Infectious and metabolic diseases: a synergistic relationship

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Abstract

Palaeopathologists have a long history of recording and interpreting evidence for infectious and metabolic diseases seen globally in preserved bodies and skeletons from archaeological sites. People today often experience co-morbidities, as did our ancestors, but little specific research in paleopathology has addressed synergies between these two categories of disease. The chapter starts by introducing these health challenges from a clinical perspective, and then considers the types of evidence used to detect them in the past, and the many methods available for recording and interpretation (macroscopic, biomolecular, histological, imaging, parasite analysis). This is followed by exploring links between leprosy and tuberculosis and vitamin D deficiency, leprosy and osteopenia/osteoporosis, the Developmental Origins Hypothesis and metabolic and infectious disease, and Paget's disease of bone and infection. It is concluded that palaeopathology is in an excellent position, theoretically and methodologically, to contribute to our understanding of disease synergies in the past, thereby providing the evolutionary time depth for present understanding.

Keywords: vitamin D, scurvy, tuberculosis, leprosy, Paget's disease, osteoporosis, co-occurrence, context, evolution, Developmental Origins Hypothesis, palaeopathology

<A> Introductory background

Infectious and metabolic diseases have been extensively explored in palaeopathology using various methods, although previous editions of this volume did not include either category of disease. To date there has been very little research synthesizing the two despite many synergies existing in the clinical literature. This chapter considers some of these synergies and highlights future research potential, rather than covering basic aspects of these two categories of disease that have been covered in many palaeopathological and clinical texts (e.g. Aufderheide and Rodríguez-Martín 1998; Ortner 2003; Roberts and Manchester 2005; and see Resnick 2002 as an example of a clinical text). In addition, this contribution attempts to consider the evidence for infections and metabolic conditions thematically, in order to understand better their aetiologies and interactions. These conditions have been identified in preserved bodies, but the evidence is less common (but see Fletcher et al. 2003 on tuberculosis in Hungarian mummies, Møller-Christensen and Hughes 1966 on leprosy in an Egyptian mummy, Stout and Teitelbaum 1976 on osteoporosis in an 'Eskimo' mummy, and Panzer et al. 2013 on rickets in a Lithuanian mummy). The focus is research on skeletal remains rather than preserved bodies, because the former is most commonly excavated from archaeological contexts.

<B> Infectious diseases

There are four types of organism that can cause infectious diseases: viruses, bacteria, eukaryotes (e.g. protozoa, fungi, and multicellular parasitic worms), and prions (e.g. Creutzfeldt-Jakob disease) (Finch et al. 2002). They can spread directly or indirectly, and cannot only be transmitted from human to human, but from other animals to humans (zoonoses). The routes for transmission include airborne, faeco-oral, vector borne, person-to-person, direct inoculation, and ingestion (ibid.). Risk factors for infections are many, but include poverty, high population density, poor housing and hygiene, dietary deficiency, specific occupations, and migration. Today many of these infections are preventable and treatable but there is increasing antimicrobial resistance (http://www.who.int/mediacentre/factsheets/fs194/en/). Organisms can adapt to environments and change their constitution so that they can resist control by drugs that

normally kill them or stop growth. This is an evolutionary process that is causing major challenges today, “but is increased and accelerated by various factors such as misuse of medicines, poor infection control practices and global trade and travel” (https://www.gov.uk/government/collections/antimicrobial-resistance-amr-information-and-resources).

Infections can be considered alongside the epidemiological transitions that have shaped who we are today; these transitions characterize relationships between the human population, their environment, and the diseases that have, and are, affecting them. However, as Barrett and Armelagos (2013, 1) suggest, “despite our reigning civilizations, it is the microbes, not the humans, who are the colonial masters of the world.” While infectious diseases overall increased during the first epidemiological transition (foraging to farming), they have declined in the third transition that started in the late 19th-mid 20th centuries. Life expectancy increased, living conditions improved, and vaccines to prevent, and drugs to treat, infections were developed. In between, or during the second transition, industrialization flourished from the mid-18th century and pandemic, epidemic and endemic infections declined. However, there were still major infectious diseases that engulfed large percentages of populations over this transition. The third, and current, epidemiological transition is now characterized by increases in globalization and urbanization, more people living longer and thus populations rising, more chronic degenerative diseases, and, in recent years, emerging and re-emerging infections (Barrett et al. 1998). For example, recent infectious diseases that have emerged to cause problems for large numbers of the population in specific regions of the world include those caused by the Zika and Ebola viruses (Laupland and Valiquette 2014; Fauci and Morens 2016). Infections shape morbidity and mortality in the human population today, as they have throughout human history; studying their origin and evolution can inform the present, such as through aDNA (ancient DNA) analysis of tuberculosis (TB) (e.g. see Müller, Roberts, and Brown 2014).

<B> Metabolic diseases

Classification of diseases is complex (Ortner 2012; Ortner 2003, 383) and the metabolic diseases have proved particularly difficult. Clinically, this term is often applied to a

group of hereditary disorders (e.g., see *Journal of Inherited Metabolic Disease*). In recent years interest in the group of cardiovascular diseases termed the 'metabolic syndrome' has also increased markedly, and this has led to further blurring of the way in which the term *metabolic* is applied to health issues. Some authors in clinical medicine and palaeopathology have used the term 'metabolic bone disease' (see Brickley and Ives 2008, 2) and others working in palaeopathology have used the term metabolic to refer to "evidence of dietary deficiency disease in the skeleton" as indirect evidence of 'indicators of stress' (Roberts and Manchester 2005, 224). For the purposes of this chapter the definition used by Brickley and Ives (2008) has been followed, but as Ortner (2012, 250) noted, classifications are artificial mental constructs and should not interfere with our understanding of disease.

A variety of risk factors put individuals at higher risk of developing metabolic diseases, many of which are linked to nutrition. The occurrence of multiple metabolic diseases has frequently been noted (Semba 2012) and, as discussed in this chapter, development of infectious disease is a common consequence of metabolic diseases with nutritional causes. The occurrence of metabolic diseases is variable across and within contemporary communities. For example, Paget's disease is suggested to be declining (Merashli and Jawad 2015), but there is widespread agreement that levels of vitamin D deficiency are rising in many areas of the world (e.g. Robinson et al. 2006; Wimalawansa 2012).

Anatomically modern humans and other primates (lemurs and lorises excluded) have lost the ability to synthesize vitamin C (Padayatty and Levine 2016). With increased urbanization and differential food access, scurvy developed in a number of past contexts (Brickley and Ives 2008) and continues to be an issue in current groups (Wijkmans and Talsma 2016). Vitamin D has also played an important role in human evolution. Vitamin D is one of the oldest known hormones, with an estimated age of 750,000,000 years (Holick 2008). Although there is some debate (discussed by Brickley, Moffat, and Watamaniuk 2014), natural selection for lighter skin pigmentation during the African diaspora is the leading hypothesis to explain the development of

lighter skin pigmentation in humans living at higher latitudes (Holick and Chen 2008; Jablonski and Chaplin 2013).

Combined with an appreciation of the synergistic relationship between infectious and metabolic diseases there is considerable potential to learn more about both past and present communities through an examination of skeletal evidence of metabolic disease. Appreciation of the importance of metabolic conditions in epidemiology and palaeopathology is far more recent than for studies of infectious diseases; therefore, large-scale diachronic studies are not yet available. Significant advances might be expected in the study of metabolic diseases over the next ten years.

<A> Palaeopathology of infections and metabolic disease

<B> Types of evidence

Human remains

As a component of bioarchaeology, palaeopathology is defined as the study of disease evident in archaeological human and non-human remains. This evidence may be seen in skeletons and in mummies (see Brickley and Ives 2008 and Roberts and Buikstra 2003 as examples). Defined as the direct evidence for disease, palaeopathology is complemented by documentary evidence describing disease and also artwork, which may include drawings, paintings, sculpture, and images on pottery. Mitchell (2011) has provided a full critique of documentary evidence, and many authors have considered the challenges of using data collected from human remains to reconstruct the history of disease (e.g. Wood et al. 1992, Wright and Yoder 2003). It is not the chapter's place to detail the general challenges of palaeopathology, but more specific considerations are provided that focus on what the evidence can tell us about the experience of infectious and metabolic diseases in the past.

Both infectious and metabolic diseases can affect the skeleton (and sometimes the teeth), but only in a small percentage of people affected. For example, only 3-5% of untreated people with leprosy or TB may have bone involvement (Resnick and Niwayama 1995a, 2462, 2487; Paterson 1961; Jaffe 1972). Reports on metabolic

diseases such as vitamin D deficiency in children (rickets) also suggest low frequency rates, for example 9% of young children in Glasgow, Scotland in the 1960s, based on radiographs (Richards, Sweet, and Arneil 1968). However, as bone changes are only detected via imaging techniques it is highly likely that these changes may be more common than clinical texts describe (and in this case less advanced imaging methods were used in the (pre-treatment) time periods that produced useful evidence for the palaeopathologist). Pathology museums with pre-treatment bones displaying these conditions can also be helpful (e.g. (e.g. https://www.rcseng.ac.uk/museums/hunterian), as might skeletal collections dated to pre-treatment eras where known medical histories are associated with specific diseases, despite the fact that those histories may not be accurate (see for example Assis, Santos, and Roberts 2012 on the Coimbra Collection).

Diagnosis of both infectious and metabolic diseases does not usually include imaging today, with the patient being subject to a wide range of tests that are used to diagnose them. When taking a clinical baseline as a starting point it is necessary to know how an infectious or metabolic disease affects the skeleton, preferably in a person who has not had access to treatment. For studies of infectious diseases that affect the skeleton it is useful to examine remains of individuals who lived before the advent of antimicrobial drugs (e.g. antibiotics, antifungals, antivirals, and anti-malarials); metabolic diseases may be investigated in populations with conditions that have not been treated/reversed. This is because the effects of treatments on the skeleton may affect the expression of characteristic bone lesions. That said, standard macroscopic recording of abnormal bone forming and destroying lesions, their characteristics, distribution pattern, and potential differential diagnoses, is the first stage in providing an opinion on the specific nature of the disease in palaeopathology.

In palaeopathology some infectious and metabolic diseases are more commonly identified. In terms of infections, the ‘specific’ conditions of leprosy, TB, and treponematosis (i.e. those where the causative organism is known) may be seen, alongside ‘non-specific’ conditions where there could be a range of organisms causing the bone changes (e.g. inflammatory lesions on the pleural surfaces of the ribs, the facial sinuses, the endocranial surface, and the lower leg bones). The metabolic

diseases are less commonly identified, but increasingly are being recognised because more nuanced diagnostic criteria have been described in recent years. Conditions that are most frequently reported include vitamin D (rickets and osteomalacia), vitamin C (scurvy) deficiency, and osteopenia and osteoporosis.

Historical and artistic evidence

Historical and artistic evidence for infections and metabolic disease may be recognized, but are challenging to interpret unequivocally. For example, the signs and symptoms of TB can overlap with other lung diseases that may be described in historical documents (Roberts and Buikstra 2003, 214), and skin lesions in artwork may not be definitive enough in appearance and distribution to differentiate leprosy from other skin diseases. As Barnett (2014, 45) outlines for skin diseases in the 19th century, "the greatest challenge was simply that of distinguishing one condition from another". There are, however, examples of historical and art evidence for some of the diseases under consideration in this chapter.

Likely evidence for TB affecting the lymph glands of the neck is described in the 1550BC Ebers Papyrus from Egypt, typical signs and symptoms documented in China in the 3rd millennium BC, and spinal TB noted by Hippocrates in Greece in the 5th/4th century BC (see Johnston 1993, 1063). Numerous depictions of people with kyphotic backs suggest the possibility of Pott's disease (e.g. Morse et al. 1964 on 5000-year-old North African evidence), but many conditions, such as age-related osteoporosis, can result in kyphosis of the spine. Leprosy can affect the skin, resulting in lesions that may be difficult to differentiate from other skin conditions such as psoriasis andeczema etc. More recent artwork, such as that from Norwegian artists who directly observed people with leprosy, is more convincing (e.g. Danielssen and Boeck 1847). Scurvy was also not readily apparent in historical sources until more recent periods. For example, Vasco da Gama, a Portuguese explorer who visited the West Indies in 1497, described scurvy in his crew (Carnemolla 2003), but it was not until 1753 that the first full description was published (Lind 1753). Early descriptions of the signs of rickets in children, deformities of the legs and spine, are seen in the writings of Soranus of Ephesus, a physician practising in Alexandria in the 2nd century AD, and in Galen's work around the same

time (Steinbock 1993). The classic description did not come until the 17th century when Glisson (1650) wrote his treatise on rickets. High frequencies of rickets in English children during and after the Industrial Revolution are also noted (Fildes 1986).

Clearly, the historical and artistic data for these conditions can range from convincing to ambiguous; nevertheless, they need to be considered, and can in some cases provide earlier evidence than that documented in human remains.

<A> Methods of analysis for metabolic and infectious diseases

<B> Macroscopic

> "The gross anatomy (as corroborated by radiographs) is often a safer guide to a correct clinical conception of the disease than the variable and uncertain structure of a small piece of tissue" (Ragsdale and Lehmer 2012, 239 citing Ewing, 1922).

This statement, made prior to many important developments in analytical techniques in biomedical research and palaeopathology, highlights an important general principal in palaeopathology. The clinical literature is an important source of information. Clinically, bone changes are rarely considered at the macroscopic level, but pathological changes to bone observed using other imaging techniques can often be observed directly in palaeopathology (e.g. 'fraying' of metaphyses seen radiologically in clinical rickets) (Thacher et al. 2000). Although care is needed as ideas develop over time, clinical reports dating to the era before the widespread use of biochemical tests, when far more information was obtained from radiological and histological analyses, are an invaluable resource for paleopathologists. Some of the earliest reports, such as Barlow's 1883 classic description of co-occurrence of rickets and scurvy, pre-dates treatments now commonly used for infectious and metabolic diseases in the developed world. Despite recent advances in other investigative techniques, macroscopic analysis is still a critical part of paleopathological assessment of human remains, as discussed above. Indeed, this initial work is the basis for developing a differential diagnosis and must come before

any destructive techniques are applied to human remains (e.g. biomolecular); however, a better appreciation of the limitations of the various techniques has developed.

Advances in diagnostic criteria used in palaeopathology continue to be made, even with conditions such as TB that have been the subject of much scrutiny. As an example, to date, much of the work undertaken has focussed on diagnosis in adults (Roberts and Buikstra 2003), but in 2011 Lewis extended research on pathological changes through a detailed investigation of TB in non-adults. There are no pathognomonic features of TB in dry bone, but possible skeletal features of the condition in non-adults were reviewed, along with careful differential diagnoses of other infectious and metabolic diseases (e.g., brucellosis and scurvy), enabling new suggestions on disease prevalence to be made. In adult skeletons, spinal damage (Pott's disease) has been the key focus for diagnosis stemming back to early work on this infection (e.g. Elliott Smith and Ruffer 1910 in Egypt), and continuing into contemporary studies. Nevertheless, there are a number of differential diagnoses for destructive lesions of the spine that must be considered (see Table 3.3 in Roberts and Buikstra 2003). Another bony change associated with TB is unilateral involvement of the joints of the arms and legs, and most frequently the hip and knee joints. Differentiating between TB of these joints and non-tuberculous septic arthritis is important, and useful differential diagnostic features are available (see Table 3.4 in Roberts and Buikstra 2003). Recent work has, however, suggested that the pattern of tuberculous bony changes has seen temporal change (Steyn et al. 2013). In this study of documented skeletons with known causes of death in South Africa, the frequency of bone lesions was seen to increase over time, particularly after the introduction of antibiotics. This increase was explained by increasing longevity, enabling the lesions to develop in spite of drug therapy.

Other areas of the skeleton have been highlighted in recent years where bone changes may also be related to TB. For example, the inflammatory-induced periosteal new bone formation (PNBF) on the pleural surfaces of the ribs has been a major discussion area in palaeopathology, initially from the research of Kelley and Micozzi (1984: Hamann Todd Skeletal Collection) – see Figure 1. Later, more nuanced contributions resulted

from research on skeletons with rib lesions from other identified skeletal collections in the USA (Robert J. Terry) and Portugal (Coimbra and Lisbon) from Roberts, Lucy, and Manchester (1994), Santos and Roberts (2006), and Matos and Santos (2006). Cause of death data for each skeleton were used in concert with evidence for rib lesions to suggest that respiratory infections, and most likely TB, caused the lesions. The often very subtle lesions identified on the ribs seen palaeopathologically have not been described in clinical literature, although one report of enlarged ribs in patients with lung diseases can be interpreted as the result of respiratory disease (Eyler et al. 1996). In this study the horizontal width of the ribs were studied from radiographs in four groups of patients: 1: 41 with chronic pleural disease, 2: 30 with a clinical diagnosis of TB of five years or more (unilateral), 3: 25 with empyema, and 4: 60 with no disease (control). Twenty-four of the 41 in Group 1 (59%) had a range of lung diseases: TB (most common), non-TB empyema, trauma or metatastic tumour, or a person who had had thoracic surgery. There was a significant difference between the size of ribs on the side of the thoracic cavity affected and the non–diseased side in groups 1 and 2, and no difference between groups 3 and 4. The difference in size is interpreted as representing varying amounts of new bone formation on the visceral surfaces of the ribs.

The common conclusion of the cited studies is that rib lesions are not pathognomonic for TB and could be caused by many lung problems, but that TB may be a cause. As a development on this idea, biomolecular studies have been used to try to prove a direct association between rib lesions and a diagnosis of TB using aDNA analysis (e.g. see Mays, Fysh, and Taylor 2002; Nicklish et al. 2012). While positive DNA results for TB were found in skeletons with rib lesions, this does not prove a direct association. It is possible than an individual with TB may have had another lung disease. Roberts, Lucy, and Manchester (1994) demonstrate this possibility: some skeletons in the Terry Collection had documented causes of death that included both TB and another pulmonary disease. In these cases, how can it be proved that TB caused the lesions and not the other respiratory disease? These cases also highlight the importance of considering the possibility that human remains examined by paleopathologists may come from individuals who would have experienced a high disease burden, including

multiple lung problems; as today, co-occurrence of conditions would have been common in past communities.

Rib lesions are not the only ‘non-specific’ bone change suggested to be related to TB. The cranium may develop destructive lesions of both tables of the skull. Hackett (1976) described and illustrated the damage, alongside showing how the lesions may be differentiated from other diseases such as venereal syphilis. Endocranially, a “maze like appearance” has been described in skeletons from the Hamann-Todd collection (Hershkovitz et al. 2002, 202), which may also be interpreted as well remodelled new bone formation. Subsequently Hershkovitz et al. (2008) published data using this diagnostic criterion as indicative of TB in 9000-year-old remains from Israel. “Granular impressions” were also described in a *Homo erectus* skeleton (Kappelman et al. 2008, 112; see also Roberts, Pfister, and Mays et al. 2009). Lewis (2004) has reviewed data on these bone lesions and concluded that significant care should be taken in diagnosing specific diseases (including TB) because of their potential multifactorial aetiology; Lewis also noted that they may represent normal bone growth in growing children (see Figure 2). Calcification of the pleura could also occur in respiratory diseases, including TB, and the long and short bone diaphyses may also be involved in a range of diseases. For example, dacylitis may be seen in people with TB, congenital syphilis, and sickle cell anaemia (Resnick and Niwayama 1995a). Finally, the condition of hypertrophic pulmonary osteoarthropathy, which involves PNBF on long bones, can be associated with TB, but also other pulmonary and non-pulmonary diseases (Resnick and Niwayama 1995b; see also Assis, Santos, and Roberts 2012).

Weston has examined the aetiology of PNBF by studying bone reactions on pathology museum bones with known medical histories (2008, 2009, 2012). Although PNBF can be associated with some infectious and metabolic diseases, this type of change develops in response to a much wider range of traumatic events and pathological conditions. It was concluded that the tissues involved dictate the type of reaction rather than the traumatic event or pathological agent. Importantly, it was also found that although some locations are more consistent with some conditions, no location is

uniquely diagnostic for a particular causative agent and factors such as age, sex, ethnicity, general health and co-occurrence of other conditions, as well as the stage of development of the condition at the time of death, will play an important role in the appearance of PNBF (Weston 2008, 56).

The research by Lewis (2011) and Weston (2008, 2009, 2012) are good examples of developments in macroscopic diagnosis in conditions that have been investigated for a considerable period of time. Lewis (2011) demonstrates how new approaches to non-adult remains, which received limited attention during the early years of palaeopathology, can provide new information whilst highlighting the potential contribution that aDNA analysis can make to the identification of less studied infectious diseases such as brucellosis. Weston's research will enable paleopathologists to evaluate what can be said with confidence, and identify areas in which further research could help increase certainty. Research that has developed the use and interpretation of non-specific markers of TB and leprosy (e.g. rib lesions in TB, and phalangeal grooves and dorsal tarsal 'bars' in leprosy – Figure 3) has also provided useful aids to strengthen diagnoses where more pathognomonic changes are present (for example, see Roberts, Lucy and Manchester 1994 for rib lesions and TB, and Andersen and Manchester 1987, 1988 for leprosy).

Paleopathological investigations of metabolic diseases have a far shorter history than the infectious diseases. Information on many of the metabolic bone diseases was compiled and published in 2008 (Brickley and Ives). This volume, along with extensive work by both Ortner and Mays since 1997 (e.g., Ortner and Mays 1998; Ortner and Ericksen 1997), has led to a number of developments (to be covered in a second edition of *The Bioarchaeology of Metabolic Bone Disease* in 2018). The topic of co-occurrence of rickets and scurvy, for which there is considerable clinical evidence and a small number of recent reports in palaeopathology, has been tackled by Schattmann et al. (2016). These authors specifically considered the effect of having both rickets and scurvy on the skeletal manifestations of pathological change (see Figure 4). Furthermore, large-scale studies, using data from well over 1000 skeletons in each

case, have been used in evaluations of osteomalacia in adults (Ives and Brickley 2014) and paleopathological evidence for hip fractures (Ives et al. in press). Similarly, Roberts and Buikstra's (2003) synthetic study of the bioarchaeology of TB explored the value of different methods of diagnosis for skeletal remains, bringing together extant published and unpublished data on TB globally. The authors further recommended close reflection on the diagnostic criteria used for identification of TB in palaeopathology in the future, and suggested that it would be beneficial to try to identify the early stage bone lesions of TB. Identification of TB before collapse of the spine in Pott's disease, which is often the 'classic' image provided in textbooks, would be beneficial (for example see Mariotti et al. 2015). Authors have tried to categorically establish early stage lesions (e.g. Baker 1999; Haas et al. 2000) and some have suggested that rib lesions may indicate that a population could have harboured TB (e.g. Larsen 1997). Roberts and Buikstra (2003) suggested that archaeozoologists might also consider exploring non-human archaeological bones for evidence of TB since it can be a zoonosis. They also highlighted the increasing contributions of aDNA analysis to understanding TB in palaeopathology, for example being able to diagnose TB in skeletons without bone changes, and differentiating between the bovine and human form of TB. This was before research commenced concerning ancient strains of the *M. tuberculosis* complex (see below).

<B> Biomolecular analysis: aDNA

Genetics have come to play an increasingly important role in the investigation of many aspects of health. A review of publication data available on PubMed reveals a rise in publications on aspects of genetics starting in the late 1980s. There has been a steady increase since this date, with genetics now constituting a significant source of information on many aspects of both metabolic and infectious diseases (e.g. see overview for TB in Galagan 2014, and Brites and Gagneux 2015). Advances in aDNA analysis in human remains have mirrored this trend, and high-throughput (or Next Generation) sequencing, from the mid-2000s has enabled whole genome sequencing from ancient DNA (e.g. Orlando et al. 2015). DNA from living communities can also be

used to investigate aspects of the origins of humans and their diseases, and has significant potential to contribute an evolutionary perspective on various conditions (e.g. see Jobling, Hurles, and Tyler-Smith 2004). For example, extensive work has been undertaken on skin pigmentation which is thought to have changed to allow adequate synthesis of vitamin D as humans moved to higher latitudes (Jablonski, 2012; Jolliffe et al. in press). Furthermore, recent developments in understanding genetic aspects of contemporary disease has considerable potential to contribute to our understanding of conditions such as Paget's disease of bone in the past (see below). Rapid advances in techniques employed mean that journal articles are the best way to access current information on available techniques and developments (e.g. see recent review of ancient pathogen genomics in Harkins and Stone 2015). However, information on basic approaches to interpreting paleopathological data and to aDNA research is provided in chapters in Grauer (2012), and more generally in Brown and Brown (2011, 242-263). There have been extensive investigations of infectious disease using biomolecuar analysis, particularly that pertaining to aDNA, in archaeological human remains. Mycolic acid (e.g. see Gernaey et al. 2001; Masson et al. 2015) and protein analyses have been studied (e.g. Boros-Major et al. 2011; Hendy et al. 2016), but the latter is only in its early stages of development.

Ancient DNA analysis has been used successfully to investigate various infectious diseases, and most commonly TB. Other infections have also been the focus of work, including leprosy and the plague, and less so for treponematosis and malaria. However, it should be noted that there are limitations to ancient DNA studies. For example, as pointed out by Roberts (2012, 436), if one of the aims of the work is to prove an association between bone lesions and a disease using aDNA analysis, a definitive link between the lesion and the pathogen cannot be established. There are wider ranges of issues to consider relating to aspects of the investigation of other infectious diseases, covered by Spigelman, Shin, and Bar Gal (2012), but also see Roberts and Ingham (2008). Whilst most work in this field has concentrated on samples of bone from skeletons or soft tissues of preserved bodies, dental calculus and coprolites are being targeted more frequently in recent years because they are believed to have "reliable

and abundant sources of directly dateable bacterial genomes from a wide variety of microorganisms" (Warinner et al. 2014, 132, and see Weyrich, Dobney, and Cooper 2015). The field is rapidly advancing and research trajectories are being shaped accordingly. Fortunately for aDNA studies, access to modern DNA data allows comparative work to be undertaken.

Research in the field of aDNA analysis can provide information that cannot necessarily be acquired through other palaeopathological methods. Establishing that a person had a specific disease even if bone changes were not evident at death (perhaps because they died in the acute stages), identifying diseases without bone changes, and indicating species or strains of pathogenic organisms are some examples. As described above, TB has been the focus for many analyses and has revealed exciting results. It is well known now that there are different phylogenetic lineages of the *Mycobacterium tuberculosis* complex (MTBC, or the organisms that cause TB in humans and other animals) associated with different geographic regions (Brites and Gagneux 2015, 21); this allows the tracking of MTBC's evolution and spread globally. In the early years, from 1993, the focus was on using the method to diagnose TB in human remains in various parts of the globe (e.g. see Salo et al. 1994: 1000 year old skeletons from Peru). Research then began to consider diagnosis in skeletons where there was no bone change (e.g. Medieval Lithuania: Faerman et al. 1997), and genotypic analysis where the infecting strain was identified to be similar to *M. tuberculosis* and not *M. bovis* (Taylor et al. 1999). This was followed in 2001 by a study of the species of TB infecting humans buried at Wharram Percy, a Medieval site in Yorkshire, England, where Mays et al. found that nine individuals all had the human form of TB. In a similar study, Zink et al. (2003) found *M. africanum* preserved in Egyptian mummies with TB. It was not until 2009 that *M. bovis* was identified (Murphy et al. 2009: Iron Age Siberia). Modern strains of TB were then found in 18th-19th century Hungarian mummies (Fletcher et al. 2003), and in Iron Age England (Taylor et al. 2005).

Since then, numerous reports of tuberculous aDNA being identified in human remains have appeared in a range of journals, and interesting developments have appeared

over the last five years or so. For example, Barnes et al. (2011) studied an allele with natural resistance to intracellular pathogens such as TB in in 12 ancient populations from different contexts. They observed that people with long histories of living in urban environments are better adapted to resisting infections such as those caused by *Mycobacteria*, which has implications for the evolution of the bacteria, and the development of resistance and susceptibility genes for infectious diseases. Of recent date there are further studies on the strains of TB in archaeological human remains, and of particular note is the documenting of TB strains in Peruvian humans, mentioned above, that are closely related to those adapted to seals and sea lions (Bos et al. 2014). It is also apparent that seals are implicated for transmission of TB to other mammals today (e.g. cattle), as seen in New Zealand where cattle have access to seals through grazing on beaches and via water courses that feed into the sea (Loeffler et al. 2014). Indeed, there is accumulating evidence that TB strains were clearly being 'transported' by humans (and other animals) as they moved around in the past. For example, a strain of TB uncommon in England in the 19th century, but present in North America at that time, was identified in a woman buried in England (Bouwman et al. 2012). Variations in strains were also found over time and in different regions of England, with two different strains being found at one Roman site, different strains in 19th century sites, and a completely different strain to all identified in England found in a person buried in Scotland (Müller, Roberts, and Brown 2014). Indeed, linking mobility with infectious disease seen in skeletal remains is beginning to become more common in palaeopathological research, although there are only a small number of extant studies. Furthermore, the trend for aDNA analysis of TB now seems to be very much focused on both macroscopic **and** aDNA analysis in attempting a diagnosis (e.g. Masson et al. 2013), the latter of which may not always be appropriate if a macroscopic diagnosis is clear.

Indirectly related to TB, and an area that has not been developed in palaeopathology, is the recent literature pertaining to lactase persistence in human populations, or the innate ability to digest the milk sugar lactose (Burger et al. 2007; Itan et al. 2009; Gerbault et al. 2011). There are some aDNA data suggesting that Neolithic skeletons

from the earliest farmers of Central Europe show the presence of the lactase persistence gene (−13.910*T); although the sample size was small it was concluded that lactase persistence was rare in these early farmers (Burger et al. 2007). Assuming that ingestion of infected milk was a route for transmission of TB to humans, it could be hypothesized that skeletons showing TB from the archaeological record may harbor the lactase persistent gene.

<B> Biomolecular analysis: Stable isotopes

Analysis of dietary and mobility stable isotopes also has the potential to make a number of important contributions to understanding and contextualising many aspects of metabolic and infectious disease in past communities. For example, nutrition is a key factor in the immune response (Krawinkel 2012) and is directly linked to the development of metabolic disease (e.g. Gennari 2001). Isotope analysis has seen a surge in popularity in the last 15 years, and Richards and Montgomery (2012) usefully provide an overview of the integration of isotopic data with evidence of disease. While there are many publications on diet **or** mobility, very few try to link those data to disease, using either macroscopic or aDNA data.

Investigation of paleodiets is an important component of stable isotope analysis (Katzenberg 2008). The field has undergone a number of significant developments since the 1970s when researchers began to address questions linked to the development of maize cultivation and consumption (e.g. Vogel and van der Merwe 1977). Stable isotopes do not provide information on specific diseases or nutrients, but when interpreted in an ecological framework, isotopic data can provide information on, for example, patterns of weaning, levels of marine resources consumed, and the contribution of protein to the diet (Katzenberg 2012). These types of data can provide strong contextual information for investigations of both metabolic and infectious diseases. Weaning patterns are known to impact the development of both rickets and scurvy and Giuffra et al. (2015) undertook isotopic analysis to establish weaning patterns in children with rickets entombed in the Medici Chapel in Florence, Italy. The

contribution of stable isotopes to investigations of vitamin D deficiency is also considered by Brickley, Moffat, and Watamaniuk (2014). Regarding vitamin C, humans can only access this nutrient from the diet, with human breast milk being an important source (Brickley and Ives 2008, 42-45); therefore, breastfeeding will be a key consideration when considering evidence for scurvy in infants. For example, Schattmann et al. (2016) and Lewis (2010) considered weaning practices in the evaluation of scurvy and rickets in skeletons of infants and children, but no isotopic analysis was carried out in these studies. However, care is required when linking weaning to conditions such as scurvy and rickets because questions have recently been raised regarding what can be said definitively about the timing of weaning (Reynard and Tuross 2015; see also Beaumont et al. 2015). Nevertheless, direct integration of isotopic data in future studies of scurvy in infants and young children would provide valuable additional data, as has been done for rickets (Giuffra et al. 2015). It has also been suggested in an *in vitro* study of *M. bovis* that bacterial growth was slower if humans were given vitamin C (Wang et al. 2012), *M. bovis* being one of the organisms in the MTBC that is responsible for TB in humans and other animals.

In contemporary societies high levels of infectious diseases such as TB (Pace-Asciak, Mamo, and Calleja 2013) and metabolic diseases, including vitamin D deficiency, have been noted in migrants and their children (Pillow, Forrest, and Rodda 1995; Hintzpeter et al. 2008). Indeed, the recent work identifying TB strains in archaeological skeletons, described above, indicates that people were likely migrating and taking their infections with them (as seen today: Gao and Rao 2015). People moved and move for many reasons, including to trade, access a better life, and move away from war torn areas of the world (e.g. see Harrison 2012 on historical perspectives of trade and its impact on disease). In addition, it is well known that migrants today often live in poverty as they travel, and may do so at their final destination, thus challenging health care settings in their chosen country (Rechel et al. 2011; Gao and Rao 2015). People travelling to TB-endemic countries are also at risk (Cobelens et al. 2000), and relevant to this discussion are the definitions today of first- and second-generation immigrants, the former being defined today as a foreign-born resident who has relocated and become a citizen or

permanent resident in a new country, and the latter being children born of at least one foreign-born parent who is a first-generation immigrant (http://immigration.about.com/od/glossary/f/How-Is-First-generation-Immigrant-Defined.htm). For example, using census data in Berlin, Germany to link immigrant status to disease, higher rates of TB in second-generation immigrants were found (Marx et al. 2015).

Isotope data in archaeological settings can provide information on patterns of migration and mobility in the past, including identifying first-generation migrants (e.g. Budd et al. 2004), but isotopic analysis may also identify second-generation migrants. For example, in a study of mobility in Roman Winchester, England it was found that up to a quarter of people buried at the Lankhills cemetery were incomers, but few likely originated outside England (Eckardt et al. 2009). There were, however, burials at Lankhills for which context suggested non-normative burial rites for this region and time period. It was suggested, though isotopic analysis, that these burials represented second-generation immigrants, but retained all the cultural behaviour of the upbringing of these people. Therefore, even though local isotopic signatures may be found for archaeological skeletons from non-normative burials in the archaeological record, this could suggest a second-generation immigrant who, at death, was afforded a burial practice of their parents' region of origin.

The relationship of mobility to the spread of disease is relevant to this chapter. For example, nutritional deficiencies associated with famine events were considered alongside isotopic evidence for migrant status in individuals buried in the East Smithfield Black Death cemetery in London, England (Kendall et al. 2013). This type of information could contribute interesting contextual information to studies of metabolic and infectious diseases related to under- and malnutrition in past groups, such as those experiencing leprosy and TB. Refinements in isotopic investigations allow for comparisons to be made between stable isotopes from teeth and bone from the same individual, thus allowing changes in diet or place of residence during the lifetime of the individual investigated. Sequential samples have also been taken from dentine,

providing a time dimension (Beaumont and Montgomery 2015). Although hair is preserved relatively rarely in archaeological contexts, recent studies have investigated stable isotopes encapsulated in archaeological hair to look at changes in diet (Webb, White, and Longstaffe 2013) and physiological stressors such as chronic infectious disease (D'Ortenzio et al. 2015). Furthermore, work by D'Ortenzio and colleagues supported suggestions made by Katzenberg and Lovell (1999) that long term illnesses may be a confounding factor that should be considered in stable isotopic investigations of diet. Related to stable isotope analysis, dental calculus can also provide information on a range of aspects of past diets and genetic data (see summary review in Salazar-García et al. 2014), and calculus has now also been analysed isotopically (Eerkens et al. 2014). However, studies have produced conflicting results and the exact nature of the correlation and offset between data from calculus and bone are not fully understood (see results and discussion in Warinner et al. 2014 and Eerkens et al. 2014). Nevertheless, dental calculus is a frequent deposit found on archaeological teeth, meaning there is potentially a wealth of information waiting to be revealed (Warinner et al. 2014, 2015).

There has been little work focusing on understanding infectious disease from a mobility perspective and to date no paleopathological work has been done on mobility and metabolic disease. Future work could enable an assessment of metabolic disease in migrants in earlier human groups. Montgomery (2002) tried to use mobility isotope analysis to track mobility and its relationship to infectious disease through studying treponematosis in late Medieval Gloucester, England – 12th-16th century AD. A more recent study by Roberts et al. (2012) also attempted to track mobility histories of individuals with treponematosis in a late Medieval cemetery associated with the port of Hull, England. Research linking mobility isotope data and TB in Roman individuals buried in England is ongoing (https://www.dur.ac.uk/archaeology/staff/?mode=staff&id=11974), and a recent paper has indicated that a person with leprosy buried in Great Chesterford, Essex, England may have originated from outside England (Inskip et al. 2015). The lack of studies in this field make it imperative that isotope specialists should work more with

palaeopathologists to explore opportunities that link both disciplines (Richards and Montgomery 2012, 725). These collaborations may reveal the dynamics of infectious disease transmission in the past. However, the data will remain challenging to interpret, without knowing when a person contracted a disease during their mobility history.

<B> Histology

With the advent of biochemical tests for many conditions the use of histological assessment in clinical work has declined considerably. However, clinically, histological assessment is still used in assessment of conditions such as renal osteodystrophy (Zang and Chouchan 2012) and older clinical work provides a valuable resource for those investigating metabolic diseases (e.g. Ranström and Von Sydow 1949; Follis, Park, and Jackson 1950). Histological analysis can provide important additional information on some aspects of metabolic and infectious diseases, but there are significant limitations in diagnosing specific diseases from dry archaeological bone (Ragsdale and Lehmer 2012, 228). As Ragsdale and Lehmer note, osteopenia is the most usual initial expression of disease, and the range of conditions that will produce this initial reaction is extensive (see review by Brickley and Ives 2008). As discussed by De Boer, Van der Merwe, and Maat (2013) and Ragsdale and Lehmer (2012), the changes used in palaeopathology will be different to those used in clinical medicine and it is likely that only individuals with more severe or longer standing conditions will be identified.

De Boer, Van der Merwe, and Maat (2013) have produced a review of research ondry bone histology undertaken in palaeopathology and addressed both its usefulness and challenges involved in the identification of infectious disease. They suggest that features previously thought to be pathognomonic for conditions such as syphilis, leprosy, and TB cannot be used as unequivocal indicators of disease (De Boer, Van der Merwe, and Maat 2013, and see Blondiaux et al. 1994; Schultz 2001; Schultz and Roberts 2002; Von Hunnius et al. 2006). Patterns of bone change observed

histologically can however be used to suggest the speed at which a lesion developed and if the features produced suggest a recurrent condition.

De Boer, Van der Merwe, and Maat (2013) also review limitations and possibilities of diagnosing various metabolic conditions using histology. They confirm that vitamin D deficiency, hyperparathyroidism, and Paget's disease can all be identified histologically. For some conditions, such as Paget's disease, the histological changes produced in bones affected are distinctive, and the presence of such features has assisted in diagnosing evidence in a number of skeletons (Bell and Jones 1991; Aaron, Rogers, and Kanis 1992). However, poor preservation and diagenetic change may be present in archaeological bone and can prevent clear conclusions being reached (Pinto and Stout 2010). For example, poor preservation limited the use of histological assessment of rickets by Schattmann et al. (2016), but histological analysis has proved effective in a number of investigations of rickets and osteomalacia (Schamall et al. 2003; Brickley, Mays, and Ives 2007; Ives and Brickley 2014, and see Figure 5). Vitamin D is essential for effective mineralisation of osteoid, and in states of deficiency the amount of osteoid present increases (St-Arnaud and Glorieux 1997). Clinically an increased volume of osteoid is the main feature used to make a diagnosis (Pitt 2002). Osteoid is unlikely to preserve in archaeological skeletal material and, as a result, some people diagnosed clinically would be missed by those working solely with dry bone. There is a range of features indicative of vitamin D deficiency that can be observed histologically, but these are likely to be found in more severe and longstanding cases of deficiency; a review of features is available in Brickley and Ives (2008, Tables 5.7 and 5.13). Features such as cloudy granular areas of bone formation (Bonucci et al. 1969) and 'buried' osteoid (Priemel et al. 2010) can be readily identified in well preserved archaeological bone. In a living person nutritionally-related vitamin D deficiency will be resolved by obtaining adequate amounts of vitamin D. In these cases the histological features that may be identified in bone will be lost through remodelling. It is not clear how long histological features linked to deficiency will remain in bone when the condition is resolved, and the length of time will vary depending on the age, sex and general health of the individual concerned.

Archaeological human remains with preserved soft tissues offer greater scope for diagnosing pathological conditions with the aid of histological assessment (Aufderheide 2003, 369-376; Grove, Peschel, and Nerlich 2015). Naturally, levels of preservation and inclusion of internal organs vary widely depending on date, mummification techniques used, or natural conditions. Evidence of parasitic infection has been identified in a number of mummies. For example, Chaga's disease (*T. cruzi* infection) has been noted in South America by Grove, Peschel, and Nerlich (2015) and Fornaciari et al. (1992). Members of the latter team have also identified various infectious diseases with the aid of histology including syphilis (Fornaciari et al.1989).

<B> Imaging

Imaging is a method of analysis applied to human remains using a range of methods, including plain film, microradiographic, and tomographic techniques (e.g. see Rockall et al. 2013, and specifically for bones and joints Section 1 of Resnick 2002 where diagnostic techniques are considered). Imaging allows for visualization of features that cannot be seen macroscopically, and has been most commonly applied to analyses of preserved bodies. However, a number of studies have suggested that x-rays can damage ancient DNA (e.g. Grieshaber et al. 2008), and therefore careful planning for future work is required (Bertrand et al. 2015) since there is more to be learned concerning the exact impact of various imaging techniques on different tissues.

Wanek, Papageorgopoulou, and Rühli (2012) provide an excellent review of the history, advantages, and disadvantages of various imaging technologies. This review examines approaches to differential diagnosis in palaeopathology using imaging technologies and outlines challenges such as equipment availability, levels of specialist knowledge required to access equipment, and relative costs involved. Plain film radiography, the simplest and most widely available imaging technique, provides important information that can assist with identification and interpretation of pathological lesions related to both metabolic and infectious diseases. CT (computed tomography) and microCT

analysis is less frequently employed compared to plain film radiographic analysis in palaeopathology, when compared to clinical medicine, but there is significant potential to obtain additional information when imaging technqiues beyond plain film radiography are employed. For example, Wanek, Papageorgopoulou, and Rühli (2012) suggest that more would be learned about subtle pathological changes such as PNBF if CT scanning was undertaken.

Even so, simple x-ray imaging can enable significantly more information to be obtained from archaeological skeletons concerning many of the metabolic diseases. In the case of rickets, as discussed by Mays, Brickley, and Ives (2006), it is possible to gather additional information that, when used in combination with some macroscopic features, can enable differentiation between active or healed rickets, or suggest an individual had experienced multiple episodes. Radiological assessment also aided in the identification of scurvy and of the co-occurrence of both rickets and scurvy (Schattmann et al. 2016). It has also been found that systematic radiological analysis can be key in accurately detecting new bone formation caused by a range of traumatic events and disease processes that had healed at the time of death (Weston 2008). Plain film radiography can also be invaluable in diagnosing Paget's disease in both clinical and paleopathological contexts ((Wittenberg 2001 and Rogers, Jeffrey, and Watt 2002, respectively). Clinically the V-shaped area of radiolucency seen in long bones, often referred to as the 'blade of grass' or 'flame' sign, is virtually pathognomonic of Paget's disease. Although the use of radiographs in the assessment of age-related bone loss and osteopenia has fallen from common use in clinical work, techniques such as metacarpal radiogrammetry, which was developed for use in clinical medicine, can be an excellent source of information on age-related cortical bone loss in palaeopathology (Ives and Brickley 2005; Mays 2001, 2006).

Plain film radiography can also be useful for nuancing a diagnosis of infections, particularly because lesions beyond the surface of bones cannot be seen macroscopically. For example, following radiography of a femur that displayed new bone formation, it was revealed that osteomyelitis was present, represented by opacity

inside the bone (Santos and Suby 2012). Likewise, multiple bone TB was diagnosed following radiographic examination shown by well-defined radiolucent lesions in the long bones (Dabernat and Crubézy 2009). Finally, in work studying preserved bodies, Elliot Smith and Dawson (1924) described leprosy in the hands and feet of a Coptic Christian mummy from El Bigha (c. 500 AD). Unerupted teeth affected by infection may also be visualised through imaging as seen in the diagnosis of congenital syphilis in a colonial period child's skeleton from Mexico City (Mansilla and Pijoan 1995).

<B> Parasite analysis

Parasites are organisms that spend all or part of their lifecycle living in or on another living organism. Humans host a wide range of parasites, and infection with these organisms has a significant effect on health and well-being today. Infection by intestinal parasites has a number of health consequences, and these include reduced nutrients for the human host. As such, they are closely linked to overall immune system strength and the metabolic diseases. As discussed by Bunnag and other members of the WHO Expert committee, there are important differences between the various intestinal parasites in communities today, and such infections can pose a significant risk to individuals with a poor nutritional and/or immune status (Bunnag et al. 1987). Due to the relatively robust nature of the eggs of intestinal parasites there has been considerable work on retrieval and analysis of their eggs from archaeological sites (Dittmar 2009). Parasite eggs may be found in a variety of archaeological contexts, for example grave soils, cess pits, latrines and sewer systems, coprolites, and intestinal contents of preserved bodies. The majority of archaeological parasites found are classed as 'souvenir' parasites ("acquired relatively recently in human prehistory" - Reinhard and Araújo 2012, 755), rather than 'heirloom' parasites ("hosted by primate common ancestors of modern apes and humans" – ibid.). While parasites not only provide evidence for infection by these organisms, and information on diet, including methods of food preparation, it has also been demonstrated that their analysis can contribute to understanding past human migrations (Le Bailly, Maicher and Dufour 2016), and modelling human adaptation (Dittmar, Araújo, and Reinhard 2012).

Some thoughts on the history of paleoparasitology and on future directions have been provided by Faulkner and Reinhard (2014), and also Reinhard and Araújo (2012). Importantly, it should be noted that researchers in different areas of the world use slightly different techniques to retrieve parasite eggs from different contexts, likely because of the robusticity and preservation of eggs encountered in these different regions (Mitchell pers com. February 2016). Dittmar, Araújo, and Reinhard (2012) provide current methodology, particularly aDNA approaches, developments that have been made, and future considerations. As discussed in this chapter, histological analysis of soft tissues from South American mummies has produced evidence of Chagas disease (leishmaniasis), caused by the parasite *Trypansosoma cruzi* carried by the triatomine bug. In the past the condition was restricted to Latin America, but recently it has spread to other areas of the world (WHO 2016). Research that looks at evolution in parasite-human host interaction demonstrates taking multiple approaches, including using aDNA and immunological techniques (Dittmar, Araújo, and Reinhard 2012), and also takes into account descriptions of lesions in mummified human remains (Araújo et al. 2009).

Although it is possible to undertake analysis of infectious and metabolic diseases using just one of the methods described in this section, it has been demonstrated by numerous studies that that it is possible to say much more about their characteristics and history when using multiple methodologies. Importantly this type of approach is more likely to produce data that can assist with suggesting the possible presence of co-occurrence.

<B> The contributions of other disciplines to understanding past metabolic and infectious disease

Palaeopathologists by necessity often work with scholars from other disciplines. These include commercial archaeologists who excavate human remains, archaeologists with specialisms in the time period being studied, and those who focus on other types of archaeological ‘materials’. Other relevant disciplines range across the arts, humanities,

social sciences, and sciences, and include anthropology, biology, chemistry, earth sciences, geography, history (including the history of medicine), medicine, and physics. However, in this section, a focus on (medical) anthropology, (medical) geography, and (evolutionary) medicine is considered.

Anthropology has a four field approach, that of archaeology, sociocultural, biological/physical anthropology, and linguistics. Palaeopathology 'sits' within biological anthropology but medical and nutritional anthropology are also found within this subdiscipline. As McElroy and Townsend state, "reaching widely across space and time for its materials, medical anthropology builds a bridge between health sciences and anthropology" (1996, xxi). It focuses on health in living populations, often in communities who live 'traditionally', which makes data from this discipline often relevant to interpreting our ancestors' lives and deaths. It furthermore engages with all aspects of anthropology to better understand those factors which influence health and well-being, the experience and distribution of illness, the prevention and treatment of sickness, healing processes, the social relations of therapy management, and the cultural importance and utilization of pluralistic medical systems (http://www.medanthro.net/9/). For example, understanding the impact of leprosy and tuberculosis on the treatment of people with these diseases in the past necessarily draws on this vast body of literature (e.g. see Roberts 2011). Understanding the 'here and now' is important, while at the same time recognising that communities that are the focus of medical anthropologists are distanced in space and time from our ancestors' worlds. Nutritional anthropology goes beyond contemporary foodways and takes a biocultural approach to considering food as a dynamic system and the development of contemporary foodways with a clear evolutionary perspective (Dufour, Goodman, and Pelto 2013). Data and approaches from these areas may not always be appropriate to use in palaeopathology, but they offer valuable perspectives.

Within geography lies the subdiscipline of medical geography, or "the comparative study of the spatial distribution of diseases and their possible causes" (Howe 1997, 1), which includes relationships between human disease and the physical and human-made environments (e.g. see Le Mare, Makungu, and Dunn 2014 on malaria). It is closely

related to epidemiology, or “the study of the distribution and determinants of health-related states or events (including disease), and the application of this study to the control of diseases and other health problems” (http://www.who.int/topics/epidemiology/en/). One of the very early and famous studies is John Snow’s investigation of the relationship of cholera victims to their water supply (public water pumps) in 1854 Soho, London. This study highlighted that one particular water pump on Broad Street was probably contaminated. On the basis of this study, Snow recommended that the pump handle be removed, which led to cholera’s decline in the area (Howe 1997, 161). This sub-discipline is of importance in palaeopathology because it can provide information that may be used to understand the distribution of disease in the past. For example, why is Paget’s disease today seen most commonly in the north-west of Europe (Mays 2010), including palaeopathological evidence (see, for example, Boylston and Ogden 2005)? In a more general sense, infections that are transmitted from person to person via droplet spread necessarily rely on high population density and people living in close contact with each other. Thus, it is to be expected that these will rise in frequency in urban situations when people have chosen to live in towns and cities. This is clearly the case for tuberculosis in the past, which became a particularly acute problem in Industrialised Europe. This transition to urban living was also noted to have led to large numbers of children developing rickets by both contemporary (Palm 1890) and later authors, and paleopathologists (Mays, Brickley, and Ives 2006). Indeed, studying global spatial frequencies of disease (essentially ‘medical geography’), integrated with genetic data, is allowing insights into epidemiology and management and control of disease in the 21st century (Pybus, Tatem, and Lemey 2015; Sintchenko and Holmes 2015).

Finally, clinical medicine is key to understanding health and well-being in people whose remains are studied by palaeopathologists (Mays 2012). Appreciating how disease affects the living person (mechanisms and its signs and symptoms), and their remains (bones, teeth, and soft tissues), is essential to appreciate how a palaeopathologist recognises and interprets disease present in skeletons and preserved bodies. However, diagnostic criteria are “usually adapted so that they are suited to the circumstances of palaeopathology rather than uncritically taken from clinical sources”

(ibid., 302). Evolutionary medicine is related to clinical medicine; the evolutionary angle is in effect a “set of concepts and approaches with which to analyse many different parts of medical science” (Stearns 2012). This is defined as placing human disease “within a framework of evolutionary thought” so that its causes can be better understood (Gluckman et al. 2011, 250). It has developed from the initial work of Williams and Nesse (1991), and explores why there is a mismatch in human evolution whereby culture is proceeding at such a fast pace that people’s bodies cannot adapt to changes quickly enough and they become sick (Gluckman and Hanson 2008). Scurvy is a key example. Humans are one of the few primates who cannot synthesise vitamin C and it is only if they are exposed to an environment lacking vitamin C that this inability is shown due to an enzyme mutation (Gluckman et al. 2011). This is why lack of access to vitamin C in the past is used to explain its presence in skeletal remains. Palaeopathology nicely complements evolutionary medicine as it provides insight to health and well-being over thousands of years. In particular the research being conducted on the evolution of pathogenic organisms found in archaeological human remains shows great promise, in concert with modern genomics, in unravelling how pathogens have changed over thousands of years (see above). In addition, medical genomics is providing essential data that will help palaeopathologists better understand disease risk (Crespi 2010).

<B> The importance of context

While research in palaeopathology crosses disciplinary divides, context is important for the interpretation of pathological conditions. Here the focus is on metabolic disease. In reviewing theory in palaeopathology, Grauer (2012, 5-6) found a tendency to take a processual approach, assuming that high frequencies of pathological conditions reflect individuals that are ‘less healthy’, these individuals being invariably those of lower social status. Taking such an approach leaves little scope for properly exploring the full range of human social possibilities (Grauer 2012). For example, there is a wide range of conditions, spatial and temporal, as well as socially and culturally mediated, that could result in the development of metabolic disease. As discussed by Brickley, Moffat, and Watamaniuk (2014), considering context and avoiding a processual approach is critical

in the case of vitamin D deficiency. Many people exploring vitamin D deficiency instantly think of rickets in the urban poor of 18th and 19th century industrial cities in northern Europe. Deficiency was indeed widespread in English communities as is evident in reports from London (e.g. Ives 2015), Birmingham (Brickley et al. 2006), Wolverhampton (Adams and Colls 2007), and North Shields (Roberts et al. 2016), and continental Europe in the inter-war years (see Figure 6). Although poor children have frequently developed rickets, the situation is more complex and context is required in considering differential diagnoses and interpreting findings.

In 18th and 19th century Birmingham, England, there were two broad social groups represented by burials made at St. Martin's Churchyard: earth-cut graves representing working-class individuals, most of whom would have been of fairly limited means, and brick-lined graves and vaults built by middle-class families. Vitamin D deficiency (active and healed cases) was evident in both adult and non-adult individuals from both social groups (Mays, Brickley, and Ives 2006; Brickley, Mays, and Ives 2007). The northerly location of Birmingham (52°N) would mean that from November to March the solar angle would be too low to produce effective vitamin D synthesis in exposed skin (latitude data from Holick 2008). The poorer individuals would also have lived in crowded housing in the city centre, in contrast to the larger houses with gardens at the edge of the city occupied by those ultimately buried in vaults. The range of socio-cultural factors operating to produce nutritional-related vitamin D deficiency in both these groups is discussed by Brickley, Moffat, and Watamaniuk (2014). Birmingham provides an example of how different socio-cultural factors could lead to the same disease outcome in both rich and poor.

In addition to nutritional-related vitamin D deficiency there are numerous pathological conditions that can cause skeletal changes associated with rickets and osteomalacia (Brickley and Ives 2008, Table 5.4; Ortner 2003, 393). Most other conditions that produce such changes are very rare and, prior to current medical treatment, few individuals would have survived for long enough for skeletal changes to manifest. The first step in determining if one of the rare conditions featured in Table 5.4 of Brickley and

Ives (2008) is present is a careful differential diagnosis. Consideration of context and socio-cultural factors can provide useful information on how likely it is that nutritional rickets or associated deficiency diseases were present. Vitamin D deficiency resulting from failure to acquire sufficient nutrients has been termed input insufficiency (Brickley, Moffat, and Watamaniuk 2014) and clinically the term 'nutritional rickets' is often used for these conditions in juveniles.

If a skeletal assemblage contains more than one individual with pathological changes associated with rickets or osteomalacia, rare aetiologies are unlikely. Given the poor preservation of skeletons with conditions causing mineralisation defects, in many cases differentiating such conditions would be impossible. Radiological assessment could assist in some conditions such as hypophosphatasia (Jaffe 1972), a genetic condition in which mineralisation of new bone is abnormal and dental development can be disrupted (Brickley and Ives 2008, 259). Genetic diagnosis of hypophosphatasia, caused by a mutation in the ALPL gene, has been undertaken clinically (Taillandier et al. 2015) and aDNA work could enable a diagnosis to be made in palaeopathology.

Scurvy is another example where development of disease can occur in a range of socio-economic groups for different reasons. The first commercially produced 'infant foods' became available in mid-19th century London. These foods would have been expensive and therefore only available to better off families. However, in the absence of firm nutritional knowledge, early commercially produced foods lacked many nutrients essential for healthy growth and development, including vitamin C. As reported by Barlow (1894) infants feeding on these foods would have been at risk of developing scurvy. In contrast, scurvy was widespread amongst the poorest members of society during the Great Irish Famine in the 1840s (Geber and Murphy 2012). The potato was an important source of vitamin C for poorer members of society and loss of this resource had massive health consequences. With the assistance of contextual information it is possible to suggest the presence of scurvy in archaeological skeletons and infer important information about contemporary socio-cultural situations in past communities.

<A> Some synergistic relationships between metabolic and infectious diseases

Using currently available techniques there are limitations to investigating relationships between infectious and metabolic disease in palaeopathology. If there was greater appreciation of the importance of exploring co-occurrence, and awareness of what can be said with confidence, this area of research could be further developed in palaeopathology. The following considers some co-morbidities.

<B> Leprosy, TB and vitamin D deficiency

Nutritional deficiency has been noted as having a particular impact on immunity (Chandra 1983), and vitamin D in particular is an immunomodulator in combatting mycobacterial and other infections (Bhutta 2008; Facchini et al. 2015). The main source of vitamin D is exposure of the skin to UVB radiation, although small amounts are available from dietary sources and are closely linked to maintaining calcium homeostasis (Holick 2008). It should also be remembered that leprosy and TB are both caused by *Mycobacteria* and have a degree of cross-immunity (Leitman, Porco, and Blower 1997). The strong link between malnutrition and many metabolic conditions means that they are closely associated with infectious disease because poor diets can affect the development and maintenance of a strong immune system that is resistant to infections.

For all pathological conditions the number of individuals that develop skeletal changes recognisable by paleopathologists will be a small proportion of individuals with the condition. For TB and leprosy there are currently no available data on what proportion of infected non-adults will develop bony changes. Some skeletal changes, such as Pott's disease of the spine, could be considered pathognomonic for TB, and the facial damage in leprosy, often described as specific to leprosy, has differential diagnostic options such as TB and treponemal disease. Nevertheless, collapse of the spine can occur in other conditions (see Roberts and Buikstra 2003: Table 3.3), and therefore

close attention to clinical descriptions of bone changes for these infections is required. Additionally, the non-specific bone changes associated with TB and leprosy could have a number of causes, making identification of individuals with these infections based on macroscopic evidence challenging (see Roberts, 2012, 435-436). The availability of prevalence data varies widely for the metabolic diseases and are dependent on the community under investigation. The international occurrence of scurvy is unknown, but a 7.1% overall prevalence of vitamin C deficiency, which can result in scurvy if severe, has been reported for the United States (Goebel 2015). Figures for Vitamin D deficiency are varied, in part because there is no internationally agreed level set for deficiency. Care in interpretation of reported figures for serum 25(OH)D levels are also required as a number of authors have pointed out the poor accuracy and issues with test result precision (e.g. Reid and Bolland 2014). Reported serum levels of 25(OH)D also vary greatly within and between communities; these issues are discussed further below, as is Paget's disease. All of these factors further confound assessing co-morbidity between infectious and metabolic disease.

If levels of vitamin D affect whether leprosy or TB develop then it is necessary to assess what levels are adequate for the prevention of vitamin D deficiency. Although there are now widespread data on levels of vitamin D evaluated from serum concentrations observed in communities around the world, there is a lack of consensus on what an individual's vitamin D level should be for sufficiency. Recommended levels of 25(OH)D are set at >50nmol/L in many countries and Spiro and Buttriss (2014) suggest levels of 25 nmol/L are the cut-off value to prevent rickets or symptomatic osteomalacia; this figure is widely used to denote deficiency. The percentage of individuals in the UK with plasma 25(OH)D of <25 nmol/L on a year-round basis ranges from 7.5% of girls and boys aged 1.5 - three years to 24.4% of girls aged 11-18 years (Spiro and Buttriss 2014, 328). It is not currently clear how low 25(OH)D levels need to be before the types of pathological changes observed by paleopathologists manifest. More research is required, as this type of information would help put the numerous current reports of vitamin D insufficiency and deficiency into context.

Investigations of adult skeletons excavated from the cemetery of St. Martin's, Birmingham, England produced a prevalence rate of 4.9% for osteomalacia (, Mays, and Ives 2007). Data pooled from a much wider range of 18th and 19th sites (including St. Martin's) produced a prevalence rate of 1.4% (Ives and Brickley 2014). The prevalence of rickets in people buried at St. Martin's was high (13% in juveniles) compared to 1.2% in the rural Medieval community from Wharram Percy examined by Ortner and Mays (1998). At St. Martin's skeletal evidence revealed that 15% of adults had evidence of a previous episode of vitamin D deficiency (Brickley, Mays, and Ives 2010), but no such evidence was identified from Wharram Percy. Conditions in Birmingham during the Industrial Revolution would have been markedly different to those experienced by the current population of the UK, although rickets, as identified by fraying of the ends of the long bones seen on radiographs, and bowed legs, is still reported, particularly amongst the immigrant community of Birmingham and the West Midlands (Callaghan et al. 2006). However, there are marked differences in patterns of vitamin D deficiency and rickets between paleopathological and clinical investigations. In the current UK population low 25(OH)D is most common in girls aged 11-18 (ibid.), but in the paleopathological investigation it was the younger children who were mstly affected. A significant factor in this difference is probably the fact that pathological changes are more likely to be seen in palaeopathology when individuals are undergoing rapid growth. Co-occurrence of vitamin D deficiency and tuberculosis has been noted at some 18th and 19th century British sites (e.g., Mays, Brickley and Ives 2006; Roberts et al. 2016 and Figure 7), but consideration of the relationship between vitamin D deficiency was not always part of the research objectives of these studies. However, a study to specifically consider co-occurrence of TB and vitamin D deficiency has recently been completed by Donoghue et al. (in press).

Limited data on 25(OH)D levels in individuals with pathological changes due to vitamin D deficiency that would be observable by paleopathologists are currently available. Holick (2007) reports that children with rickets often have 25(OH)D serum levels of <15ng/ml (37.44nmol/L (all figures converted using Oxford University Press 2016), and notes that skeletal deformities are usually a result of long standing rickets. In a report of symptomatic rickets in juveniles from the UK, bowed legs were reported in an 11-year-

old individual with 25(OH)D of 8.99 nmol/L, and severe knock-knee deformities in two individuals aged 14 years, both with 25(OH)D of <4.99 nmol/L (Crocombe, Mughal, and Berry 2004). Symptomatic osteomalacia was also reported in a young adult female (21 years) from Japan with serum 25(OH)D of <5 ng/mL (Watanabe pers. com. May 2016). In the person reported by Watanabe, Hotta and Ichihara (2015), thoracic deformities were observed of the type reported by Mensforth (2002) in the Hamann-Todd collection (kyphosis, scoliosis and deformities of the clavicles, sternum and ribs). There were multiple vertebral fractures, but no typical pseudofractures observed. This individual also had secondary hyperparathyroidism, and it was determined that the cause of the deficiency was nutritional rather than one of the rare conditions known to result in osteomalacia (ibid. See Brickley and Ives 2008, Table 5.4). Useful information on the lifestyle and diet of the individual and the development of the skeletal changes over two years are provided by this study.

Improvements in testing quality and data standardisation, as well as additional data, would be required for greater certainty, but it seems likely that many individuals reported as being deficient in 25(OH)D levels <25 nmol/L in the clinical literature would not be identifiable in palaeopathology using currently available analytical techniques. It is unlikely that as many skeletons with vitamin D deficiency are missed as skeletons with TB where, as discussed, the percentage of individuals with the condition that display skeletal changes is very low, but a substantial proportion of people will not be identified and levels will be higher for adults. Of course, with tuberculosis and leprosy there is the possibility of using aDNA analysis to identify individuals with no skeletal changes.

It is possible that episodes of vitamin D deficiency *in utero* and during the first few years of life may have significant consequences for health in later life (Barker 1994; Holick 2007). There is also good evidence that vitamin D deficiency after birth will affect an individual's immune response (Wei and Christakos 2015). Positive effects of vitamin D on the health of individuals with TB have been noted for some time, and there are reports from the 1940s of treating skin lesions caused by TB infection with vitamin D

(e.g. Dowling, Gauvain, and Macrae 1948). In a review undertaken by Peterlik (2012), tuberculosis was the only infectious disease with good evidence that vitamin D reduces the infectivity of the pathogen from large cohort studies. More recently Facchini and co-workers (2015) found a significant association between TB and low vitamin D levels in a review of 147 published studies. There is still much to learn about the relationship between TB and vitamin D and some authors have urged caution regarding claims that vitamin D could be used in the prevention or treatment of TB (e.g. Miragliotta and Miragliotta 2015). It is, however, clear that there is a synergistic relationship between these two conditions and future studies using paleopathological data may be able to provide additional information.

Leprosy and Vitamin D deficiency

While the full role of vitamin D and its receptor (VDR) in the control of Mycobacteria is not fully understood yet, it is clear that the VDR influences the cellular immune response to leprosy (Fernando and Britton 2006). It can affect the type of leprosy a person develops, and the complexity and severity in how a leprosy reaction progresses (see Mandal et al. 2015). Vitamin D has been associated with modulating the immune system to protect against the development of either multibacillary or paucibacillary leprosy, confirmed by Lu'o'ng and Nguyê Combining Tilde (2012). Furthermore, recent work indicates that two VDR gene polymorphisms place people at risk from developing leprosy (Neela et al. 2015). As yet, there is no work in palaeopathology that has tried to identify the VDR genes.

Leprosy and osteopenia/osteoporosis

Osteopenia is diagnosed when bone mineral density is between 1-2.5 standard deviations lower than the mean for a healthy young adult reference group, and osteoporosis is significant bone loss (> 2.5 standard deviations below the mean), with associated with bone fragility which can predispose to fracture (Kanis 1990; WHO 2003)". Osteoporosis may affect most of the skeleton (e.g. in scurvy or rickets and osteomalacia), one part (e.g. paralysis following a 'stroke'), or be very localised and

related to infection and tumours. It can also co-occur with other conditions. For example, rheumatoid arthritis may be accompanied by osteoarthritis (Resnick and Niwayama 1995c, 823), and osteoporosis can be related to health problems that have led to limb disuse, such as in the viral infection of poliomyelitis (Resnick 1995:3365).

Osteoporosis will predispose bones to subsequent fractures, especially in those experiencing age-related changes (e.g. post-menopausal women). A genetic predisposition needs also to be considered when aetiological factors are being assessed because “genes may act variably on bone metabolism” (Brickley and Ives 2008, 157). However, bone loss has been noted in infectious diseases (see Brickley and Ives 2008, 192-197), including leprosy, and it has been suggested that infections can “upset the balance between bone deposition and bone resorption” (Trevathan, Smith, and McKenna 2008, 205).

While clinical reports of osteoporosis associated with leprosy are quite scarce, perhaps because leprosy is usually the focus of treatment and not any associated osteopenia/osteoporosis, it is clear that one of the complications of leprosy is endocrine dysfunction (Leal and Foss 2009), including hypogonadism, sterility, and osteoporosis (Ishikawa et al. 1999). In hypogonadism the body does not produce enough testosterone (for male growth and development during puberty), or it has an impaired ability to produce sperm, or both. The consequence of osteoporosis in leprosy is fracture, and a high proportion of people with leprosy have been found to have associated osteoporosis (Choudhuri et al. 1999) and low mineral density. Indeed, osteoporosis has been implicated in tarsal disintegration in leprosy (Patil and Jacob 2000). This suggests that hypogonadism in men with the infection is a risk factor for this condition. In addition, it has been noted that there is variation in the vitamin D receptor (VDR) gene in relation to susceptibility to osteoporosis, and the type of immune response to leprosy (Roy et al. 1999). A poor prenatal environment may also lead to lower bone mineral deposition and people will be predisposed to osteoporosis later in life (Cooper et al. 2006). In leprosy, localised osteoporosis can also occur in people with the low resistant form (lepromatous leprosy) as osteolytic lesions, and can be present

underneath active localised skin lesions. The relationship between leprosy, osteoporosis, and vitamin D deficiency in the past has not been explored in any detail.

In a study of one archaeological site, however, 65 skeletons with osteoporosis were noted from the late Medieval leprosarium at Chichester, England (Lee and Boylston 2008), with 12 of 29 males (41%) having osteopenia. The association of osteoporosis, trauma and leprosy has also not been considered very frequently. However, of relevance here is the study of Judd and Roberts (1998) who considered evidence for fractures in people with and without leprosy buried in the same leprosarium cemetery. Males in particular may develop leprosy-associated osteoporosis, as discussed above, and people with leprosy may become blind, and could develop impairments leading to clumsiness, and loss of proprioception; they thus might be more likely to experience falls and fractures. In this study twenty-seven males (12.7% of 212 sexed individuals) and five females (2.4%) had fractures. Firstly, the frequency of fractured bones (2.6%) and the fracture rate among individuals (15.1%) were much higher than data from other urban medieval cemetery sites, and secondly, males exhibited more fractures. However, although fractures were seen at Chichester, people with leprosy did not have a higher frequency of fractures than those without leprosy, but it is possible that those with tuberculoid leprosy (high resistance, with no identifiable bone changes) could have been represented by some skeletons identified with fractures.

While this archaeological site produced a high frequency of people with fractures overall when compared to other contemporary urban late Medieval sites, the impact of leprosy on fracture rates in particular is apparent (one third of long bone fractures were seen in people with bone changes of leprosy). However, diagnosing osteoporosis in archaeological remains can be challenging because of diagenetic changes that can make a bone appear to be osteoporotic. If the bones of the skeleton are affected by post-mortem changes, leading to loss of bone mass and/or thinning of the bone cortex, this may be mistaken for osteoporosis (but cortical erosion may indicate diagenesis). Furthermore, suggesting that bone loss was the result of leprosy in archaeological skeletal remains is complicated by the fact that it cannot be known whether bone loss occurred first or leprosy, and of course bone loss may purely be an indicator of increasing age. Nevertheless, this is an area of study that is worth pursuing in palaeopathology,

considering the strong link between osteoporosis and leprosy, and between vitamin D deficiency and leprosy.

<B> The Developmental Origins Hypothesis, metabolic and infectious disease

In 1990 David Barker proposed the Developmental Origins Hypothesis, suggesting that intrauterine growth retardation, low birth weight, and premature birth predisposed people later in life to metabolic syndrome diseases, such as cardiovascular disease and diabetes (Barker 1990). As a result, structures in organs can change, systems that control hormone levels, normal metabolism may alter, and enhanced stress responses may develop, making people more likely to react to psychosocial stress (Godfrey and Hanson 2009). The immune system changes over the life course, varying the strength of an individual's resistance to infections. It is suggested that young adults adapt better to deal with chronic infections, when compared to the very young and very old; this adaptation reflects the importance of young adults in '…the survival of the species...' (Simon, Hollander, and McMichael 2015, 7). Therefore, taking a life course approach to understand past and present health and well-being makes sense.

Palaeopathologists in particular have been adopting this working hypothesis in their research in recent years, and linking evidence for height and various health indicators with age at death (Gowland 2016). For example, short stature was found to be associated with a higher risk of death in people living in London during the Black Death (DeWitte and Hughes-Morey 2012). While genetics and environmental (mostly) factors contribute to attained adult stature, in palaeopathology it is not possible to be specific about what might have caused short stature, but childhood diseases such as infections and nutritional deficiency are implicated. Infection that affects the teeth and alveolar bone (caries and periodontitis) has also been used to explain a higher risk for death in another Medieval cemetery in London, when compared to people buried there without these conditions (DeWitte and Bekvalac 2010). However, perhaps the most common health indicator used in palaeopathology to test the Developmental Origins Hypothesis has been linear enamel hypoplastic defects, or defects in the enamel of the teeth that

can develop during growth of the teeth if a person is stressed due to a childhood disease (possibly an infection) and/or a nutritional deficiency (Hillson 1996). For example, early work relating to infectious disease found that people with TB in their skeletons had more frequent and severe enamel hypoplasia than those without TB, and the defects developed "prior to circa seven years of age" (Knick 1981, 136). Duray (1990, 1996) also found more hypoplasias in people from the 800-1100 AD Libben site, Ottawa County, Ohio, USA who died early. More recent research confirms these findings from other geographic regions (e.g. Miszkiewicz 2012).

Directly correlating early life 'stress' markers in the skeleton, indicative of metabolic or infectious disease, or both, with disease later in life is a challenge for palaeopathology. Determining the precise early life health problem that has led to a specific metabolic or infectious disease evident in the skeleton would be impossible. However, this type of research shows how palaeopathologists must take a whole life course approach to appreciating morbidity and mortality in past communities.

<B> Paget's disease of bone and infection

Paget's disease of bone (PDB) is a metabolic disease in which there is focal disruption of normal bone remodelling. Localised areas of increased resorption develop in association with extensive defective bone formation (Galson and Roodman 2014). The pelvis, skull, long bones (particularly the femur and tibia) and the lumbar vertebrae are commonly involved (Figure 8), although other skeletal areas can be impacted (see review in Brickley and Ives 2008). Histological (De Boer, Van der Merwe, and Maat 2013) and radiological (Brickley and Ives 2008) techniques have significant potential to assist in suggesting paleopathological evidence of PDB with confidence. It has been suggested that the prevalence of PDB has declined in recent years (Galson and Roodman 2014; Merashli and Jawad 2015) and it has been proposed that environmental triggers, including infectious diseases, have played a role. Changes in prevalence are not, however, completely clear-cut because in earlier stages the condition is often asymptomatic. The development of PDB is strongly age-related and

is often diagnosed in individuals over the age of 55 years (Merashli and Jawad 2015); thus, there will be difficulties in accurately establishing the incidence of the condition in clinical medicine. Prevalence is highly variable geographically (Cundy and Bolland 2008), but has been reported to be 0.71% ± 0.18% in the United States (Altman et al. 2000). Recent work on archaeological evidence of PDB provides strong evidence to support the theory that the disease originated in populations in Northwest Europe (Mays 2010). Clinical evidence appears to show that there has been a marked reduction in the prevalence of PDB (Bolland and Cundy 2016) and paleopathological work could provide valuable data on the epidemiology of the condition.

In some pathological conditions there is a genetic factor that is only expressed if there is a triggering agent (Ortner 2012, 251). In the case of PDB there are clearly underlying genetic factors (Ralston and Albagha 2014), but there has been considerable debate about what the possible triggering agents could be. There are clear regional differences in the prevalence of PDB. Individuals from Britain currently have the highest levels, with high rates in other areas of Northern Europe, and regions of the world that have experienced high levels of immigration from these areas (Merashli and Jawad 2015). High on the list of possible suspects for triggering agents are viral infections. Various viral infections have been suggested (Cundy and Bolland 2008), and these infectious conditions may have a zoonotic component. Studies have noted that individuals with PDB are more likely to have owned a dog than control individuals, and possible links with canine distemper virus have been investigated (Ralston 2008). However, the debate regarding a possible relationship between PDB and viral infections remains unresolved. Some viruses have been demonstrated to impact aspects of bone remodelling, but such observations do not mean there is a direct link (Ralston 2008, 822). It is likely that the development of PDB varies between individuals with different genetic mutations. In some individuals a specific viral infection may be a required co-factor in the development of PDB, and in others carrying the $p62^{P392L}$ gene (a result of the commonest genetic mutation in PDB) a second genetic mutation could be required, or an additional viral trigger (Galson and Roodman 2014, 87-88). With advances in

high-throughput sequencing for DNA studies it may be possible to investigate genetic mutations in paleopathological skeletons showing PDB.

<A> Conclusions

With recent developments in the field, paleopathologists are now on the cusp of being able to fully investigate the synergistic relationships known to exist between infectious and metabolic diseases. Developments in analytical techniques outlined in this chapter mean it is now possible to investigate a much wider range of questions, and biomolecular analysis is likely to remain particularly important. A number of important areas have emerged where combining information from both infectious and metabolic diseases offers the potential to not only understand more about the lives of those who experienced these diseases in the past, but also contribute clearer answers to questions in current clinical medicine. For example, palaeopathology has the potential to contribute information on the epidemiology of Paget's disease of bone and assist in determining the role of infectious disease in its development. Much clearer information could also be provided on the nature of the co-occurrence of infectious and metabolic conditions such as TB and vitamin D deficiency. More can be also learned about the way in which bone loss develops, through systematic studies of the development of osteopenia and osteoporosis in skeletons diagnosed with leprosy. Importantly, consideration of both infectious and metabolic disease in the context of the Developmental Origins Hypothesis would make a significant area of research that has considerable potential to contribute to a number of important debates on current health. A theme that emerges is the important need for curation of archaeological human remains in a careful manner, and planning for their long-term study. It is clear that restudies of human remains with new methods and questions generate new outcomes (Buikstra and Gordon 1981). Reburial of human remains prevents their study in the future as sophistications in methodology develop and new theories about the history of disease emerge. Thus, the potential is lost for using palaeopathology's deep time perspective which enables the present to learn from the past.

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References

Aaron JE, Rogers J, Kanis JA. 1992. Paleohistology of Paget's disease in two medieval skeletons. Am J Phys Anthropol 89:325–331.

Adams J, Colls K. 2007. Out of darkness cometh light. Life and death in nineteenth century Wolverhampton. British Archaeological Reports British Series 442. Oxford: Archeopress.

Altman RD, Bloch DA, Hochberg MC, Murphy WA. 2000. Prevalence of pelvic Paget's disease of bone in the United States. J Bone Miner Res 15:461-465.

Andersen J, Manchester K. 1987. Grooving of the proximal phalanx in leprosy: a palaeopathological study. J Archaeol Sci 14:77-82.

Andersen J, Manchester K. 1988. Dorsal tarsal exostosis in leprosy: a palaeopathological and radiological study. J Archaeol Sci 15:51-56.

Araújo A, Jansen A, Reinhard K, Ferreira L. 2009. Paleoparasitology of Chagas disease - a review. Mem Inst Oswaldo Cruz 104 Suppl 1:9-16.

Assis S, Santos AL. Roberts CA. 2012. Evidence of hypertrophic osteoarthropathy in inidviduals from the Coimbra Skeletal Identified Collection (Portugal). Int J Paleopathology 2:155-163

Aufderheide A. 2003. The scientific study of mummies. Cambridge: Cambridge University Press.

Aufderheide AC, Rodríguez-Martín C.1998. The Cambridge encyclopedia of human paleopathology. Cambridge: Cambridge University Press.

Baker BJ. 1999. Early manifestations of tuberculosis in the skeleton. In: Pálfi G, Dutour O, Deák J, Hutás, editors. Tuberculosis. Past and present. Budapest/Szeged: Golden Book Publishers and Tuberculosis Foundation. p 301-307.

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

Barker DJP. 1990. The fetal and infant origins of adult disease. Brit Med J 301:1111.

Barlow T. 1883. On cases described as "acute rickets" which are probably a combination of scurvy and rickets, the scurvy being the essential, and the rickets a variable, element. Medico-Chirurgical Transactions 66:159-219.

Barlow T. 1894. The Bradshaw lecture on infantile scurvy and its relation to rickets: delivered before the Royal College of physicians of London. Brit Med J 2:1029-1034.

Barnes I, Duda A, Pybus OG, Thomas MG. 2011. Ancient urbanization predicts genetic resistance to tuberculosis. Evolution 65:842–848.

Barnett R. 2014. The sick rose or; Disease and the art of medical illustration. London: Thames and Hudson.

Barrett R, Armelagos GJ. 2013. An unnatural history of emerging infections. Oxford: Oxford University Press.

Barrett R, Kuzawa CW, McDade T, Armelagos G. 1998. Emerging and re-emerging infectious diseases: the third epidemiologic transition. Annu Rev Anthropol 27:247-271.

Beaumont J, Montgomery J. 2015. Oral histories: a simple method of assigning chronological age to isotopic values from human dentine collagen. Ann Hum Biol 42:407-414.

Beaumont J, Montgomery J, Buckberry J, Jay M. 2015. Infant mortality and isotopic complexity: new approaches to stress, maternal health, and weaning. Am J Phys Anthropol 157:441-457.

Bell LS, Jones SJ, 1991. Macroscopic and microscopic evaluation of archaeological pathological bone: backscattered electron imaging of putative Pagetic bone. Int J Osteoarchaeol 1:179–184.

Bertrand L, Schöeder S, Anglos D, Breese MBH, Janssens K, Moini M, Simon A. 2015. Mitigation strategies for radiation damage in the analysis of ancient materials. Trac-Trend Anal Chem 66:128-145.

Bhutta ZA. 2008. Vitamin D and child health: some emerging issues. Matern Child Nutr 4:83-85.

Blondiaux J, Duvette J-F, Vatteon S, Eisenberg L. 1994. Microradiographs of leprosy from osteoarchaeological contexts. Int J Osteoarchaeol 4:13-20.

Bolland MJ, Cundy T. 2016. Paget's disease of bone: clinical review and update. J Clin Pathol 66(11):924-927.

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

Boros-Major A, Bona A, Lovasz G, Molnár E, Marcsik A, Pálfi G, Mark L. 2011. New perspectives in biomolecular palaeopathology of ancient tuberculosis. A proteomic approach. J Archaeol Sci 38:197-201.

Bos KI, Harkins KM, Herbig A, Coscolla M, Weber N, Comas I, Forrest SA, Bryant JM, Harris SR, Schuenemann VJ, Campbell TJ, Majander K,Wilbur AK, Guichon RA, Wolfe Steadman DL, Cook DC, Niemann S, Behr MA, Zumarraga M, Bastida R, Huson D, Nieselt K, Young D, Parkhill J, Buikstra JE, Gagneux S, Stone AC. 2014. Pre-Columbian mycobacterial genomes reveal seals as a source of New World human tuberculosis. *Nature* 514:494-497.

Boylston A, Ogden A. 2005. A study of Paget's disease at Norton Priory, Cheshire. A medieval religious house. In: Zakrewski SR, Clegg M. editors. Proceedings of the fifth annual conference of the British Association for Biological Anthropology and Osteoarchaeology. British Archaeological Reports International Series 1383. Oxford: Archaeopress. p.69-76.

Bouwman AS, Kennedy SL, Muller R, Stephens RH, Holst M, Caffell AC, Roberts CA, Brown TA. 2012. Genotype of a historic strain of *Mycobacterium tuberculosis*. Proc Nat Acad Science USA 109:18511-18516.

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

Brickley M, Buteux S, Adams J, Cherrington R. 2006. St. Martin's uncovered: investigations in the churchyard of St. Martin's-in-the-Bull Ring, Birmingham, 2001. Oxford: Oxbow Books.

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

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

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

Brites D, Gagneux S. 2015. Co-evolution of *Mycobacterium tuberculosis* and Homo sapiens. Immunol Rev 264:6-24.

Brown T, Brown K. 2011. Biomolecular archaeology. An introduction. Oxford: Wiley-Blackwell.

Budd P, Millard A, Chenery C, Lucy S, Roberts CA. 2004. Isotopic evidence for archaeological immigration and residential mobility in the U.K. Antiquity 78:127-141.

Buikstra JE, Gordon CC. 1981. The study and restudy of human skeletal series: the importance of long-term curation. Ann Ny Acad Sci 376:449-465.

Bunnag D, Cabrera BD, Crompton DWT, Farid Z, Gilles HM, Kale OO, Kan-Chua SP, Kilama WL, Marsden PD, Martinez-Palomo A, Ozeretskovskaya N, Schultz MG. 1987. Public health significance of intestinal parasitic infections. B World Health Organ 65: 575-588.

Burger J, Kirchner M, Bramanti B, Haak W, Thomas MG. 2007. Absence of the lactase-persistence-associated allele in early Neolithic Europeans. Proc Nat Acad Sci USA 104:3736-3741.

Callaghan AL, Moy RJ, Booth IW, Debelle G, Shaw NJ. 2006. Incidence of symptomatic vitamin D deficiency. Arch Dis Child 91:606–607.

Carnemolla SE. 2003. Death from scurvy on Vasco da Gama's first journey to India. Med Secoli 15:615-630.

Chandra RK. 1983. Nutrition and immune responses. Can J Physiol Pharm 61:290-294.

Choudhuri H, Thappa DM, Kumar RH, Elangovan S. 1999. Bone changes in leprosy patients with disabilities/deformities (a clinico-radiological correlation). Indian J Leprosy 71:203–215.

Cobelens FGJ, van Deutekom H, Draayer-Jansen IWE, Schepp-Beelen ACHM, van Gerven PJHJ, van Kessel RPM, Mense MEA. 2000. Risk of infection with *Mycobacterium tuberculosis* in travellers to areas of high tuberculosis endemicity. Lancet 356:461-465.

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

Crespi BJ. 2010. The emergence of human-evolutionary medical genomics. Evol Appl 4:292-314.

Crocombe S, Mughal M, Berry J. 2004. Symptomatic vitamin D deficiency among non-Caucasian adolescents living in the United Kingdom. Arch Dis Child 89:197–199.

Cundy T, Bolland M. 2008. Paget's disease of bone. Trends Endocrin Met 19:246-253.

Dabernat H, Crubézy É. 2009, Multiple bone tuberculosis in a child from predynastic Upper Egypt (3200 BC). Int J Osteoarchaeol 20:719-730.

Danielssen D-C, Boeck CW. 1847. Øm spedalskhed. Christiania: Christian Grøndahl.

De Boer HH, Van der Merwe AE, Maat GJR. 2013. The diagnostic value of microscopy in dry bone palaeopathology: A review. Int J Paleopathology 3:113-121.

DeWitte S, Bekvalac J. 2010. Oral health and frailty in the medieval English cemetery of St Mary Graces. Am J Phys Anthropol 142:341-354.

DeWitte SN, Hughes-Morey G. 2012. Stature and frailty during the Black Death: the effect of stature on risks of epidemic mortality in London, A.D. 1348-1350. J Archaeol Sci 39:1412-1419.

Dittmar K. 2009. Old parasites for a new world: the future of paleoparasitological research. A review. J Parasitol 95:365-371.

Dittmar K, Araújo A, Reinhard KJ. 2012. The study of parasites through time: archaeoparasitology and paleoparasitology. In: Grauer AL, editor. A companion to paleopathology. Chichester: Wiley-Blackwell. p 170-190.

D'Ortenzio L, Brickley M, Schwarcz H, Prowse T. 2015. You are not what you eat during physiological stress: isotopic evaluation of human hair. Am J Phys Anthropol 157:374–388.

Donoghue HD, Bekvalac J, Redfern RC. In press. Molecular evidence of tuberculosis from mid-19th century human remains without typical palaeopathology, from the Cross Bones Burial site, Redcross Way, London. In: Nystrom P, Swales DM, editors. Trends in Biological Anthropology 3. Oxford: Oxbow.

Dowling GB, Gauvain S, Macrae DE. 1948. Vitamin D in treatment of cutaneous tuberculosis. Brit Med J 1:430-435.

Dufour D, Goodman AH, Pelto GH, editors. 2013. Nutritional anthropology: biocultural perspectives in food and nutrition. 2nd edition. New York: Oxford University Press.

Duray S. 1990. Deciduous enamel defects and caries susceptibility in a prehistoric Ohio population. Am J Phys Anthropol 81:27-34.

Duray SM. 1996. Dental indicators of stress and reduced age at death in prehistoric Native Americans. Am J Phys Anthropol 99:275-286.

Eckardt H, Chenery C, Booth P, Evans JA, Lamb A, Müldner G. 2009. Oxygen and strontium isotope evidence for mobility in Roman Winchester. J Archaeol Sci 30:1-10.

Eerkens JW, de Voogt A, Dupras TL, Rose SC, Bartelink EJ, Francigny V. 2014. Intra- and inter-individual variation in d13C and d15N in human dental calculus and comparison to bone collagen and apatite isotopes. J Archaeol Sci 52:64-71.

Elliott Smith G, Ruffer MA. 1910. Pottsche krakheit an einer ägyptischen mumie aus der zeit der 21 dynastie (um 1000 v. Chr.). In: Zur historichen biologie der Kranzheit serreger. Leipzig. p 9-16.

Elliot Smith G, Dawson WR. 1924. Egyptian mummies. London: George Allen and Unwin Ltd.

Ewing J. 1922. A review and classification of bone sarcomas. Arch Surg 4:485–533.

Eyler WR, Monsein LH, Beute GH, Tilley B, Schultz LR, Schmitt WGH. 1996. Rib enlargement in patients with chronic pleural disease. Am J Roentgenol 167:921-926.

Facchini L, Venturini E, Galli L, Martino Md, Chiappini E. 2015. Vitamin D and tuberculosis: a review of a hot topic. J Chemotherapy 27:128-138.

Faerman, M, Jankauskas R, Gorski A, Bercovier H, Greenblatt ChL. 1997. Prevalence of human tuberculosis in a Medieval population of Lithuania studied by ancient DNA analysis. Ancient Biomolecules 1:205-214.

Fauci AS, Morens DM. 2016. Zika virus in the Americas — yet another Arbovirus threat. New Engl J Med 374:601-604.

Faulkner CT, Reinhard KJ. 2014. A retrospective examination of paleoparasitology and its establishment in the Journal of Parasitology. J Parasitol 100:253-259.

Fernando SI, Britton WJ. 2006. Genetic susceptibility to mycobacterial disease in humans. Immunol Cell Biol 84:125-137.

Fildes VA. 1986. The English disease: Rickets and scurvy in pre industrial England. In: Cule J, Turner T, editors. Child care through the centuries: an historical survey from papers given at the tenth British congress on the history of Medicine at Clyne Castle, Swansea. Cardiff: STS publishing for The British Society of the History of Medicine. p 121-134.

Finch RG, Moss P, Jeffries DJ, Anderson. J. 2002. Infectious diseases, tropical medicine and sexually transmitted diseases. In: Kumar P, Clark M, editors. Clinical medicine. 5th edition. Edinburgh: WB Saunders. p 21-151.

Fletcher HA, Donoghue HD, Holton J, Pap I, Spigelman M. 2003, Widespread occurrence of *Mycobacterium tuberculosis* DNA from 18th-19th century Hungarians. Am J Phys Anthropol 120:144-152.

Follis BH, Park EA, Jackson D. 1950. The prevalence of scurvy at autopsy during the first two years of age. B Johns Hopkins Hosp 87:569-591.

Fornaciari, G, Castagna M, Tognetti A, Tornaboni D, Bruno J. 1989. Syphilis in a Renaissance Italian mummy. Lancet 334:614.

Fornaciari G, Castagna M, Viacava P, Tognetti A, Bevilacqua G, Segura EL. 1992. Chagas' disease in Peruvian Inca mummy. *The Lancet* 339, No. 8785:128–129**.**

Galagan JE. 2014. Genomic insights into tuberculosis. Nature Reviews. Genetics 15:307-320.

Galson GL, Roodman GD. 2014. Pathobiology of Paget's disease of bone. J Bone Metab 21:85-98.

Gao XF, Rao Y. 2015. Quality of life of a migrant population with tuberculosis in western China. Int J Tuberc Lung D 19:223-230.

Geber J, Murphy E. 2012. Scurvy in the great Irish famine: evidence of vitamin C deficiency from a mid-19th century skeletal population. Am J Phys Anthropol 148:512-524.

Gennari C. 2001. Calcium and vitamin D nutrition and bone disease of the elderly. Public Health Nutr 4:547-559.

Gerbault P, Liebert A, Itan Y, Powell A, Currat M, Burgere J, Swallow DM, Thomas MG. 2011. Evolution of lactase persistence: an example of human niche construction. Philos T R Soc B 366:863-877.

Gernaey AM, Minnikin DE, Copley M, Dixon R, Middleton JC, Roberts CA. 2001 Mycolic acids and ancient DNA confirm an osteological diagnosis of tuberculosis. Tuberculosis 81:259-265.

Giuffra V, Vitiello A, Caramella D, Fornaciari A, Giustini D, Fornaciari G., 2015. Rickets in a high social class of Renaissance Italy: the Medici children. Int J Osteoarchaeol 25:608–624.

Glisson F. 1650. A treatise of the rickets: being a disease common to children. Translated and edited by Culpeper N. London: P Cole.

Gluckman P, Hanson M. 2008. Mismatch. The lifestyle diseases timebomb. Oxford: University Press.

Gluckman PD, Low FM, Buklijas T, Hanson MA, Beedle AS. 2011. How evolutionary principles improve the understanding of human health and disease. Evol Appl 4:249-263.

Godfrey K, Hanson M. 2009. The developmental origins of health and disease. In: Panter-Brick C, Fuentes A, editors. Health, risk and adversity. Oxford: Berghahn Books. p 185-208.

Goebel L. 2015. Scurvy. http://emedicine.medscape.com/article/125350-overview#a6. Accessed April 2016.

Gowland RL. 2016. Entangled lives: implications of the developmental origins of health and disease hypothesis for bioarchaeology and the life course. Am J Phys Anthropol 158:530-540.

Grauer AL. 2012. Introduction: The scope of paleopathology. In: Grauer AL, editor. A companion to paleopathology. Chichester: Wiley-Blackwell. p 1-14.

Grieshaber BM, Osborne DL, Doubleday AF, Kaestle FA. 2008. A pilot study into the effects of X-ray and computed tomography exposure on the amplification of DNA From bone. J Archaeol Sci 35:681–687.

Grove C, Peschel O, Nerlich AG. 2015. A systematic approach to the application of soft tissue histopathology in paleopathology. Biomed Res Int. August 6 doi: 10.1155/2015/631465.

Haas CJ, Zink A, Molnár E, Szeimies U, Reischel U, Marcsik A, Ardagna Y, Dutour O, Pálfi G, Nerlich A. 2000. Molecular evidence for different stages of tuberculosis in ancient bone samples from Hungary. Am J Phys Anthropol 113:293-304.

Hackett CJ. 1976. Diagnostic criteria of syphilis, yaws and treponarid (treponematoses) and of some other diseases in dry bone. New York and Heidelberg: Springer.

Harkins KM, Stone AC. 2015. Ancient pathogen genomics: insights into timing and adaptation. J Hum Evol 79:137-149.

Harrison M. 2012. Contagion. How commerce has spread disease. London: Yale University Press.

Hendy J, Collins M, Tech KY, Ashford DA, Thomas-Oates J, Donoghue HD, Pap I, Minnikin DE, Spigelman M, Buckley M. 2016. The challenge of identifying tuberculosis proteins in archaeological tissues. J Archaeol Sci 66:146-153.

Hershkovitz I, Greenwald C, Latimer B, Jellema LM, Wish-Baratz S, Eshed V, Dutour O, Rothschild B. 2002. Serpens endocrania symmetrica (SES): a new term and possible clue for identifying intrathoracic disease in skeletal populations. Am J Phys Anthropol 118:201-216.

Hershkovitz I, Donoghue HD, Minnikin DE, Besra GS, Lee OY-C, Gernaey AM, Galili E, Eshed V, Greenblatt CL, Lemma E, Bar-Gal GK, Spigelman M. 2008. Detection and molecular characterization of 9000-Year-Old *Mycobacterium tuberculosis* from a Neolithic settlement in the Eastern Mediterranean. PLoS One 3:e3426. http://dx.doi.org/10.1371/journal.pone.0003426

Hillson S. 1996. Dental anthropology. Cambridge: Cambridge University Press.

Hintzpeter B, Scheidt-Nave C, Muller MJ, Schenk L, Mensink GBM. 2008. Higher prevalence of vitamin D deficiency is associated with immigrant background among children and adolescents in Germany. J Nutr 138:1482–1490.

Holick MF. 2007. Vitamin D deficiency. New Engl J Med 357:266-281.

Holick MF. 2008. Vitamin D: a D-Lightful health perspective. Nutr Rev 66:S182–S194.

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

Howe GM. 1997. People, environment, disease and death. Cardiff: University of Wales Press.

Inskip SA, Taylor GM, Zakrzewski SR, Mays SA, Pike AWG, Llewellyn G, Williams CM, Lee OY-C, Wu HHT, Minnikin DE, Besra GS, Stewart GR. 2015. Osteological, biomolecular and geochemical examination of an early Anglo-Saxon case of lepromatous leprosy. PLoS One: doi.org/10.1371/journal.pone.0124282.

Ishikawa S, Ishikawa A, Yoh K, Tanaka H, Fujiwara M. 1999. Osteoporosis in male and female leprosy patients. Calcified Tissue Int 64:144-147.

Itan Y, Powell A, Beaumont MA, Burger J, Thomas MG. 2009. The origins of lactase persistence in Europe. PLoS Comput Biol: doi.org/10.1371/journal.pcbi.1000491.

Ives R. 2015. Insights into health, life and death in Victorian London's East End. London Archaeologist 14:150-154.

Ives R, Brickley M. 2005. Metacarpal radiogrammetry. A useful indicator of bone loss throughout the skeleton? J Archaeol Sci 32:1552-1559.

Ives R, Brickley M. 2014. New findings in the identification of adult vitamin D deficiency osteomalacia: results from a large-scale study. Int J Paleopathology 7:45-56.

Ives R, Mant M, de la Cova C, Brickley M. In press. Large-scale palaeopathological study of hip fractures from Post-Medieval urban England. Int J Osteoarchaeol.

Jablonski NG. 2012. The evolution of human skin colouration and its relevance to health in the modern world. J R Coll Physicians Edinb 42:58-63.

Jablonski NG, Chaplin G. 2013. Epidermal pigmentation in the human lineage is an adaptation to ultraviolet radiation. J Hum Evol 65:671–675.

Jaffe HL. 1972. Metabolic, degenerative, and inflammatory diseases of bones and joints. Philadelphia: Lea & Febiger.

Jobling MA, Hurles ME, Tyler-Smith C. 2004. Human evolutionary genetics: origins, peoples and disease. New York: Taylor & Francis.

Johnston WD. 1993. Tuberculosis. In: Kiple K, editor. The Cambridge world history of human disease. Cambridge: Cambridge University Press. p 1059-1068.

Jolliffe DA, Hanifa Y, Witt KD, Venton TR, Rowe M Timms PM, Hyppönen E, Walton RT, Griffiths CJ, Martineau AR. In press. Environmental and genetic determinants of vitamin D status among older adults in London, UK. J Steroid Biochem doi: 10.1016/j.jsbmb.2016.01.005.

Judd M, Roberts CA. 1998. Fracture patterns at the Medieval leper hospital in Chichester. Am J Phys Anthropol 105:43-55.

Kanis JA. 1990. Osteoporosis and osteopenia. J Bone Miner Res 5:209–211.

Kappelman J, Alçiçek MC, Kazanci N, Schultz M, Ozkul M, Sen SS. 2008. First Homo erectus from Turkey and implications for migrations into temperate Eurasia. Am J Phys Anthropol 135:110–116.

Katzenberg MA. 2008. Stable isotope analysis: a tool for studying past diet, demography and life histories. In: Katzenberg MA, Saunders SR, editors. Biological anthropology of the human skeleton. 2nd edition. Hoboken: John Wiley and Sons. p 413-442.

Katzenberg MA. 2012. The ecological approach: understanding past diet and the relationship between diet and disease. InL Grauer AL, editor. A companion to paleopathology. Chichester: Wiley-Blackwell. p 97-113.

Katzenberg MA, Lovell NC. 1999. Stable isotope variation in pathological bone. Int J Osteoarchaeol 9:316–324.

Kelley M, Micozzi M. 1984. Rib lesions in pulmonary tuberculosis. Am J Phys Anthropol 65:381-386.

Kendall EJ, Montgomery J, Evans JA, Stantis C, Mueller V. 2013. Mobility, mortality, and the middle ages: identification of migrant individuals in a 14th century black death cemetery population. Am J Phys Anthropol 150:210-222.

Knick SG. 1981. Linear enamel hypoplasia and tuberculosis in the pre-Columbian North America. Ossa 8:131-138.

Krawinkel MB. 2012. Interaction of nutrition and infections globally: an overview. Ann Nutr Metab 61(suppl, 1):39-45. doi:1159/000345162.

Larsen CS 1997. Bioarchaeology. Interpreting behavior from the human skeleton. Cambridge: Cambridge University Press.

Laupland KB, Valiquette L. 2014. Ebola virus disease. Can J Infect Dis Med 25:128–129.

Leal AM, Foss NT. 2009. Endocrine dysfunction in leprosy. Eur J Clin Microbiol 28:1-7.

Lee F, Boylston A. 2008. Other pathological conditions. In: Magilton JR, Lee F, Boylston A, editors. 'Lepers outside the gate'. Excavations at the cemetery of hospital of St James and St Mary Magdalene, Chichester, 1986-87 and 1993. York: Council for British Archaeology, Research Report 158 and Chichester Excavations Vol 10. p 252-259.

Leitman T, Porco T, Blower S. 1997. Leprosy and tuberculosis: the epidemiological consequences of cross-immunity. Am J Public Health 87:1923-1927.

Le Bailly M, Maicher C, Dufour B. 2016. Archaeological occurrences and historical review of the human amoeba, Entamoeba histolytica, over the past 6000 years. Infect Genet Evol 42:34-40.

Le Mare A, Makungu C, Dunn C. 2014. "Yes we are here, living, but malaria is surrounding us" sustainable livelihoods and malaria in Tanzania. Development in Practice 24:216-233.

Lewis ME. 2004. Endocranial lesions in non-adult skeletons: understanding their aetiology. Int J Osteoarchaeol 14:82-97.

Lewis ME. 2010. Life and death in a civitas capital: metabolic disease and trauma in the children from late Roman Dorchester, Dorset. Am J Phys Anthropol 142:405-416.

Lewis ME. 2011. Tuberculosis in the non-adults from Romano-British Poundbury Camp, Dorset, England. Int J Paleopathology 1:12-23.

Lind J. 1753. A treatise of the scurvy. Edinburgh: A Kincaid and A Donaldson.

Loeffler SH, de Lisle GW, Neill MA, Collins DM, Price-Carter M, Paterson B, Crews KB. 2014. The seal tuberculosis agent, *Mycobacterium pinnipedii*, infects domestic cattle in New Zealand: epidemiologic factors and DNA strain typing. J Wildlife Dis 50:180-187.

Lu'o'ng KV, Nguyê Combining Tilde NT. 2012. Role of vitamin D in leprosy. Am J Med Sci 343:471-482.

Mandal D, Reja AH, Biswas N, Bhattacharyya P, Patra PK, Bhattacharyya B. 2015. Vitamin D receptor expression levels determine the severity and complexity of leprosy reaction patients. New Microbes New Infect 6:35-39.

Mansilla J, Pijoan C.1995. Brief communication: a case of congenital syphilis during the colonial period in Mexico City. Am J Phys Anthropol 97:187-195.

Mariotti V, Zuppello M, Pedrosi ME, Bettuzzi M, Brancaccio R, Peccenini E, Morigi MP, Belcastro MG. 2015. Skeletal evidence of tuberculosis in a modern identified human skeletal collection (Certosa cemetery, Bologna, Italy). Am J Phys Anthropol 157:389-401.

Marx FM, Fiebeg L, Hauer B, Brodhun B, Glaser-Paschke G, Magdorf K, Haas W. 2015. Higher rate of tuberculosis in second generation migrants compared to native residents in a metropolitan setting in Western Europe. PloS One: doi: 10.1371/journal.pone 0119693.

Masson M, Molnar E, Donoghue HD, Besra GS, Minnikin DE, Wu HHT, Lee OY-C, Bull ID, Pálfi G. 2013. Osteological and biomolecular evidence of a 7000 year old case of hypertrophic pulmonary osteopathy secondary to tuberculosis from Neolithic Hungary. PLoS One 8:e78252.

Masson M, Bereczki Z, Molnar E, Donoghue HD, Lee OY-C, Wu HH, Houdini HT, Besra GS, Minnikin DE, Bull I, Palfi G 2015 7000 year-old tuberculosis cases from Hungary e Osteological and biomolecular evidence. Tuberculosis 96:S13-S17.

Matos V, Santos AL. 2006. On the trail of pulmonary tuberculosis based on rib lesions: results from the Human Identified Skeletal Collection from the Museu Bocage (Lisbon, Portugal). Am J Phys Anthropol 130:190-200.

Mays S. 2001. Effects of age and occupation on cortical bone in a group of 18th-19th century British men. Am J Phys Anthropol 116: 34-44.

Mays S. 2006. Age-related cortical bone loss in women from a 3rd-4th century AD population from England. Am J Phys Anthropol 129: 518-528.

Mays S. 2010. Archaeological skeletons support a northwest European origin for Paget's disease of bone. J Bone Min Res 25:1839-1841.

Mays S. 2012. The relationship between palaeopathology and the clinical sciences. In: Grauer AL, editor. A companion to paleopathology. Cambridge: Cambridge University Press. p 285-309.

Mays S, Brickley M, Ives R. 2006. Skeletal manifestations of rickets in infants and young children in an historic population from England. Am J Phys Anthropol 129:362-374.

Mays S, Taylor GM, Legge AJ, Young DB, Turner-Walker G. 2001. Paleopathological and biomolecular study of tuberculosis in a Medieval skeletal collection from England. Am J Phys Anthropol 114: 298-311.

Mays S, Fysh E, Taylor E. 2002. Investigation of the link between visceral surface rib lesions and tuberculosis in a Medieval skeletal series from England using ancient DNA. Am J Phys Anthropol 119:27-36.

McElroy A, Townsend PK. 1996. Medical anthropology in ecological perspective. Boulder: Westview Press.

Mensforth RP. 2002. Vitamin D deficiency mortality: impaired immune response in infants and elevated cancer risk in adults. Am J Phys Anthropol 34(suppl.):112.

Merashli M, Jawad A. 2015. Paget's disease of bone among various ethnic groups. Sultan Qaboos University Med J 15:22–26.

Miragliotta G, Miragliotta L. 2014. Vitamin D and infectious diseases. Endocr Metab Immune Disord Drug Targets 14:267-71.

Miszkiewicz JJ. 2015. Linear enamel hypoplasia and age-at-death at Medieval (11th–16th Centuries) St. Gregory's Priory and cemetery, Canterbury, UK. Int J Osteoarchaeol 25:79-87.

Mitchell PD. 2011. Retrospective diagnosis, and the use of historical texts for investigating disease in the past. Int J Paleopathology 1:81-88.

Møller-Christensen V, Hughes DR. 1966. An early case of leprosy from Nubia. Man 62:177-179.

Montgomery J. 2002. Lead and strontium isotope compositions of human dental tissues as an indicator of ancient exposure and population dynamics. PhD thesis, University of Bradford.

Morse D, Brothwell DR, Ucko PJ. 1964. Tuberculosis in ancient Egypt. Am Rev Respir Dis 90:526-541.

Müller R, Roberts CA, Brown TA. 2014. Genotyping of ancient Mycobacterium tuberculosis strains reveals historic genetic diversity. P R Soc B 281:20133236. doi: 10.1098/rspb.2013.3236.

Murphy EM, Chistov YK, Hopkins R, Rutland P, Taylor GM. 2009. Tuberculosis among Iron Age individuals from Tyva, South Siberia: Palaeopathological and biomolecular Findings. J Archaeol Sci 36:2029-2038.

Neela VS, Suryadevara NC, Shinde VG, Pydi SS, Jain S, Jonnalagada S, Singh SS, Valluri VL, Anandaraj MP. 2015. Association of Taq I, Fok I and Apa I polymorphisms in Vitamin D receptor (VDR) gene with leprosy. Hum Immunol 76:402-405.

Nicklish N, Maixner F, Ganslmeier R, Friederich S, Dresely V, Meller H, Zink A, Alt KW. 2012. Rib lesions in skeletons from early Neolithic sites in central Germany: on the trail of tuberculosis at the onset of agriculture. Am J Phys Anthropol 149:391-404.

Orlando L Thomas M, Gilbert P Willerslev E. 2015. Reconstructing ancient genomes and epigenomes. Nat Rev Genet 16:395-408.

Ortner DJ. 2003. Identification of pathological conditions in human skeletal remains. London: Academic Press.

Ortner DJ. 2012. Differential diagnosis and issues in disease classification. In: Grauer AL, editor. A companion to paleopathology. Chichester: Wiley-Blackwell. p 250-267.

Ortner DJ, Ericksen M. 1997. Bone changes in infancy in the human skull probably resulting from scurvy in infancy and childhood. Int J Osteoarchaeol 7:212-220.

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

Oxford University Press. 2016 http://www.amamanualofstyle.com/page/si-conversion-calculator, American Medical Association. Accessed January 2016.

Pace-Asciak A, Mamo J, Calleja N. 2013. Tuberculosis among undocumented boat migrants to Malta: implications for a migrant tuberculosis policy. Int J Tuberc Lung D.17:1065-1070.

Padayatty SJ, Levine M. 2016. Vitamin C: the known and the unknown and Goldilocks. Oral Dis. In press doi: 10.1111/odi.12446.

Palm TA. 1890. The geographical distribution and etiology of rickets. Practitioner 45: 270-279.

Panzer S, Tamošiūnas A, Valančius R, Jankauskas R, Piombino-Mascali D. 2013. Radiological evidence of rickets in a Lithuanian child mummy. Rofo 185:670-672.

Paterson DE. 1961. Bone changes of leprosy. Their incidence, progress, prevention and arrest. Int J Leprosy 29:393-422.

Patil KM, Jacob S. 2000. Mechanics of tarsal disintegration and plantar ulcers in leprosy by stress analysis in three dimensional foot modesl. Indian J Leprosy 72:69-86.

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

Pinto DC, Stout SD. 2010. Paget's disease in Pre-Contact Florida? Revisiting the Briarwoods Site in Gulf Coast Florida. Int J Osteoarchaeol 20:572-578.

Pillow JJ, Forrest PJ, Rodda CP. 1995. Vitamin D deficiency in infants and young children born to migrant parents. J Paediatr Child H.31:180-184.

Pitt MJ. 2002. Rickets and osteomalacia. In: Resnick DL, editor. Diagnosis of bone and joint disorders. 4th ed. Edinburgh: WB Saunders. p 1901-1945.

Priemel M, von Domarus C, Klatte TO, Kessler S, Schlie J, Meier S, Proksh N, Pastor F, Netter C, Streichert T, Püschel K, Amling M. 2010. Bone mineralization defects and vitamin D deficiency: histomorphometric analysis of iliac crest bone biopsies and circulating 25-hydroxyvitamin D in 675 patients. J Bone Miner Res 25:305-312.

Pybus OG, Tatem AJ, Lemey P. 2015. Virus evolution and transmission in an ever more connected world. P R Soc B 282:doi.org/10.1098.

Ragsdale BD, Lehmer LM. 2012. A knowledge of bone at the cellular (histological) level is essential to paleopathology. In: Grauer AL, editor. A companion to paleopathology. Chichester: Wiley-Blackwell. p 227-249.

Ralston SH. 2008. Pathogenesis of Paget's disease of bone. Bone 43:819-825.

Ralston SH, Albagha OME. 2014. Genetics of Paget's disease of bone. Curr Osteoporos Rep 12:263–271.

Ranström S, Von Sydow G. 1949. Rickets in newborn infants: clinical and histologic study. Pediatrics 4:406-411.

Rechel B, Mladovsky P, Devillé W, Rijks B, Petrova-Benedict R, McKee M, editors. 2011. Migration and health in the European Union. Maidenhead: McGraw Hill for Open University Press.

Reid IR, Bolland MJ. 2014. Skeletal and nonskeletal effects of vitamin D: is vitamin D a tonic for bone and other tissues? Osteoporosis Int 25:2347–2357.

Reinhard K, Araújo A. 2012. Synthesizing parasitology with archaeology in paleopathology. In: Buikstra JE, Roberts CA, editors. The global history of palaeopathology. Pioneers and prospects. Oxford: University Press. p. 750-764.

Resnick D, editor. 2002. Diagnosis of bone and joint disorders. 4th edition. Edinburgh: W.B. Saunders

Resnick D. 1995. Neuromuscular disorders. In: Resnick D, editor. Diagnosis of bone and joint disorders. 3rd edition. Edinburgh: WB Saunders, p3365-3412.

Resnick D, Niwayama G. 1995a. Osteomyelitis, septic arthritis, and soft tissue Infection: organisms. In: Resnick D, editor. Diagnosis of bone and joint disorders. 3rd edition. Edinburgh: WB Saunders, p 2448–2558.

Resnick D, Niwayama G. 1995b Osteomyelitis, septic arthritis, and soft tissue infection: axial skeleton. In: Resnick D, editor. Diagnosis of bone and joint disorders. 3rd edition. Edinburgh: WB Saunders, p 2419-2447.

Resnick D, Niwayama G. 1995c. Rheumatoid arthritis and the seronegative spondyloarthropathies: radiographic and pathologic concepts. In: Resnick D, editor. Diagnosis of bone and joint disorders. 3rd edition. Edinburgh: WB Saunders, p 807-865.

Reynard LM, Tuross N. 2015. The known, the unknown and the unknowable: weaning times from archaeological bones using nitrogen isotope ratios. J Archaeol Sci 53:618-625.

Richards M, Montgomery J. 2012. Isotope analysis and paleopathology: a short review. In: Buikstra JE, Roberts CA, editors. The global history of palaeopathology. Pioneers and prospects. Oxford: Oxford University Press. p 718-731.

Richards IDG, Sweet EM, Arneil GC. 1968. Infantile rickets persists in Glasgow. Lancet 1:803-805.

Roberts CA. 2011. The bioarchaeology of leprosy and tuberculosis in late Medieval England: a comparative study of perceptions, stigma, diagnosis and treatment. In: Glencross B, Agarwal S, editors. Handbook of social archaeology. Blackwell Studies in Global Archaeology. Chichester: Wiley Blackwell. p 252-281.

Roberts CA. 2012. Re-emerging Infections: developments in bioarchaeological contributions to understanding tuberculosis today. In: Grauer AL, editor. A companion to paleopathology. Chichester: Wiley-Blackwell. p.434-457.

Roberts CA, Buikstra JE. 2003. The bioarchaeology of tuberculosis: A global view on a reemerging disease. Gainsville: University Press of Florida.

Roberts CA, Ingham S. 2008. Using ancient DNA analysis in palaeopathology: a critical analysis of published papers with recommendations for future work. Int J Osteoarchaeol 18:600-613.

Roberts C, Manchester K. 2005. The archaeology of disease. 3rd edition. Stroud: Sutton Publishing.

Roberts CA, Pfister L, Mays S. 2009. Letter to the editor: Was tuberculosis present in *Homo erectus* in Turkey? Am J Phys Anthropol 139:442-444.

Roberts C, Lucy D, Manchester K. 1994. Inflammatory lesions of ribs: an analysis of the Terry Collection. Am J Phys Anthropol 95:169-182.

Roberts CA, Millard AR, Nowell GM, Grocke DR, MacPherson CG. 2012. Isotopic tracing of the impact of mobility on infectious disease: the origin of people with treponematosis buried in Hull, England, in the late Medieval period. Am J Phys Anthropol 150:273-285.

Roberts CA. Caffell A, Filipek-Ogden KL, Gowland R, Jakob T. 2016. 'Til poison phosphorous brought them death': a potentially occupationally-related disease in a post-medieval skeleton from north-east England. Int J Paleopathology 13:39–48.

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

Rockall A, Hatrick A, Armstrong P, Wastie M. 2013. Diagnostic imaging. Chichester: Wiley-Blackwell.

Rogers J, Jeffrey DR, Watt I. 2002. Paget's disease in an archeological population. J Bone Miner Res 17:1127-1134.

Roy S, Frodsham A, Saha B, Hazra SK, Mascie-Taylor CG, Hill AV. 1999. Association of vitamin D receptor genotype with leprosy type. J Infect Dis 179:187-191.

Salazar-García DC, Richards MP, Nehlich O, Henry AG. 2014. Dental calculus is not equivalent to bone collagen for isotope analysis: a comparison between carbon and nitrogen stable isotope analysis of bulk dental calculus, bone and dentine collagen from same individuals from the Medieval site of El Raval (Alicante, Spain). J Archaeol Sci 47:70-77.

Salo WL, Aufderheide A, Buikstra JE, Holcomb TA. 1994. Identification of *Mycobacterium tuberculosis* in a pre-Columbian Peruvian mummy. P Natl Acad Sci USA 91:2091-2094.

Santos AL, Roberts CA. 2006. Anatomy of a serial killer: differential diagnosis of tuberculosis based on rib lesions of adult individuals from the Coimbra Identified Skeletal Collection, Portugal. Am J Phys Anthropol 130:38-49.

Santos AL, Suby JA. 2012. Skeletal and surgical evidence for acute osteomyelitis in non-adult individuals. Int J Osteoarchaeol 25:110-118.

Semba RD. 2012. The historical evolution of thought regarding multiple micronutrient nutrition. J Nutr 142:143S-56S.

Schamall D, Teschler-Nicola M, Kainberger F, Tangl ST, Brandstatter F, Patzak B, Muhsil J, Plenk Jr, H. 2003. Changes in trabecular bone structure in ricketsand osteomalacia: the potential of a medico-historical collection. Int J Osteoarchaeol 13:283–288.

Schattmann A, Bertrand B, Vatteoni S, Brickley M. 2016. Approaches to co-occurrence: Scurvy and rickets in infants and young children of 16th – 18th century Douai, France. Int J Paleopathology 12:63-75.

Schultz M. 2001. Paleohistology of bone: a new approach to the study of ancient diseases. Yearb Phys Anthropol 44:106-147.

Schultz M, Roberts CA. 2002. Diagnosis of leprosy from an English later Medieval leprosy hospital using histological analysis. In: Roberts CA, Lewis ME, Manchester K, editors. The past and present of leprosy. Archaeological, historical, palaeopathological and clinical approaches. Proceedings of the International Congress on the Evolution and palaeoepidemiology of the infectious diseases 3 (ICEPID), University of Bradford, 26th-31st July 1999. British Archaeological Reports. International Series 1054. Oxford: Archaeopress. p 89-110.

Simon AK, Hollander GA, McMichael A. 2015. Evolution of the immune system in humans from infancy to old age. Proc Roy Soc B 282:doi.org/10.1098/rspb.2014.3085.

Sintchenko V, Holmes EC. 2015. The role of pathogen genomics in assessing disease transmission. Brit Med J 350:doi: http://dx.doi.org/10.1136/bmj.h1314.

Spigelman M, Shin DH, Bar Gal GK. 2012. The promise, the problems, and the future of DNA analysis in paleopathology studies. In: Grauer AL, editor. A companion to paleopathology. Chichester: Wiley-Blackwell. p 133-151.

Spiro A, Buttriss JL. 2014. Vitamin D: an overview of vitamin D status and intake in Europe. Nutr Bull 39:322-350.

St-Arnaud R, Glorieux FH. 1997. Vitamin D and bone development. In: Feldman D, Glorieux F, Pike J, editors. Vitamin D. San Diego: Elsevier Academic Press. p 239-303.

Stearns SC. 2012. Evolutionary medicine: its scope, interest and potential. *Philos T R Soc B* 279:4305-4321.

Steinbock RT. 1993. Rickets and osteomalacia. In: Kiple K, editor. The Cambridge world history of human disease. Cambridge: Cambridge University Press. p 978-980.

Steyn M, Scholtz Y, Botha D, Pretorius S. 2013. The changing face of tuberculosis: trends in tuberculosis-associated skeletal changes. Tuberculosis 93:467-474.
Stout SD, Teitelbaum SL. 1976. Histomorphometric determination of formation rates of archaeological bone. Calcified Tissue Int 21:163-169.

Stout SD, Teitelbaum SL. 1976. Histomorphometric determination of formation rates of archaeological bone. Calcified Tissue Int 21:163-169.

Taillandier A, Domingues C, De Cazanove C, Porquet-Bordes V, Monnot S, Kiffer-Moreira T, Rothenbuhler A, Guggenbuhl P, Cormier C, Baujat G, Debiais F, Capri Y, Cohen-Solal M, Parent P, Chiesa J, Dieux A, Petit F, Roume J, Isnard M, Cormier-Daire V, Linglart A, Millán JL, Salles JP, Muti C, Simon-Bouy B, Mornet E. 2015. Molecular diagnosis of hypophosphatasia and differential diagnosis by targeted Next Generation Sequencing. Mol Genet Metab 116:215-220.

Taylor GM, Young DB, Mays S. 2005. Genotypic analysis of the earliest known prehistoric case of tuberculosis in Britain. J Clin Microbiol 43:2236-2240.

Taylor GM, Goyal M, Legge AJ, Shaw RJ, Young D. 1999. Genotypic analysis of *Mycobacterium tuberculosis* from Medieval human remains. Microbiology 145:899-904.

Thacher TD, Fischer PR, Pettifor JM, Lawson JO, Manaster BJ, Reading JC. 2000. Radiographic scoring method for the assessment of the severity of nutritional rickets. J Trop Pediatrics 46:132–139.

Trevathan WR, Smith EO, McKenna JJ. 2008. Evolutionary medicine and health. New perspectives. Oxford: Oxford University Press.

Vogel JC, Van der Merwe NJ, 1977. Isotopic evidence for early maize cultivation in New York State. Am Antiquity 42:238-242.

Von Hunnius TE, Roberts CA, Boylston A, Saunders SR. 2006. Histological identification of syphilis in pre-Columbian England. Am J Phys Anthropol 129:559-566.

Wanek J, Papageorgopoulou C, Rühli F. 2012. Fundamentals of paleoimaging techniques: bridging the gap between physicists and paleopathologists. In: Grauer AL, editor. A companion to paleopathology. Chichester: Wiley-Blackwell. p 324-338.

Wang J, Zhou X, Zhang Z, Xu L, Yin X, Yang L, Zhao D. 2012. Effect of interaction of vitamin C on macrophage immune response to infection with *Mycobacterium bovis*. Cell Mol Biol (Noisy-le-grand) 58(suppl.):OL1688-94.

Warinner C, Speller C, Collins MJ, Lewis CM. 2015. Ancient human microbiomes. J Hum Evol 79:125-136.

Warinner C, Rodrigues JF, Vyas R, Trachsel C, Shved N, Grossmann J, Radini A, Hancock Y, Tito RY, Fiddyment S, Speller C, Hendy J, Charlton S, Luder HU, Salazar-Garcia DC, Eppler E, Seiler R, Hansen LH, Castruita JA, Barkow-Oesterreicher S, Teoh KY, Kelstrup CD, Olsen JV, Nanni P, Kawai T, Willerslev E, von Mering C, Lews CM Jr, Collins MJ, Gilbert MT, Rühli F, Cappellini E. 2014. Pathogens and host immunity in the ancient human oral cavity. Nat Genet 46:336-344.

Watanabe D, Hotta M, Ichihara A. 2015. Osteomalacia, severe thoracic deformities and respiratory failure in a young woman with anorexia nervosa. Internal Med 54:929-934.

Webb E, White C, Longstaffe F. 2013. Dietary shifting in the Nasca Region as inferred from the carbon- and nitrogen-isotope compositions of archaeological hair and bone. J Archaeol Sci 40:129–139.

Wei R, Chrystakos S. 2015. Mechanisms Underlying the Regulation of Innate and Adaptive Immunity by Vitamin D. Nutrients 7: 8251-8260.

Weston DA. 2008. Investigating the specificity of periosteal reactions in pathology museum specimens. Am J Phys Anthropol 137:48-59.

Weston DA. 2009. Brief communication: Paleohistopathological analysis of pathology museum specimens: can periosteal reaction microstructure explain lesion etiology? Am J Phys Anthropol 140:186-193.

Weston DA. 2012. Non-specific infection in paleopathology: interpreting periosteal reactions. In: Grauer AL, editor. A companion to paleopathology. New York: Academic Press. p 492-512.

Weyrich LS, Dobney K, Cooper A. 2015. Ancient DNA and dental calculus. J Hum Evol 79:119-224.

Wijkmans RAA, Talsma K. 2016. Modern scurvy. J Surg Case Rep 1:1-3. DOI: http://dx.doi.org/10.1093/jscr/rjv168.

Williams GC, Nesse RM. 1991. The dawn of Darwinian medicine. Q Rev Biol 66:1-22.

Wimalawansa, 2012. Vitamin D in the new millennium. Curr Osteoporos Rep 10:4-15.

Wittenberg K. 2001. The blade of grass sign. Radiology 221:199-200.

World Health Organisation. 2003. Prevention and management of osteoporosis, Report of a WHO Scientific Group. http://www.who.int/chp/topics/Osteoporosis.pdf. Retrieved March 2011.

World Health Organisation. 2016. Chagas disease (American trypanosomiasis). Factsheet. http://www.who.int/mediacentre/factsheets/fs340/en/. Accessed May 2016.

Wood JW, Milner GR, Harpending HC, Weiss KM. 1992. The osteological paradox. Problems of inferring health from skeletal samples. Curr Anthropol 33:343-370.

Wright LE, Yoder CJ. 2003. Recent progress in bioarchaeology: approaches to the osteological paradox. J Archaeol Res 11:43-70.

Zang R, Chouhan KK. 2012. Metabolic bone disease in kidney transplant recipients. World J Nephrol 1:127-133.

Zink AR, Sola C, Udo R, Grabner W, Rastogi N, Wolf H, Nerlich AG. 2003. Characterisation of *Mycobacterium tuberculosis* complex DNAs from Egyptian mummies by spoligotyping. J Clin Microbiol 41:359-367.

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