current study, only 6.6% had moderate to severe anemia, and only 7.2% had IDA. 15.6% (n = 26) of anemia cases were classified as iron-deficiency anemia (IDA). A further 17% (n = 27) of anemia cases met our criteria for anemia of inflammation (AI), with ESR in the

fourth quartile and no indication of iron deficiency, though the most severe anemia cases were IDA accompanied by high ESR. Our conservative approach to classifying anemia leaves most anemia cases unclassified, but many are likely to be caused by the combined effects of marginal iron deficiency and universally elevated inflammation. No anemia cases presented with a mean corpuscular hemoglobin concentration above 35 g/dL, making B-vitamin deficiency an unlikely cause of anemia for individuals in this analysis.

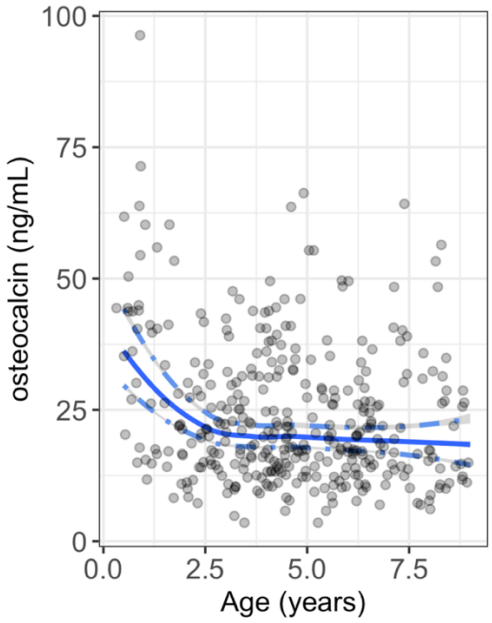

Figure 4. 1 Predicted mean osteocalcin for age from a univariate gam (blue line). Dashed lines are 95% confidence intervals for predicted mean. Points are raw values.

4.3.2 Inflammation does not predict osteocalcin (P1, P2, P3).

Contrary to our expectation that CRP (**P1**)—and to a lesser extent, ESR (**P2**)— would be negatively associated with osteocalcin, we found that neither CRP (model 1: p = 0.44) nor ESR (model 2: p = 0.11) was significantly associated with osteocalcin-for-age (Table 4.3; Figure 2). High ESR had a weak negative association with hemoglobin (Figure S4.6), so we controlled for hemoglobin to remove mediating effects of anemia on the relationship between ESR and

osteocalcin. Furthermore, body energy stores neither moderate the relationship between inflammation and bone turnover (P3): weight-for-height did not alter the association between osteocalcin and CRP (model 3: p = 0.26) or osteocalcin and ESR (model 4: p = 0.99).

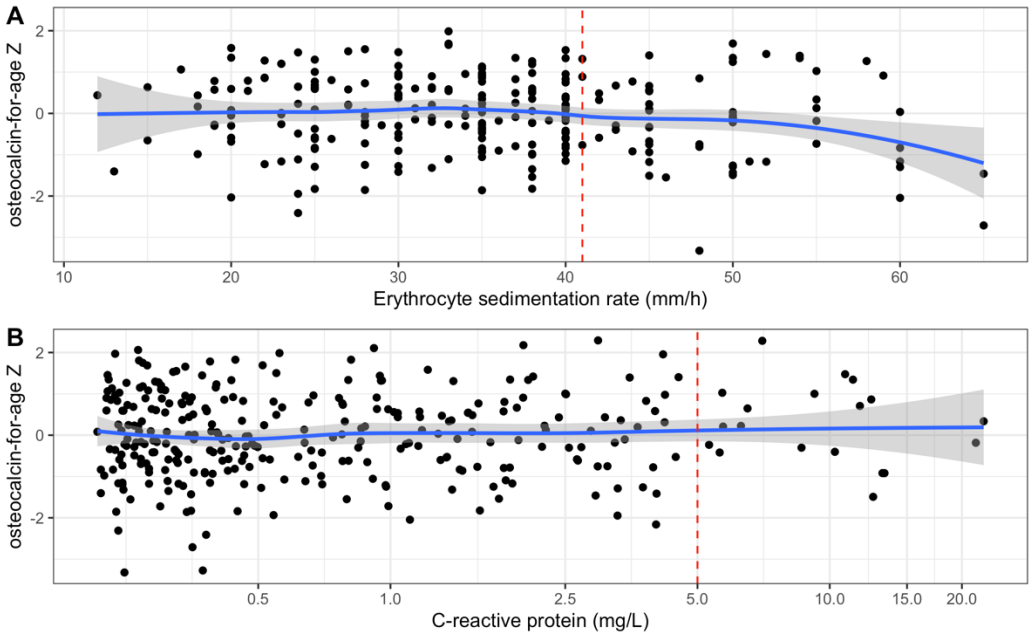

Figure 4. 2 Scatter plots with Loess smooths overlaid showing the relationships between osteocalcin-for-age and inflammatory markers CRP and ESR. A) red dashed line indicates fourth quartile ESR threshold used for classifying anemia of inflammation. B) red dashed line indicates CRP threshold high enough to suggest active infection.

4.3.3. Moderate to severe anemia is associated with lower osteocalcin (**P4**).

Compared to non-anemic individuals, those with moderate to severe anemia have lower osteocalcin-for-age (model 5: β = -0.57; SE = 0.215; p = 0.0085) (Figure 4.3A; Table 4.3). Moderate to severe anemia was also marginally associated with lower height-for-age (Figure S8). Mild anemia was not significantly associated with either osteocalcin or height-for-age. 58.3% of moderate-to-severe anemia was IDA, compared to only 8.2% of mild anemia. Perhaps unsurprisingly then, IDA was associated with lower osteocalcin-for-age compared to non-anemic individuals (model 6: β = -0.44; SE = 0.209; p = 0.036), but AI was not associated with osteocalcin-for-age (p = 0.58) (Figure 4.4).

Table 4. 3 Standardized effects from models of age-adjusted osteocalcin.Outcome variable for all models is standardized log-osteocalcin-for-age.

	Question	**n**	**predictor**	**beta**	**SE**	**p-value**
model **1**	Is acute inflammation (CRP) associated with differences in osteocalcin-for-age?	306	(Intercept)	0.012	0.059	0.836
			log-CRP	0.045	0.059	0.442
model **2**	is chronic inflammation (ESR) associated with differences in osteocalcin-for-age (after controlling for hemoglobin)?	245	(Intercept)	-0.410	0.537	0.446
			ESR	-0.101	0.063	0.112
			hemoglobin	0.036	0.048	0.463
model **3**	Do body energy stores (weight-for-height) moderate the relationship between CRP and osteocalcin-for-age?	212	(Intercept)	-0.045	0.173	0.797
			log-CRP	0.208	0.202	0.304
			WFH medium	-0.122	0.192	0.524
			WFH low	0.259	0.280	0.356
			log-CRP:WFH medium	-0.128	0.220	0.560
			log-CRP:WFH low	-0.310	0.276	0.263
model **4**	Do body energy stores (weight-for-height) moderate the relationship between ESR and osteocalcin-for-age?	166	(Intercept)	0.464	0.601	0.442
			WFH medium	-0.467	0.673	0.488
			WFH low	0.490	1.043	0.639
			ESR	-0.018	0.017	0.270
			WFH medium:ESR	0.016	0.018	0.390
			WFH low:ESR	0.000	0.029	0.987
model **5**	Is anemia associated with lower osteocalcin-for-age?	362	(Intercept)	0.054	0.072	0.452
			mild anemia	-0.040	0.109	0.712
			moderate-severe anemia	**-0.569**	**0.215**	**0.009**
model **6**	Does the association between osteocalcin-for-age and anemia differ for AI and IDA?	362	(Intercept)	0.054	0.072	0.455
			anemia: IDA	**-0.439**	**0.209**	**0.036**
			anemia: AI	-0.109	0.199	0.583
			anemia: unclassified	-0.043	0.117	0.714

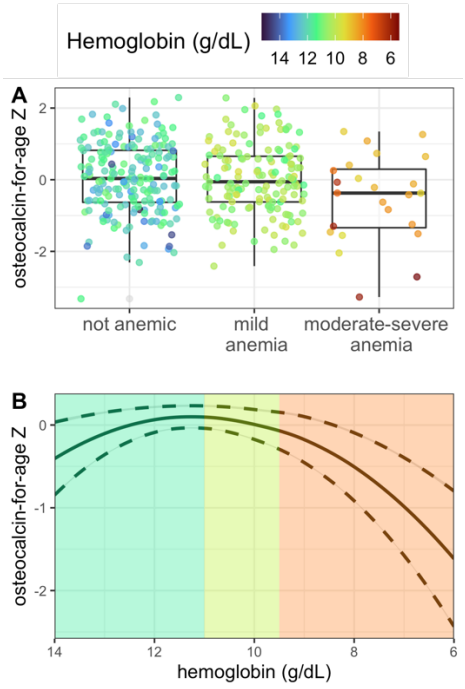

Figure 4. 3 Relationship between anemia severity and standardized age-adjusted osteocalcin(OCN). A) Points show individual osteocalcin-for-age z-scores, with colors mapped to hemoglobin concentration. B) Univariate gam-predicted mean (solid line) and 95% confidence interval (dashed lines) of standardized age-adjusted osteocalcin across varying hemoglobin concentrations.

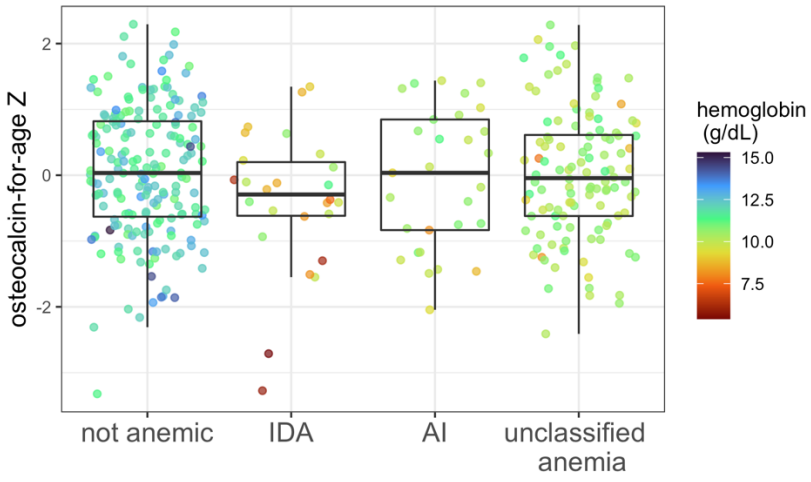

Figure 4. 4 Comparison of osteocalcin-for-age across categories of anemia. Iron-deficiency anemia (IDA), but not anemia of inflammation (AI), is associated with lower osteocalcin-for-age compared to non-anemic individuals. The dashed line extends the median value for IDA osteocalcin-for-age values to facilitate visual comparison with central tendencies of osteocalcin in other anemia categories.

4.4 Discussion

The current study investigated the impacts of anemia and immune activation on skeletal metabolism in children from the Tsimane of lowland Bolivia, a tropical subsistence population. We find no evidence of an energetic tradeoff between energy expenditure on innate immune activity and skeletal metabolic activity (bone turnover) in Tsimane children. Contrary to our expectations, concentrations of the bone turnover marker osteocalcin are not associated with either the acute inflammatory marker CRP or the level of generalized systemic inflammation indicated by ESR, even among children with low body energy stores, where evidence of such a tradeoff is expected to be most apparent. We also find no evidence that anemia of inflammation, a feature of sustained innate immune activity, depresses skeletal metabolism by prioritizing iron's role in immune defenses over its role in other physiological functions. However, moderate to severe anemia, which tended to be accompanied by iron deficiency rather than driven solely by inflammation, was associated with lower osteocalcin in prepubertal Tsimane children.

4.4.1 Inflammation and bone turnover in different ecological contexts

Environmental exposures shape individual investments in immune function and population-level variation in immune profiles (French, Moore, & Demas, 2009). Many aspects of skeletal morphology and physiology are highly heritable (Lovejoy, McCollum, Reno, & Rosenman, 2003), but there is great potential for disease ecology to shape skeletal morphology or skeletal integration with other physiological systems. The skeletal and immune systems are intimately connected—all immune cells are produced within the bone marrow—but bone-immune interactions are a largely unexplored facet of variation in human immune function across diverse ecological contexts. Results of the current study provide a starting point for

ecological osteoimmunology, a crucial lens for understanding the range of biological normalcy in these integrated physiological systems.

The relationship between inflammation and bone turnover in Tsimane children differs from patterns observed in other populations. Whereas we find no support for any association between concentrations of CRP or ESR and osteocalcin in Tsimane children, studies of industrialized populations consistently report an inverse association between bone formation and inflammation, independent of body energy stores; findings in non-industrialized settings are more varied. Among Gambian children experiencing seasonal food insecurity (Munday, Ginty, Fulford, & Bates, 2006), higher levels of the inflammatory markers alpha1- antichymotrypsin and sialic acid—but not CRP—were significantly associated with lower levels of the bone formation markers procollagen type I N propeptide (P1NP) and serum C-terminal telopeptide of type 1 collagen (S-CTX). The authors interpret as evidence of skeletal growth faltering in response to infection, compatible with a skeletal-immune tradeoff in energetic investment. However, Shuar children in Ecuador (aged 2-15 years; n = 67) exhibit a positive association between CRP and osteocalcin (Madimenos et al., 2020).

Cross-sectional studies of industrialized populations report an inverse relationship between osteocalcin and CRP that is moderated by body energy reserves (Fatahi et al., 2019), but these results cannot be interpreted as evidence of an energetic tradeoff between the skeletal and immune systems. These studies generally focus on a context of high body energy reserves (weight, body mass index (BMI), or adiposity) and report the greatest negative association between osteocalcin and inflammation in the presence of obesity or metabolic syndrome (Albadah et al., 2015; Giudici, Fisberg, Marchioni, Peters, & Martini, 2017; Oh, Lee, Nam, Rhie, & Lee, 2019; Reinehr & Roth, 2010; Wang, Tang, Ruan, & Cai, 2014). Among US adults, a pathway analysis of body fat, inflammation, and bone mineral density suggests that central

adiposity leads to decreased BMD by driving both sedentary behavior and systemic inflammation (CRP) (Griffin, Dent, & Berger, 2021), a scenario distinct from inflammatory immune activation in high-pathogen, energy-limited contexts.

Skeletal metabolism in Tsimane children may be fittingly calibrated for maintaining skeletal tissue in a high-pathogen environment. Given that both chronic and acute inflammation have been linked to decreased bone formation and increased resorption via direct and indirect pathways (Griffin et al., 2021; Hardy & Cooper, 2009), we expected that inflammation would play a prominent role in driving patterns of skeletal metabolism in a population whose immune profile is characterized by elevated inflammatory markers. Instead, we find that CRP and ESR are unrelated to osteocalcin concentrations. In the face of an immune system attuned to handle the persistent and manifold attacks present in a tropical environment, the structural integrity of skeletal tissues may be best maintained by dampening bone's responsivity to inflammatory signaling.

The sampling design for the current study intentionally avoided the influence of the adolescent growth spurt in order to highlight the roles of infection and nutrient deficiency on skeletal metabolism, but tradeoffs between competing physiological demands for energy may be most likely to manifest in adolescence when energetic demands of immune function are negotiated in a context of simultaneous rapid growth and sexual maturation. Moreover, the extent to which maintenance of skeletal tissues might be expected to trade off against immediate energy requirements of immune activation is likely to vary across the life course.

4.4.2 Anemia

We found that anemia in the presence of iron deficiency, but not in the presence of elevated inflammation without iron deficiency, was associated with lower osteocalcin-for-age.

Given that lower osteocalcin concentrations are a consistent finding in inherited and acquired anemias with varied causes and also in chronic inflammatory conditions, the absence of an association between osteocalcin and anemia of inflammation is somewhat surprising (Abd El-Moneim, Zolaly, Al-Hawsawi, Abdelmoneim, & Abosdera, 2018; Hardy & Cooper, 2009; Katsumata, Tsuboi, Uehara, & Suzuki, 2006; Katsumata, Katsumata-Tsuboi, Uehara, & Suzuki, 2009; Moreau et al., 2012; Tsay et al., 2010). It is, however, in alignment with the expectation that disruptions to bone formation from micronutrient shortfalls such as iron deficiency are likely to be greater than any effects that fall within the limits of immune-mediated iron sequestration.

It is unclear whether osteocalcin is affected by iron deficiency itself or by the degree of anemia. Cases of IDA identified in this sample tended to be moderate to severe anemia, while AI cases are all mild to moderate. This pattern is consistent with the hypothesis that the functional iron deficiency of AI is an adaptive feature of a plastic immune system while the true iron deficiency of IDA an externally imposed deficit with greater potential for pathological outcomes, but it also precludes a comparison of the effects of iron deficiency and inflammation on bone turnover independent of anemia severity.

4.4.3 Is osteocalcin primarily measuring bone formation in Tsimane children?

In cross-sectional studies osteocalcin concentrations across childhood commonly align with the growth velocity curve, a pattern also seen in the current study (Blackwell et al., 2017; Johansen et al., 1988; Kanbur, Derman, Şen, & Kinik, 2002; Low & Lau, 1992). Considering prior findings that high CRP in Tsimane children is inversely associated with linear growth velocity (McDade et al., 2008), we expected osteocalcin to reveal costs of immune activation to bone formation including and extending beyond linear growth, but osteocalcin was not

associated with same-day CRP. This may reflect the fact that measures of bone turnover, the energy spent *by* bone cells, do not necessarily translate into energy spent *on* bone. Bone turnover markers facilitate investigations of the skeleton's role as an integrated physiological system, beyond the formation and maintenance of skeletal structures.

The current study measured total serum osteocalcin, but much of the circulating osteocalcin in Tsimane children may be in its uncarboxylated form, which does not play a role in bone formation but appears to mediate energy uptake by skeletal muscle and regulate insulin sensitivity, among other things (Ferron & Karsenty, 2020). There is growing evidence of uncarboxylated osteocalcin's involvement in many physiological processes beyond building bone, and high osteocalcin in childhood is sometimes associated with diminished bone mineral accrual (Slemenda, Peacock, Hui, Zhou, & Johnston, 1997). In addition to active processes of energy metabolism, nutritional constraints on bone mineralization may be relevant here; the Tsimane diet is low in several micronutrients critical for bone formation, including vitamin K. Estimates of vitamin K consumption based on dietary recall fall short of recommended daily intake by more than an order of magnitude (Kraft et al., 2018). Osteocalcin requires vitamin K to transform into its carboxylated state, in which it contributes to building mineralized skeletal tissue (Cole, Carpenter, & Gundberg, 1985).

4.4.4 Comparison with the Shuar of Ecuador

Tsimane bone mineral density (BMD) is low when contextualized against other populations. Tsimane men have BMD values comparable to age-matched US cohorts and Tsimane women have lower BMD even in their twenties, despite markedly higher levels of daily physical activity (Stieglitz, Beheim, et al., 2015; Stieglitz, Madimenos, Kaplan, & Gurven, 2016; Stieglitz et al., 2019). High calcium demands of extended breastfeeding across multiple births

may explain lower bone mass in Tsimane women (Stieglitz, Beheim, et al., 2015). More striking is the recent observation using comparable methods that Tsimane estimated BMD using calcaneal quantitative ultrasound is lower than that of the Shuar population of Ecuador, despite living a similar subsistence lifestyle in the neotropics (Madimenos et al., 2020; Stieglitz et al., 2019). While BMD has not been reported for Tsimane children, differences between Tsimane and Shuar bone density are apparent in adolescence, pointing to childhood as a critical period for shaping lifelong trajectories of skeletal health.

Madimenos and coauthors suggested that lower BMD among Tsimane compared to Shuar is consistent with patterns of energy allocation predicted by life history theory: in the higher-pathogen Tsimane environment greater investment in immune function might trade off against not only linear growth but also investment in bone mineral acquisition (2020). While we found no association between osteocalcin and CRP or ESR, other skeletal-immune interactions might contribute to population-level differences in BMD. Key ways in which disease burden in Tsimane children differs from Shuar childhood disease experience include higher prevalence of helminth infection—most notably hookworm, which is absent from Shuar territory—and higher levels of multiple markers of inflammation at all ages (Blackwell et al., 2016; Blackwell et al., 2011; Madimenos et al., 2020). Levels of circulating Immunoglobulin-E (IgE), an antibody involved in immune defense against helminths, are low at birth and peak in childhood or adolescence. Tsimane IgE levels are higher than Shuar levels and peak earlier in childhood, suggesting a greater and more rapid investment in helminth defense in the Tsimane environment (Blackwell et al., 2011). Among Shuar children, IgE is positively associated with the bone resorption marker TRAcP-5b, and helminth-driven bone resorption remains an unexplored pathway for skeletal-immune tradeoffs in the Tsimane context.

4.4.5 Study limitations

Skeletal phenotype and bone health outcomes are difficult to predict using a single marker of bone turnover; ultimately the net balance between bone formation and resorption may be more informative about bone health trajectories. Pathological conditions associated with both high rates of bone turnover (e.g., parathyroidism, Paget's disease) and low rates of bone turnover (e.g., irritable bowel disease) both lead to diminished bone mass (Guo, Thomas, al-Dehaimi, Assiri, & Eastell, 1996; Pappa et al., 2011). Osteocalcin concentrations are therefore best interpreted alongside additional markers of bone formation and resorption and within the physiological context of different life stages.

Because trajectories of bone loss and acquisition result from the balance between bone formation and resorption, we had initially planned to interpret osteocalcin concentrations together with the bone resorption marker tartrate-resistant acid phosphatase 5b (TRAcP-5b). TRAcP-5b concentrations are at their lifetime peak in infancy and early childhood and are known to be increased by inflammatory cytokines (Chen et al., 2005; De Benedetti, 2009; Epsley et al., 2021; Rauchenzauner et al., 2007). We therefore anticipated that in Tsimane children skeletal metabolic activity would be characterized by high circulating concentrations of this osteoclast-produced enzyme. However, the preliminary test plate for TRAcP-5b instead revealed that most samples fell below the assay's threshold of detection, making quantification of this biomarker unfeasible for the current study. TRAcP-5b is sensitive to repeat freezing and thawing of serum samples, but its sensitivity to freeze-thaw cycles in dried blood spots has not been established, precluding any conclusions about Tsimane TRAcP-5b levels.

We were also unable to control for several factors known to influence serum osteocalcin concentrations. Most notably, osteocalcin has a circadian rhythm with a nocturnal peak, and osteocalcin also increases markedly in response to physical activity (Chahla et al., 2015; Diemar

et al., 2022; Heshmati et al., 1998). Blood draws were typically obtained within a window of several hours in the morning, but precise time of blood draw and same-day physical activity prior to sample collection are unknown. Additionally, the saltatory nature of growth itself is a source of noise; some individuals in the sample may be at quiescent moments of the growth process.

Finally, the available tools for measuring aspects of anemia are likely to introduce some noise into anemia classifications. On the one hand, clinical anemia thresholds based on age may slightly over-diagnose the prevalence of mild anemia in this population. Hemoglobin is positively correlated with fat-free body mass, and Tsimane children tend to be small for their age compared to WHO growth references. Their hemoglobin levels may in fact meet oxygen requirements for smaller body size despite falling slightly below established clinical thresholds for anemia. On the other hand, the sTfR cutoff of 5 mg/L likely underdiagnoses iron deficiency in this group. Visual examination of the correlation between hemoglobin and sTfR (Figure S4.9) suggests that the sTfR cutoff used to identify iron deficiency is conservatively high. If iron-deficiency is the initial cause of anemia, iron-deficiency precedes decreases in hemoglobin. Suggested cutoffs for sTfR vary, and in the absence of an additional iron marker to examine alternative cutoffs we can determine the level of iron deficiency at which anemia starts to occur but are unable to identify non-anemic cases of iron deficiency (Hadley & Decaro, 2015).

4.5 Conclusion

Though mild anemia is common among Tsimane children, iron-deficiency anemia appears to be relatively rare, and anemia is associated with reduced skeletal metabolism only in moderate to severe cases. Though inflammation and osteocalcin appear unrelated among Tsimane children, the potential for tradeoffs between the immune and skeletal systems changes

across a lifetime just as the development and maintenance of both these systems is a dynamic lifelong process. Among Tsimane adults aged 50+ white blood cell counts are inversely associated with calcaneal stiffness, a pattern consistent with the presence of skeletal-immune tradeoffs during a life stage in which the costs of skeletal fragility tend to manifest (Stieglitz et al., 2016). To disambiguate potential tradeoffs of immune investment with both linear growth and bone mineral acquisition, future studies could combine baseline measures of multiple immune activation and bone turnover markers with follow-up measures of height gain and calcaneal sonometry measures of bone mineral density at multiple timescales.

The skeleton is revealing itself to be an active participant in whole organism biology. It has long been known that the actions of bone cells are influenced by nutrition, infection, sex hormones, and physical activity, but understanding the ways in which these forces act in concert to shape skeletal structures across the life course requires attention to the skeleton as a dynamic system in populations with diverse life histories and disease ecologies. The population-level variation in human immune function uncovered by ecological immunology implies unexplored variation in how the skeleton interfaces with the immune system and how these interactions affect skeletal structures and disease susceptibility.

4.6 References

Abd El-Moneim, E. S., Zolaly, M. A., Al-Hawsawi, Z. M., Abdelmoneim, A. A., & Abosdera, M. M. (2018). Age-related changes in biochemical bone profile in thalassemic children. *Pediatrics and Neonatology*, *59*(2), 189–197. https://doi.org/10.1016/j.pedneo.2016.08.012

Alami, S., von Rueden, C., Seabright, E., Kraft, T. S., Blackwell, A. D., Stieglitz, J., … Gurven, M. (2020). Mother's social status is associated with child health in a horticulturalist population. *Proceedings of the Royal Society B: Biological Sciences*, *287*(1922).

https://doi.org/10.1098/rspb.2019.2783

Albadah, M. S., Dekhil, H., Shaik, S. A., Alsaif, M. A., Shogair, M., Nawaz, S., & Alfadda, A. A. (2015). Effect of weight loss on serum osteocalcin and its association with serum adipokines. *International Journal of Endocrinology*, *2015*, 508–532. https://doi.org/10.1155/2015/508532

Allen, M. R., & Burr, D. B. (2014). *Chapter 4 - Bone Modeling and Remodeling* (D. B. Burr & M. R. B. T.-B. and A. B. B. Allen, Eds.). https://doi.org/https://doi.org/10.1016/B978-0-12-416015-6.00004-6

Anderson, A. S., Trumble, B. C., Hové, C., Kraft, T. S., Kaplan, H., Gurven, M., & Blackwell, A. D. (2019). Old friends and friendly fire: Pregnancy, hookworm infection, and anemia among tropical horticulturalists. *American Journal of Human Biology*, *32*(2), e23337. https://doi.org/10.1002/ajhb.23337

Angeles, I. T., Schultink, W. J., Matulessi, P., Gross, R., & Sastroamidjojo, S. (1993). Decreased rate of stunting among anemic Indonesian preschool children through iron supplementation. *The American Journal of Clinical Nutrition*, *58*(3), 339–342.

Beguin, Y., Clemons, G. K., Pootrakul, P., & Fillet, G. (1993). Quantitative assessment of erythropoiesis and functional classification of anemia based on measurements of serum transferrin receptor and erythropoietin. *Blood*, *81*(4), 1067–1076. https://doi.org/10.1182/blood.v81.4.1067.bloodjournal8141067

Blackwell, A. D., Martin, M., Kaplan, H., & Gurven, M. (2013). Antagonism between two intestinal parasites in humans: the importance of co-infection for infection risk and recovery dynamics. *Proceedings of the Royal Society B: Biological Sciences*, *280*(1769), 20131671–20131671. https://doi.org/10.1098/rspb.2013.1671

Blackwell, Aaron D., Gurven, M. D., Sugiyama, L. S., Madimenos, F. C., Liebert, M. A., Martin,

M. A., … Snodgrass, J. J. (2011). Evidence for a peak shift in a humoral response to helminths: Age profiles of IgE in the shuar of Ecuador, the Tsimane of Bolivia, and the U.S. NHANES. *PLoS Neglected Tropical Diseases*, *5*(6). https://doi.org/10.1371/journal.pntd.0001218

Blackwell, Aaron D., Snodgrass, J. J., Madimenos, F. C., & Sugiyama, L. S. (2010). Life history, immune function, and intestinal helminths: Trade-offs among immunoglobulin E, C-reactive protein, and growth in an Amazonian population. *American Journal of Human Biology*, *22*(6), 836–848. https://doi.org/10.1002/ajhb.21092

Blackwell, Aaron D., Urlacher, S. S., Bret, B., von Rueden, C., Jaeggi, A., Stieglitz, J., … Kaplan, H. (2017). Growth references for Tsimane forager-horticulturalists of the Bolivian Amazon. *American Journal of Physical Anthropology*, *162*(3), 441–461. https://doi.org/10.1002/ajpa.23128.Growth

Blackwell, Aaron D, Trumble, B. C., Suarez, I. M., Stieglitz, J., Beheim, B., Snodgrass, J. J., … Gurven, M. (2016). Immune function in Amazonian horticulturalists. *Annals of Human Biology*, *43*(4), 382–396.

Brindle, E., Fujita, M., Shofer, J., & O'Connor, K. A. (2010). Serum, plasma, and dried blood spot high-sensitivity C-reactive protein enzyme immunoassay for population research. *Journal of Immunological Methods*, *362*(1), 112–120. https://doi.org/https://doi.org/10.1016/j.jim.2010.09.014

Cappellini, M. D., Comin-Colet, J., de Francisco, A., Dignass, A., Doehner, W., Lam, C. S., … Group, on behalf of the I. C. (2017). Iron deficiency across chronic inflammatory conditions: International expert opinion on definition, diagnosis, and management. *American Journal of Hematology*, *92*(10), 1068–1078. https://doi.org/https://doi.org/10.1002/ajh.24820

Chahla, S. E., Frohnert, B. I., Thomas, W., Kelly, A. S., Nathan, B. M., & Polgreen, L. E. (2015). Higher daily physical activity is associated with higher osteocalcin levels in adolescents. *Preventive Medicine Reports*, *2*, 568–571. https://doi.org/https://doi.org/10.1016/j.pmedr.2015.06.017

Chen, C. J., Chao, T. Y., Janckila, A. J., Cheng, S. N., Ku, C. H., & Chu, D. M. (2005). Evaluation of the activity of tartrate-resistant acid phosphatase isoform 5b in normal Chinese children - A novel marker for bone growth. *Journal of Pediatric Endocrinology and Metabolism*, *18*(1), 55–62. https://doi.org/10.1515/JPEM.2005.18.1.55

Chowdhury, S., Schulz, L., Palmisano, B., Singh, P., Berger, J. M., Yadav, V. K., … Karsenty, G. (2020). Muscle-derived interleukin 6 increases exercise capacity by signaling in osteoblasts. *Journal of Clinical Investigation*, *130*(6), 2888–2902. https://doi.org/10.1172/JCI133572

Cole, D. E. C., Carpenter, T. O., & Gundberg, C. M. (1985). Serum osteocalcin concentrations in children with metabolic bone disease. *The Journal of Pediatrics*, *106*(5), 770–776. https://doi.org/10.1016/S0022-3476(85)80351-6

De Benedetti, F. (2009). The Impact of Chronic Inflammation on the Growing Skeleton: Lessons from Interleukin-6 Transgenic Mice. *Hormone Research*, *72*, 26–29. https://doi.org/10.1159/000229760

DeLouize, A. M., Liebert, M. A., Madimenos, F. C., Urlacher, S. S., Schrock, J. M., Cepon-Robins, T. J., … Sugiyama, L. S. (2022). Low prevalence of anemia among Shuar communities of Amazonian Ecuador. *American Journal of Human Biology*, *34*(1), e23590. https://doi.org/10.1002/ajhb.23590

Diemar, S. S., Dahl, S. S., West, A. S., Simonsen, S. A., Iversen, H. K., & Jørgensen, N. R. (2022). A Systematic Review of the Circadian Rhythm of Bone Markers in Blood. *Calcified Tissue International*. https://doi.org/10.1007/s00223-022-00965-1

Eick, G. N., Devlin, M. J., Paul, T. J. C., Sugiyama, L. S., & Snodgrass, J. J. (2019). A dried blood spot-based method to measure levels of tartrate-resistant acid phosphatase 5b (TRACP-5b), a marker of bone resorption. *American Journal of Human Biology*, *31*(3), e23240. https://doi.org/10.1002/ajhb.23240

Eick, G. N., Kowal, P., Devlin, M. J., & Sugiyama, L. S. (2020). *Validation of an enzyme-linked immunoassay assay for osteocalcin , a marker of bone formation , in dried blood spots.* (January), 1–11. https://doi.org/10.1002/ajhb.23394

Epsley, S., Tadros, S., Farid, A., Kargilis, D., Mehta, S., & Rajapakse, C. S. (2021). The Effect of Inflammation on Bone. *Frontiers in Physiology*, *11*(January). https://doi.org/10.3389/fphys.2020.511799

Fatahi, S., Ghaedi, E., Mohammad-Mousavi, S., Bawadi, H., Rahmani, J., Pezeshki, M., & Kord-Varkaneh, H. (2019). The Association between osteocalcin and C-Reactive Protein; A relation of bone with inflammation: A systematic review and meta-analysis. *Hormonal and Metabolic Research*, *51*(6), 353–361.

Ferron, M., & Karsenty, G. (2020). Regulation of energy metabolism by bone-derived hormones. *Principles of Bone Biology*, 1931–1942. https://doi.org/10.1016/b978-0-12-814841-9.00084-1

Ferron, M., Wei, J., Yoshizawa, T., Del Fattore, A., DePinho, R. A., Teti, A., … Karsenty, G. (2010). Insulin Signaling in Osteoblasts Integrates Bone Remodeling and Energy Metabolism. *Cell*, *142*(2), 296–308. https://doi.org/10.1016/j.cell.2010.06.003

French, S. S., Moore, M. C., & Demas, G. E. (2009). *Ecological immunology : The organism in context*. *49*(3), 246–253. https://doi.org/10.1093/icb/icp032

Fulzele, K., Riddle, R. C., DiGirolamo, D. J., Cao, X., Wan, C., Chen, D., … Clemens, T. L. (2010). Insulin Receptor Signaling in Osteoblasts Regulates Postnatal Bone Acquisition and Body Composition. *Cell*, *142*(2), 309–319. https://doi.org/10.1016/j.cell.2010.06.002

Garcia, A. R. (2018). *The Embodiment of Stress : Do cortisol-immune interactions moderate social influences on health ?* University of California Santa Barbara.

Garcia, A. R., Blackwell, A. D., Trumble, B. C., Stieglitz, J., Kaplan, H., & Gurven, M. D. (2020). Evidence for height and immune function tradeoffs among preadolescents in a high pathogen population. *Evolution, Medicine, and Public Health and Public Health*, *2020*(1), 86–99.

Giudici, K. V., Fisberg, R. M., Marchioni, D. M. L., Peters, B. S. E., & Martini, L. A. (2017). Crosstalk Between Bone and Fat Tissue: Associations Between Vitamin D, Osteocalcin, Adipokines, and Markers of Glucose Metabolism Among Adolescents. *Journal of the American College of Nutrition*, *36*(4), 273–280. https://doi.org/10.1080/07315724.2016.1274923

Griffin, J. S., Dent, S. C., & Berger, S. M. (2021). Pathways linking activity, adiposity, and inflammation to bone mineral density in women and men from NHANES 2007 to 2010. *American Journal of Human Biology*, *33*(2), e23583. https://doi.org/10.1002/ajhb.23583

Guo, C. Y., Thomas, W. E., al-Dehaimi, A. W., Assiri, A. M., & Eastell, R. (1996). Longitudinal changes in bone mineral density and bone turnover in postmenopausal women with primary hyperparathyroidism. *The Journal of Clinical Endocrinology & Metabolism*, *81*(10), 3487–3491. https://doi.org/10.1210/jcem.81.10.8855790

Gurven, M. D., Trumble, B. C., Stieglitz, J., Yetish, G., Cummings, D., Blackwell, A. D., … Pontzer, H. (2016). High resting metabolic rate among Amazonian forager- horticulturalists experiencing high pathogen burden. *American Journal of Physical Anthropology*, *161*(3), 414–425. https://doi.org/10.1002/ajpa.23040

Gurven, M., Kaplan, H., & Supa, A. Z. (2007). *Mortality Experience of Tsimane Amerindians of Bolivia : Regional Variation and Temporal Trends*. *398*(September 2006), 376–398. https://doi.org/10.1002/ajhb

Gurven, M., Stieglitz, J., Trumble, B., Blackwell, A. D., Beheim, B., Davis, H., … Kaplan, H. (2017). The Tsimane Health and Life History Project: Integrating anthropology and biomedicine. *Evolutionary Anthropology*, *26*(2), 54–73. https://doi.org/10.1002/evan.21515

Hadley, C., & Decaro, J. A. (2015). *Brief Communication : Does Moderate Iron Deficiency Protect Against Childhood Illness ? A Test of the Optimal Iron Hypothesis in Tanzania*. *679*(April), 675–679. https://doi.org/10.1002/ajpa.22756

Hardy, R., & Cooper, M. S. (2009). Bone loss in inflammatory disorders. *Journal of Endocrinology*, *201*(3), 309–320. https://doi.org/10.1677/JOE-08-0568

Hauschka, P. V, Lian, J. B., Cole, D. E., & Gundberg, C. M. (1989). Osteocalcin and matrix Gla protein: vitamin K-dependent proteins in bone. *Physiological Reviews*, *69*(3), 990–1047. https://doi.org/10.1152/physrev.1989.69.3.990

Hauschka, P. V, Lian, J. B., & Gallop, P. M. (1975). Direct identification of the calcium-binding amino acid, gamma-carboxyglutamate, in mineralized tissue. *Proceedings of the National Academy of Sciences*, *72*(10), 3925–3929. https://doi.org/10.1073/pnas.72.10.3925

Heshmati, H. M., Riggs, B. L., Burritt, M. F., Mcalister, C. A., Wollan, P. C., & Khosla, S. (1998). Effects of the circadian variation in serum cortisol on markers of bone turnover and calcium homeostasis in normal postmenopausal women. *Journal of Clinical Endocrinology and Metabolism*, *83*(3), 751–756. https://doi.org/10.1210/jc.83.3.751

Ivaska, K. K., Hentunen, T. A., Väräniemi, J., Ylipahkala, H., Pettersson, K., & Väänänen, H. K. (2004). Release of Intact and Fragmented Osteocalcin Molecules from Bone Matrix during Bone Resorption in Vitro. *Journal of Biological Chemistry*, *279*(18), 18361–18369. https://doi.org/10.1074/jbc.M314324200

Johansen, J. S., Giwercman, A., Hartwell, D., Nielsen, C. T., Price, P. A., Christiansen, C., & Skakkebaek, N. E. (1988). Serum Bone Gla-Protein as a Marker of Bone Growth in

Children and Adolescents: Correlation With Age, Height, Serum Insulin-Like Growth Factor I, and Serum Testosterone. *The Journal of Clinical Endocrinology & Metabolism*, *67*(2), 273–278. https://doi.org/10.1210/jcem-67-2-273

Kanbur, N. Ö., Derman, O., Şen, T. A., & Kinik, E. (2002). Osteocalcin: A biochemical marker of bone turnover during puberty. *International Journal of Adolescent Medicine and Health*, *14*(3), 235–244. https://doi.org/doi:10.1515/IJAMH.2002.14.3.235

Karsenty, G. (2003). The complexities of skeletal biology. *Nature*, *423*(6937), 316–318. https://doi.org/10.1038/nature01654

Katsumata, S. I., Tsuboi, R., Uehara, M., & Suzuki, K. (2006). Dietary iron deficiency decreases serum osteocalcin concentration and bone mineral density in rats. *Bioscience, Biotechnology and Biochemistry*, *70*(10), 2547–2550. https://doi.org/10.1271/bbb.60221

Katsumata, S., Katsumata-Tsuboi, R., Uehara, M., & Suzuki, K. (2009). Severe Iron Deficiency Decreases Both Bone Formation and Bone Resorption in Rats. *The Journal of Nutrition*, *139*(2), 238–243. https://doi.org/10.3945/jn.108.093757

Korkmaz, U., Korkmaz, N., Yazici, S., Erkan, M., Baki, A. E., Yazici, M., … Ataoğlu, S. (2012). Anemia as a risk factor for low bone mineral density in postmenopausal Turkish women. *European Journal of Internal Medicine*, *23*(2), 154–158. https://doi.org/10.1016/j.ejim.2011.11.009

Kraft, T. S., Stieglitz, J., Trumble, B. C., Martin, M., Kaplan, H., & Gurven, M. (2018). Nutrition transition in two lowland Bolivian subsistence populations. *The American Journal of Clinical Nutrition*, *108*(6), 1183–1195. https://doi.org/10.1093/ajcn/nqy250

Lanyon, L. E. (1984). Functional strain as a determinant for bone remodeling. *Calcified Tissue International*, *36*(1), S56–S61. https://doi.org/10.1007/BF02406134

Lovejoy, C. O., McCollum, M. A., Reno, P. L., & Rosenman, B. A. (2003). Developmental

biology and human evolution. *Annual Review of Anthropology*, *32*, 85–109. https://doi.org/10.1146/annurev.anthro.32.061002.093223

Low, L. C. K., & Lau, Y. L. (1992). Serum osteocalcin in normal and short Chinese children. *Journal of Paediatrics and Child Health*, *28*(6), 432–435. https://doi.org/10.1111/j.1440-1754.1992.tb02711.x

Madimenos, F. C., Urlacher, S. S., Eick, G. N. Liebert, M. A., Cepon-Robins, T. J., Gildner, T. E., Sugiyama, L. S., & Snodgrass, J. J. (2020). Bone metabolism & immune function: A preliminary cross-sectional analysis of immune markers, osteocalcin, and TRACP-5b among indigenous Shuar youth. *American Journal of Human Biology*, *32*.

Madimenos, Felicia C., Liebert, M. A., Cepon-Robins, T. J., Urlacher, S. S., Josh Snodgrass, J., Sugiyama, L. S., & Stieglitz, J. (2020). Disparities in bone density across contemporary Amazonian forager-horticulturalists: Cross-population comparison of the Tsimane and Shuar. *American Journal of Physical Anthropology*, *171*(1), 50–64. https://doi.org/10.1002/ajpa.23949

Martin, M. A., Garcia, G., Kaplan, H. S., & Gurven, M. D. (2016). Conflict or congruence? Maternal and infant-centric factors associated with shorter exclusive breastfeeding durations among the Tsimane. *Social Science and Medicine*, *170*, 9–17. https://doi.org/10.1016/j.socscimed.2016.10.003

Martin, M., Blackwell, A. D., Gurven, M., & Kaplan, H. (2013). Make new friends and keep the old? Parasite coinfection and comorbidity in Homo sapiens. In J. F. Brinkworth & K. Pechenkina (Eds.), *Primates, Pathogens, and Evolution* (pp. 363–387). https://doi.org/10.1007/978-1-4614-7181-3

McDade, T. W., Leonard, W. R., Burhop, J., Reyes-Garcìa, V., Vadez, V., Huanca, T., & Godoy, R. a. (2005). Predictors of C-reactive protein in Tsimane' 2 to 15 year-olds in lowland

Bolivia. *American Journal of Physical Anthropology*, *128*(4), 906–913. https://doi.org/10.1002/ajpa.20222

McDade, Thomas W., Reyes-García, V., Tanner, S., Huanca, T., & Leonard, W. R. (2008). Maintenance versus growth: Investigating the costs of immune activation among children in lowland Bolivia. *American Journal of Physical Anthropology*, *136*(4), 478–484. https://doi.org/10.1002/ajpa.20831

McDade, Thomas W., Williams, S., & Snodgrass, J. J. (2007). What a drop can do: Dried blood spots as a minimally invasive method for integrating biomarkers into population-based research. *Demography*, *44*(4), 899–925. https://doi.org/10.1353/dem.2007.0038

McDade, Thomas W, & Shell-Duncan, B. (2002). Whole blood collected on filter paper provides a minimally invasive method for assessing human transferrin receptor level. *The Journal of Nutrition*, *132*(12), 3760–3763.

Mei, J. V, Alexander, J. R., Adam, B. W., & Hannon, W. H. (2001). Use of Filter Paper for the Collection and Analysis of Human Whole Blood Specimens 1. *The Journal of Nutrition*, *131*, 1631–1636.

Moreau, R., Tshikudi Malu, D., Dumais, M., Dalko, E., Gaudreault, V., Roméro, H., … Scorza, T. (2012). Alterations in Bone and Erythropoiesis in Hemolytic Anemia: Comparative Study in Bled, Phenylhydrazine-Treated and Plasmodium-Infected Mice. *PLoS ONE*, *7*(9), 1–11. https://doi.org/10.1371/journal.pone.0046101

Munday, K., Ginty, F., Fulford, a., & Bates, C. J. (2006). Relationships between biochemical bone turnover markers, season, and inflammatory status indices in prepubertal gambian boys. *Calcified Tissue International*, *79*(1), 15–21. https://doi.org/10.1007/s00223-005-0276-4

Oh, S. Bin, Lee, W. Y., Nam, H. K., Rhie, Y. J., & Lee, K. H. (2019). Serum osteocalcin levels in overweight children. *Annals of Pediatric Endocrinology and Metabolism*, *24*(2), 104–107.

https://doi.org/10.6065/apem.2019.24.2.104

Oury, F., Sumara, G., Sumara, O., Ferron, M., Chang, H., Smith, C. E., … Karsenty, G. (2011). Endocrine regulation of male fertility by the skeleton. *Cell*, *144*(5), 796–809. https://doi.org/10.1016/j.cell.2011.02.004

Pappa, H., Thayu, M., Sylvester, F., Leonard, M., Zemel, B., & Gordon, C. (2011). Skeletal Health of Children and Adolescents With Inflammatory Bowel Disease. *Journal of Pediatric Gastroenterology and Nutrition*, *53*(1), 11–25. https://doi.org/10.1097/MPG.0b013e31821988a3

Rauchenzauner, M., Schmid, A., Heinz-Erian, P., Kapelari, K., Falkensammer, G., Griesmacher, A., … Högler, W. (2007). Sex- and age-specific reference curves for serum markers of bone turnover in healthy children from 2 months to 18 years. *Journal of Clinical Endocrinology and Metabolism*, *92*(2), 443–449. https://doi.org/10.1210/jc.2006-1706

Reinehr, T., & Roth, C. L. (2010). A new link between skeleton, obesity and insulin resistance: relationships between osteocalcin, leptin and insulin resistance in obese children before and after weight loss. *International Journal of Obesity*, *34*(5), 852–858. https://doi.org/10.1038/ijo.2009.282

Ryan, A. S. (1997). Iron-Deficiency Anemia in Infant Development: Implications for Growth, Cognitive Development, Resistance to Infection, and Iron Supplementation. *Yearbook of Physical Anthropology*, *40*, 25–62.

Scrimshaw N. S. (1992). Effect of infection on nutritional status. *Proceedings of the National Science Council, Republic of China. Part B, Life Sciences*, *16*(1), 46–64.

Shattuck-Heidorn, H., Reiches, M. W., Prentice, A. M., Moore, S. E., & Ellison, P. T. (2017). Energetics and the immune system: Trade-offs associated with non-acute levels of CRP in adolescent Gambian girls. *Evolution, Medicine and Public Health*, *2017*(1), 27–38.

https://doi.org/10.1093/emph/eow034

Slemenda, C. W., Peacock, M., Hui, S., Zhou, L., & Johnston, C. C. (1997). Reduced rates of skeletal remodeling are associated with increased bone mineral density during the development of peak skeletal mass. *Journal of Bone and Mineral Research*, *12*(4), 676–682. https://doi.org/10.1359/jbmr.1997.12.4.676

Stieglitz, J., Beheim, B. a., Trumble, B. C., Madimenos, F. C., Kaplan, H., & Gurven, M. (2015). Low mineral density of a weight-bearing bone among adult women in a high fertility population. *American Journal of Physical Anthropology*, *156*(4), 637–648. https://doi.org/10.1002/ajpa.22681

Stieglitz, J., Jaeggi, A. V., Blackwell, A. D., Trumble, B. C., Gurven, M., & Kaplan, H. (2014). Work to Live and Live to Work : Productivity , Transfers , and Psychological Well-Being in Adulthood and Old Age. In *Sociality, Hierarchy, Health, Comparative Demography: Papers from a Workshop* (pp. 197–221). https://doi.org/10.17226/18822

Stieglitz, J., Madimenos, F., Kaplan, H., & Gurven, M. (2016). Calcaneal quantitative ultrasound indicates reduced bone status among physically active adult forager-horticulturalists. *Journal of Bone and Mineral Research*, *31*(3), 663–671. https://doi.org/10.1002/jbmr.2730

Stieglitz, J., Trumble, B. C., Finch, C. E., Li, D., Budoff, M. J., Kaplan, H., & Gurven, M. D. (2019). Computed tomography shows high fracture prevalence among physically active forager-horticulturalists with high fertility. *ELife*, *8*, e48607. https://doi.org/10.7554/elife.48607

Tsay, J., Yang, Z., Ross, F. P., Cunningham-Rundles, S., Lin, H., Coleman, R., … Vogiatzi, M. G. (2010). Bone loss caused by iron overload in a murine model: Importance of oxidative stress. *Blood*, *116*(14), 2582–2589. https://doi.org/10.1182/blood-2009-12-260083

Urlacher, S. S., Ellison, P. T., Sugiyama, L. S., Pontzer, H., Eick, G., Liebert, M. A., …

Snodgrass, J. J. (2018). Tradeoffs between immune function and childhood growth among Amazonian forager-horticulturalists. *Proceedings of the National Academy of Sciences*, 201717522. https://doi.org/10.1073/pnas.1717522115

Wander, K., Shell-duncan, B., & McDade, T. W. (2009). *Evaluation of Iron Deficiency As a Nutritional Adaptation to Infectious Disease : An Evolutionary Medicine Perspective*. *179*(August 2008), 172–179. https://doi.org/10.1002/ajhb.20839

Wang, J.-W., Tang, Q.-Y., Ruan, H.-J., & Cai, W. (2014). Relation Between Serum Osteocalcin Levels and Body Composition in Obese Children. *Journal of Pediatric Gastroenterology and Nutrition*, *58*(6). Retrieved from https://journals.lww.com/jpgn/Fulltext/2014/06000/Relation_Between_Serum_Osteocalcin_Levels_and_Body.14.aspx

Wei, J., & Karsenty, G. (2015). An Overview of the Metabolic Functions of Osteocalcin. *Current Osteoporosis Reports*, *13*(3), 180–185. https://doi.org/10.1007/s11914-015-0267-y

Weiss, G. (2009). Iron metabolism in the anemia of chronic disease. *Biochimica et Biophysica Acta (BBA) - General Subjects*, *1790*(7), 682–693. https://doi.org/10.1016/j.bbagen.2008.08.006

Wetteland, P., Roger, M., H, S. E., & Iversen, O. H. (1996). Population-based erythrocyte sedimentation rates in 3910 subjectively healthy Norwegian adults. A statistical study based on men and women from the Oslo area. *Journal of Internal Medicine*, *240*(3), 125–131. https://doi.org/https://doi.org/10.1046/j.1365-2796.1996.30295851000.x

WHO. (2011). Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. In *Vitamin and Mineral Nutrition Information System*. https://doi.org/2011

Wong, P., Fuller, P. J., Gillespie, M. T., & Milat, F. (2016). Bone disease in thalassemia: A molecular and clinical overview. *Endocrine Reviews*, *37*(4), 320–346. https://doi.org/10.1210/er.2015-1105

Wood, S. N. (2011). Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models. *Journal of the Royal Statistical Society (B)*, *73*(1), 3–36.

Zarychanski, R., & Houston, D. S. (2008). Anemia of chronic disease : A harmful disorder or an adaptive , beneficial response ? *Canadian Medical Association Journal*, *179*(4), 333–337.

Zhang, Q., Riddle, R. C., & Clemens, T. L. (2015). Bone and the regulation of global energy balance. *Journal of Internal Medicine*, *277*(6), 681–689. https://doi.org/10.1111/joim.12348

Zoch, M. L., Clemens, T. L., & Riddle, R. C. (2016). New insights into the biology of osteocalcin. *Bone*, *82*, 42–49. https://doi.org/10.1016/j.bone.2015.05.046

V. CONCLUSION

This dissertation presents an interdisciplinary case study of skeletal manifestations of childhood health. As such, it moves across multiple temporal and physiological scales. To improve the potential for understanding deep temporal patterns of human health in prehistory, it engages with skeletal plasticity at the cellular and gross morphological levels, as well as adult health outcomes associated with skeletal indicators of childhood. I wrote it to bring longstanding questions in bioarchaeology to a setting in which they might finally be answered (Wood et al., 1992). I have focused on the knowledge gap surrounding porous cranial lesions, a skeletal finding notable for its centrality in bioarchaeological studies of population health and its obscurity in contemporary medical practice (DeWitte & Stojanowski, 2015). I argue that this discrepancy results not from stark differences in health between past and contemporary populations nor the irrelevance of porous cranial lesions to individual health but rather from disciplinary blind spots that highlight the value of interdisciplinary approaches.

5.1 Summary of findings

Chapter II established that porous cranial lesions, as defined using paleopathological criteria, can be identified on clinical computed tomography (CT) scans—but only when both the scans and the lesions meet certain criteria.

To be suitable for evaluating PCLs, clinical CT scans must include a bone-optimized (sharp kernel/algorithm) reconstruction and must be obtained using a slice width <1mm (the thinner, the better). In clinical settings, the highest probability of scans that meet these criteria comes from assessments of cranial fracture following head trauma, which are often obtained in tandem with scans calibrated to catch evidence of intracranial bleeding.

To have a high probability of being identified on such CT scans, the cortical surface porosity of porous cranial lesions must include individual foramina with a diameter greater than the CT scan's image resolution, a metric which is not directly provided in a scan's metadata but can be calculated from the values provided for the scan's slice width and field of view. Volume-rendered reconstructions are most sensitive for identifying the presence of cranial vault lesions, but assessments should always be verified by the visibility of cortical porosity or pitting in multiple viewing planes of a multiplanar reconstruction (MPR). Conversely, MPR provides the clearest view of orbital roof lesions but should be cross-checked against the appearance of the orbits in volume-rendered reconstructions.

Additionally, several other characteristics of PCLs increase their visibility on CT scans. Cranial vault lesions with expansion of the marrow space or hair-on-end orientation of trabecular bone are easily identified in MPR. Porosity or pitting of the occipital squama is clearly visible in volume-rendered reconstructions. This is partly because patients are in a supine position for most CT scans, and thin axial slices can create higher image resolution in the cranio-caudal direction. But it is also partly due to a feature of lesion morphology: individual foramina occurring on the occipital squama tend to be larger than individual foramina on the superior or anterior aspects of the cranial vault.

For lesions of the orbital roof, porosity in the cortical bone of the orbital roofs can be positively identified with a high degree of certainty **only if** it occurs in orbital roofs with substantial thickness of trabecular bone and without substantial thinning of the cortical bone surrounding individual foramina. And finally, widened intertrabecular spacing in the trabecular bone of the orbital roofs is clearly visible in MPR.

All of this ultimately means that individual observers can only reliably identify a subset of PCLs on CT scans of living adults: PCLs with larger individual foramina, or with pronounced

changes to the structure of the underlying trabecular bone. Conversely, lesions comprised of superficial vascular impressions or 'pinprick' porosity (Buikstra & Ubelaker, 1994), and lesions in advanced stages of remodeling—which together constitute a major portion of lesions in some archaeological assemblages—are unlikely to be visible with the current scanning parameters of clinical CT. This lower sensitivity may be an advantage compared to traditional osteological observations, as it removes much of the noise that exists in paleopathological data from interobserver disagreement over what constitutes sufficiently noticeable porosity to categorize as pathological, as well as the accidental inclusion of post-mortem damage in counts of pathological lesions.

Chapter III was then able to bridge the study of developmental approaches to health and disease in past and contemporary populations by exploring adult health outcomes associated with PCLs, which are interpreted by bioarchaeologists as skeletal indicators of childhood stress. After examining a population-representative sample of 375 cranial CT scans of adults aged 40+ years from the Tsimane population of lowland Bolivia, we have the first results on the relationship between PCLs and adult health outcomes.

A first novel finding of this investigation is simply the demographic patterning of PCLs among older adults in a living population. Small sample sizes and large age ranges limit the statistical power of archaeological analyses to detect demographic patterning of lesions among older adults. This study is unique in being a population-representative sample of several hundred older adults with precise estimates for age and sex. We find that PCL frequencies do not differ significantly by sex, and the frequency of cranial vault porosity does not differ by age. However, the frequency of orbital roof porosity is inversely associated with age. This is a finding frequently reported in archaeological assemblages, though it is typically a pattern driven by high frequency

of orbital roof lesions in juvenile skeletal remains and never before documented among older adults.

This chapter tested whether orbital roof or cranial vault porosity were associated with a broad range of medical diagnoses, functional disability assessments, white blood cell profiles, anemia, and overall physiological dysregulation. We found that cranial vault porosity was associated with lower vertebral bone mineral density. It is unclear what direction causality might flow here: Does the cranial vault porosity observed in this sample develop in adulthood as a manifestation of systemic bone loss? Do causes of cranial vault porosity in early life also predispose individuals to lower peak bone mass or more rapid bone loss in adulthood? Cranial vault porosity was not associated with differences in any of the other health outcomes measured.

Orbital roof porosity was associated with a higher hazard of developing symptomatic respiratory tuberculosis and with a lower CD4/CD8 ratio. These may be causally related, though again the direction of causality is unclear. Low CD4/CD8 ratio is a characteristic of immune senescence and is considered an immune risk phenotype. Symptomatic tuberculosis in older adults typically results from the opportunistic activation of asymptomatic TB acquired in childhood, particularly in populations where TB is endemic. Individuals with low CD4/CD8 ratios are more susceptible to symptomatic tuberculosis, but mounting an immune response to a chronic active TB infection can also lead to a low CD4/CD8 ratio.

In combination with the evidence for higher risk of symptomatic tuberculosis and greater immunosenescence, the negative association between orbital roof porosity and age suggests that older Tsimane adults with orbital roof lesions may face higher mortality risk than their lesion-less peers and that susceptibility to infectious disease may be the mechanism for this frailty. However, our findings do not universally support a conceptual model of orbital roof lesions as skeletal indicators of adult disease susceptibility; orbital roof porosity was not

associated with significantly higher incidence of respiratory infections other than TB. Continued collection of Tsimane demography will ultimately provide data that can speak directly to the relative mortality risk of individuals in the current study with and without PCLs.

Finding correlations between these skeletal lesions and adult health outcomes is a necessary first step in the larger project of understanding their relationship to health over the life course. But PCLs' association with morbidity among the sample of adults in this study also rests on a slew of factors that each present a substantial and challenging area of research that must be addressed before causal relationships can be evaluated. Immediate questions that spring to mind include, for a start: Does the association between orbital roof lesions and health outcomes observed here diminish, accumulate, or remain constant across the life course? To what extent does lesion-associated mortality risk prior to age 40 shape the observable associations between lesions and health among adults aged 40+ years? What is the window of opportunity for PCL development, and how does lesion formation timing affect lifelong health outcomes associated with lesions? What are the necessary and sufficient conditions for PCL formation? What is the full range of specific causes of PCLs, and how does heterogeneity in the causes of PCLs contribute to aggregate patterns of lesion-associated morbidity and mortality? How big is the role of heterogeneity in individual genetic predisposition to forming lesions (heritability)? And what is the probability that skeletal remodeling erases observable evidence of PCLs among some survivors?

5.1.1 The anemia question

Though some bioarchaeological papers assume that these lesions should also be connected to anemias with onset in adulthood (Godde & Hens, 2021; Hens, Godde, & Macak,

2019), we find no association between the presence of PCLs and adult hemoglobin concentrations. The bulk of archaeological and clinical studies conclude that PCLs develop in childhood and remain observable in adult crania. Among Tsimane adults PCLs were neither associated with same-day hemoglobin measurements accompanying the cranial CT scans from which individual lesion status was assessed nor with longitudinal averages of each individual's hemoglobin values across multiple years.

Even the single individual whose cranial vault porosity was accompanied by marked expansion of the underlying marrow space and hair-on-end appearance of cranial vault trabeculae—considered a pathognomonic characteristic of lesions caused by childhood anemia—had no documented instances of anemia among seven measures of hemoglobin obtained in across the sixth and seventh decades of life.

Childhood anemia is often cited as a major cause of PCLs, but the specific causes of anemia capable of causing PCLs are heavily debated. In the absence of radiological data on Tsimane children, **Chapter IV** tested whether anemia was associated with measurable differences in skeletal metabolism among children <9 years, as measured by the biomarker osteocalcin. In addition to hemoglobin, we also assessed iron status using serum transferrin receptor in order to test whether iron-deficiency anemia and anemia of inflammation differ in their effects on osteocalcin. We find that among children in the current study (aged 0.3-8 years), though elevated ESR was universal and almost 50% were anemic, iron-deficiency anemia was relatively rare (7.2%).

The majority of anemia cases were mild (hemoglobin > 10 g/dL). Cases of iron deficiency tended to be moderate to severe anemia, so the effects of iron deficiency per se were not able to be assessed independent of anemia severity. Iron-deficiency anemia, but not anemia

of inflammation, was associated with significantly lower osteocalcin, though it is unclear whether the degree of anemia or iron deficiency itself is the salient variable.

Ultimately, these data speak to overall patterns in skeletal cellular metabolism in the presence of anemia and inflammation but are not well positioned to evaluate whether childhood anemia is a cause of specific gross skeletal outcomes, such as PCLs, in the Tsimane population. Serum osteocalcin may pick up on local or systemic shifts in bone turnover. Rather than identifying PCL formation, lower osteocalcin in children with IDA may indicate disruptions in linear growth, or diminished bone formation throughout the skeleton, which would be consistent with reports of growth stunting and generalized osteopenia in anemic children (N. Agarwal et al., 1970; Aksoy, Muzaffer; Camli, Necdet; Erdem, 1966; Ryan, 1997).

We can, however, say that compared to non-anemic individuals, anemia in the absence of iron deficiency does not appear to be associated with any difference in skeletal metabolism. This is the case even when ESR values fall in the fourth quartile for study participants, our conservative threshold for classifying anemia of inflammation in a sample of children who all have elevated ESR according to US clinical references (Hollinger & Robinson, 1953). In fact, neither ESR nor the acute inflammatory marker CRP were significantly associated with osteocalcin-for-age. This finding is consistent with the lack of erythroid hyperplasia in anemia of inflammation, and perhaps suggests that gross skeletal changes such as PCLs are unlikely to be caused by inflammation in this population.

5.1.2 Causes and correlates of PCLs in Tsimane forager-horticulturalists—what have we learned?

Part of the initial impetus for assessing PCLs in the Tsimane was based on the high prevalence of childhood anemia in this population. Given that PCLs are so rarely reported in

contemporary populations but commonly reported in archaeological assemblages, with a latitudinal distribution that correlates with the global patterning of acquired anemia (Hengen, 1971), the tropical subsistence ecology of the Tsimane appeared to present one of the highest-probability settings for finding PCLs in a contemporary population.

However, the fact that we did indeed observe PCLs among older Tsimane adults should not be taken as additional evidence that childhood anemia is responsible for the formation of these skeletal lesions. It is not even clear that Tsimane prevalence of PCLs is higher than other contemporary populations. Recent anthropological studies of human skeletal remains from modern historic and contemporary populations report PCL frequencies in line with archaeological contexts (Beatrice & Soler, 2016; David, 2018; O'Donnell et al., 2020; Steyn, Voeller, Botha, & Ross, 2016; Wright & Chew, 1998).

Instead, the finding that orbital roof porosity is associated with tuberculosis and immunosenescence among older Tsimane adults adds to a growing body of research on the relevance of this skeletal marker for health across the life course.

5.1.3 Respiratory infections: A possible mechanism for PCL-associated mortality risk

Since PCLs are so often attributed to childhood anemia, the mortality impact of childhood anemia has often been an implicit or explicit part of bioarchaeological narratives of population health (Mcfadden & Oxenham, 2020). Yet, epidemiological analyses of anemia-associated childhood mortality risk are largely inconclusive outside of severe (<5 g/dL) anemia in malarial regions (Brabin, Premji, & Verhoeff, 2001). Most studies fail to scale hemoglobin to infant body size, conflating the risks of supposed anemia with the well-established mortality risks associated with low birth weight. Archaeological analyses have reported elevated mortality risk

for young adults with PCLs; yet, a case-control study of Malawian children aged 6 months to 5 years found that the mortality risk associated with even severe anemia appeared to plateau 8 months after in-hospital treatment, and much of the anemia-associated mortality in this study was attributable to HIV infection (Phiri et al., 2008). Among Tsimane, a very preliminary exploration of childhood hemoglobin and mortality data did not find higher mortality risk associated with ever having had anemia, but future work will formally assess anemia's role as a predictor of all-cause mortality in this population across the life course.

Where skeletal indicators of childhood stress predict higher mortality risk in childhood and young adulthood, the pathway for this relationship is presumably through increased susceptibility to the primary causes of death among these age groups in past environments. In light of recent reports that porous lesions of the orbital roofs are significantly more common in children with fatal respiratory infections (Gomes et al., 2022; O'Donnell et al., 2020), higher lifetime susceptibility to infection, and perhaps respiratory infections in particular, presents a plausible pathway between lesion-causing stress and sustained mortality risk. Respiratory infections are consistently a leading cause of death in historic records and in contemporary low-income groups (Mathers et al., 2009), including Tsimane (M. Gurven et al., 2007), and initial infection appears to have far-reaching effects on subsequent morbidity.

Other recent work has found support for a connection between PCLs and fatal respiratory infections, particularly in connection with orbital roof lesions. Perhaps the most direct evidence to date that PCLs are associated with physiological stress, elevated morbidity, and elevated mortality risk comes from a recent study using digital autopsies of contemporary New Mexican children (O'Donnell et al., 2020). Controlling for age, PCLs of the orbits and cranial vault were both significantly more common among children who died of natural (congenital, infectious) causes than those who died abruptly from violence or accidents,

suggesting that even in the contemporary United States PCLs are an indicator of premature mortality risk. When specific causes of death were considered, porous lesions of the orbital roofs—and, to a lesser extent, the cranial vault—were associated with fatal respiratory infections, particularly pneumonia. A study designed to replicate this analytical approach with a sample of children <18 years from a historic Portuguese cemetery similarly found that orbital roof porosity was associated with deaths from respiratory tuberculosis (Gomes et al., 2022).

5.2 Directions for Future Research

5.2.1 A case for interdisciplinary osteology

Human bodies are constantly shaped by the environments in which we live. Every human has a skeleton that is built by interactions with our environment from before we are born until the day we die. Of course, after death, human bodies are also disassembled by our environment, but bones turn to soil more slowly than the rest of us. The result is that human skeletal remains provide a window into lives long since lived, just as the bones inside you or I have been and continue to be shaped by the process of living. The skeletal record is remarkably continuous, stretching from prehistory to the present day. Yet, there are noticeable gaps in our knowledge of human skeletal biology that stem from the disparate priorities, methods, and constraints of different communities of researchers interested in the skeleton's relationship to health and disease.

For the bioarchaeologist interested in understanding largescale temporal and geographic patterns of population health, the skeleton is a cipher of human experience and decoding its relationship to the life lived is a project of central importance. Yet for the contemporary human biologist, who is also deeply interested in human health, disease, and the embodiment of experience, the skeleton is only one of many sources of information—often not the most

informative and certainly not the most accessible. This is true too for the physician, whose inquiry is focused on identifying and treating the suffering of individual patients.

The research gap resulting from these different orientations to the relationship between health and the skeleton has long been recognized in bioarchaeology. As Douglas Ubelaker put it:

> "…no single professional field offers the type of comprehensive training needed to address all of the issues involved…. The need is for broader training in all areas, or at least enhanced communication and collaboration among participants. The intellectual minefields along disciplinary borders must be defused to approach the goal of understanding the complexities of the past disease experience" (p.98, Ubelaker, 2003).

The inescapable confounding factors inherent to reconstructing population health from the dead, collectively known as the osteological paradox (J. W. Wood et al., 1992), are magnified when the relationship between skeletal expression and disease experience is unknown among the living, as is the case for PH and CO. Among other avenues forward, the authors of both the paradox and of more recent reviews of the paradox's impact on the field (DeWitte & Stojanowski, 2015a; Wright & Yoder, 2003) call for a stronger connection between modern health research and paleopathology as a means of advancing anthropological studies of health in past societies. This dissertation was designed as a case study of a cross-disciplinary approach to the relationship between health and the skeleton. It provides a proof of concept for numerous productive avenues of future research in human skeletal biology to bridge the gap between understandings of health in the past and the present.

5.2.2 Expanding the validation of lesion visibility in clinical radiology

The 22 archaeological crania scanned for Chapter II were initially intended to be a pilot sample, but due to Covid-19 closures in 2020-21 I was unable to return to the NMNH collections to expand the sample. Establishing the clinical appearance of archaeologically defined skeletal phenomena is a foundational project for investigating the relationship between these skeletal lesions and health. Future work is still needed to further characterize the relationship between directly observable lesion morphology in dry bone and observable lesion morphology on clinical CT scans. An expanded sample should include more cases of PCLs, particularly orbital roof lesions, as well as a higher proportion of crania without lesions, in order to calculate the sensitivity and specificity of lesion identification from CT. Investigation of PCLs in contemporary populations will need to be conducted using radiological imaging, and the findings of such studies will need to be interpreted with an understanding of the sensitivity and specificity of data obtained from CT and other imaging modalities. Future work should also expand the age range of archaeological reference crania to establish age-related variation in lesion morphology and the limits of lesion visibility in clinical CT scans of non-adults.

Once the lesions can be reliably identified from CT scans of living individuals, the appearance of lesions on magnetic resonance imaging (MRI) should also be investigated. Identification of PCLs on MR images will enable investigation of how these lesions are related to the composition (red or yellow) of adjacent bone marrow, a long overdue test of the commonly invoked idea that lesion formation is a consequence of red marrow expansion.

5.2.3 Quantitative approaches to analyzing CT scans

Chapter II also makes a case for the utility of quantitative approaches for analyzing CT evidence of PCLs. The investigation of PCLs—or other phenomena primarily encountered by paleopathologists—in living people will call for collaboration between paleopathologists and radiologists. The major strength of semi-automated quantitative analyses that engage directly with the three-dimensional matrices of density values in a CT scan is that they are replicable and do not rely solely on subjective assessments from individual observations. For one thing, the results of this dissertation demonstrate how variable individual assessments can be. For another, quantitative assessments of density values can be used to mediate cross-disciplinary disagreement in scan evaluations based on subjective observations.

5.2.4 Leveraging existing data sets to answer outstanding questions in human skeletal biology

To address many questions in human skeletal biology, ideal data would come from longitudinal collection of radiological imaging, medical histories, family circumstances, individual exposures, and genetic variability in samples of living children. However, before we can marshal the resources necessary for such prospective cohort studies, we need pilot data demonstrating the relevance of skeletal indicators to health in living people. If the skeletal lesions that comprise the bulk of data on health in prehistory are meaningful indicators of disease experience and mortality risk, then they should also be critical markers of individual health risks in contemporary populations. This is not to suggest that clinical protocols increase patient radiation exposure through unessential imaging, but rather that incidental findings on otherwise indicated scans could provide additional information on patient medical history and current risks. Conversely, if features that are interpreted as skeletal indicators of stress cannot be meaningfully

linked to health experiences in contemporary populations, their use by bioarchaeologists should be reevaluated.

Chapter III relies on the unique data infrastructure of a long-running health and anthropology project to investigate associations between skeletal lesions and individual health outcomes, but it models an approach that can be applied to other settings to explore human skeletal biology with existing data. Published clinical imaging may focus on unusual disease presentations, but there is untapped potential for fertile collaborations between anthropologists and physicians using existing imaging and associated medical data. While even unpublished clinical imaging is not population-representative, CT scans are often conducted to rule out internal injuries after traumatic events, and scans from minor trauma might serve as a control sample for studies of chronic or systematic processes affecting the skeleton. Incidental skeletal data are regularly collected in the course of clinical practice, and anthropologists can leverage such existing data on health in contemporary populations to answer questions about the relationship between health and skeletal phenotype that are motivated by observations of archaeological skeletal remains—an endeavor that I term ethnopaleopathology.

An ethnopaleopathological approach, in which skeletal lesions in living individuals are mapped onto lived experience and disease diagnoses, is a particularly promising route for interrogating the relationship between visible skeletal features and individual experiences. It stands in contrast to the traditional relationship between paleopathology and the clinical sciences, which advances paleopathological inference through a diagnosis-centered approach that asks what skeletal features are associated with conditions diagnosed from non-skeletal signs and symptoms. Instead, ethnopaleopathology uses a lesion-centered model that starts with a skeletal feature and asks what diagnoses or disease experiences are associated with a particular skeletal phenotype.

Such a lesion-centered approach requires the creation of directly comparable data from archaeological remains and living individuals. X-ray and CT are imaging modalities that can be applied to both in vivo and dry skeletal material, but CT provides the most informative images. The first task for an ethnopaleopathological inquiry involves clearly delineating the strengths and limitations of identifying a skeletal feature of interest from radiological images obtained with scanning parameters used for scans of living individuals in clinical contexts—as demonstrated here in Chapter II. The sensitivity and specificity of skeletal indicators of disease experience has occasionally been investigated in living populations. One such inquiry found that individuals with and without lower back pain have no noticeable difference in degenerative vertebral changes (Jensen et al., 1994), a result that highlights the potential for misunderstanding the skeletal manifestations of disease when only individuals with specific symptoms are sent for diagnostic imaging.

Additionally, among the numerous aspects of hidden heterogeneity in prehistoric mortality samples that can be productively addressed with studies of living populations is the role of genetic variation in predisposing some individuals to developing skeletal lesions. The current project did attempt to estimate the heritability of PCLs in the Tsimane population using a kinship matrix for all individuals with CT scans, but ultimately the current sample did not provide sufficient data for reliable heritability estimates. Future work may be able to produce heritability estimates from an expanded sample of cranial scans as the work of the THLHP continues.

5.2.5 Do PCLs ever heal completely?

The rate at which PCLs might become unobservable to due total remodeling of the affected area is unknown because it cannot be calculated from the inherently cross-sectional data of mortality samples. Where PCLs are associated with younger age at death this relationship has been interpreted to mean that individuals with PCLs face a higher risk of mortality than their peers without PCLs—but this is also the pattern we should observe if skeletal remodeling obscures PCLs in some individuals who survive to later ages, regardless of individual mortality risk. The extent to which remodeling contributes to the age distribution of PCLs in the archaeological record is yet unquantified, but, given that these lesions are observable in older adults, the process of total lesion remodeling is either very slow or highly variable. The rate of cortical turnover in adults is estimated at 2-4% annually, and the occipital bone of the cranium exhibits slower turnover than much of the rest of the skeleton (Clarke, 2008; Fahy, Deter, Pitfield, Miszkiewicz, & Mahoney, 2017). Slow annual turnover rates could nevertheless potentially add up to noticeable declines in observable lesion frequencies across decades of life and at present is unaccounted for in bioarchaeological analyses.

5.2.6 Are skeletal lesions correlated with lived health experience?

Paleopathological interest in skeletal remains does not end with diagnosis. The connection between skeletal phenotype and disease experience is often the more salient issue for addressing the cost of stress to individuals and communities. For instance, how does skeletal involvement differ in symptomatic and asymptomatic chronic infections? What are the downstream consequences of early life stress as written in the skeleton, not just for mortality but also for resilience and susceptibility to future stress? Perhaps more challenging than the task of

diagnosis, these questions have been productively addressed in recent years with the emergence of the bioarchaeology of care (Tilley, 2015)(and the application of the DOHaD paradigm and life course approaches to skeletal lesions (S. C. Agarwal, 2016; Goodman & Armelagos, 1988; Roberts & Steckel, 2019; Temple, 2018). Yet, considerations of the adult outcomes predicted by skeletal indicators of childhood stress have been predominantly carried out in archaeological contexts in which mortality, not morbidity, is the primary health outcome. The role of the skeleton as a record of experience and predictor of subsequent health can be clarified by working with samples of living people, allowing consideration of skeletal plasticity in the context of other organ systems.

5.2.7 Ecological osteoimmunology

And finally, the findings in Chapter IV illustrate both the promise and the problems of biomarkers of skeletal metabolism. It adds to a growing body of human biology work demonstrating that the relationships between markers of inflammation and skeletal metabolism appear to differ across populations (Fatahi et al., 2019; F. C. Madimenos et al., 2020; Munday et al., 2006), a finding that highlights the productive and underexplored interdisciplinary space between the two relatively new fields of ecological immunology and osteoimmunology. The extent to which local disease ecology drives variation in the interplay between the skeletal and immune systems is underexplored, and it joins the long list of variables contributing to hidden heterogeneity in the determinants of observable skeletal phenotypes and their relationships to morbidity and mortality.

5.3 References

Agarwal, N., M, S., Dhar, N., & Bhardwaj, P. (1970). Roentgenologic changes in iron deficiency anemia. *American Journal of Roentgenology*, *110*(3), 635–637.

Agarwal, S. C. (2016). *Bone Morphologies and Histories : Life Course Approaches in Bioarchaeology*. *149*, 130–149. https://doi.org/10.1002/ajpa.22905

Aksoy, Muzaffer; Camli, Necdet; Erdem, S. (1966). Roentgenographic Bone Changes in Chronic Iron Deficiency Anemia: a study in twelve patients. *Blood*, *27*(5).

Beatrice, J. S., & Soler, A. (2016). Skeletal indicators of stress: A component of the biocultural profile of undocumented migrants in southern Arizona. *Journal of Forensic Sciences*, *61*(5), 1164–1172. https://doi.org/10.1111/1556-4029.13131

Brabin, B. J., Premji, Z., & Verhoeff, F. (2001). An Analysis of anemia and childhood mortality. *The Journal of Nutrition*, *131*(2), 636S-648S.

Buikstra, J. E., & Ubelaker, D. H. (1994). *Standards for data collection from human skeletal remains: Proceedings of a seminar at the Field Museum of Natural History*. Fayetteville, AR: Arkansas Archeological Survey.

Clarke, B. (2008). Normal bone anatomy and physiology. *Clinical Journal of the American Society of Nephrology*, *3*(Supplement 3), S131–S139.

David, M. (2018). *Traumatic predictors of femicide: A forensic anthropological approach to domestic violence* (Florida Gulf Coast University). Retrieved from https://fgcu.digital.flvc.org/islandora/object/fgcu%3A32589

DeWitte, S. N., & Stojanowski, C. M. (2015). The Osteological Paradox 20 Years Later: Past Perspectives, Future Directions. *Journal of Archaeological Research*, *23*(4), 397–450. https://doi.org/10.1007/s10814-015-9084-1

Fahy, G. E., Deter, C., Pitfield, R., Miszkiewicz, J. J., & Mahoney, P. (2017). Bone deep:

Variation in stable isotope ratios and histomorphometric measurements of bone remodelling within adult humans. *Journal of Archaeological Science*, *87*(November), 10–16. https://doi.org/10.1016/j.jas.2017.09.009

Fatahi, S., Ghaedi, E., Mohammad-Mousavi, S., Bawadi, H., Rahmani, J., Pezeshki, M., & Kord-Varkaneh, H. (2019). The Association between osteocalcin and C-Reactive Protein; A relation of bone with inflammation: A systematic review and meta-analysis. *Hormonal and Metabolic Research*, *51*(6), 353–361.

Godde, K., & Hens, S. M. (2021). An epidemiological approach to the analysis of cribra orbitalia as an indicator of health status and mortality in medieval and post-medieval London under a model of parasitic infection. *American Journal of Physical Anthropology*, *174*(4), 631–645. https://doi.org/10.1002/ajpa.24244

Gomes, R. A. M. P., Petit, J., Dutour, O., & Santos, A. L. (2022). Frequency and co-occurrence of porous skeletal lesions in identified non-adults from Portugal (19th to 20th centuries) and its association with respiratory infections as cause of death. *International Journal of Osteoarchaeology*, (January), 1–12. https://doi.org/10.1002/oa.3132

Goodman, A. H., & Armelagos, G. J. (1988). Childhood Stress and Decreased Longevity in a Prehistoric Population. *American Anthropologist*, *90*(4), 936–944. https://doi.org/10.1525/aa.1988.90.4.02a00120

Gurven, M., Kaplan, H., & Supa, A. Z. (2007). *Mortality Experience of Tsimane Amerindians of Bolivia : Regional Variation and Temporal Trends*. *398*(September 2006), 376–398. https://doi.org/10.1002/ajhb

Hengen, O. P. (1971). Cribra orbitalia pathogenesis and probable etiology-annotated. *HOMO*, *22*, 57–75.

Hens, S. M., Godde, K., & Macak, K. M. (2019). Iron deficiency anemia , population health and

frailty in a modern Portuguese skeletal sample. *PLoS ONE*, *14*(3), e0215235. Retrieved from https://doi.org/10.1371/journal.pone.0215235

Hollinger, N., & Robinson, S. (1953). A study of the erythrocyte sedimentation rate for well children. *Journal of Pediatrics*, *42*, 304–319.

Jensen, M. C., Brant-Zawadzki, M. N., Obuchowski, N., Modic, M. T., Malkasian, D., & Ross, J. S. (1994). Magnetic resonance imaging of the lumbar spine in people without back pain. *The New England Journal of Medicine*, *331*(2), 69–73.

Madimenos, F. C., Urlacher, S. S., Eick, G. N. Liebert, M. A., Cepon-Robins, T. J., Gildner, T. E., Sugiyama, L. S., & Snodgrass, J. J. (2020). Bone metabolism & immune function: A preliminary cross-sectional analysis of immune markers, osteocalcin, and TRACP-5b among indigenous Shuar youth. *American Journal of Human Biology*, *32*.

Mathers, C. D., Boerma, T., & Ma Fat, D. (2009). Global and regional causes of death. *British Medical Bulletin*, *92*(1), 7–32. https://doi.org/10.1093/bmb/ldp028

Mcfadden, C., & Oxenham, M. F. (2020). A paleoepidemiological approach to the osteological paradox : Investigating stress , frailty and resilience through cribra orbitalia. *American Journal of Physical Anthropology*, *173*(2), 205–217. https://doi.org/10.1002/ajpa.24091

Munday, K., Ginty, F., Fulford, a., & Bates, C. J. (2006). Relationships between biochemical bone turnover markers, season, and inflammatory status indices in prepubertal gambian boys. *Calcified Tissue International*, *79*(1), 15–21. https://doi.org/10.1007/s00223-005-0276-4

O'Donnell, L., Hill, E. C., Anderson, A. S., & Edgar, H. J. H. (2020). Cribra orbitalia and porotic hyperostosis are associated with respiratory infections in a contemporary mortality sample from New Mexico. *American Journal of Physical Anthropology*, *173*(4), 721–733. https://doi.org/10.1002/ajpa.24131

Phiri, K. S., Calis, J. C. J., Faragher, B., Nkhoma, E., Ng'oma, K., Mangochi, B., … van

Hensbroek, M. B. (2008). Long term outcome of severe anaemia in Malawian children. *PLoS ONE*, *3*(8). https://doi.org/10.1371/journal.pone.0002903

Roberts, C. A., & Steckel, R. H. (2019). The Developmental Origins of Health and Disease: Early Life Health Conditions and Adult Age at Death in Europe. In R. H. Steckel, C. S. Larsen, C. A. Roberts, & J. Baten (Eds.), *The Backbone of Europe: Health, diet, work and violence over two millennia* (1st ed., pp. 325–351). Cambridge: Cambridge University Press.

Ryan, A. S. (1997). Iron-Deficiency Anemia in Infant Development: Implications for Growth, Cognitive Development, Resistance to Infection, and Iron Supplementation. *Yearbook of Physical Anthropology*, *40*, 25–62.

Steyn, M., Voeller, S., Botha, D., & Ross, A. H. (2016). Cribra orbitalia: Prevalence in contemporary populations. *Clinical Anatomy*, *830*(April), 823–830. https://doi.org/10.1002/ca.22734

Temple, D. H. (2018). Bioarchaeological evidence for adaptive plasticity and constraint : Exploring life-history trade-offs in the human past. *Evolutionary Anthropology*, *28*(September), 34–46. https://doi.org/10.1002/evan.21754

Tilley, L. (2015). Accommodating difference in the prehistoric past: Revisiting the case of Romito 2 from a bioarchaeology of care perspective. *International Journal of Paleopathology, 8*, 64–74.

Ubelaker, D. H. (2003). Anthropological Perspectives on the Study of Ancient Disease. In C. Greenblatt & M. Spigelman (Eds.), *Emerging Pathogens, Archaeology, Ecology and Evolution of Infectious Disease* (pp. 93–102). Oxford: Oxford University Press.

Wood, J. W., Milner, G. R., Harpending, H. C., Weiss, K. M., Cohen, M. N., Eisenberg, L. E., … Weiss, K. M. (1992). The osteological paradox : Problems of inferring prehistoric health from skeletal samples. *Current Anthropology*, *33*(4), 343–370.

Wright, L. E., & Chew, F. (1998). Porotic hyperostosis and paleoepidemiology: A forensic perspective on anemia among the ancient Maya. *American Anthropologist*, *100*(4), 924–939. https://doi.org/10.1525/aa.1998.100.4.924

Wright, L. E., & Yoder, C. J. (2003). Recent progress in bioarchaeology: Approaches to the Osteological Paradox. *Journal of Archaeological Research*. https://doi.org/10.1023/A:1021200925063

APPENDIX

Supplemental Information for Chapter II

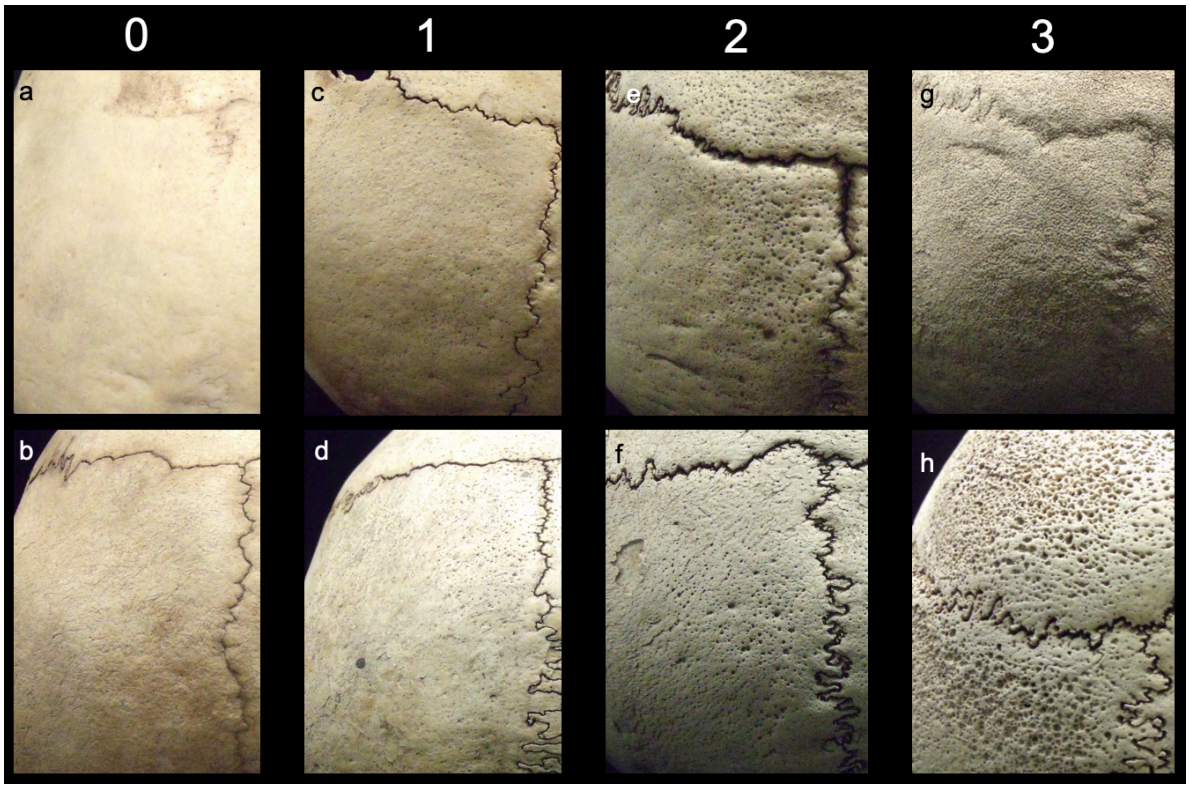

Figure S2. 1 Examples of Stuart-Macadam lesion classification applied to study sample during direct in-person observation. a-b) 0 – absence of lesion; c-d) 1 – scattered fine foramina; e-f) 2 – large and small isolated foramina; g-h) 3 – foramina coalescing in a trabecular structure. Image g shows a lesion presumed to be in advanced stages of healing; superficial trabecular impressions predominate, with complete infilling of any associated foramina. During direct observation no crania were scored as 4 – 'outgrowth in trabecular structure from the normal contour of the outer bone table.' This extreme type of lesion expression is more commonly seen in non-adults.

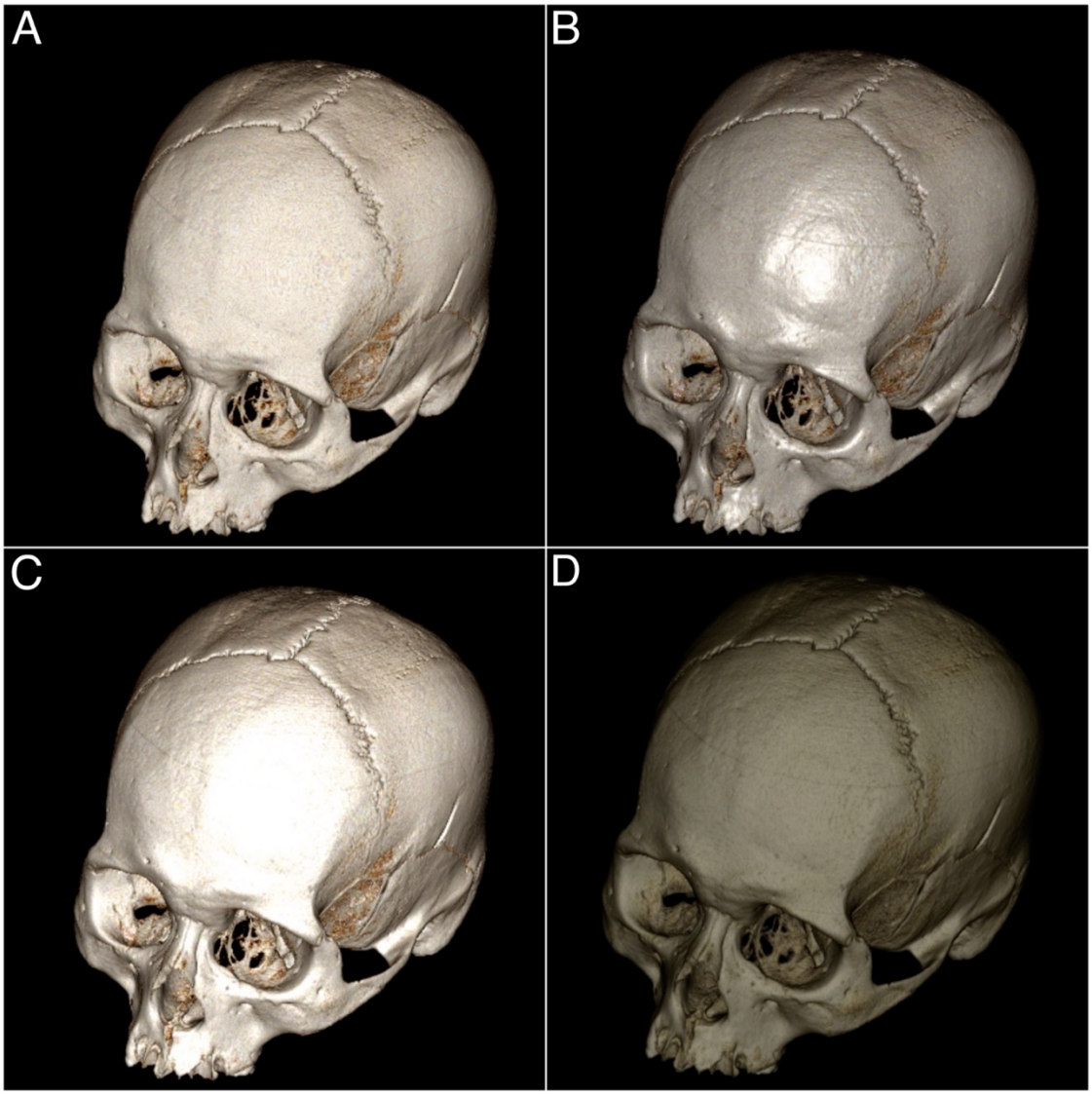

Figure S2. 2 Differences in observer settings independently chosen for viewing 3-D volume-rendered images.

A) WW/WL – default setting; 3-D preset – Basic, High contrast; Opacity – Logarithmic Inverse Table; rendering color (CLUT) – VR Muscles-Bones; Shading – customized: specular coefficient = 0, ambient coefficient = 1, diffuse coefficient = 1.

B) WW/WL – Full dynamic; 3-D preset – Basic, High contrast; Opacity – Logarithmic Table; CLUT – VR Muscles-Bones; Shading: customized – ambient 0.4; diffuse 0.7; specular 0.6-50.0

C) WW/WL – default setting; 3-D preset – Basic, High contrast; Opacity – Logarithmic inverse; color – VR Muscles-Bones; Shading – default setting.

D) WL/WW – CT-Bone; Opacity – Linear Table; CLUT – VR Bone; Shading – customized: ambient = 0.25, diffuse = 0.28, specular = 0.27, specular Power = 2.02

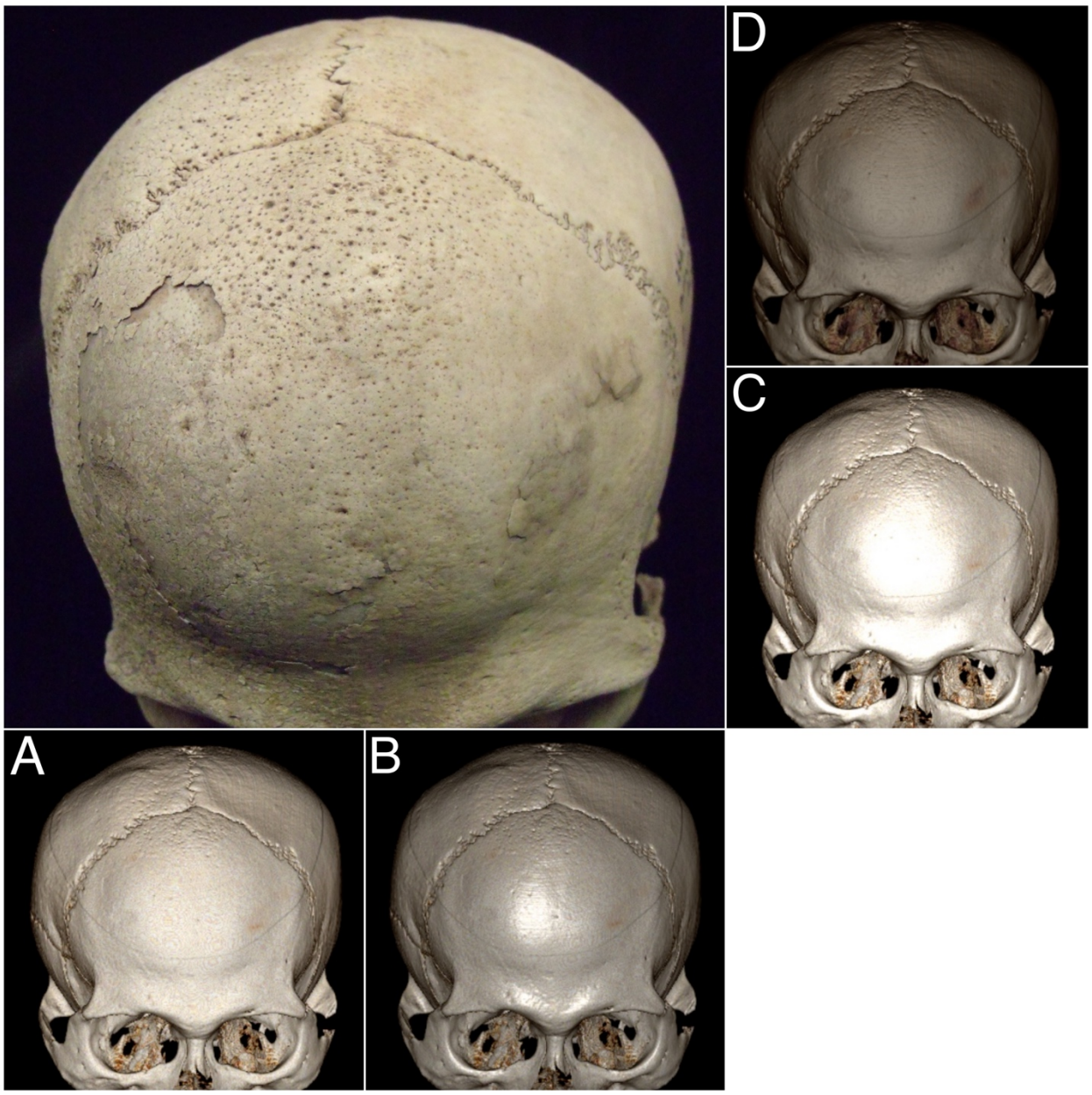

Figure S2. 3 Additional comparison of observer settings for 3-D volume-rendered images, with photographic view for reference. Letters correspond to the same settings as Fig. S2.

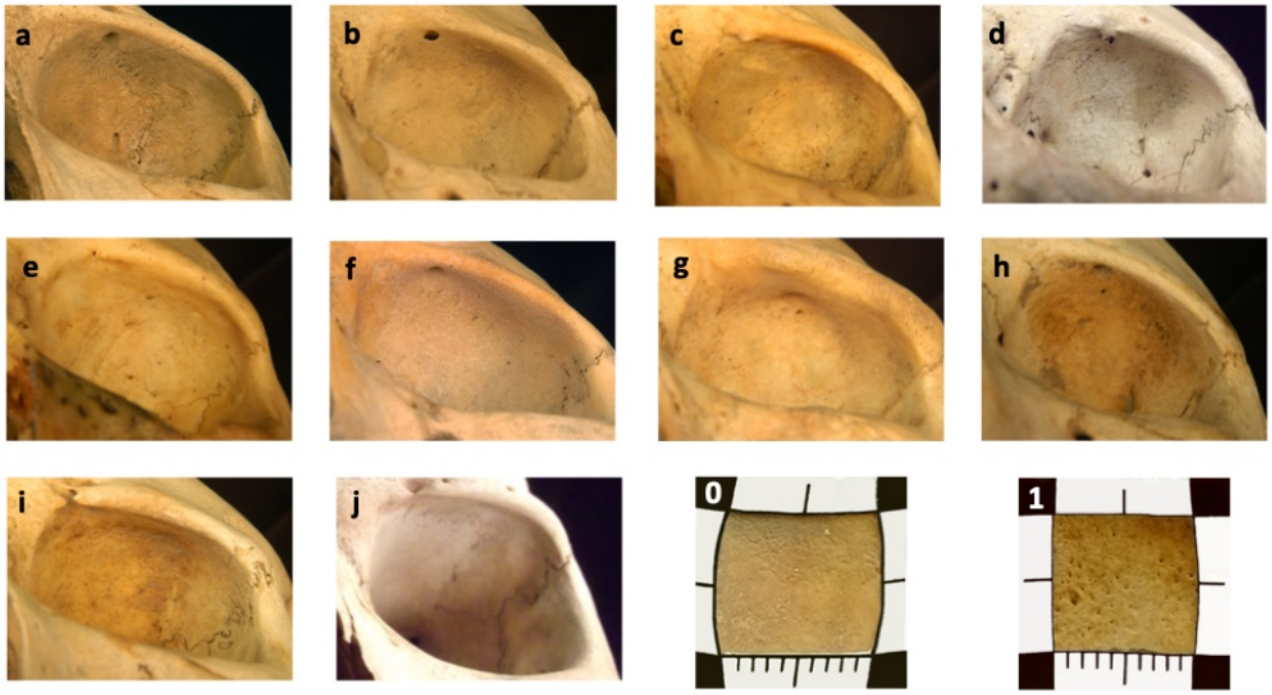

Figure S2. 4 Orbital roofs for which observers disagreed on the presence/absence of lesions from photographic assessment. In the bottom right are the visual references for categories 0 – absence of lesion, and 1— scattered fine foramina (from Rinaldo and Zedda, 2019) used during lesion assessment.

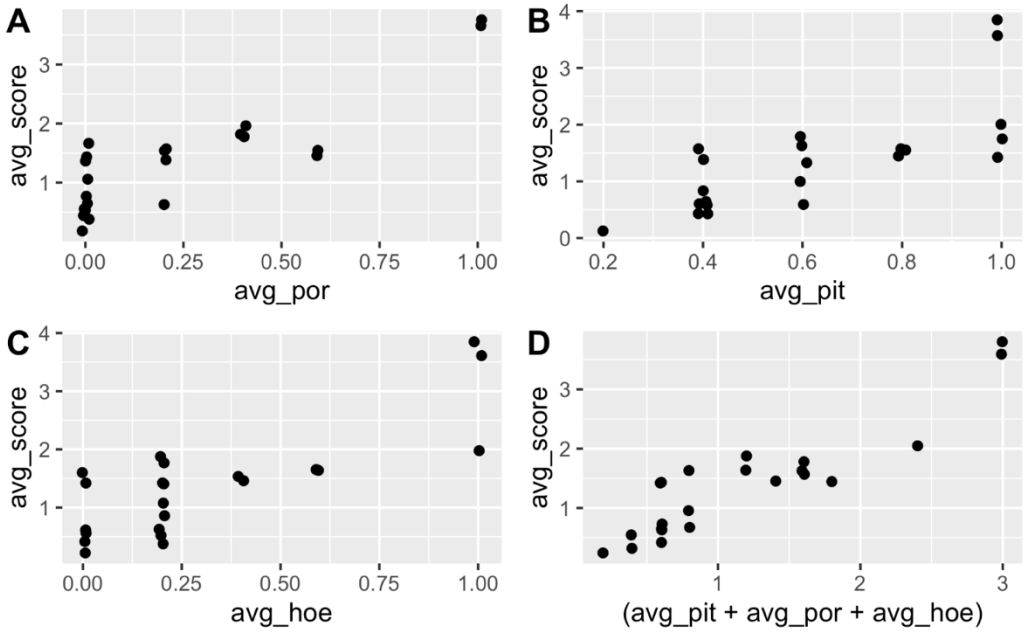

Figure S2. 5 Correlation of average observer scores for each lesion-related trait on 2-D CT MPR with average observer scores for lesion morphology on 3-D CT volume rendering. A) Porosity was a reliable indicator of lesion visibility on 3-D volume rendering but was only present in a minority of cases. B) Observer agreement on presence of ectocranial pitting is a clear positive predictor of lesion visibility on 3-D volume rendering. C) Hair-on-end sign (radial orientation of diploic trabeculae) is an indicator of extreme cranial changes. Attempts to identify subtle hair-on-end expression do not produce clear results. D) The strongest relationship between lesion-related traits and lesion visibility on 3-D volume rendered images is apparent when lesion-related traits are combined to create an aggregate lesion score with the 2-D MPR view.

Interobserver Agreement in MPR:

Scoring biases of individual observers are apparent in 2-D MPR. For assessments of orbital roof porosity, ectocranial pitting, and hair-on-end sign, one observer's scores differed distinctly from others, though the rogue observer was a different individual in each case. Of the lesion-related traits, interobserver agreement was most consistent for presence of vault porosity, with 73-82% agreement between any two observers (Fig. S6). This evaluation of observer agreement includes a fifth observer (here, observer 2), an inexperienced observer whose data were not included in the main text of the paper. Removing this observer's data did not change the results of the analyses, but including them introduced observer experience as a variable with an n of 1.

When lesion presence on CT 2-D MPR was defined by the identification of either porosity or ectocranial pitting, this viewing modality had the widest divergence of observer opinion on lesion presence (Fig. 5A). Observers agreed that ectocranial pitting was present in five cases, all of which had average scores > 1.5 from 3-D volume-rendered assessments. However, there was no case in which all observers agreed that pitting was absent (Fig. S5); observers had difficulty distinguishing between normal and pathological patterns of cortical surface texture in 2-D MPR's cross-sectional views.

Observer 1 tended to over-score orbital roof porosity relative to other observers. Observer 2 (inexperienced observer) over-scored hair-on-end sign. Observer 3 (radiologist) under-scored ectocranial pitting relative to individuals with osteological training. Observers 4 and 5 had remarkably high agreement on assessments of all lesion-related traits, which likely reflects their shared experience scoring cranial lesions from CT scans together on a previous project.

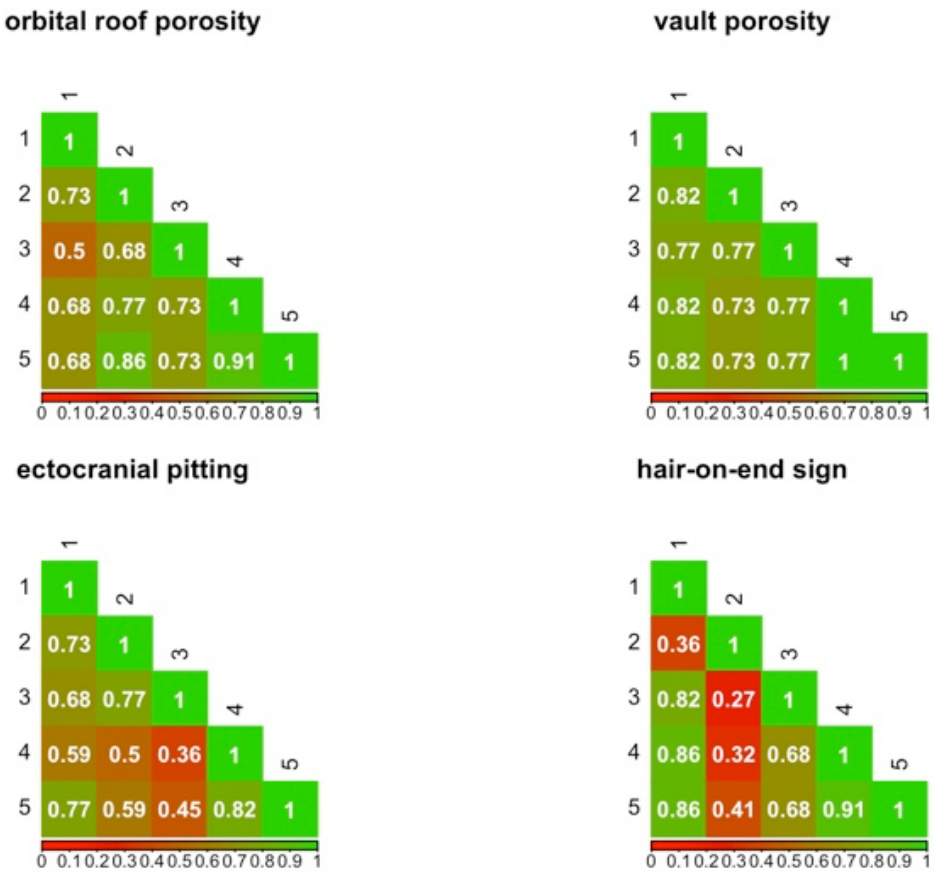

Figure S2. 6 Agreement between pairs of observers on presence/absence of lesion-related traits on CT 2-D MPR. Expected agreement by chance is 0.5. For each square in the correlation plot, border numbers to the left and above indicate which two observers' scores are being compared within the square. The fifth observer, not reported in the main text, was an undergraduate research assistant.

Table S2. 1 Estimated sample demographics and classification of porous orbital and vault lesions from multiple viewing modalities.

0 = no pathological lesion present; 1 = scattered fine foramina; 2 = large and small isolated foramina; 3 = foramina coalescing in a trabecular structure. Sex estimates are male (M), female (F), indeterminate (I).

Catalog No.	Sex	Age	Direct Observation		Photographs				3-D CT				2-D CT MPR		
					Orbits		Vault		Orbits		Vaults		Orbits	Vaults	
			Orbit score	Vault score	mean	SD	mean	SD	mean	SD	mean	SD	% obs. (n = 4) Reporting porosity	% obs. (n = 4) Reporting porosity	% obs. Reporting ectocranial pitting
264543	I	Young-Middle	2	3	1.75	0.96	3.75	0.5	2.25	2.06	4	0	100	100	100
266027	I	Young-Middle	0	3	1.75	0.5	3.75	0.5	2.75	1.26	3.75	0.5	100	100	100
266562	M	Older	0	3	0	0	3.25	0.5	1	0	0.75	0.5	100	0	25
267171	I	Middle-Older	0	3	1	0	2.5	1	0.33	0.58	2	0	50	25	50
267641	M	Older	1	3	1.5	0.58	2	0	1	1	1.5	1.29	75	25	75
268028	M	Older	0	3	1.5	1.29	3.5	0.58	1	0	1.75	0.5	75	50	25
266148	M	Older	2	2	1.75	0.5	2	0	1.75	1.5	1.5	0.58	100	0	50
266399	M	Older	1	2	1.5	1.29	2	0	2.75	1.26	2	0	100	25	100
266487	M	Middle	0	2	1	1.41	2	0	1.5	0.58	1.75	0.5	50	50	100
266741	M	Older	0	2	1	0	2	0	1.67	0.58	1.75	0.5	100	0	50
266748	M	Middle	3	2	3.5	0.58	2	0	3	0.82	1.75	0.5	100	0	75
267071	M	Middle-Older	0	2	0.25	0.5	2	0	1	0.82	1.75	0.5	25	0	75
267107	M	Middle	0	2	0.25	0.5	2	0	1	0.82	1.25	0.5	100	0	50
267150	I	Older	0	2	0.25	0.5	2	0	0.67	1.15	1.75	0.5	50	25	100

267200	M	Middle	2	2	2.25	0.5	1.5	0.58	0.33	0.58	1.5	0.58	100	0	25
267186	M	Young-Middle	1	1	1	0.82	1.25	0.5	1	1	0.5	0.58	100	0	25
267208	F	Young	0	1	1.25	0.96	1.75	0.5	0	0	0.75	0.5	0	0	25
266316	M	Older	3	0	2.25	0.5	1.5	0.58	1.5	1.29	0.75	0.96	100	0	25
266926	M	Older	0	0	0	0	0.75	0.5	0.67	0.58	1	1.41	50	0	25
267097	M	Middle-Older	0	0	0.25	0.5	1	0	1.33	1.53	0.5	0.58	25	0	25
267182	F	Older	0	0	0.25	0.5	0.75	0.5	0.75	0.96	0.75	0.5	50	25	50
267197	F	Older	0	0	0.5	0.58	1.25	0.5	0	0	0.25	0.5	75	0	0

Supplemental Information for Chapter III

Pitting/porosity was most common on the occipital bone (14.75%) and least common on the frontal bone (0.54%). When porous changes were present, the frontal and superior parietal bones invariably presented with fine, 'pinprick' porosity or pitting and were thus considered ambiguous cases, while larger more distinct foramina were seen on the posterior aspect of the parietal bones and on the squamous occipital bone. Cases of ambiguous fine porosity were noted almost exclusively among women, while larger more distinct foramina on the posterior parietal bones or occipital bones were more common among men, though this pattern was not statistically significant. While the limitations of CT visualization do lead to greater underreporting of lesions on the superior frontal and parietal bones (Anderson et al., 2021), other studies of cranial cribra in South American populations have also reported the posterior parietal bones and central occipital squama in particular as the most common sites of vault lesions (Beatrice, Soler, Reineke, & Martínez, 2021; Blom et al., 2005; Suby, 2014). Because radiological cross sections are more sensitive to expansive changes in the diploic space of the cranial vault than to surface porosity per se, clinical cases do not report much in the way of changes in the occipital squama, which does not have the same capacity as the frontal and parietal bones for expansion of the diploic space. In cases of acquired iron-deficiency anemia cranial changes are reported most often in the frontal squama, followed by the parietal bones (Stuart-Macadam, 1987).

Ectocranial pitting vs. true porosity: Ectocranial pitting is widely interpreted by bioarchaeologists as an advanced stage of healing porosity. Mann and Hunt (Mann & Hunt, 2005) note that it appears primarily in middle-aged adults. It can be difficult to distinguish pitting from porosity using traditional osteological methods of macroscopic observation, but CT provides a clear way to identify when apparent porosity on the cranial vault's outer table truly is the surface manifestation of perforations that extend into the diploe, and when porosity is more superficial. If ectocranial pitting is an advanced healing stage of cranial porosity acquired in early childhood, we should expect the proportion of porous cranial lesions that manifest as pitting rather than true porosity to grow increasing more common in older adults.

Ectocranial pitting is generally considered a more advanced stage of porous lesion remodeling than cranial vault porosity (Mensforth et al., 1978). Given this interpretation, we might expect that the proportion of lesions showing true porosity will decline with age and that ectocranial pitting will account for a correspondingly larger proportion of lesions among older individuals, but this was not the pattern observed in the current study. The proportion of pitting to porosity was roughly equivalent at all ages.
Assuming the lesions identified in Tsimane adults developed during childhood, several inferences can be drawn. First, ectocranial porosis is not associated with higher mortality risk for Tsimane adults older than 40 years. If it were, overall lesion frequency would decline with age. Second, the absence of an age-related shift from porosity to pitting suggests that individual variability may play a relatively large role in determining the retention of lesions over the lifespan and that complete remodeling (disappearance) of cranial vault lesions is unlikely at older ages.

Demographic patterns

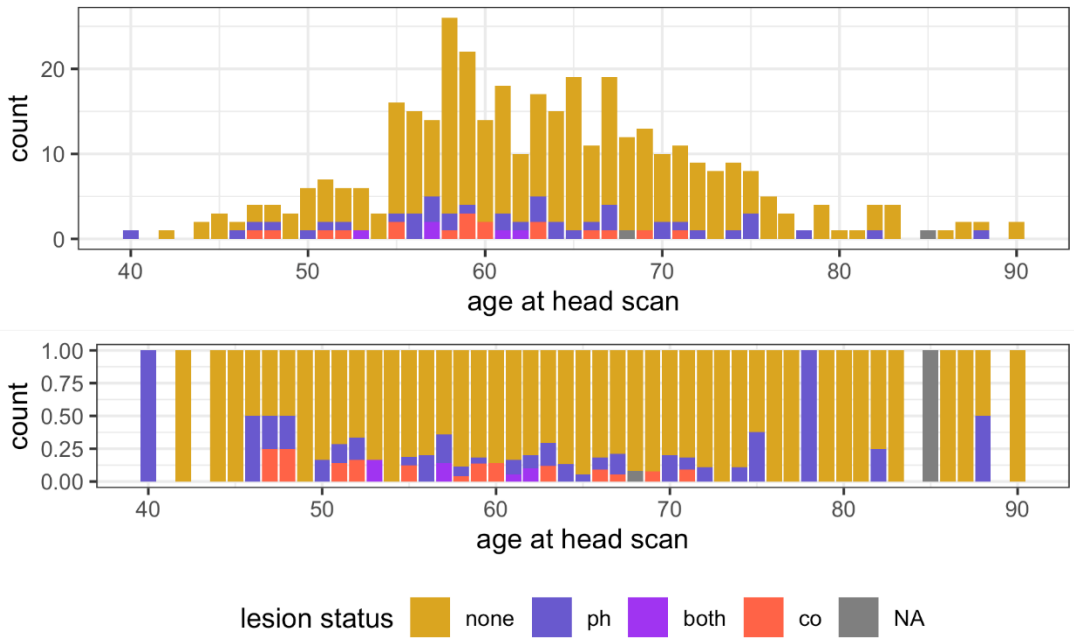

Figure S3. 1 Age distribution of porous cranial lesions in study sample. Top = raw counts, bottom = proportion of individuals at each age (single year intervals) with a given lesion status.

There are several possible explanations for cribra orbitalia's negative relationship with age. Lesions could be less observable at older ages because individuals with lesions face higher overall mortality risks than their non-lesioned peers and are thus less well represented in older cohorts. This is the interpretation typically made in archaeological contexts when cribra orbitalia is more common among younger individuals. Lesions in some individuals might also be obscured over a lifetime by skeletal remodeling. The rate at which lesions are obscured by remodeling has not yet been established, and skeletal remodeling likely renders lesions unobservable on CT scans faster than they become undetectable to direct observation of skeletal remains.

Between the date of scanning (2017-18) and March 2022, 11 of 375 study participants (aged 59-84 years) had died. Of these, one (aged 66 years) had cribra orbitalia and another (62 years) had cranial vault porosity.

Cribra orbitalia and thoracic BMD

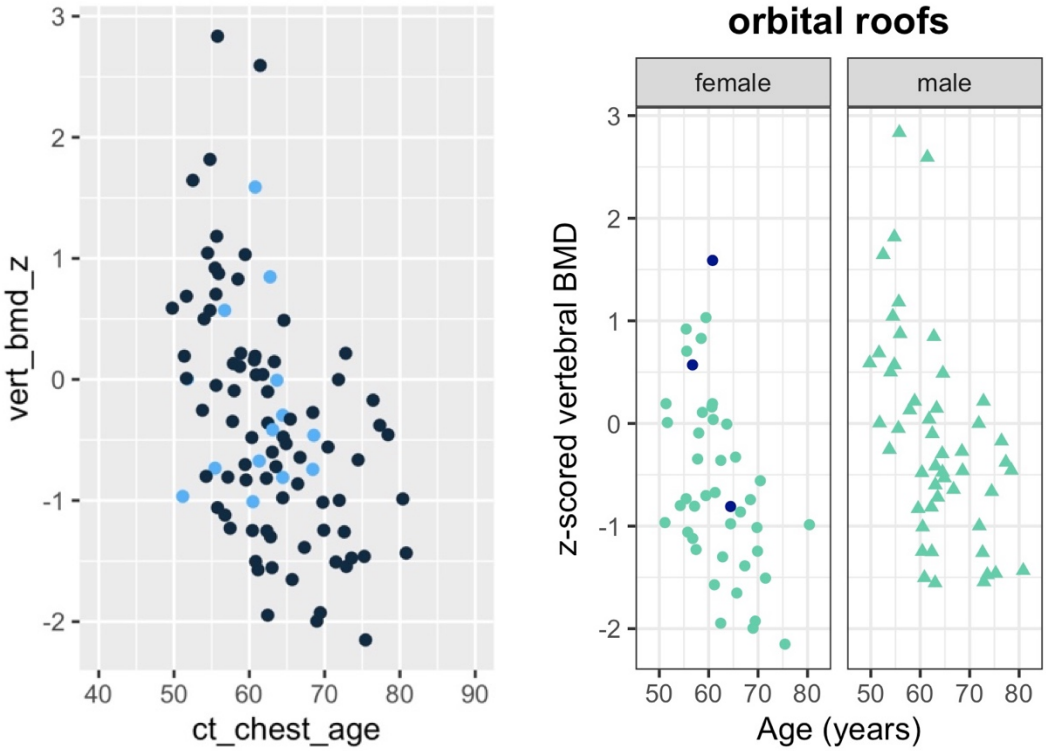

Figure S3. 2 Scatter plot of BMD in individuals with/without orbital roof porosity.When marginal/ambiguous cases of orbital roof porosity are considered in the lesion-present category (left: light blue), the distribution of thoracic BMD is consistent with the existence of a CT visibility threshold for thin orbital roofs, if low thoracic BMD is indicative of systemic bone loss that affects the cranial bones. Not even 'possible cribra orbitalia' cases were identified in individuals with thoracic vertebral bone mineral density less than 1 SD below the sample mean. Age-related systemic bone loss may contribute to this effect, and the absence of identified cases among low-BMD individuals may be due to the limitations of the scanner rather than a true absence of orbital roof porosity in this group. Unambiguous/clear cases are represented in dark blue on the righthand plot (n = 3).

Clear cases of cribra orbitalia were associated with vertebral BMD 1.04 (0.1-2.0) standard standard deviations higher, but only three individuals with BMD measures had certain cases of cribra. When 'possible' cribra cases were removed from the analysis rather than coded as absent of lesions, the effect size remained stable but the credibility interval crossed zero (-0.01, 2.06). When 'possible' cribra cases were included as positive cases, the effect disappeared, but a visual exploration does appear to show a threshold at 1 SD below mean BMD. For individuals with vertebral BMD lower than this, not even possible cases were reported.

Finally, the lower frequency of lesions at older ages corresponds with greater numbers of ambiguous cases of orbital roof lesions and may be a product of the limitations of CT, exacerbated by age-related thinning of the orbital roofs. Thinning of the orbital roof is a known change in cranial morphology associated with ageing, and regions of the skull substantially

thinner than the scans' slice width (here, 0.625 mm) are not captured well by CT. As a result, it may be more difficult to visualize orbital lesions in older individuals.

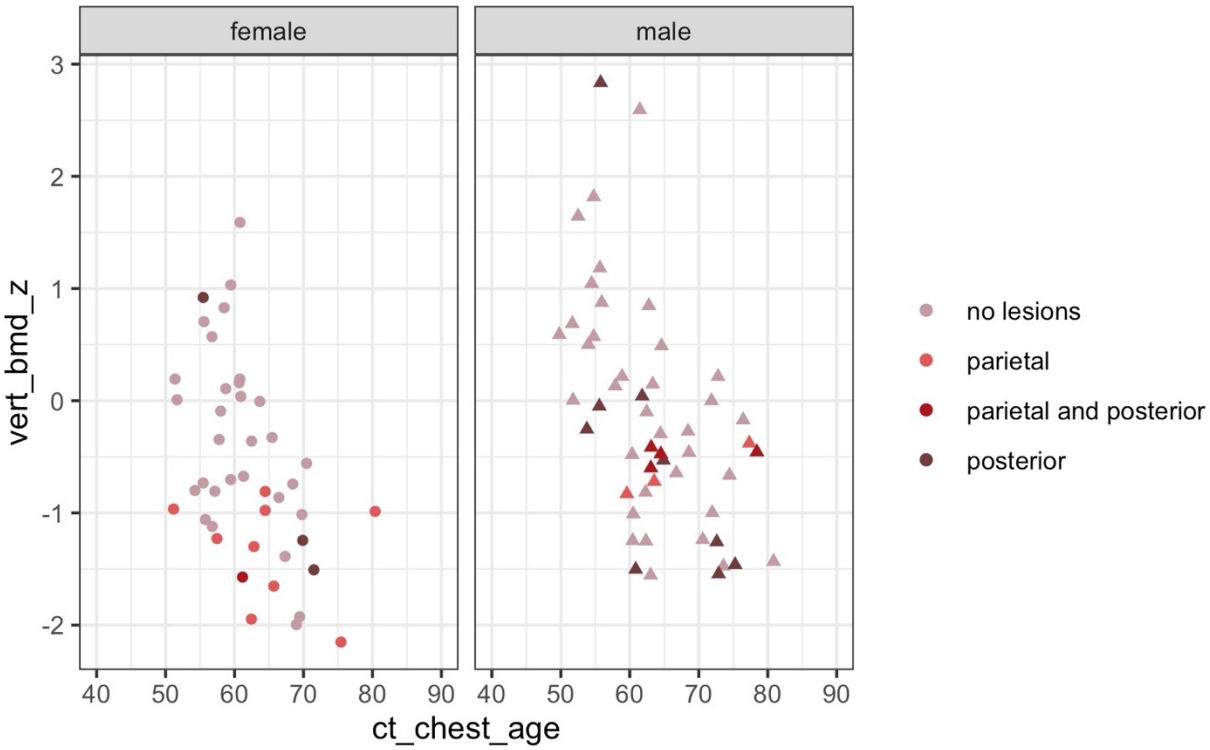

Figure S3. 3 Scatter plot of vertebral BMD and cranial porosity by affected bone. The relationship between cranial vault porosity and vertebral BMD does not differ by sex and shows clear pattern based on the spatial distribution of porosity.

Tuberculosis

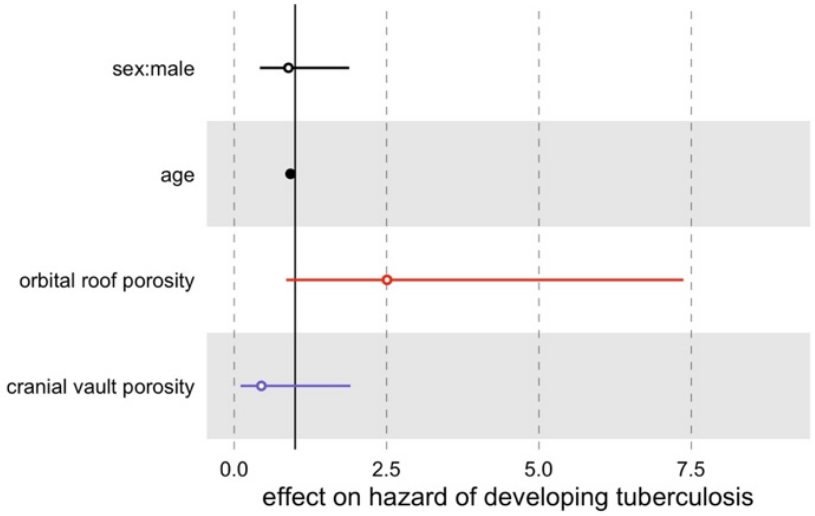

Figure S3. 4 Estimated effect (and 95% CI) of covariates on time to first diagnosis of tuberculosis.

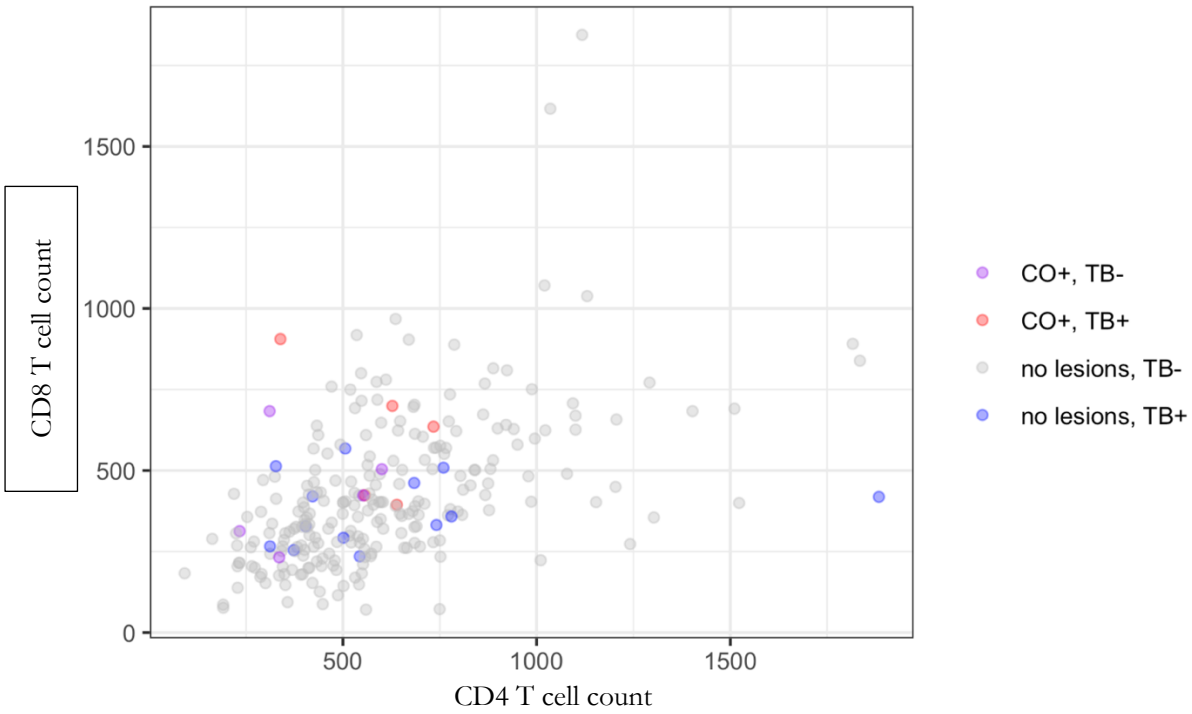

Figure S3. 5 Scatter plot of CD4 and CD8 T cell counts, by tuberculosis/cribra status

Diagnoses of the head and eyes

In addition to respiratory conditions, we examined associations with diagnoses of the eyes and head. Cranial vault porosity is associated with double the odds of reporting headaches during

medical visits, though in general headaches were rarely reported (Table S3.1). This finding is surprising in light of the wide array of conditions capable of causing headaches, and no concurrent diagnoses could explain this association.

Of course, lesions comprised of new bone formation in response to local inflammation, infection, or trauma need not be confined to childhood (Ortner, 2003), though bone is more responsive to stimuli during growth and development. Because it is possible (though not probable) for PCLs to be caused at later ages by porous new bone, we tested associations between PCLs and medical conditions of the eyes and head. Localized responses should be considered particularly in the case of unilateral lesions. However, because orbital lesion presence on the scans can be determined with higher certainty than orbital lesion absence, a clear distinction between unilateral and bilateral cases could not be made using CT scans.

To assess whether localized eye conditions might be the primary cause of cribra orbitalia among the adults in this study, we tested whether orbital lesions were associated with eye infections or pterygium. Pterygium is a highly vascularized fleshy growth on the conjunctival surface of the eye. Risk factors for pterygium are high chronic levels of exposure to sun, wind, and dust (Solomon, 2006). While it has not been clinically linked to orbital roof porosity, the increased ocular angiogenesis involved in its development and the high prevalence of pterygium in the study population make a possible connection worth investigating. Nevertheless, cribra orbitalia was not associated with any diagnosed eye conditions (Table S3.2; Fig. S3.4).

Table S3. 1 Odds ratios and 95% credibility intervals showing the strength and certainty of association between porous cranial lesions and diagnoses of respiratory infection and conditions of the eyes and head. Models controlled for age and sex.

		Orbital roof porosity		*Cranial vault porosity*	
Diagnosis	% of medical visits with diagnosis	OR	95% CI	OR	95% CI
respiratory infection	5.72	0.7	(0.3, 1.53)	1.18	(0.7, 1.93)
pterygium	4.97	1.16	(0.47, 2.64)	0.78	(0.42, 1.4)
eye infection	2.06	1.33	(0.36, 4.22)	1.13	(0.48, 2.54)
headache	1.97	1.53	(0.51, 3.95)	1.98	(1.03, 3.85)

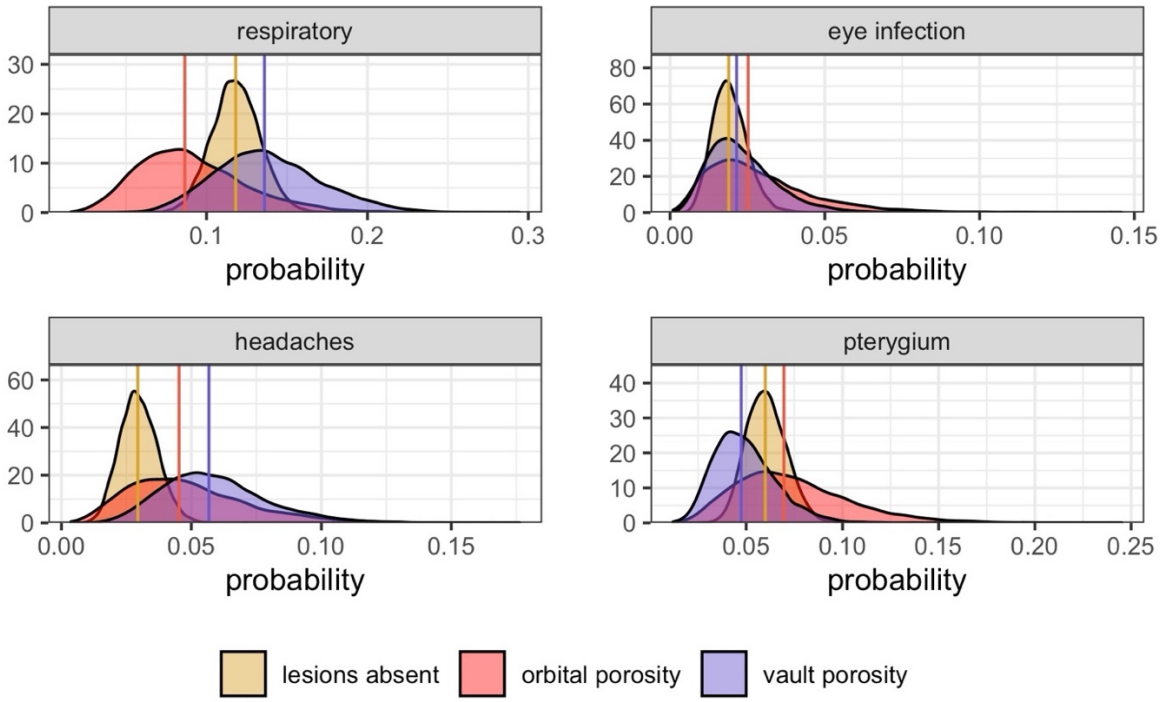

Figure S3. 6 Distributions for posterior predicted mean probabilities of medical diagnoses, conditional on lesion status. Vertical lines indicate the median estimate of the posterior mean. Values were predicted for a female with age at the sample mean.

For all outcome variables we report the median values and 5th and 95th quantile of age- and sex-standardized posterior parameter distributions for each lesion status (no cranial lesions, orbital porosity, vault porosity). The effect of cranial lesions on medical diagnoses is reported as the predicted probability of diagnosis, given lesion status (Table S3.2), while the predicted effects of lesions on hematological measures are reported in each outcome variable's unit of measure

Table S3. 2 Mean predicted probabilities for models of disease incidence and the magnitude of predicted differences (deltas) with or without cranial lesions.

	lesions absent		*orbital porosity*		*vault porosity*		*delta, orbital porosity*		*delta, vault porosity*	
	Predicted probability	95% CI	Predicted probability	95% CI	Predicted probability	95% CI	Predicted probability	95% CI	Predicted probability	95% CI
respiratory infection	0.12	(0.09, 0.15)	0.09	(0.03, 0.16)	0.14	(0.08, 0.21)	-26.50%	(-76.3%, 33.8%)	15.20%	(-31.2%, 70.5%)
pterygium	0.06	(0.04, 0.08)	0.07	(0.02, 0.13)	0.05	(0.02, 0.08)	16.30%	(-59.5%, 125%)	-20.60%	(-64.4%, 30%)
eye infection	0.02	(0.01, 0.03)	0.03	(0, 0.06)	0.02	(0, 0.05)	33.20%	(-93.9%, 237.3%)	13.40%	(-64.1%, 125.8%)
headache	0.03	(0.02, 0.04)	0.05	(0.01, 0.1)	0.06	(0.02, 0.1)	52.90%	(-65.2%, 222%)	91.10%	(-9.3%, 223.6%)

Table S3. 3 Predicted mean values and 95% credibility interval for a female at the average sample age, given varying lesion status (no lesions, orbital porosity, cranial vault porosity). Deltas provide an intuitive way to interpret effect size, showing the difference between the distributions of predicted means for individuals with and without lesions. (e.g., the predicted mean B cell count for an average-aged woman in the sample is 30% (3.4-56%) lower if she has orbital roof porosity than if she has no porous cranial lesions).

	lesions absent		*Orbital roof porosity*				*Cranial vault porosity*			
	predicted mean	95% CI	predicted mean	95% CI	delta	95% CI	predicted mean	95% CI	delta	95% CI
hemoglobin (g/dL)	12.69	(12.55, 12.85)	12.56	(12.1, 12.99)	-1%	(-4.5%, 2.5%)	12.55	(12.23, 12.88)	-1.10%	(-3.5%, 1.3%)
ESR (mm/hr)	29.7	(27.46, 32.02)	25.01	(19.79, 31.14)	-15.80%	(-33.5%, 5.4%)	28.9	(24.36, 33.67)	-2.60%	(-17.2%, 12.4%)
eosinophils (cells/uL)	1316	(1212, 1417)	1199	(950, 1495)	-8.80%	(-27.8%, 14.4%)	1262	(1063, 1480)	-4.10%	(-19.5%, 10.9%)
neutrophils (cells/uL)	4476	(4327, 4639)	4098	(3695, 4528)	-8.40%	(-18.1%, 1.3%)	4327	(4015, 4658)	-3.30%	(-10.1%, 3.4%)
lymphocytes (cells/uL)	2508	(2428, 2603)	2633	(2368, 2919)	5%	(-6%, 16.2%)	2535	(2352, 2731)	1.10%	(-6.1%, 8%)
Natural Killer cells (cells/uL)	518	(466, 575)	587	(392, 813)	13.40%	(-24.7%, 57.3%)	574	(444, 717)	10.80%	(-14.7%, 35.9%)

B cells (cells/uL)	350	(313, 388)	244	(163, 336)	-30%	(-56%, -3.4%)	335	(261, 419)	-4.20%	(-25.3%, 17.5%)
naive CD4 T cells (cells/uL)	76	(62, 91)	38	(18, 68)	-49.10%	(-82.2%, -6.4%)	75	(46, 110)	-1.10%	(-38.4%, 43.2%)
naive CD8 T cells (cells/uL	193	(165, 227)	229	(120, 364)	18.70%	(-37.6%, 87.9%)	208	(141, 288)	7.30%	(-24.8%, 47.7%)
total CD4:CD8 ratio	1.61	(1.44, 1.76)	0.99	(0.69, 1.34)	-38.30%	(-58.6%, -16%)	1.41	(1.12, 1.71)	-12.30%	(-30%, 5.7%)

Table S3. 4 Standardized betas and 95% CI's of models from Table 3.4, controlling for current infection

(logged neutrophil count).

	Orbital roof porosity		*Cranial vault porosity*	
	beta	95%CI	beta	95%CI
Dm	0.01	(-0.16, 0.17)	0.01	(-0.11, 0.13)
hemoglobin	-0.08	(-0.36, 0.2)	-0.06	(-0.26, 0.15)
ESR	-0.17	(-0.48, 0.15)	0.03	(-0.18, 0.24)
WBC	-0.22	(-0.49, 0.05)	0	(-0.19, 0.19)
eosinophils	-0.22	(-0.51, 0.07)	0.01	(-0.19, 0.2)
neutrophils	-0.25	(-0.52, 0.02)	0.02	(-0.16, 0.19)
lymphocytes	-0.03	(-0.28, 0.22)	0.07	(-0.09, 0.24)
NK cells	-0.26	(-1.11, 0.56)	0.21	(-0.25, 0.66)
B cells	-0.5	(-1.14, 0.14)	-0.08	(-0.48, 0.32)
total CD4 cells	-0.81	(-1.59, -0.05)	-0.22	(-0.68, 0.23)
naive CD4 cells	-0.7	(-1.48, 0.07)	-0.1	(-0.57, 0.36)
non-naive CD4 cells	-0.71	(-1.5, 0.07)	-0.27	(-0.74, 0.2)
total CD8 cells	0.42	(-0.4, 1.22)	-0.11	(-0.57, 0.35)
naive CD8 cells	0.04	(-0.79, 0.88)	-0.04	(-0.5, 0.42)
non-naive CD8 cells	0.72	(-0.14, 1.57)	-0.1	(-0.57, 0.39)
CD4/CD8 ratio	-0.78	(-1.52, -0.06)	-0.19	(-0.61, 0.24)

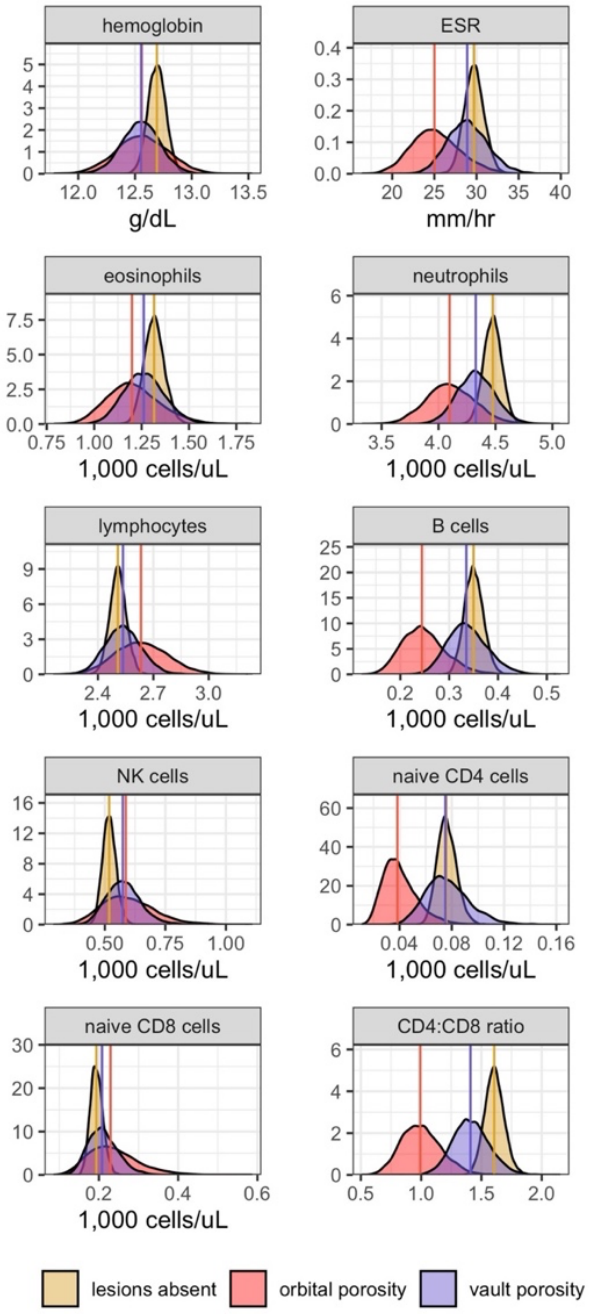

Figure S3. 7 Distributions for posterior predicted mean probabilities of hematological measures, with and without porous cranial lesions. Vertical lines indicate the median estimate of the posterior mean. Values were predicted for a female with age at the sample mean.

Table S3. 5 Descriptive statistics for continuous dependent variables

variable	***median***	***(5th, 95th) percentile***	***n (obs)***	***n (people)***	***median obs/person***	***SD obs/person***	***dates***
physiological dysregulation (Dm)	44.1	(11.9, 136.6)	3045	374	7	4.84	2002 - 2020
n biomarkers contributing to Dm	17	(2, 31)	3045	374	7	4.84	2002 - 2020
hemoglobin (g/dL)	13.2	(10.9, 15.4)	2248	374	6	2.97	2004 - 2020
ESR (mm/h)	25	(6, 73)	1960	374	5	2.87	2004 - 2020
total leukocytes (cells/uL)	9000	(5500, 14100)	2238	374	6	2.95	2004 - 2020
neutrophils (cells/uL)	4678	(2688.4, 8213.8)	1984	374	5	2.82	2004 - 2020
eosinophils (cells/uL)	1328	(318.3, 3552.7)	1984	374	5	2.82	2004 - 2020
lymphocytes (cells/uL)	2500	(1365.4, 4099.4)	1984	374	5	2.82	2004 - 2020
natural killer cells (cells/uL)	596.2	(230.7, 1313.2)	252	195	1	0.52	2011 - 2013
B cells (cells/uL)	320.5	(135.3, 752.8)	252	195	1	0.52	2011 - 2013
CD4 T cells (cells/uL)	552	(258.7, 1122.8)	253	196	1	0.52	2011 - 2013
naive CD4 cells (cells/uL)	66.8	(12, 277.7)	244	190	1	0.51	2011 - 2013
non-naive CD4 cells (cells/uL)	466.7	(226.4, 978.9)	244	190	1	0.51	2011 - 2013
CD8 T cells (cells/uL)	386.6	(152.9, 803.8)	253	196	1	0.52	2011 - 2013
naive CD8 cells (cells/uL)	186	(66.8, 504.7)	244	190	1	0.51	2011 - 2013
non-naive CD8 cells (cells/uL)	186.3	(65.7, 391.1)	244	190	1	0.51	2011 - 2013
thoracic vertebral BMD (mg/cm^3)	143.5	(97.4, 216.7)	94	94	1	0	2014 - 2015

Table S3. 6 Descriptive statistics for presence/absence variables

Physician Diagnosis	***period prevalence***	***n (obs)***	***n (people)***	***median obs/person***	***SD obs/person***	***dates (period years)***
headache (yes/no)	4.20%	29171	371	55	66.87	2005 - 2020
eye infection/inflammation (yes/no)	4.60%	29171	371	55	66.87	2005 - 2020
pterygium (yes/no)	10%	29171	371	55	66.87	2005 - 2020
respiratory infection (yes/no)	13.40%	29228	371	55	66.99	2002 - 2020
Functional disability assessment						
cannot maintain stability in tandem stance w/eyes closed	28.90%	1341	349	4	1.87	2007 - 2020
cannot balance steadily on one leg (other foot)	16.30%	1329	347	4	1.88	2007 - 2020
cannot balance steadily on one leg	16.00%	1336	350	4	1.87	2007 - 2020
needs multiple chances to stand up from chair	10.30%	1307	350	4	1.88	2007 - 2020
cannot maintain stability in tandem stance w/eyes open	5.70%	1341	349	4	1.88	2007 - 2020
cannot complete short rapid walk	3.30%	1349	348	4	1.89	2007 - 2020
cannot bend to pick up a pencil on the ground	2.70%	1351	349	4	1.89	2007 - 2020
cannot maintain stability in semi-tandem stance	1.60%	774	293	3	1.27	2007 - 2013

Table S3. 7 Fixed effects from hazard model of functional disability displayed in Fig. 3.5:
coxph(formula = Surv(start_age, age, disability, type = "counting") ~ co + ph_sure + sex + task, data = frailty, cluster = pid)

	coef	exp(coef)	se(coef)	robust se	z	Pr(>|z|)	
Orbital roof porosity	0.116	1.123	0.168	0.213	0.542	0.588	

Cranial vault porosity	-0.102	0.903	0.097	0.216	-0.470	0.638	
Sex:male	-0.388	0.678	0.062	0.116	-3.334	0.001	***
taskdisability_semitandem	-1.281	0.278	0.302	0.326	-3.936	<0.001	***
taskdisability_tandem	-0.581	0.559	0.145	0.152	-3.831	<0.001	***
taskdisability_blindtandem	1.051	2.861	0.101	0.088	11.898	<0.001	***
taskdisability_legstand1	0.459	1.583	0.111	0.112	4.117	<0.001	***
taskdisability_legstand2	0.485	1.624	0.111	0.116	4.179	<0.001	***
taskdisability_pencil_pickup	-1.400	0.247	0.191	0.165	-8.502	<0.001	***
taskdisability_rapidwalk	-1.240	0.289	0.179	0.193	-6.414	<0.001	***

Table S3. 8 Mixed effect cox proportional hazards model with random effects for individual identity and specific task in the battery of functional ability assessments. Note a slightly greater effect of both orbital and vault porosity on functional disability, but the result is still far from statistical significance. This model is perhaps a better fit for the structure of the data than the model reported above but does not lend itself as easily to plotting model results.

Model: Surv(start_age, age, disability, type = "counting") ~ co + ph_sure + sex + (1 | pid) + (1 | task)

Fixed coefficients					
	coef	exp(coef)	se(coef)	z	p
Orbital roof porosity	0.331	1.392	0.368	0.900	0.370
Cranial vault porosity	-0.160	0.852	0.328	-0.490	0.630
Sex:male	-0.625	0.535	0.189	-3.310	0.001
Random effects					
Group	Variable	Std Dev	Variance		
Personal identity	Intercept	1.170	1.368		
Disability assessment task	Intercept	0.927	0.860		

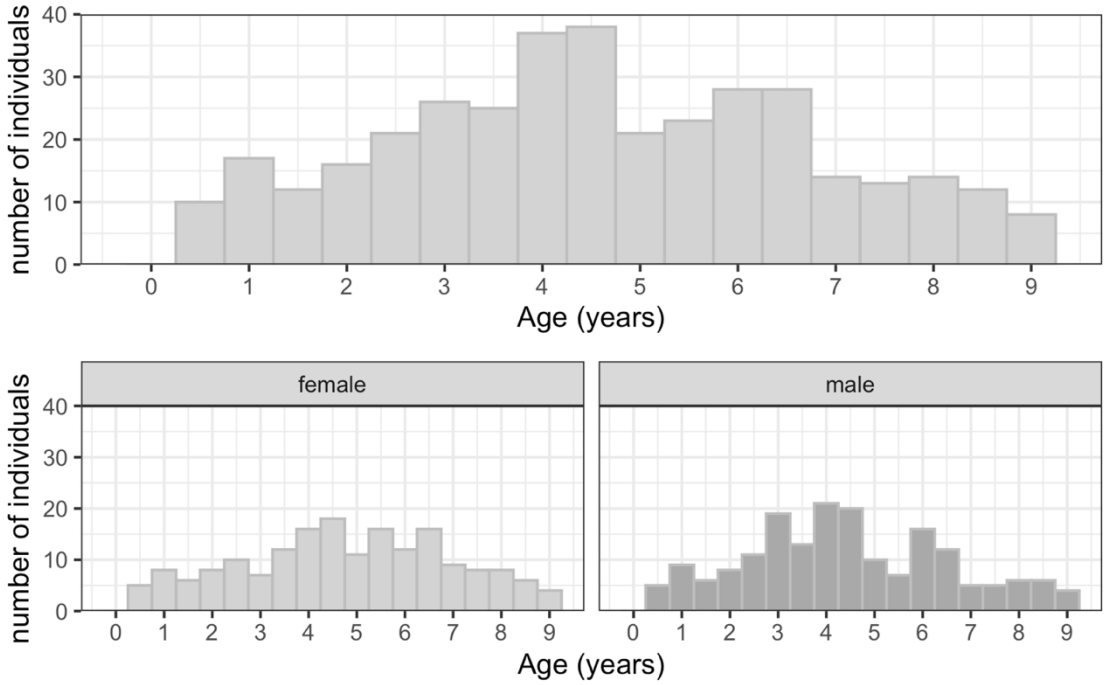

Figure S4. 1 Age and sex distribution of study participants.

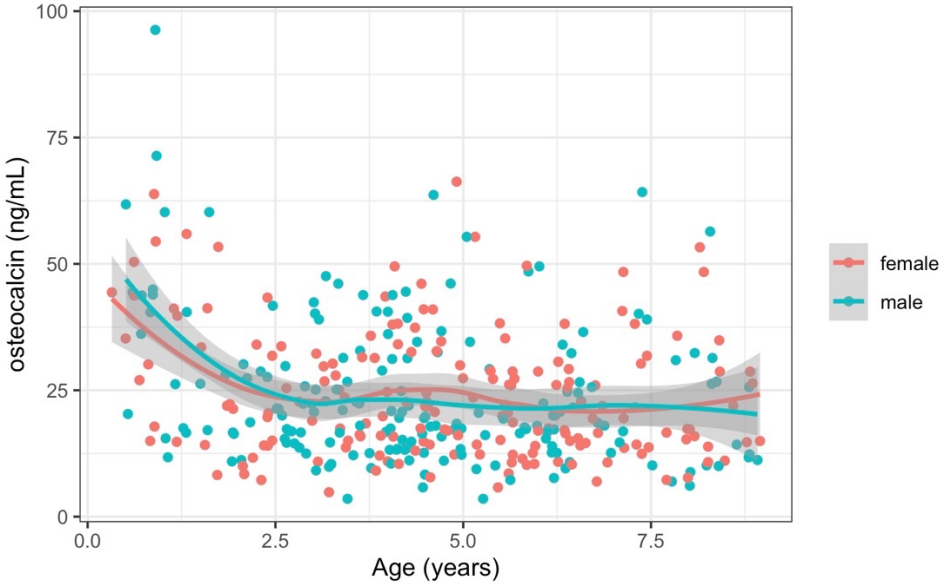

Figure S4. 2 Scatterplot and smoothed fit (Loess) for osteocalcin as a function of age. Results do not vary by sex.

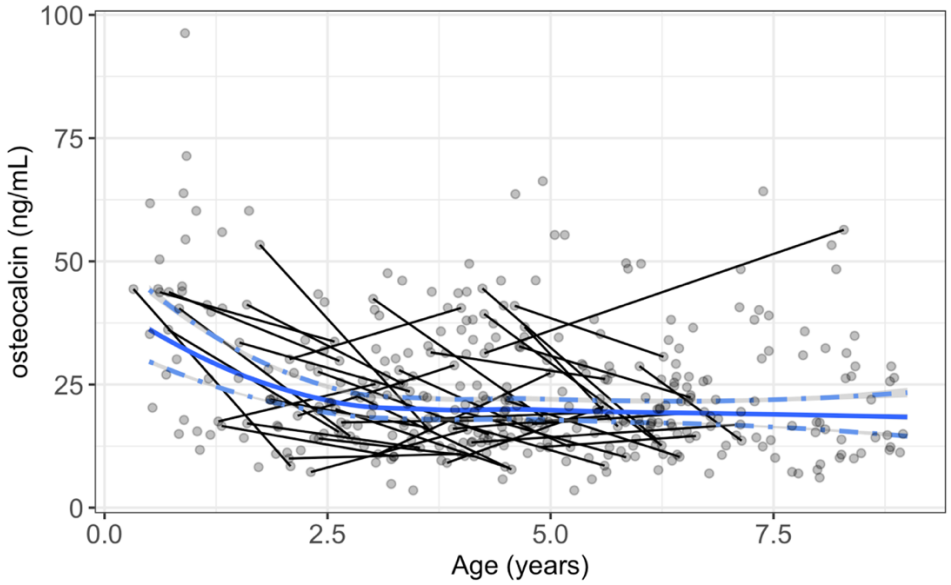

Figure S4. 3 Relationship between age and osteocalcin. Blue solid and dashed lines show model-predicted mean and 95% CI (see Fig. 1). Points are raw data, with black lines connecting repeat observations on the same individuals at different ages.

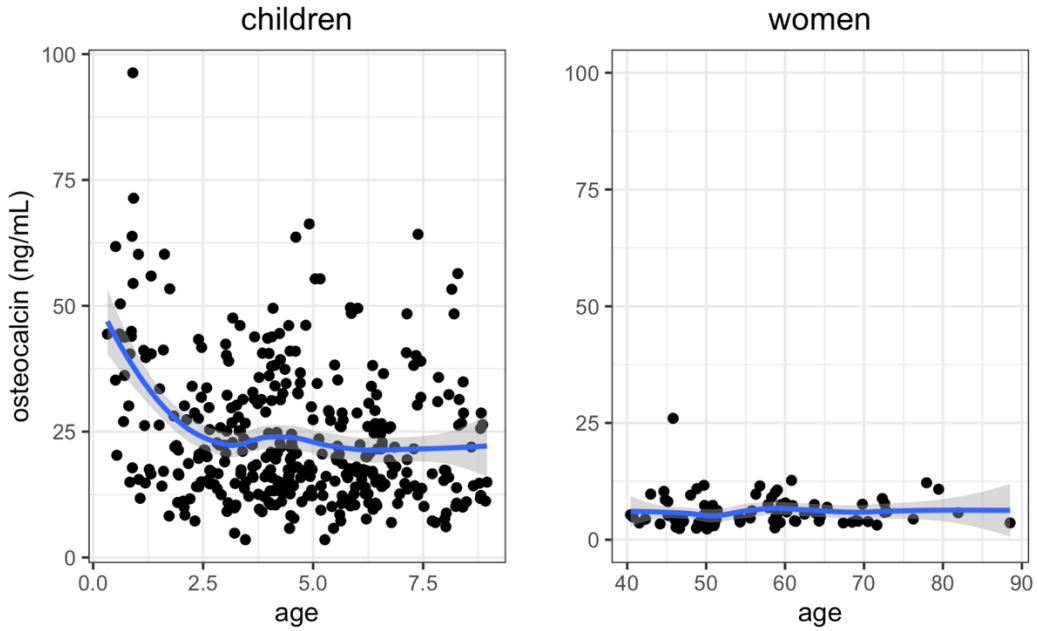

Figure S4. 4 Osteocalcin values in samples of Tsimane children (left) and older Tsimane women. (right).Child values are consistently higher than adult values, but some overlap with adult values exists in children older than two years.

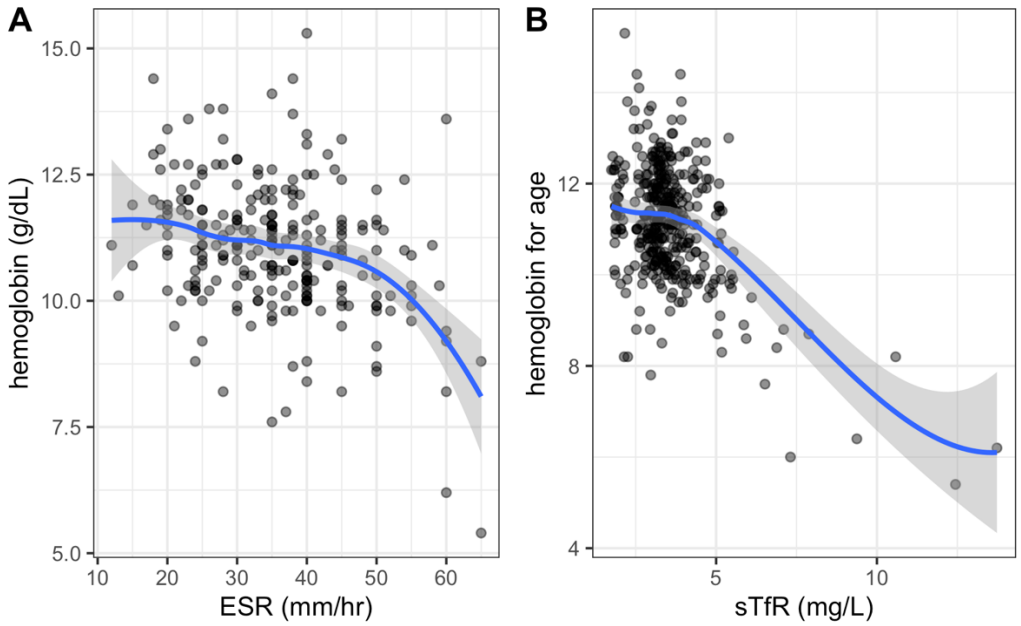

Figure S4. 5 Scatter plots and smoothed fit (Loess smooth) for both erythrocyte sedimentation rate (ESR) and serum transferrin receptor (sTfR) show an inverse association with hemoglobin.

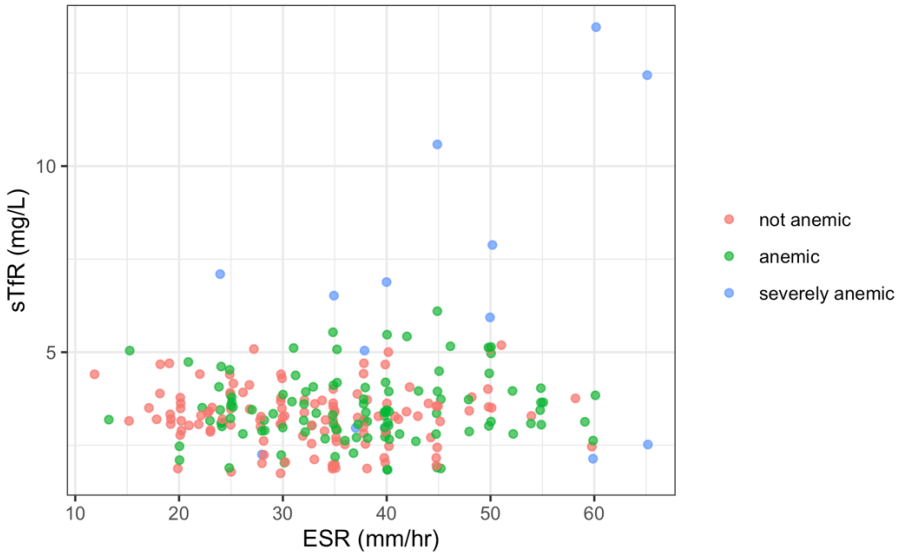

Figure S4. 6 Jittered scatter plot showing the joint contributions of inflammation (high ESR) and iron deficiency (high sTfR) to anemia severity.

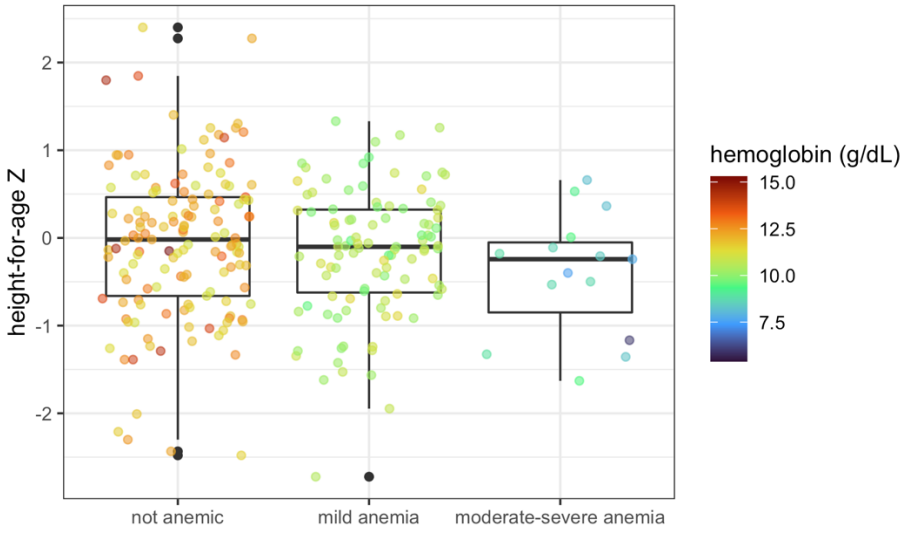

Figure S4. 7 Relationship between anemia and height. Individuals with moderate-severe anemia have an average height-for-age 0.35 SDs lower than non-anemic individuals, though this difference is not statistically significant (p = 0.11).

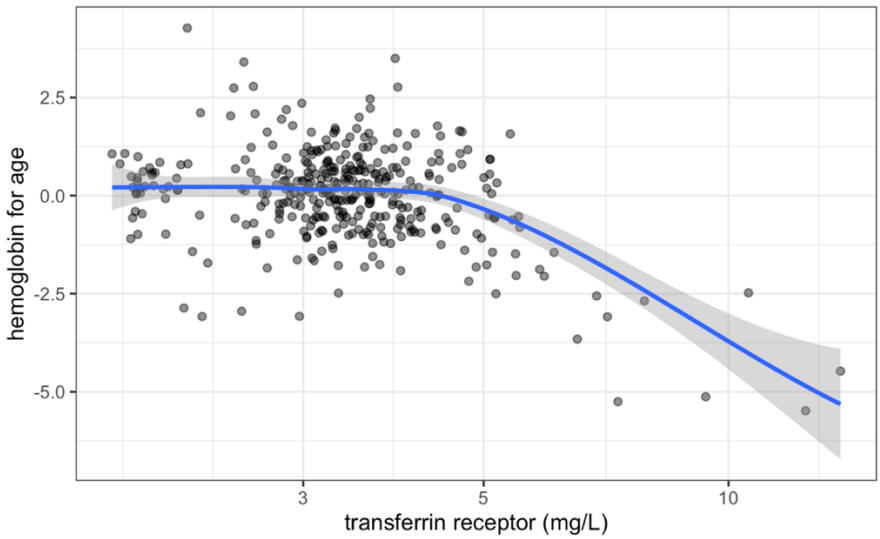

Figure S4. 8 After adjusting for the linear relationship between hemoglobin and age, the negative effect of iron deficiency on expected hemoglobin for age becomes visible at a TfR concentration of ~4.5 mg/L. Y-axis values are difference from predicted mean hemoglobin for age, in g/dL. This pattern indicates that the 6.7 mg/L TfR threshold for diagnosing iron deficiency used by McDade and Shell-Duncan (2002) is conservative for this sample. Since iron deficiency occurs significantly before its effects on hemoglobin start to manifest, this plot also suggests that iron deficiency occurs at a TfR value less than 4.5 mg/L.

Table S1.

Table S4. 1 Spearman's correlation coefficients for continuous variables. Blue cells in upper right show correlations for children aged 0.5-2.5 years. White cells in bottom left show correlations for children aged 2.5-8 years.

	Age	hemoglobin (g/dL)	sTfR (mg/L)	ESR (mm/hr)	CRP (log-mg/L)	osteocalcin (log-ng/mL)	height-for-age Z	weight-for-height Z
Age	1	0.234	0.065	-0.264	-0.134	-0.463	-0.046	0.214
hemoglobin	0.211	1	-0.580	-0.389	-0.114	0.053	0.146	-0.035
sTfR (mg/L)	-0.066	-0.438	1	0.317	0.136	-0.171	0.131	0.013
ESR (mm/hr)	-0.257	-0.304	0.113	1	0.526	0.119	0.107	-0.043
CRP (log-mg/L)	0.001	-0.081	0.073	0.056	1	-0.034	-0.084	0.009
osteocalcin (log-ng/mL)	-0.062	0.059	-0.116	-0.174	0.070	1	-0.024	-0.263
height-for-age Z	0.105	0.111	0.040	-0.020	-0.070	0.021	1	-0.501
weight-for-height Z	-0.061	-0.044	0.025	-0.011	-0.058	0.024	-0.004	1

Table S4. 2 Generalized linear model of log-osteocalcin-for-age as a function of standardized weight-for-height and height-for-age.

Question	**n**	**predictor**	**beta**	**SE**	**p-value**
Are height-for-age or weight-for-height associated with osteocalcin?	256	(Intercept)	-0.086	0.065	0.182
		WFHz	0.013	0.061	0.829
		HFAz	0.027	0.079	0.736

Studies of obese adolescents report a negative association between BMI and osteocalcin, but studies of children with more moderate BMIs do not tend to find an association between osteocalcin and weight-for-height (Ambroszkiewicz, Gajewska, Rowicka, Klemarczyk, & Chelchowska, 2018; Bayer, 2014; F. C. Madimenos et al., 2020; Rauchenzauner et al., 2007). We found no evidence of an association with weight-for-height among Tsimane children, among whom overweight BMIs are exceedingly rare.

Positive correlation with height-for-age is often reported during the adolescent growth spurt (Johansen et al., 1988; Paldánius PM, Ivaska KK, Mäkitie O, 2021), though not universally (Abdul-Hussein et al., 2021; Rauchenzauner et al., 2007). Among Tsimane children aged 4 months to 8 years, osteocalcin concentrations are not associated with height-for-age (Table S2), even for rapidly growing individuals younger than 2.5 years. In Shuar children aged 2-15 years osteocalcin is positively associated with height-for-age (F. C. Madimenos et al., 2020). This difference is consistent with a greater role for osteocalcin as a moderator of energy availability in

the high-pathogen Tsimane environment, but it may also be due to the influence of the adolescent growth spurt among the older Shuar participants.

Appendix References

Abdul-Hussein, A., Kareem, A., Tewari, S., Bergeron, J., Briollais, L., Challis, J. R. G., … Connor, K. L. (2021). Early life risk and resiliency factors and their influences on developmental outcomes and disease pathways: A rapid evidence review of systematic reviews and meta-analyses. *Journal of Developmental Origins of Health and Disease*, *12*(3), 357–372. https://doi.org/10.1017/S2040174420000689

Ambroszkiewicz, J., Gajewska, J., Rowicka, G., Klemarczyk, W., & Chelchowska, M. (2018). Assessment of Biochemical Bone Turnover Markers and Bone Mineral Density in Thin and Normal-Weight Children. *Cartilage*, *9*(3), 255–262. https://doi.org/10.1177/1947603516686145

Anderson, A. S., Sutherland, M. L., O'Donnell, L., Hill, E. C., Hunt, D. R., Blackwell, A. D., & Gurven, M. D. (2021). Do computed tomography findings agree with traditional osteological examination? The case of porous cranial lesions. *International Journal of Paleopathology*, *33*(April), 209–219. https://doi.org/10.1016/j.ijpp.2021.04.008

Bayer, M. (2014). Reference values of osteocalcin and procollagen type I N-propeptide plasma levels in a healthy Central European population aged 0-18 years. *Osteoporosis International*, *25*(2), 729–736. https://doi.org/10.1007/s00198-013-2485-4

Beatrice, J. S., Soler, A., Reineke, R. C., & Martínez, D. E. (2021). Skeletal evidence of structural violence among undocumented migrants from Mexico and Central America. *American Journal of Physical Anthropology*, *176*(4), 584–605. https://doi.org/10.1002/ajpa.24391

Blom, D. E., Buikstra, J. E., Keng, L., Tomczak, P. D., Shoreman, E., & Stevens-Tuttle, D. (2005). Anemia and childhood mortality: Latitudinal patterning along the coast of pre-Columbian Peru. *American Journal of Physical Anthropology*, *127*(2), 152–169. https://doi.org/10.1002/ajpa.10431

Johansen, J. S., Giwercman, A., Hartwell, D., Nielsen, C. T., Price, P. A., Christiansen, C., & Skakkebaek, N. E. (1988). Serum Bone Gla-Protein as a Marker of Bone Growth in Children and Adolescents: Correlation With Age, Height, Serum Insulin-Like Growth Factor I, and Serum Testosterone. *The Journal of Clinical Endocrinology & Metabolism*, *67*(2), 273–278. https://doi.org/10.1210/jcem-67-2-273

Madimenos, F. C., Urlacher, S. S., Eick, G. N. Liebert, M. A., Cepon-Robins, T. J., Gildner, T. E., Sugiyama, L. S., & Snodgrass, J. J. (2020). Bone metabolism & immune function: A preliminary cross-sectional analysis of immune markers, osteocalcin, and TRACP-5b among indigenous Shuar youth. *American Journal of Human Biology*, *32*.

Mann, R. W., & Hunt, D. R. (2005). *Photographic regional atlas of bone disease: a guide to pathologic and normal variation in the human skeleton*. Springfield, Illinois: Charles C Thomas Publisher.

McDade, T. W., & Shell-Duncan, B. (2002). Whole blood collected on filter paper provides a minimally invasive method for assessing human transferrin receptor level. *The Journal of Nutrition*, *132*(12), 3760–3763.

Mensforth, R. P., Lovejoy, C. O., Lallo, J. W., & Armelagos, G. J. (1978). Part Two: The role of constitutional factors, diet, and infectious disease in the etiology of porotic hyperostosis and periosteal reactions in prehistoric infants and children. *Medical Anthropology*, *2*(April 2016), 1–59. https://doi.org/10.1080/01459740.1978.9986939

Ortner, D. J. (2003). *Identification of Pathological Conditions in Human Skeletal Remains* (2nd ed.). San Diego: Academic Press.

Paldánius PM, Ivaska KK, Mäkitie O, V. H. (2021). Serum and Urinary Osteocalcin in Healthy 7- to 19-Year-Old Finnish Children and Adolescents. *Frontiers in Pediatrics*, *24*(9), 610227. https://doi.org/10.3389/fped.2021.610227

Rauchenzauner, M., Schmid, A., Heinz-Erian, P., Kapelari, K., Falkensammer, G., Griesmacher, A., … Högler, W. (2007). Sex- and age-specific reference curves for serum markers of bone turnover in healthy children from 2 months to 18 years. *Journal of Clinical Endocrinology and Metabolism*, *92*(2), 443–449. https://doi.org/10.1210/jc.2006-1706

Rinaldo, N., Zedda, N., Bramanti, B., Rosa, I., & Gualdi-Russo, E. (2019). How reliable is the assessment of Porotic Hyperostosis and Cribra Orbitalia in skeletal human remains ? A methodological approach for quantitative verification by means of a new evaluation form. *Archaeological and Anthropological Sciences*, *11*(7), 3549–3559.

Solomon, A. S. (2006). Pterygium. *British Journal of Ophthalmology*, *90*(6), 664–665. https://doi.org/10.1136/bjo.2006.091892

Stuart-Macadam, P. (1987). A radiographic study of porotic hyperostosis. *American Journal of Physical Anthropology*, *74*(4), 511–520. https://doi.org/10.1002/ajpa.1330740409

Suby, J. a. (2014). Porotic hyperostosis and cribra orbitalia in human remains from southern Patagonia. *Anthropological Science*, *122*(July), 1–11. https://doi.org/10.1537/ase.140430