

# Diet, Tuberculosis, and the Paleopathological Record

by A. K. Wilbur, A. W. Farnbach, K. J. Knudson, and J. E. Buikstra

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Osseous manifestation of infectious disease is of paramount importance to paleopathologists seeking to interpret ancient health, but the relationships among infectious agent exposure, development of disease, and skeletal involvement are complex. The outcome of an exposure strongly depends on multiple factors, including ecology, diet, nutrition, immune function, and the genetics of pathogen and host. Mycobacterial diseases are often studied in ancient remains but also are especially influenced by these factors; individual and population differences in severity and course are apparent following onset of active disease. The osteological record for these diseases represents the complex interplay of host and pathogen characteristics influencing within- and among-individual skeletal lesion prevalence and distribution. However, many of these characteristics may be assessed independently through the archaeological record. Here, we explore the contributions of dietary protein and iron to immune function, particularly the course and outcome of infection with *Mycobacterium tuberculosis*. We emphasize how nutrition may influence the dissemination of bacilli to the skeleton and subsequent formation of diagnostic lesions. We then generate models and hypotheses informed by this interplay and apply them to four prehistoric New World areas. Finally, discrepancies between our expectations and the observed record are explored as a basis for new hypotheses.

The interpretation of human health in prehistory has been of interest since at least the eighteenth century (Roberts and Manchester 1995), and modern paleopathological analyses have provided rich resources for studies of past disease as well as human adaptation, migration, identity, and other anthropological issues. A majority of paleopathological analyses relies on the skeletal record, which presents a unique set of problems for diagnosis of ancient infectious diseases. The outcome of exposure to many infectious agents—including the development of skeletal lesions—strongly depends on multiple factors including ecology, diet, nutrition, immune function, and genetics of pathogen and host.

In their formulation of the “osteological paradox,” Wood and colleagues (1992) called attention to several key issues surrounding the interpretation of skeletal lesions. While their examples are drawn primarily from among the suite of non-specific indicators of stress, Wood and colleagues’ admoni-

tions are no less apt for those interpreting disease-specific paleopathology. They write that estimations of disease prevalence are impossible if based solely upon skeletal lesion frequency. They also argue that while many of the tangled issues surrounding such factors as hidden heterogeneity of risk await biomedical insight, anthropologists can contribute significantly to a better understanding of “the role played by cultural context in determining heterogeneous frailty and the level of selective mortality.” They further call for “a better understanding of the details of various pathological processes at the cell, tissue, and organ levels” (pp. 357–8).

The improved understanding sought by Wood and colleagues (1992) is crucial to interpret skeletal pathology resulting from the mycobacterial diseases tuberculosis and leprosy. Mycobacterial diseases hold particular interest for paleopathologists because they afford the opportunity to examine the interactions between an infectious agent and human migration and settlement patterns (Buikstra 1977; Buikstra and Cook 1981; Formicola, Milanese, and Scarsini 1987; Jankauskas 1998). The mycobacterial diseases tuberculosis and leprosy have plagued *Homo sapiens*, and probably our hominid ancestors, for millennia. Despite our long coevolutionary history, however, these pathogens have not reduced in virulence to a state of benign commensalism with humans.

The development of effective chemotherapy in the early to

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**A. K. Wilbur** is a postdoctoral research associate, **A. W. Farnbach** is a Ph.D. candidate, **K. J. Knudson** is Assistant Professor, and **J. E. Buikstra** is Professor and Director of the Center for Bioarchaeological Research in the School of Human Evolution and Social Change at Arizona State University (Tempe, AZ 85287, U.S.A. [alicia.wilbur@asu.edu]). This paper was submitted 11 XII 06 and accepted 3 IV 08.

mid-twentieth century held great promise to eradicate tuberculosis and leprosy, but by the late twentieth century it was clear that the relationship between pathogenic mycobacteria and humans would not be easily dissolved. In 1993, the World Health Organization (WHO) declared tuberculosis a global emergency; the disease has reemerged as a leading cause of mortality, responsible for an estimated 1.6 million deaths per year (WHO 2007). Devastating to human health and social systems, tuberculosis has been the subject of research efforts throughout the world. Expression of mycobacterial disease is especially influenced by multiple host, pathogen, and environmental factors (Nicod 2007), some of which can be independently assessed from the archaeological record.

We apply this knowledge of host-pathogen interactions to clarify the development of tuberculous skeletal lesions, responding to Wood and colleagues' (1992) call for improved understanding of paleopathological processes. We first review the influence of two nutrients, protein and iron, on the course and outcome of tuberculosis. We discuss the roles of each in the immune response to tuberculosis, emphasizing the way each may influence the metastasis of bacilli to the skeleton and subsequent formation of lytic lesions. We then present models for the formation of tuberculosis paleopathology informed by this complex interplay of nutrition, immune function, and infectious disease. Finally, we compare expectations based on our models to observed paleopathological evidence for tuberculosis and nutritional stress from four different areas of the New World. Our goal is to better inform interpretation of disease in the ancient past while also more generally addressing issues of diet and health that are relevant today.

## Identification of Tuberculosis in Past Populations

The "best" approach to understanding the prevalence of tuberculosis in past populations remains debated. Where historical documents are extant, diagnostic imprecision and a lack of standardization in recording cause of death can render identification of pulmonary tuberculosis somewhat unreliable in medical records before the mid-nineteenth century (McKeown and Record 1962). Tuberculosis does not occur as a diagnosis until the late 1830s (Keers 1978), and earlier terms like "consumption" could subsume a wide range of conditions in addition to tuberculosis. In the United Kingdom, Morton (1720) describes consumptions resulting from bloody flux, breast-feeding with inadequate diet, scurvy, and diabetes in addition to pulmonary consumptions that bear a closer resemblance to tuberculosis. Hardy (1988) notes that in the London Bills of Mortality, deaths from chronic bronchitis are misallocated to consumption from at least 1810 to the 1830s, when the Bills ceased to be published. For many regions of the world, such systematically recorded medical data are not available.

Molecular methods are increasingly used to identify the causative agents of tuberculosis—*Mycobacterium tuberculosis*

and other members of the *M. tuberculosis* complex (MTBC, comprising *M. canettii*, *M. microti*, *M. bovis*, and *M. africanum*)—in ancient remains, but these studies commonly rely on the preidentification of potentially tuberculous bone samples through traditional osteological techniques or from historically documented cemetery populations. The DNA from ancient remains holds promise to identify the causative agent in cases where skeletal or soft tissue signals are ambiguous and even to elucidate the evolution of the infectious agent, but there are serious limitations as well. Preservation of ancient DNA is variable, and even under the best conditions, the molecules degrade (Handt et al. 1994; Höss et al. 1996; Pääbo 1989), leaving only very small (<500 bp) fragments.

In some aspects, tuberculosis is ideal for ancient DNA studies. Mycobacteria replicate, sometimes in large numbers, within lesions generated by the disease's osseous form. These lesions are classically located in vertebral bodies but can be present in other bones. The pulmonary form of the disease may affect the internal aspects of ribs, but this type of lesion is also found with other severe pulmonary infections (Buikstra and Williams 1991; Pfeiffer 1991; Roberts, Lucy, and Manchester 1994; Lambert 2002) and is probably secondary to the inflammatory process rather than directly due to mycobacterial replication at the site. However, some studies have reported successful recovery of MTBC DNA from affected ribs (Mays, Fysh, and Taylor 2002; Raff, Cook, and Kaestle 2006).

Several challenges of diagnosing ancient tuberculosis using DNA have yet to be entirely overcome, however. Among the most significant of these is the abundance of nontuberculous mycobacteria throughout nature, including burial sites. Strong genetic similarities between species, compounded by the small DNA fragments obtainable, make species and subspecies identification difficult. However, the presence of repetitive elements unique to the MTBC genome has aided identification to at least the level of the complex. One of these, the IS6110 insertion sequence (Thierry et al. 1990a, 1990b) present in multiple copies in many strains, leading IS6110 to be the earliest—and still the most common—amplified target for ancient tuberculosis (Salo et al. 1994; Arriaza et al. 1995). Subsequent studies have reported MTBC-specific DNA amplification from nonpathological bones of documented tuberculosis cases (Baron, Hummel, and Hermann 1996) and from skeletons with nonspecific lesions or no lesions (Haas et al. 2000; Zink et al. 2001).

Many molecular studies of ancient tuberculosis have met with skepticism because they lack discussion of adherence to proper authentication procedures. Cooper and Poinar (2000) set forth minimal criteria for scientific investigation of ancient DNA that focus primarily upon two issues: control of contamination and independent reproducibility of results. In addition, samples available for ancient DNA studies are limited, and the small amplicons produced are difficult to place in phylogenetic context. These and practical issues such as the expense and time necessary for analysis currently render mo-

lecular methods at best supplementary to traditional paleopathological ones.

Given our present technology, osteolytic lesions indicative of tuberculosis may present a more reliable picture of ancient tuberculosis morbidity and mortality than molecular methods. Nonetheless, these lesions can identify only a subset of affected individuals in whom disease disseminated to the skeleton. Those who survived sufficiently long to develop skeletal lesions may have had the best immunity to the disease (Wood et al. 1992), and the sample of skeletons examined may or may not be representative of the original population (Waldron 1994; Roberts and Buikstra 2003).

Estimates of tuberculosis prevalence from such a subset are also problematic because risk for extrapulmonary disease is multifactorial. Exposure to *M. tuberculosis* does not always lead to infection, and infection does not always lead to active disease. Clinical studies on modern individuals of European ancestry indicate that only approximately 5%–10% of infected persons develop active disease (Medical Research Council 1972), though this risk of disease is not uniform across all modern populations (e.g., Hurtado et al. 2003). Of individuals who develop active tuberculosis, only a portion of cases will disseminate from the initial site of infection, and of these, only approximately 2%–4% will experience migration of the bacteria to osseous tissues (Cailhol, Decludt, and Che 2005; Lee and Abramson 1996). Thus, relationships among risks of pulmonary infection, skeletal involvement, and death are not intuitive, and it is difficult to translate prevalence of tuberculosis paleopathology into an explicit understanding of the extent to which a population was affected by this disease.

Knowledge of the macroscopic and microscopic processes of immune reaction to mycobacterial infection, especially of tuberculosis lesion formation in bone, may lead to a more complete understanding of which members of a population are likely to exhibit skeletal involvement and thus the extent to which the prevalence of skeletal indicators reflects the prevalence of tuberculosis in the living population. Many factors determining inter- and intrapopulation differences are invisible in a skeletal sample; however, examination of the immune response to pulmonary *M. tuberculosis* infection reveals that mechanisms related to protein and iron metabolism are likely to affect the dissemination of bacteria to the skeleton and destruction of bone at the site of skeletal involvement.

## The Human Immune Response to *Mycobacterium tuberculosis*

Interactions between mycobacteria and the immune system are complex and still being elucidated (Kaufmann 2001; Quesniaux et al. 2004; Houbin, Nguyen, and Pieters 2006; Nicod 2007), but it is clear that both the innate and acquired immune systems respond using a variety of interrelated signaling mechanisms. Pathogenic mycobacteria, in turn, have evolved strategies to circumvent host immune responses at several steps along a progression from exposure to infection to con-

tainment (latency) to active disease. Figure 1 is a simplified diagram of stages in the human immune response to *M. tuberculosis*.

In humans, the typical route of exposure and infection with *M. tuberculosis* is via inhalation of droplets discharged from the lungs of infected individuals. Biological, social, and environmental factors influence susceptibility to mycobacteria, and the susceptible portion of a population varies. Among immune-competent individuals of European ancestry, only 10% of exposed individuals are expected to become infected (Medical Research Council 1972); of those infected, approximately 50% may ultimately develop active disease. However, Hurtado et al. (2003) found that 64% of Paraguayan Aché became infected following exposure, and 30% of those infected developed active disease within 10 years of the study onset.

Following entry into the lungs, mycobacteria encounter alveolar macrophages and monocytes such as dendritic cells (Reddy and Anderson 1998). These phagocytic cells bear receptors to bind infectious agents, engulf them in an organelle called a phagosome, and digest them following fusion of the mature phagosome with a lysosome, an organelle containing acid hydrolases. Fragments (antigens) from this digestion are presented on the outside of the macrophage, and cell-signaling molecules called cytokines are released (see Kaufmann 2001 for further review). The cytokines recruit T-cells, a component of the acquired immune system, which can recognize the antigens exhibited by the phages.

Recruited T-cells produce cytokines that activate macrophages, enhancing their bactericidal and antigen presentation capacities (Cosma, Sherman, and Ramakrishnan 2003). During chronic *M. tuberculosis* infection, activated macrophages enclose the focus of infection within a granuloma. This structure creates an environment that contains and restricts growth of the bacilli, resulting in latency; fibrotic or calcified tissue surrounds and stabilizes the granuloma over time. Most immune-competent individuals are able to control the infection at this stage without any signs of illness, but they do not generally eradicate the bacteria from their system (Flynn and Ernst 2000).

Sometimes, however, the bacilli evade the granuloma response, replicate freely, and disseminate from the initial site of infection. If the latent state is disturbed (or never attained), the infection enters a progressive state characterized by uncontrolled bacterial replication and dissemination. The focus of infection becomes liquefied and remains exposed to oxygen, providing a rich medium for bacterial growth. As the bacteria replicate, destroying the infected tissue (McDonough and Kress 1995), the expanding, structurally compromised granuloma may erode into an airway, allowing infection to spread quickly to a new host as the contents are shed. Erosion of the liquefied granuloma into blood or lymph vessels results in metastasis of the infection to extrapulmonary sites, including the bones and joints (Granger, Hibbs, and Broadnax 1991).

Mycobacteria have evolved mechanisms to thwart the host

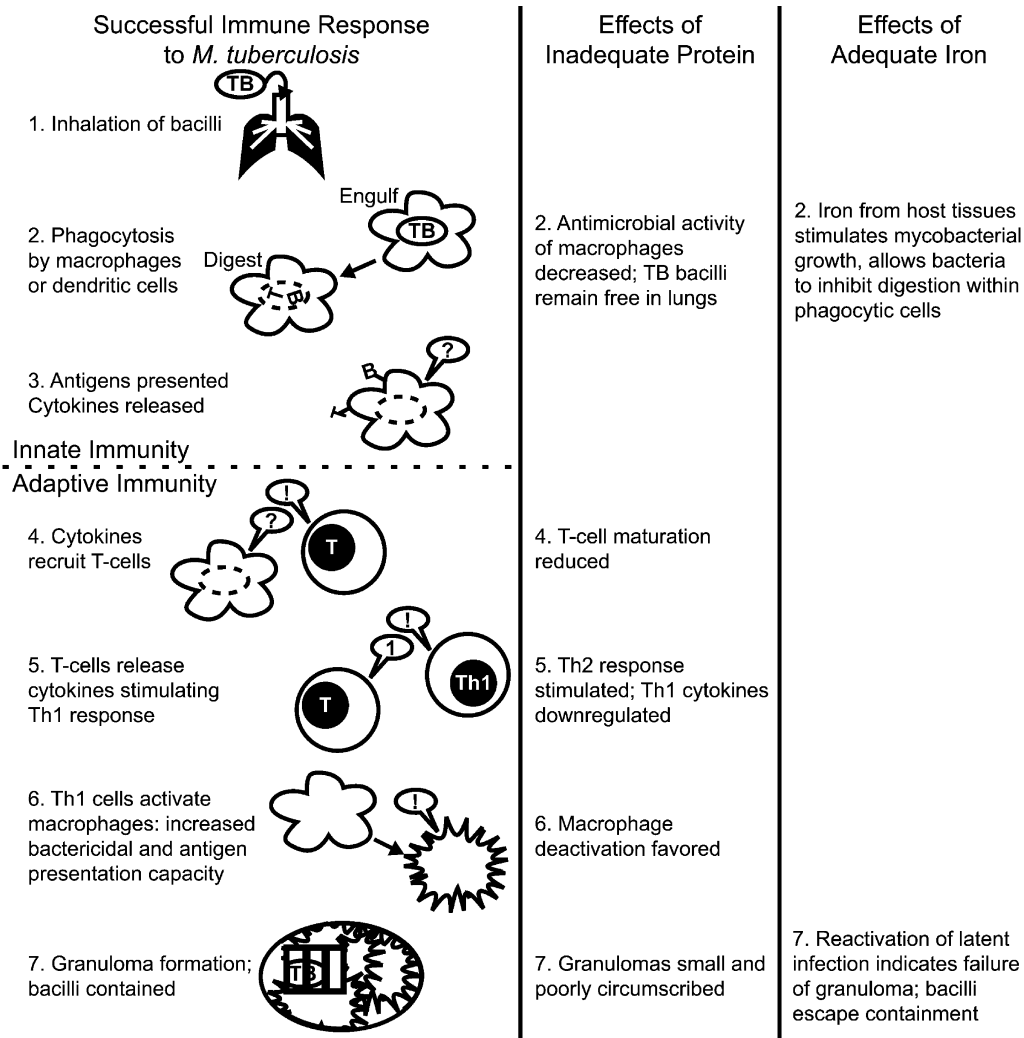


Figure 1. Simplified flow chart of the human immune response to inhalation of *M. tuberculosis*, and the individual effects of inadequate protein and adequate iron on that response.

immune system. During the early encounters of phagocytic cells and mycobacteria, for example, receptor binding and phagosome-lysosome fusion play key roles in the development of a maximally effective host immune response. Pathogenic mycobacteria are able to manipulate the phagosome membrane to prevent phagosome-lysosome fusion (reviewed in Houbin, Nguyen, and Pieters 2006; Kaufmann 2001), preventing antigen presentation, blocking the Th1 response, and barring macrophage activation.

The interplay between host innate (phagocytic cells) and adaptive (T cells) immune cells is also intricate. The adaptive immune response—as opposed to the innate immune response emphasized thus far—is characterized by the differentiation of precursor T-helper cells into T-helper type 1 (Th1) or T-helper type 2 (Th2) cells (Mosmann et al. 1986). The cell-mediated Th1 response is the most effective against

intracellular pathogens such as *M. tuberculosis*, while the antibody-dominated Th2 response is more effective against extracellular pathogens such as macroparasites. Each of these responses downregulates the other (Sander et al. 1995), but both are necessary to maintain health.

The differentiation of T-helper cells is mediated at least in part by Toll-like receptors (TLR), proteins on the surfaces of phagocytic cells that recognize various types of microbial molecules. The TLR2 specifically recognizes mycobacteria (Basu and Fenton 2004; Quesniaux et al. 2004; Houbin, Nguyen, and Pieters 2006); recognition by TLR2 stimulates dendritic cells to produce cytokines invoking a Th1 response. Because *M. tuberculosis* lives preferentially in macrophages, the Th1 response activating these cells is key: unactivated macrophages digest the mycobacteria much less effectively, allowing *M. tuberculosis* to replicate.

## Diet and Tuberculosis

Relationships among diet, nutrition, and the course of tuberculosis have been known since the nineteenth century and were suspected even earlier. In his dissertation on the causes of a predisposition to consumption, Francis Bowes Sayre (1790) recommended a balanced diet for those displaying the earliest stages of tuberculosis. Armand Trousseau (cited in Murray et al. 1978) warned in 1872 against iron supplementation for recovering patients, as this had been observed to cause a relapse of active disease. Writing to *Science* in 1886, N.W. “emphatically denie(d)” that “consumption is contagious in the ordinary sense of the word” and argued that “the ultimate cause of the disease (is) impaired nutrition” (1886, 87–88). Diets were carefully controlled in tuberculosis sanatoria (Santos 1999; Roberts and Buikstra 2003).

In the first half of the twentieth century, the importance of diet was confirmed. Johnston (1951) examined nutrition and tuberculosis in adolescent girls. Depressed levels of nitrogen (i.e., protein) storage were associated with prolonged disease and sometimes with dissemination, while adequate calcium was required for maintenance of the primary tuberculosis granuloma and prevention of reactivation. Getz, Long, and Henderson (1951) found a statistically significant relationship between low initial levels of vitamin A and C and development of tuberculosis among adult males. Downes (1950) found a clear trend toward decreasing incidence of tuberculosis in low socioeconomic status African-American families who received nutritional supplementation.

Today, malnutrition is the commonest cause of immune deficiency in the world, and it is known to influence the course and outcome of tuberculosis infection (Chandra 1996). Many micro- and macronutrients affect immunocompetency, including vitamin D, calcium, iron, and protein, which are especially important for responses to intracellular pathogens like mycobacteria. Here, we address the influence of protein and iron, which have the best-understood effects on mycobacterial infection.

### Protein

Protein malnutrition is linked with tuberculosis morbidity and mortality; experimental support for these interactions is reviewed in CA+ online supplement A, and the effects of insufficient protein on tuberculosis are summarized in figure 1. When protein is inadequate, macrophage antimicrobial activity decreases; furthermore, T-cell maturation is hindered, and the Th1 response is depressed while Th2 is favored. As a result, macrophages remain deactivated and granulomas ill-formed, allowing mycobacterial replication and dissemination.

Thus, among protein malnourished individuals, one might expect *Mycobacterium tuberculosis* infection to cause fulminant disease—characterized by extensive tissue destruction and relatively rapid death—due to a poorly organized gran-

uloma response and ineffective induction of the Th1 immune response. While extrapulmonary dissemination might result, it is possible that in such a rapidly progressive disease state, osseous manifestation would be limited to rib lesions from lung inflammation.

### Iron

CA+ online supplement B reviews in detail the support for iron’s influence on tuberculosis that is described briefly here; figure 1 summarizes iron’s effects on tuberculosis. Mycobacteria and mammalian hosts compete to obtain iron for bacterial use or to sequester the mineral where bacteria cannot access it. Where mycobacteria obtain adequate iron, replication is stimulated and macrophage bactericidal activity subverted, favoring bacterial dissemination. Indeed, mild host iron deficiency is protective against active tuberculosis, while iron supplementation favors reactivation of latent infections.

In sum, available iron supports *Mycobacterium tuberculosis* multiplication within host macrophages, favoring active disease and dissemination from the initial site of infection. Thus, for both iron-replete and protein-deficient individuals, fulminant, disseminated disease is expected following infection with *M. tuberculosis*.

## Implications for Paleopathology: Hypotheses

Immunological, epidemiological, and archaeological data can be integrated to develop predictions for tuberculosis in skeletal samples. These hypotheses assume that basic immune biology is similar among all human groups throughout time, although the possibility of host genetic differences should be considered.

*Hypothesis 1.* If chronic, severe protein malnutrition leads to high mortality from pulmonary tuberculosis, then rapid death before osseous involvement is expected among most members of an infected, protein-deficient group. Because fulminant pulmonary tuberculosis may still leave porosity or new bone formation on ribs (Roberts, Lucy, and Manchester 1994), the signature of pulmonary tuberculosis and protein malnutrition might appear in the form of new bone formation on visceral surfaces of ribs. Some segments of the population may show disseminated tuberculosis, especially if there is differential access to protein. A high carbohydrate diet deficient in protein could be inferred from presence of high rates of caries and abscesses, indicators of carbohydrate-rich diets; another indication could be high rates of dental enamel hypoplasias, nonspecific indicators of chronic stressors in early life.

*Hypothesis 2.* If dietary iron insufficiency restricts growth of intracellular mycobacteria and contains the organisms, then infection may remain latent without progression to disease in most members of an iron-deficient group. However, members of the population with higher access to iron-rich foods

could experience pulmonary tuberculosis and occasionally extrapulmonary tuberculosis. Porotic hyperostosis, observed as cribra orbitalia and cribra cranii, is expected as indicative of iron-deficiency anemia, but latent *Mycobacterium tuberculosis* infection would leave no osseous signature.

This may be confounded in the Old World, where genetic anemias may give the same osseous signature as iron-deficiency anemia, and in regions where anemia occurs secondarily to macroparasitic infestation. Iron absorption will be fairly high among populations with adequate dietary iron but high parasite loads, because iron absorption ability is inversely related to serum iron concentrations (MacPhail and Bothwell 1992). In this case, at least some serum iron is available to mycobacteria. The tendency for parasite infection to favor Th2 responses would favor active tuberculosis. This result is expected to be similar to that of protein deprivation, in which a Th2 response renders macrophages unable to contain bacilli, but the ability of mycobacteria to reproduce and disseminate will be dependent on diet and severity of macroparasitic infection. If parasite infestation is endemic and ubiquitous, porotic hyperostosis could develop in children and be retained in adult crania. Dental enamel hypoplasias are expected in children who survive serious parasite infection, but unlike protein malnutrition, caries may not be frequent unless the diet also contains a large amount of carbohydrates. Hereditary anemias and malaria-induced hemolytic anemias, not incorporated here, are discussed below.

Table 1 presents a model of expectations for the immune response and skeletal manifestation of tuberculosis based on protein and iron status. For ease of reference, we divide each possible dietary combination of iron and protein into categories 1 through 4, with category 5 representing protein and iron repletion but parasitic loss of iron. In table 2, we incorporate other skeletal/dental indicators of stress.

Considering the extremes of protein and iron availability, dissemination of *M. tuberculosis* to the spine is expected in categories 1, 4, and 5. In category 1, protein and iron are replete in the general population. This allows for Th1 re-

sponses, but availability of iron to mycobacteria also allows for bacterial growth and dissemination to other organ systems, including the skeleton. Response to infection and course of disease will depend on host factors such as genetics, reduced immunity due to trauma or other infectious agents, and intensity of exposure to the pathogen. Occurrence of enamel hypoplasias will depend on timing of other health insults. Caries rates are assumed to be low unless the population had a carbohydrate-rich diet. Porotic hyperostosis is unlikely to be present. Occurrence of Pott's disease and porosity on internal aspect of ribs will depend on the course of infection but are possible.

Category 5 also features sufficient dietary protein and iron, but endemic and ubiquitous macroparasite infection reduces serum iron to some degree and pushes immunity toward the Th2 response. Thus, macrophages will be unable to contain bacilli. If the diet contributes some iron, mycobacteria will be able to replicate and disseminate; the course and severity of disease will depend on the combination of diet and severity of parasite infection. Caries frequency in the population will depend on the amount of carbohydrates in the diet, but enamel hypoplasias and porotic hyperostosis are expected in the young. Porosity on internal aspect of ribs is expected due to pulmonary tuberculosis, and Pott's disease is possible.

Category 4 results from severe restriction of protein and iron, making disseminated osseous tuberculosis possible. This may seem counterintuitive, given the necessity of iron for mycobacterial growth and dissemination, but when protein levels are also insufficient, macrophages tend to remain unactivated and are unable to contain bacilli. Bacterial growth is therefore expected to be very slow, but this situation may lead to dissemination of the organism and osseous manifestation over time. Because this is expected to be a slow and chronic process, skeletal lesions would not occur until after some time had passed, and thus are not expected in the very young. High levels of hypoplasias, caries and porotic hyperostosis are expected.

In categories 2 and 3, dissemination of mycobacteria to the

Table 1. Expectations for Immune Status and Pott's Disease Based on Protein and Iron Status

Category	Protein	Iron	Immune Status	Pott's Disease?	Rib Lesions
1	+	+	Th1 immunity but iron available to mycobacteria; growth and dissemination possible	Possible in all age groups	Possible
2	+	-	Th1 immunity and no iron for mycobacteria; latent tuberculosis	No	No
3	-	+	Th2 immunity, iron for mycobacteria; fulminant pulmonary disease (rib lesions), no dissemination, or dissemination but death before formation of osseous lesions	No	Yes
4	-	-	Th2 immunity, macrophage inability to contain bacilli, but no iron available so slow growth; chronic disease may allow dissemination to skeleton and formation of characteristic lesions	Yes, but unlikely in young children	Yes
5	+	+ diet - parasites	Th1 immunity, some iron for mycobacteria	Depends on iron levels	Yes

Note: Th1 = T-helper type 1; TH2 = T-helper type 2.

Table 2. Population Expectations for Skeletal Indicators of Stress and Tuberculosis Based on Dietary Protein and Iron

Category	Protein	Iron	DEHs	Caries	Porotic Hyperostosis	Rib Porosity	Pott's Disease
1	+	+	O.c.	Low	No	Possible	Possible
2	+	−	Yes	High	Yes	No	No
3	−	+	Yes	High	No	Yes	No
4	−	−	High	High	Yes	Yes	Yes
5	+	±	Yes	Diet <sup>a</sup>	Yes	Yes	Possible

Note: DEHs = dental enamel hypoplasias; O.c. = other causes possible.

<sup>a</sup>Depends on diet.

skeleton is not expected. In both situations, enamel hypoplasias and caries are expected to be elevated due to high dietary carbohydrates. In category 2, under conditions of protein sufficiency but iron deprivation, Th1 immunity is possible and limited iron causes the mycobacteria to remain latent unless other circumstances trigger activation. Porotic hyperostosis is expected in response to iron deficiency, but osseous manifestations of tuberculosis are unlikely. In category 3, when protein is insufficient but iron is replete, fulminant pulmonary disease leading to rapid mortality may occur before dissemination and osseous reaction is possible. Only new bone and porosity on internal aspects of ribs are expected in reaction to the severe pulmonary infection.

The preceding discussion presents simplified scenarios in which only the extremes of nutritional status are considered. Our model makes assumptions on three broad—and not mutually exclusive—levels: environment, pathogen, and host. At the level of the environment, we assume that all sites have a similar potential for animal reservoirs of tuberculosis, that influences of climate on the pathogen and host are negligible, and that effects of other infectious diseases (except for macroparasites) are negligible. We assume that closely related species of mycobacterium are involved and that if different strains affect different populations or individuals within a population, they have similar transmission, growth, and dissemination capabilities (i.e., virulence). At the level of the host, we assume uniform exposure and transmission opportunities for all individuals within a population, similar immune genetics within and among populations, similar immune capabilities within and among populations, similar responses to diet within and among populations, and that food preparation techniques do not influence dietary response to protein and/or iron. We do not incorporate age or sex of hosts into our model. Given the complex and multifactorial nature of tuberculosis manifestation in human populations, our aim is to provide a framework in which at least one archaeologically recognizable factor, nutritional status, can be controlled. The predicted patterns can then be used to formulate intrasite expectations—where factors such as status may alter diet to some degree—and populational or regional expectations, where geography, trade routes, or other factors might also influence diet. Discrepancies between our model-derived expectations and observations from the archaeological record

may highlight other potential paleoepidemiological factors and lead to new hypotheses.

## Diet and Pre-Columbian Tuberculosis in the Americas

The pattern of presence and absence of ancient tuberculosis in the New World exemplifies how diet can influence the paleopathological record. In this section, we develop expectations for the presence of skeletal tuberculosis in the prehistoric New World, based on archaeological and paleopathological evidence of diet and nutrition. Four case studies—the ancient Maya, Andean South America, the North American southwest, and the lower Illinois River Valley—are discussed. In each, population aggregation and trade routes could have supported transmission and maintenance of tuberculosis. Table 3 summarizes the available isotopic and archaeological evidence concerning the diet of these groups.

### *Maize and the Maya: Why Is Evidence of Skeletal Tuberculosis Lacking?*

Many convincing cases of skeletal tuberculosis have been documented throughout the pre-Columbian Americas (Buikstra 1999; Roberts and Buikstra 2003). However, ancient Mayan skeletons present few or no convincing cases of disseminated tuberculosis (Buikstra 1999; Roberts and Buikstra 2003), despite large population aggregates and long-distance trade since pre-Classic times (Sharer 1994). Although Maya sites are noted for poor bone preservation, skeletons are available for observation: the record from the site of Copán includes approximately 900 skeletons, roughly 30% of which have at least some vertebral fragments preserved and 16% of which have more than 25% of each vertebral class preserved (K. A. Miller, personal communication). Because Pott's disease primarily manifests as lytic lesions of the vertebral bodies, some differential preservation is expected; however, it seems unlikely that proliferative ankylosis subsequent to vertebral collapse would not have been preserved somewhere in the Maya realm.

Published sources related to pathology and/or diet and nutrition define two general patterns of nutritional stress common among the ancient Maya. The first is very high reliance on maize agriculture during the Classic period. There was

Table 3. Dietary Inferences from Reported Isotopic Composition of Human Bone Collagen or Bone Apatite

	Freshwater Fish Meat	Marine Fish Meat	Animal Meat	C <sub>3</sub> Plants	C <sub>4</sub> Plants	References
Maya	Little	Little	Little	Little	High	Wright and White 1996
Peru:						
Chen Chen	None	None	Moderate	Moderate	High	Tomczak 2003
Estuquina	Moderate	Moderate	Moderate	Moderate	Moderate	Tomczak 2001
San Geronimo	None	High	Little	Moderate	Little	Tomczak 2003
El Yaral	None	Little	High	Little	High	Tomczak 2003
Chiribaya Alta	None	High	Moderate	Moderate	Moderate	Tomczak 2003
San Cristobal	Little	None	Little	Little	High	
Illinois:						
Middle Woodland	Moderate	None	Moderate	High	No	Buikstra et al. 1987; Styles and Buikstra 2006
Early Late Woodland	High	None	High	High	No	Buikstra et al. 1987; Styles and Buikstra 2006
Late Late Woodland	Moderate	None	High	Moderate	Moderate	Buikstra and Wil- liams 1991; Styles and Buikstra 2006
Mississippian	High	None	High	Low	High	Buikstra and Wil- liams 1991; Styles and Buikstra 2006

Note: C<sub>3</sub> and C<sub>4</sub> plants use different photosynthetic pathways. C<sub>4</sub> plants include tropical grasses such as maize, while the majority of other Maya dietary items were C<sub>3</sub> plants.

some temporal and regional variation (Whittington 1989; Wright and White 1996; White 1997), though, and most sites also show evidence of supplementation with beans and peppers, as well as occasional terrestrial and aquatic resources.

The second pattern among ancient Maya sites is high prevalence of porotic hyperostosis (Saul 1972; Whittington 1989; Whittington and Reed 1997a). The condition is present in juveniles and remains unremodeled through adulthood. This pathology is typically attributed to dietary iron-deficiency anemia secondary to heavy reliance on maize (Whittington 1989; Wright 1994; Wright and White 1996; Chase 1997; Massey and Steele 1997; Saul and Saul 1997; Whittington and Reed 1997a). Maize is relatively low in bioavailable nutrients, especially iron and protein (Katz, Hediger, and Valleroy 1974; Young and Pellett 1994; Hunt 2003; Mangels, Messina, and Melina 2003), and experimental studies have shown that absorption tends to be very low relative to other foods (Layrisse et al. 1969). Maize supplemented with meat, fish, or foods with high ascorbic acid content may supply an intermediate level of iron, which will probably meet the needs of at least 50% of adult females (MacPhail and Bothwell 1992). Limited supplementation, however, will not increase the bioavailable iron to a level sufficient for most adults.

The elevated prevalence of dental caries at several sites indicates high reliance on carbohydrates (Chase 1997; Massey and Steele 1997), although some between- and within-site differences are noted (Saul and Saul 1997; White 1997). Table

4 presents the observed and expected population-level rates of skeletal indicators of stress and tuberculosis. In general, rates of porotic hyperostosis (described separately as cribra orbitalia and cribra cranii) are reported for juveniles only, as it is in juveniles that marrow hyperplasia results in erosion of the cranial vault cortex to increase oxygen levels. Evidence for marrow hyperplasia in juveniles is fairly common in all of the American samples examined here, perhaps because of young children's high iron requirements (Stoltzfus and Dreyfuss 1998). By adulthood these lesions are often remodeled, but many Mayan sites such as Copán (Whittington and Reed 1997b) show high frequencies of lesions unremodeled into adulthood.

It seems clear that throughout the Classic period, a large segment of the Maya suffered iron deficiency beginning in early childhood. Maize is also limited in lysine, an essential amino acid, but supplementation with foods containing lysine—in this case, legumes—and/or certain types of processing can provide complete dietary protein (Katz, Hediger, and Valleroy 1974; Young and Pellett 1994; Mangels, Messina, and Melina 2003). This exemplifies category 2 from table 2, in which adequate protein allows Th1 immunity, while lack of iron inhibits mycobacterial growth. Among the Maya, then, exposure to *Mycobacterium tuberculosis* may have led to latent infection among large segments of the community, but disseminated tuberculosis would not be expected.

Socioeconomic differences in reliance on maize (Wright

Table 4. Observed and Expected Population Level Rates of Skeletal Indicators of Stress and Tuberculosis (TB) Compared to Diet

Area	Stress Indicators Observed (Expected)					TB Indicators Observed (Expected)					References
	Protein	Iron	Category	Enamel Hypoplasias (%)	Caries (%)	Cribra Orbitalia Juveniles (%)	Cribra Cranii Juveniles (%)	Rib Lesions	Vertebral Lesions	Other TB	
Maya	±	-	2	42.3 (yes)	1.8-24.5 <sup>a</sup> (high)	21-31.5 <sup>b</sup> (yes)	12.5-77.8 <sup>c</sup> (yes)	No (no)	No (no)	No	Whittington 1989; Wright 1994; Chase 1997; Massey and Steele 1997; Saul and Saul 1997; Whittington and Reed 1997 <sup>a</sup>
Peru:											
Chen Chen	+	±	5			58.8 (yes)	63.7 (yes)	High (yes)	High (possible)	Low	Blom et al. 2005
Estuquina	+	±	1			44.4 (no)	45 (no)	Low (possible)	High (possible)	Low	Buikstra and Williams 1991; Tomczak 2001, 2003
San Geronimo	+	+	5		12.9 (diet)	56.7 (yes)	41.9 (yes)	Low (yes)	Medium (possible)	Medium	Buikstra and Williams 1991; Tomczak 2001, 2003
El YaraI	+	+	1		7.2 (low)	60.5 (no)	58.1 (no)	High (possible)	Medium (possible)	Medium	Buikstra and Williams 1991; Tomczak 2001, 2003
Chiribaya Alta	+	+	1		12.7 (low)	60.4 (no)	52.7 (no)	Medium (possible)	Medium (possible)	Medium	Buikstra and Williams 1991; Tomczak 2001, 2003
San Cristobal Illinois: <sup>c</sup>	+	-	2	6 (yes)	57 (high)	90 <sup>d</sup> (yes)	8 (yes)	No (no)	Low (no)	No	Stodder 1990, 1996
Middle Woodland	+	+	1		16 (low)	0-40 <sup>f</sup> (no)			No (possible)	No	
Early Late Woodland	+	+	1		22 (low)	5-55 <sup>f</sup> (no)			No (possible)	No	
Late Woodland	±	+	1 or 3		24 (low or high)	15-60 <sup>f</sup> (no)			No (possible or no)	Low	
Mississippian	±	±	1?		42 (medium?)	30-65 <sup>f</sup> (no)			High (possible)	High	

Note: Plus sign = replete, plus/minus = moderate, and minus sign = deficient.

<sup>a</sup>White 1997; Lamanai, classic periods.

<sup>b</sup>Whittington and Reed (1997b) report that for individuals with more than 50% of cranial sites observable, 64% show evidence of anemia; lesions also remain unremodeled in adults at very high frequencies.

<sup>c</sup>Summarized in Wright and White (1996) from many sites.

<sup>d</sup>Includes unremodeled and remodeled lesions in all age groups; most lesions remodeled by adulthood.

<sup>e</sup>References for Illinois data: Cook and Buikstra 1979; Buikstra and Cook 1981; Buikstra 1984; Cook 1984.

<sup>f</sup>Frequencies of cribra orbitalia were reported by infant age categories; thus, the entire range is given here.

and White 1996; White 1997) and variation in porotic hyperostosis and dental enamel hypoplasia prevalence (Massey and Steele 1997; Saul and Saul 1997) have been reported at some ancient Maya sites. It is probable that individuals in communities or social groups with reduced reliance on maize or greater access to iron-rich foods may be more appropriately placed between categories 1 and 2. In these groups, protein access allowed for Th1 immunity, but there may have been sufficient bioavailable iron to allow mycobacterial growth; the outcome of *M. tuberculosis* infection would depend largely on other stressors such as severe trauma or concurrent illnesses, especially those that shift immunity to Th2. In those cases, pulmonary tuberculosis and resulting rib lesions or miliary tuberculosis with possible osseous lesions could arise.

These expected outcomes may explain the paucity of evidence for tuberculosis among the Maya. At sites exemplifying category 2, lesions on internal rib surfaces are the most likely osseous signature, and given these elements' fragility, this is unlikely to be preserved. Only a small portion of the population at some sites is expected to have experienced pulmonary tuberculosis; an even smaller fraction of those would have experienced dissemination of mycobacteria to bones. These are more likely to be found at sites that display lower evidence of maize reliance, and concomitant lower prevalence of stress indicators.

#### *The South American Andes: Contrasting Ancient Diets*

The long occupation history and exceptional preservation in Andean South America, particularly on the coast, provide another record with which to examine the relationship between paleodiet and osteological indicators of tuberculosis. Tuberculosis has been identified in archaeological sites throughout the Andes, particularly in Peru and Chile (Allison, Mendoza, and Pezzia 1973; Allison et al. 1981; Buikstra and Milner 1991; Williams 1991; Arriaza et al. 1995; Burgess 1999). We focus on well-documented cemetery populations in southern Peru for which detailed paleopathological and paleodietary information is available.

During the Middle Horizon (AD 500–1000) and the Late Intermediate Period (AD 1000–1350), the Osmore Drainage, or Ilo and Moquegua valleys, of southern Peru was home to many different archaeological cultures. Tiwanaku-affiliated sites like Chen Chen and Estuquiña were located in the Upper Osmore Drainage during the Middle Horizon and Terminal Late Intermediate periods, respectively. Chiribaya-affiliated sites were located in both the Upper and Lower Osmore Drainage: Chiribaya Alta and San Gerónimo were located on the coast in the Lower Osmore Drainage, while El Yaral was located further inland in the Upper Osmore Drainage. Although these sites were occupied roughly concurrently, the foods consumed at each site varied. This variability is further reflected in the rates of skeletal tuberculosis in the human remains from these sites.

Some of the earliest osseous evidence for tuberculosis in

the southern Andes comes from the site of Chen Chen. Although only approximately 80 km from the Pacific Ocean, carbon and nitrogen isotope analysis on human remains from Chen Chen show a dietary reliance on maize and protein from terrestrial animals such as camelids rather than on marine or lacustrine foods (Sandness 1992; Tomczak 2001, 2003). Data presented in table 2 indicate that protein consumption would have been relatively high at Chen Chen, but iron intake may have been moderate, corresponding to category 1. Table 4 shows that more than half of all juvenile skeletons evidence porotic hyperostosis; Blom et al. (2005) report that 36.4% of adults also displayed cribra orbitalia lesions. Evidence for extensive irrigation at Chen Chen leads Blom et al. (2005) to suggest that macroparasites were endemic. In this case, we expect the pattern of indicators of stress and tuberculosis to be more similar to category 5 than to category 1, and indeed, the observed data are consistent with category 5.

During the Terminal Late Intermediate Period, at least 10 individuals with pathologies indicative of tuberculosis were buried at the site of Estuquiña (Buikstra and Milner 1991; Williams 1991; Roberts and Buikstra 2003). Paleodietary analyses at Estuquiña show higher within-site variability in the amount of C<sub>3</sub> and C<sub>4</sub> plants consumed, and unlike the individuals buried at Chen Chen, those at Estuquiña ate Pacific Ocean or lacustrine resources (Tomczak 2001, 2003). We interpret the diet at Estuquiña to be high in protein and iron. Levels of childhood porotic hyperostosis are lower than at the other South American sites (table 4). Estuquiña's diet corresponds closely to category 1, in which tuberculosis would disseminate to the skeleton. Tomczak (2001) found no statistically significant dietary differences between the sexes, but interestingly, males at Estuquiña exhibit much higher prevalence of osseous tuberculosis. Buikstra and Williams (1991) attribute this to occupational factors, particularly exposure to camelids: these animals may have been a reservoir for MTBC bacteria.

In contrast to individuals buried at Chen Chen and Estuquiña, those interred at Chiribaya-affiliated sites had access to goods from a variety of ecological zones within the Chiribaya polity (Buikstra 1995; Lozada Cerna and Buikstra 2002, 2005; Tomczak 2003; Blom et al. 2005). Although foodstuffs were clearly being traded, subsistence choices at each site indicate socioeconomic specialization (Buikstra 1995; Lozada Cerna and Buikstra 2002, 2005; Tomczak 2003; Blom et al. 2005). For example, isotopic analysis shows that individuals buried at the coastal site of San Gerónimo consumed the greatest amount of marine products (Sandness 1992; Tomczak 2001, 2003). Although physiological stress can be seen in the presence of porotic hyperostosis (Burgess 1999), this is thought to result from helminthic infestation (Martinson et al. 2003) rather than dietary factors (Blom et al. 2005) and thus exemplifies category 5. At San Gerónimo, the number of individuals exhibiting skeletal pathologies consistent with tuberculosis infection is lower than the Upper Osmore Drain-

age sites, but disseminated tuberculosis is present (Burgess 1999) as expected.

In contrast, individuals buried at the inland Upper Osmore Drainage, Chiribaya-affiliated site of El Yaral consumed fewer marine products and relied more heavily on terrestrial products including maize and animals such as camelids (Sandness 1992; Tomczak 2001, 2003). Iron and protein levels are expected to have been adequate, as in category 1. While individuals buried at El Yaral show fewer skeletal lesions in general, the number of osseous lesions consistent with tuberculosis is significantly increased, and the number of skeletal elements affected is greater when compared to the contemporaneous coastal sites of San Gerónimo and Chiribaya Alta (Burgess 1999). Therefore, at Chiribaya-affiliated sites, there is a correlation between greater evidence of tuberculosis and a greater reliance on maize, camelids, and other terrestrial products rather than marine resources (Burgess 1999).

Chiribaya Alta also preserves evidence for more heterogeneous diets (Tomczak 2001, 2003), cranial modification styles, and mortuary treatments (Buikstra 1995; Lozada Cerna and Buikstra 2002, 2005; Blom et al. 2005). We interpret the diet of the individuals buried at Chiribaya Alta as containing moderate to high levels of protein and iron, although we expect within-site variability in both. In general, however, we expect this site to resemble category 1, and disseminated tuberculosis is indeed present here.

#### *Colonialism and Southwestern Pueblo Populations*

Puebloan populations from southwestern North America engaged in extensive maize agriculture supplemented by beans, amaranth, and other gathered and hunted resources (Stodder 1990). An abundance of crops and game was apparent to Europeans on first contact, although there was probably considerable local variation in availability of resources such as pinyon nuts, waterfowl, fish, bison, and antelope (Stodder 1990). At San Cristobal Pueblo, surplus corn was traded for buffalo meat, which provided up to 20% of protein in protohistoric times. Following the arrival of the Spanish in the early 1500s, trade shifted to nonsubsistence items, and governors appropriated corn surpluses. Food shortages resulted, and a series of famines and disease epidemics swept the New Mexico pueblo and mission communities (Stodder 1990, 1996). Stodder's (1990) paleopathological analysis of the San Cristobal remains found evidence for developmental arrest in dentition and long bones, dental pathology, iron-deficiency anemia, and infectious diseases including tuberculosis. Instances of possible tuberculosis were recently reanalyzed by J. E. Buikstra, with the new data presented here.

Fifty-seven percent of San Cristobal adults exhibit dental caries, and rates are somewhat higher in young to middle adults. This age range also shows high rates of occlusal surface wear, lending significance to the high rate of caries given the negative correlation between these lesions and dental attrition. Stodder (1990) attributes early, rapid dental wear to abrasive

maize-grinding lithics and supports a general model of maize-dominated subsistence through comparison to other southwestern precontact and protohistoric sites. Porotic hyperostosis is present in 89%–90% of adult and subadult individuals; these lesions have been attributed to iron-deficiency anemia, which is a secondary effect of high reliance on maize. Skeletal lesions that initially were thought to indicate disseminated tuberculosis occur in areas associated with later occupation of the site. Reanalysis, however, suggests that none of these are unequivocally tuberculosis; only a single young female subadult (no. 8708) has spinal lesions that may indicate tuberculosis.

Precontact San Cristobal people are expected to have adequate protein levels but moderate to severe iron-deficiency anemia. This corresponds to category 2, and as in the ancient Maya, Pott's disease and rib porosity are not expected. Stodder (1990) emphasizes that these skeletons are from the late protohistoric component of the cemetery and may postdate colonization. If so, following Spanish occupation, trade disruption, food appropriation, and repeated famines would have resulted in protein and iron deficiency for many native Puebloan people. In this case, the diet would instead correspond to category 4, in which the lack of protein pushes the immune response toward Th2 and macrophages are unable to effectively inhibit the bacilli. However, the lack of iron would result in slow growth of mycobacteria. In these situations, chronic disease could facilitate dissemination to various organ systems, including bone. Thus osseous lesions in the form of Pott's disease and rib porosity are expected in a small percentage of individuals. Because the process is slow, skeletal tuberculosis is not expected in the youngest individuals, although other nonspecific signs of infection may occur. Interestingly, there are three extremely young individuals (nos. 8634, 8644, and 8648) who show nonspecific signs of systemic infection. Based on our model, it seems more likely then that this sample postdates Spanish occupation, but other types of research are required in order to ascertain whether this is indeed the case.

#### *Dietary Change in West-Central Illinois*

The lower Illinois River valley provides an excellent context in which to examine the effects of dietary changes on health. West-central Illinois was occupied nearly continuously from as early as Paleoindian times, around 8000 BC (Buikstra 1984). During the Woodland period (600 BC–AD 1000), populations were concentrated in forested areas rich in deer, turkey, small mammals, and edible plants. The floodplains of the Illinois and Mississippi rivers provided fish and mussels and supported wetlands that contained roots, tubers, and migratory waterfowl (Asch, Farnsworth, and Asch 1979). Change in subsistence patterns over time has been well documented (Asch and Asch 1978; Asch, Farnsworth, and Asch 1979; Buikstra 1984; Styles and Buikstra 2006); an estimation of

relative usage of various resources over time is shown in table 5.

Through the Middle Woodland period (50 BC–AD 250), subsistence focused on hunting and gathering of wild resources. Various nuts provided a high-quality resource, rich in protein and fat (Buikstra 1984). Seed cultivation, including gourd and squash, began during the Middle Woodland. Marsh elder, an oily, nutritious plant gathered since Archaic times, became domesticated during the Middle Woodland; by Early Late Woodland, however, nuts and marsh elder become more sparse in the archaeological record. Starchy seeds such as those of knotweed, maygrass, and goosefoot become more prominent; these seeds contain less protein and fat and more carbohydrate. By the Late Late Woodland period, maize cultivation becomes important, and consumption increases to up to 55% of the dietary carbon through the Mississippian (AD 1000–1300; van der Merwe and Vogel 1978). During the Late Late Woodland, terrestrial mammal protein decreases but is replaced by aquatic resources. Thus, over time there is a trend for plant foods rich in proteins and fats to be replaced by those higher in carbohydrates. However, supplementation by terrestrial mammals and/or aquatic animals provided both protein and iron throughout all periods.

During most of the prehistoric period, the primary mortuary practice was mound burial, which results in generally excellent preservation of skeletal remains; the osteological record for this area is thus relatively complete (Buikstra 1984). Extensive excavation has been conducted on regional mortuary sites that date from the Archaic (8000–600 BC) through the Mississippian. Here we focus on the time period that begins in the Middle Woodland and continues through the Mississippian periods. During this span, subsistence shifted from solely hunting and foraging to supplementation with seed cultivation and on to high reliance on maize agriculture (Asch and Asch 1977, 1978; Asch, Farnsworth, and Asch 1979). Cook (1984) examined osteological and dental evidence (Cook and Buikstra 1979) for changes in health and found that Late Late Woodland children experienced retarded growth relative to children in earlier or later periods. Cribra orbitalia is common only among juveniles in this region, and frequencies are relatively low throughout the entire Woodland period, with a modest increase during Mississippian times. Cook (1984) summarizes the effects of change in subsistence

on health as complicated and resulting from trade-offs: hunting and foraging provided balanced nutrition and a varied diet, but seasonality of resources led to seasonal nutritional stresses. The development of food production and the focus on dependable, storable items during Woodland times (Buikstra 1984) buffered against seasonal stress. However, the introduction of maize agriculture and increasing dependence on it negatively impacted childhood health. Further, maize agriculture allowed increases in population sizes and aggregation and, with these, an increase in infectious disease.

From these data, indicators of stress and tuberculosis for Middle Woodland and Early Late Woodland sites should resemble those expected for category 1 due to the mixed hunting and gathering diet. During the Late Late Woodland, as increasing components of the diet come from maize and other carbohydrates while terrestrial mammal exploitation is replaced by exploitation of aquatic animals, adequate iron levels and possibly decreased protein levels should be apparent—the assemblage should resemble a transition between category 1 and category 3. From table 5 it is apparent that the rate of caries observed is somewhat higher than would normally be expected under category 1; this probably reflects growing utilization of starchy seeds, although oily seeds and nuts are still utilized in abundance during this period. By Mississippian agricultural periods, although there is high reliance upon maize as a staple, supplementation with aquatic and terrestrial animals provided some protein and iron. Although there is no category in our simplified model for moderate levels of protein and iron, one would expect slow growth of mycobacteria on infection, and it is exactly under these conditions that osseous tuberculosis is expected.

These expectations can be compared to data from the existing paleopathological literature. In 1981, Buikstra and Cook examined 1,403 skeletal individuals from eight lower Illinois River Valley region mortuary sites spanning the Middle Woodland, Late Woodland, and Mississippian time periods. Macroscopic examination of all skeletons with two or more observable elements was conducted, with special emphasis on pathology characterized by vertebral body destruction in the thoracolumbar spine with little or no proliferative response or involvement of other elements. In children, fusiform expansion of diaphyses was also considered as possibly indicative of tuberculosis.

Table 5. Relative Usage of Various Dietary Resources by Cultural Period in the Lower Illinois River Valley

	Middle Woodland	Early Late Woodland	Late Late Woodland	Mississippian
Terrestrial mammals	Moderate	Moderate	Moderate	Low
Aquatic animals	High	High	High	High
Nuts	High	High	Low	Moderate
Oily seeds	Low	Low	Low	Low
Starchy seeds	High	High	High	High
Maize	No	No	Medium	High

The eight sites were divided into Middle Woodland, Early Late Woodland, Late Late Woodland, and Mississippian time components. Using the above criteria, no evidence of tuberculosis was present in any of the 216 Middle Woodland individuals examined. A single individual in the Early Late Woodland displayed a possible calcified nodule resulting from pulmonary tuberculosis, but no other evidence of tuberculosis was found in this or other Early Late Woodland individuals.

Possible evidence for disseminated tuberculosis appears in six children from the Ledders, Helton, and Schild sites' Late Late Woodland components, all of whom exhibit diaphyseal modeling consistent with (but not diagnostic of) tuberculosis, and a single adult displays a destructive lesion of the left auricular surface of the ilium. Only one of these—the Helton child—is considered to be a convincing case of tuberculosis (Buikstra and Cook 1981; Roberts and Buikstra 2003). Both Mississippian sites, however, show evidence of vertebral destruction and/or classic Pott's disease, with other axial involvement in adults and juveniles, and a single possible calcified pleural nodule. Buikstra and Cook (1981) also demonstrate changes in the relative frequencies of cribra orbitalia over time, with lower rates in Middle and Early Late Woodland followed by increasing rates in Late Late Woodland and generally higher rates in Mississippian individuals. An important deviation from our expectations is the lack of tuberculosis found in Middle and Early Late Woodland, although it should theoretically be possible. This is discussed in detail below.

## Discussion

The model of expectations for paleopathological indicators of diet and disease presented in tables 1 and 2 is based upon experimental and epidemiological studies of the effects of nutrition on immune function. Its utility lies in its ability to predict when paleopathological evidence of tuberculosis should be present, providing that the population was exposed to the disease. Of necessity, the model is general, but its application to the skeletal record can serve to suggest potentially productive avenues for future research in instances when indicators of disease do not match expectations based on archaeological evidence of diet. In many cases, further hypotheses can be formulated, and while not all of these will be testable in the archaeological record, more may be in the future as technology develops.

One overall problem is the potentially limited utility of porotic hyperostosis as an indicator of iron-deficiency anemia. To some extent this is probably attributable to the age-specific nature of porotic hyperostosis; of necessity, we used data on juvenile rates because those data were most readily available. The true reflection of long-term, population-level iron-deficiency anemia is more likely to be seen in the frequencies of unremodeled porotic hyperostosis in adulthood, which is noted at high frequencies at some Maya sites as well as at San Cristobal. Most importantly, it is crucial to keep in mind the

osteological paradox when observing indicators of stress in order to make inferences about the health status of the population. Moderate to high levels of porotic hyperostosis occur in children from all of the New World series examined here, and it seems reasonable to infer that these children were the most susceptible to the diseases that killed them. However, the interpretation of the adult skeletons showing high levels of unremodeled porotic hyperostosis is the opposite: these individuals seem to represent those best able to resist the detrimental effects of anemia and other health problems. Below, we provide a few further examples of factors that may come into play when comparing observed indicators of tuberculosis and stress to our model's expectations.

### *Categories 1 and 5*

In these two categories, osseous tuberculosis can be expected if the population was exposed to *Mycobacterium tuberculosis*. When protein and iron are adequate, development of tuberculosis following infection, as well as dissemination to extrapulmonary locations in the body, largely depends on both host and pathogen factors. Even in the absence of extreme nutritional deficiencies, the host may experience immune stressors such as trauma or other infections, and these can affect an entire population—in war, for example. Host genetics also plays an important role in susceptibility and resistance to tuberculosis (Bellamy and Hill 1998). In a small, largely endogamous population, a similar level of susceptibility or resistance among all individuals is expected because of reduced heterozygosity, while in a much larger exogamous population, variation in susceptibility is expected.

Pathogen factors such as dose of infectious organism, number of episodes of exposure, and the pathogenic strain involved also influence host susceptibility to infection and disease (Read et al. 1999) and probably the course of disease. Exposure to high doses of mycobacteria causes inefficient immune responses, and it is thought that crowded living conditions may be more likely to lead to infection and disease (Power, Wei, and Bretscher 1998). The *M. tuberculosis* strain also influences the course of disease following infection (e.g., Arvanitakis et al. 1998).

One important host factor that has not been incorporated in our model is coinfection with *Plasmodium* species, the causative agents of malaria. This is an enormous world health problem (WHO 2002) and is caused by mosquito-borne parasites that are well adapted to their hosts. Malaria has a number of effects on the human immune system (Boutlis, Yeo, and Anstey 2006; Riley et al. 2006; Coban et al. 2007), but one commonality is that the plasmodium has evolved to live in red blood cells. The rupture of these cells causes many complications, including hemolytic anemia. Unlike iron-deficiency anemias secondary to diet, iron is present in the host and is released upon rupture of the red blood cells. Incorporating this disease in a very simple way into our model would place it into category 5, but this is probably a gross

and misleading oversimplification. The complicated effects of the malaria parasite on the human immune system at various stages in the parasitic life cycle are difficult to predict, especially in conjunction with other infections (Page et al. 2005), and may be compounded by factors such as host age, genetics, and pregnancy status (Stevenson and Zavala 2006).

#### Category 2

Disseminated disease is not expected in this situation, at least not at high frequency. Pott's disease in some individuals may indicate dietary differences by social groups. In large numbers, it is possible that the iron deficiency is in fact the result of parasitic infections, and thus the population fits into category 5. Only latent or pulmonary tuberculosis is expected in category 2—lack of evidence for osseous tuberculosis here would say nothing about whether the disease was present in the population.

#### Category 3

In this case, Th2 immunity combined with adequate iron for mycobacterial growth suggests that fulminant pulmonary disease will result in the large majority of individuals who become infected. This should result in porosity and new bone formation on the internal aspect of ribs (Roberts, Lucy, and Manchester 1994), but sufficient time for development of osseous disease is not expected. If Pott's disease is present, especially in a large number of individuals, this may indicate that the population was sufficiently healthy to withstand the bacteria.

#### Category 4

A diet poor in protein and iron provides the most likely situation in which osseous tuberculosis will develop. In this case, macrophages will tend to remain unactivated and unable to contain bacilli. Although very little serum iron will be available for the pathogens, extremely slow growth is possible, and this may lead over time to dissemination and osseous manifestation of tuberculosis. If there is evidence of exposure to *M. tuberculosis* and no evidence of spinal involvement, it may be that individuals were dying of tuberculosis of other organ systems or of something other than tuberculosis—and with this level of malnutrition, that is not surprising.

#### Goodness of Fit

In our analysis of 11 New World skeletal series, we found a reasonable fit between our model-based expectations and stress indicators among the three Chiribaya-affiliated sites. An exception to the fit lies in the caries level reported for San Gerónimo and Chiribaya Alta, where very little maize was consumed; the level was higher than the one at El Yaral. Burgess (1999) finds a generally higher level of dental health

at El Yaral than at other Chiribaya sites, and she suggests possible environmental trace mineral or behavioral differences as the cause. Given the presence of ceramic *keros* and maize at sites like El Yaral (Lozada and Buikstra 2002) and the importance of consumption of maize beer (*chicha*) in the Andes (Isbell 1978; Weismantel 1988; Allen 2002; Jennings 2004), it is possible that maize was often consumed as *chicha* in the Moquegua Valley. If most maize consumed at El Yaral was in liquid form, it may have inhibited the deposition of carbohydrates on the teeth, which mostly were used for chewing meat products. A number of factors may contribute to the difference in dental health between El Yaral and the other two Chiribaya sites, but it is interesting that the caries rates among these South American groups consuming *chicha* are quite low.

The model expectations and observed skeletal indicators also fit well in all but three North American series. At San Cristobal, precontact peoples are expected to have had sufficient protein, with moderate to severe iron deficiency corresponding to category 2. Disseminated disease is not expected in this situation, and indeed, no classic cases of osseous tuberculosis are observed. However, the presence of possible Pott's disease in a subadult female as well as systemic infection in three extremely young children could indicate tuberculosis infection with slow disease development and dissemination due to extreme protein and iron deficiencies as in category 4. This discrepancy is intriguing because these skeletons are from the late protohistoric component of the cemetery, and could be contemporary with Spanish occupation and the extreme repeated famines documented.

Perhaps an even more intriguing lack of fit between our model-based expectations occurs in the Illinois series during the Middle and Early Late Woodland periods. Based on the adequate dietary levels of both protein and iron, disseminated tuberculosis should theoretically be possible, but none is observed. The discrepancy noted in this small area of North America hints at a much larger question relevant to the entire world: why does evidence for osseous tuberculosis appear so late in time?

## Conclusions

*Mycobacterium tuberculosis* has been a human pathogen for millennia. A comparison of differences in the DNA between two closely related strains of modern *M. tuberculosis* conservatively estimated a minimum age for the complex of 35,000 years (Hughes, Friedman, and Murray 2002), and phylogenetic evidence from molecular studies of the entire MTBC indicates an African origin perhaps more than 2.5 million years ago (Gutierrez et al. 2005). Although this number may be an overestimate due to the incorporation of recombining portions of the genes (Smith 2006), it is clear that the association between humans and tuberculosis is ancient. Given that the New World pathogen has also been identified as a member of the MTBC (Arriaza et al. 1995; Braun, Cook, and

Pfeiffer 1998; Salo et al. 1994), the bacteria must have been present in human and/or animal migrants from the Old World during the peopling of the Americas.

It follows from our model that groups with adequate iron and protein stores (category 1) were susceptible to disseminated tuberculosis. Certainly many hunter-gatherer populations of the New World had an adequate diet, but there is no osseous evidence for tuberculosis anywhere in the New World until approximately AD 300 (Allison et al. 1981; Roberts and Buikstra 2003). This issue is not unique to a study of the disease in the Americas, because neither does skeletal tuberculosis appear in the Old World until relatively late. The earliest paleopathological evidence comes from 5800 BC in Neolithic Italy (Canci, Minozzi, and Borgognini Tarli 1996).

How can we account for the absence of tuberculosis among skeletal remains until 5800 BC in the Old World and AD 300 in the New World? How could the disease have been maintained in small populations of hunter-gatherers? Was the common ancestor of the MTBC maintained instead in animal populations until humans began living in large, permanent settlements? The latter seems unparsimonious, as at least two zoonotic transmissions into human would then have to be posited—one in the Old World, and one in the New World. One such transmission was already hypothesized for the Old World (Rich 1944), with *M. bovis* suggested as the ancestral organism (Cockburn 1963) that gave rise to human tuberculosis following cattle domestication. However, strong genetic evidence from multiple research groups and multiple types of genetic polymorphisms indicates that the common ancestor of the complex gave rise to the human pathogens *Mycobacterium canettii* and *M. tuberculosis*, with the other species arising later (Brosch et al. 2002; Gutacker et al. 2002; Baker et al. 2004; Gutierrez et al. 2005).

Despite the many studies that have been conducted on *M. tuberculosis*, important questions remain regarding the origin and coevolution of humans and pathogenic mycobacteria. Considering the complexity and antiquity of this relationship, answers will probably come from diverse areas of research, including phylogenetics, microbiology, immunology, epidemiology, paleopathology, history, and mathematical modeling. Our purpose in this paper has been to elucidate areas of possible future research by highlighting areas in which the tuberculosis observed in past populations does not fit with our theoretical expectations.

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## Comments

### Bernardo Arriaza

Instituto de Alta Investigación, Centro de Investigaciones del Hombre en el Desierto—Corporación Regional de Desarrollo Científico y Tecnológico (CIHDE-CODECITE), Departamento de Antropología, Universidad de Tarapacá, Arica, Chile (barriaza@uta.cl). 21 VIII 08

Wilbur et al. propose a methodological approach to test osseous tuberculosis in ancient populations. The authors want to shed light on the osteological paradox and to discover those individuals who died of fulminant tuberculosis, leaving minimal osseous lesions. There is increasing evidence that dissemination of *Mycobacterium tuberculosis* is reduced by interrupting iron availability (Karyadi et al. 2000; Boelaert et al. 2007) and that bacteria have developed genes to collect iron from a human host (Lensbouer et al. 2008). Thus, the authors suggest correlating osteopathological tuberculosis findings with stress markers, diet, and the way the immune system responds to tuberculosis. A low protein diet will lead to rapid death if one is infected with tuberculosis. Low iron in the diet may decrease tuberculosis and create a Th1 cell-mediated immune response.

To develop new methodologies is a challenge. The paper could benefit by incorporating epidemiological information on native populations and a biocultural approach as well. Certainly, as highlighted by the authors, tuberculosis infection and disease progression depends on multiple factors such as the particular strain of tuberculosis and immune status. However, ancient osteological remains are not isolated findings, and most came from well-known archaeological contexts. Thus, key demographic and cultural variables, such as population density, number of habitational sites, room sizes, and subsistence need consideration too. Many of these variables can be inferred from the archaeological record. Thus, the authors should debate on how social and environmental factors influence susceptibility to tuberculosis and how these variables may increase the prevalence of this disease.

At least for the Andean region, it seems subsistence strategy was an important factor, either minimizing or triggering tuberculosis. Early coastal fishing-gathering populations such as the Chinchorro show no sign of skeletal tuberculosis. They had a steady source of marine food resources, a low animal reservoir (sources) for tuberculosis, and a low population density. These early coastal populations were also endemically affected by parasites such as *Dipyllobothrium pacificum* causing long-term dietary iron deficiency anemia. In populations infected by parasites, said the authors, tuberculosis may shift into a state of latency, generating a Th2 antibody-mediated response. Bioarchaeological studies undertaken using Andean mummies are useful to debate the model. It is interesting to highlight the absence of skeletal and soft tissue tuberculosis

lesions in radiographs and autopsies of preceramic Andean mummies. It seems that tuberculosis did not affect early coastal populations at all. On the other hand, one could predict that late agropastoral populations, particularly those showing intensive social conflicts and living in more enclosed houses, for example, should have greater evidence of tuberculosis. This seems to be the case in the Andean region. Later agropastoral mummies show a variety of lesions ranging from primary healed fibrous, calcified lesions (Ghon complex), miliary tuberculosis, and Pott's disease. The Ghon complex and Pott's disease are visible on chest x-rays of mummies (Allison et al. 1981).

The need to study those dying of tuberculosis is paramount (Devi et al. 2003). Tuberculosis causes consumption, loss of appetite, and wasting away. Compared with controls, tuberculosis patients had a significantly lower body mass index, reduced skinfold thicknesses (triceps and biceps), and smaller mid-upper arm circumference (Karyadi et al. 2000). Thus, overall robusticity and cross-sectional geometry of long bones within a population (controlling by sex, age, and subsistence) could be another variable to explore. Individuals dying or suffering from tuberculosis should be emaciated and their bones thinner.

The authors posed the question why tuberculosis rose late in both the New World and the Old World. Tuberculosis reigns in crowded living conditions. The microscopic-droplet nuclei infected with tuberculosis are catapulted by sneezing and coughing to nearby individuals. These droplet nuclei remain airborne for a long time and can be easily inhaled. Therefore, given this mode of transmission, analysis of population aggregation in antiquity should be an important factor to consider as well. There is a good general trend between the appearance of tuberculosis in both the New World and the Old World and population increase, nucleation of villages, and intensive herding and farming. With those conditions, if farmers had extended families and were living in small houses, the creation of a social milieu in which to become infected with tuberculosis is more likely.

The authors state that the mycobacterium is ideal for ancient DNA studies, but perhaps they should explain a bit more about why and in addition give the readers some recommendations on how to minimize contamination and preserve samples for future DNA testing. Perhaps it is the right time to create an ancient tuberculosis sample collection center.

Quantifying how much protein and iron was available in antiquity and its sources is relevant to the model. Iron from meat is more easily broken down and absorbed than iron found in grains. Certainly, studying isotopes and using today's technology, such as laser ablation inductive coupled mass spectrometry, individuals' dietary habits can be analyzed and used to test the various-alternatives model proposed by the authors. Finally, as suggested by the authors, integrating diet, stress indicators, parasites, and mycobacterium adaptation gives us a cultural- and ecologically oriented model to work with and from which to debate tuberculosis in antiquity.

### Deborah Blom

Department of Anthropology, University of Vermont,  
Williams Hall 508, 72 University Place, Burlington, VT  
05405-0168, U.S.A. (deborah.blom@uvm.edu). 4 IX 08

This welcome contribution to the field of paleopathology is especially exciting because it brings together decades of data and will prove extremely fruitful for future research. The authors build a theoretical model using well-documented immunological and epidemiological data on tuberculosis outcome, and where the paleopathological data do not result as expected, we are asked to reconsider initial conclusions about the nutritional and health status of a particular group (e.g., the explanation of goodness of fit at San Cristobal or the suggestion that if a case that is originally designated as category 2 does not meet the expectations, "it is possible that the iron deficiency in fact is due to parasitic infections, and thus this is truly a case of category 5"). Inherent in the model is the assumption that the populations studied here were all exposed to tuberculosis (i.e., a lack of lesions is the result of disease outcome rather than lack of exposure). I am willing to accept that assumption for now but wonder how it will be received in general. The approach taken here, rather than a more traditional testing and tweaking of the model, is productive, and it, as well as the nuanced treatment of disease outcome, will prove an invaluable example for future studies into a wide range of paleopathological conditions.

This paper presents very relevant information about the effect of macroparasites on iron availability and immune response, and I think this can be developed further, especially in building models. The authors point out that if anemia is the result of abdominal bleeding caused by parasites, iron may still be available to mycobacteria. However, this may not be the case if the anemia secondary to parasites is due to diarrhea and nutrients passing too rapidly for sufficient iron absorption to occur. As it stands, the model contains one category (5) that incorporates some of the expectations for populations with high parasite loads. However, we can imagine categories with other iterations of the variables considered, and the expectations generated for category 5 currently do not to include the suggestion that we might expect a Th2 immune response in the presence of chronic macroparasites.

The effects of iron levels is important for predicting expected outcomes for many paleopathological conditions, and the authors found porotic hyperostosis to be of limited use for determining iron deficiency anemia. I agree that only using the porotic hyperostosis prevalence in children is problematic, but I am not yet convinced that using unremodeled lesions in adulthood is the way to measure iron deficiency overall. Many other factors could determine whether lesions will remodel or develop in adulthood, such as plasticity of the cranial vault cortex and the distribution of red marrow. In fact, many other factors may be in play, including those involving parasites. Chronic disease can cause anemia, yet high iron levels

can contribute to disease. Dietary protein and iron are not completely independent because diets deficient in protein can limit the body's access to iron (perhaps important for category 3), and food processing techniques might raise levels of one nutrient to the detriment of another. Consideration of these factors may aid in the development of model to further include expectations for parasites.

A few clarifications about the model and presented data would also be helpful. For one, in the draft I reviewed, the caries expectations presented in table 2 need more explanation. Why are the expectations different across categories when the protein dietary information is identical? What other factors are being considered? Additionally, little information on expectations or observations for prevalence of lesions is presented in some cases. Is this a relevant and feasible aspect to consider in the model? Finally, more information on the unpublished South American tuberculosis data (similar to that described for the North American sites) would be welcome, as would definitions for "low," "medium," and "high" in the last three columns of table 4. These clarifications can only add to the worth of the paper.

In closing, I have to say again that this article is a seminal contribution to the field, building beautifully on Wood *et al.* (1992). I look forward to seeing the wealth of scholarship that it is certain to generate, especially on intraregional and intrapopulation analyses of differential access to resources and exposure/risk factors, such as population movement. The authors should be congratulated.

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#### **Piers D. Mitchell**

College of Medical and Dental Sciences, University of Birmingham, Birmingham B15 2TT, UK (p.mitchell@clara.co.uk). 14 VIII 08

The aim of this paper is to highlight the complex interplay between infectious disease, diet, and immune function, so allowing a more nuanced understanding of disease in past populations. The authors chose tuberculosis as their model infectious disease because it can be diagnosed in human skeletal remains, and a large amount of modern clinical research has been undertaken on this pathogen. This article models expectations of tuberculosis prevalence in 11 archeological populations from the Americas that are believed to have varied in their dietary intake of protein and iron. Modern clinical research on people with TB suggests that protein energy malnutrition reduces resistance to the infection by impairing the immune system, while iron deficiency anemia may increase resistance by reducing iron available for mycobacterial replication. The model suggests that populations with both protective factors and good protein and energy intake in their diet but little iron should show no sign of TB, because immunity should be optimal. Groups with neither protective factor, having protein energy malnutrition but normal iron intake, should die quickly from pulmonary TB and so have

rib lesions but no dissemination to the skeleton. Communities with just one of the protective factors should have spinal involvement, as they would live long enough for the disease to affect bones such as the spine. The article concludes that the model does fit the data in the majority of populations analyzed but not all. This would suggest that both iron deficiency and protein energy malnutrition sometimes had a profound influence on the prevalence of TB in the past in the way the authors expected. The sites where the data does not fit the model may imply that anthropological assessment of past iron and protein intake are incorrect or that other factors that influence susceptibility to TB (such as genetics or disease comorbidity) may be more dominant in those communities.

The authors do state that there are many limitations to their study, and they have tried hard to allow for these. However, a major limitation to the approach used is one that would appear to have been correctable. There is no statistical analysis to determine whether the prevalence of TB in each group was significantly different enough to actually mean anything. Stating that some cases were found at a site where the model predicted a high prevalence of TB compared with few or no cases found at sites where the model predicted little TB is tantalizing but does not in itself prove much. Data is rarely given as to how many individuals were found at each site. If the number of individuals excavated from one site was five times that recovered at another, then five times the number of cases of TB found at the larger site does not signify any difference in prevalence of the disease. Similarly, data is only occasionally given regarding the completeness of the skeletal material at each site. Sites with better skeletal preservation may appear to have more cases of TB merely because more bone has survived for us to inspect for lesions. It may be that the number of skeletons from each region are similar and that preservation at all sites was similar, but without knowing that and quantifying it in a standardized way we can never really be sure of the influence that may have on the results. While the quality of preservation is often not given in a quantifiable manner in excavation reports, the number of skeletons from each site is usually available.

This study has many laudable elements, including a sensible hypothesis, good modern biomolecular evidence, data from a broad range of skeletal series, and a well-researched bibliography. However, I would argue that the research does not prove their hypothesis. In my mind, the important message of the article is that expansive studies such as this do raise exciting hypotheses and can provide supportive evidence. However, in the absence of any statistical analysis of the data, I feel we should stop short of claiming that anything has been proved, just that the hypothesis is plausible.

The article closes with a number of questions left unanswered by this study. One of them is particularly apt for all of us interested in disease in the past. If genetic studies suggest that TB is at least 35,000 years old, why does the earliest case in the Americas date to AD 300? One might suggest that it

could well be the limited number of well-preserved skeletons dating from before AD 300 is just too small for us to have randomly encountered one with classic lesions. This reminds us once again of the difficulties in studying the past prevalence of a disease in which only a small fraction of infected individuals develop skeletal lesions we can confidently diagnose today.

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**Ekaterina A. Pechenkina**

Department of Anthropology, Queens College of the City University of New York, Powdermaker Hall, 314 65-30 Kissena Boulevard, Flushing, NY 11367, U.S.A. (ekaterina.pechenkina@qc.cuny.edu). 2 IX 08

*Mycobacterium tuberculosis* is a curious pathogen. It is neither as old as some tropical pathogens that have circulated among primates for tens of millions of years (Martin 2003) nor as recent as pathogens acquired during the Holocene from domesticated animals. Members of the human lineage first encountered pathogens of the tuberculosis complex as early as 2.5 million years ago, when our ancestors spread into open habitats harboring abundant reservoirs of mycobacteria (Gutierrez et al. 2005). Surprisingly, tuberculosis remained invisible in the paleopathological record for almost the entirety of its coexistence with humans. Except for a single case of *Lep-tomeningitis tuberculosa* reported for a *Homo erectus* cranium from Turkey by Kappelman et al. (2008), no skeletal manifestations of tuberculosis have been found on human remains dating to before 8,000 years ago. Thereafter, tuberculosis-related lesions became fairly common, although with considerable interpopulation variation in their location and frequency.

Wilbur et al.'s paper is a thought-provoking meta-analysis of the effects of diet and nutrition on the epidemiology of tuberculosis in past populations. The authors propose a model linking manifestations of the disease to the levels of two dietary components: iron and protein. Their model postulates that diet-related anemia would curb the progression of tuberculosis infection, while protein deficiencies would elicit an aggressive form of the disease. Wilbur et al.'s approach has great potential. Using the known effects of dietary composition on disease progression to construct models for paleo-epidemiology ought to help resolve multiple conundrums in the human skeletal record with respect to tuberculosis, as well as to other diseases.

Their proposed model is simple by necessity: the low resolution of the paleopathological record prohibits addressing variation in individual human responses to pathogens or variations in the virulence of particular pathogens. Of greater concern is whether dietary levels of iron and protein are really the major factors affecting the progression of mycobacterial infection and whether the effects of variation in the availability of these two nutrients can be reduced to the tenets of the proposed model. The relationship between iron intake and

the progression of tuberculosis infection may not be as straightforward as the authors presume.

As Wilbur et al. suggest, extreme iron deficiency would impede the reproduction of mycobacteria. However, this shortage of iron would also compromise immune response by diminishing respiratory burst and nitrogen oxide production in macrophages, thus increasing the efficiency of initial infection (Schaible and Kaufman 2004, 948; Ekiz et al. 2005). Since low levels of dietary iron may produce such opposing effects on the progression of tuberculosis, it is not clear at what levels the benefits of dietary anemia in curbing mycobacterial reproduction would outweigh the disadvantages of compromised immune response.

Furthermore, chronic disease can cause anemia even when dietary iron is sufficient; this is probably a component of the innate response restricting pathogen proliferation (Zarychanski and Houston 2008). Patients suffering from tuberculosis often develop anemia as a result of the infection, even if their levels of dietary iron are normal. Successful treatment of these patients for tuberculosis restores their serum iron without iron supplementation (Lee et al. 2004; Sahiratmadja et al. 2007). Skeletal indicators of anemia interpreted as diet-linked could be the result of anemia due to infection instead (Stuart-Macadam 1992). It is possible that Wilbur et al. were unable to find a clear inverse relationship between skeletal manifestations of tuberculosis and anemia evidenced by porotic hyperostosis, not because porotic hyperostosis is an indicator of "limited utility" but rather because the relationship between tuberculosis epidemiology and anemia is nonlinear.

In addition, their proposed model seems to underplay the role of stress in the progression of tuberculosis. Persistent stresses in general, including physiological stress caused by malnutrition or nutrient deficiencies, result in immunosuppression by activating the hypothalamic-pituitary-adrenal axis, increasing both susceptibility to new pathogens and the reactivation of latent ones (Rhen and Cidlowski 2005, 1714). With specific respect to the progression of tuberculosis, almost any form of stress, including protein or caloric deficiency (Zachariah et al. 2002), lack of vitamins (Chan 2000), or even extreme psychological stress due to war, deprivation, migration, or natural disaster (Barr and Menzies 1994; Lerner 1996; Pavlovic et al. 1998; Ponticciello et al. 2005), correlates positively with increased morbidity and mortality.

We should acknowledge that the epidemiology of tuberculosis is an outcome of complex interactions among multiple factors having continuous variation, including levels of various nutrients, the virulence of particular strains, individual susceptibility, and so on. With all that in mind, it may well be that stress accounted for much of the recognized variation in the distribution of tuberculosis-related lesions in past populations. The transition to agriculture and population growth during the early Holocene introduced a whole series of new stressors into the human condition. One possible result was that the pathogenesis of tuberculosis became aggressive enough to cause skeletal lesions.

### Susan Pfeiffer

Department of Anthropology and School of Graduate Studies, 65 St. George Street, University of Toronto, Toronto, Ontario M5S 2Z9, Canada (susan.pfeiffer@utoronto.ca). 25 VIII 08

The contribution of Wilbur and colleagues is a valuable thought piece, presented with maturity and balance. It draws the reader into thinking about whether their perspective enriches our understanding of tuberculosis patterns in geographic areas beyond those discussed. My comments apply their insights to our understanding of tuberculosis lesions among Iroquoian peoples of the upper Great Lakes (chiefly what is now southern Ontario) from AD 1400 to 1650. The people of this region, at that time, lived in multifamily long houses, practiced maize horticulture, and interred most their dead in ossuaries. Analysis of remains from these large, secondary burial pits is complex (Pfeiffer and Fairgrieve 1994; Williamson and Steiss 2003), but probable tuberculosis is commonly seen. I will focus on those ossuaries I have studied myself. The Moatfield (minimum number of individuals [MNI] = 87, ca. AD 1300) and Uxbridge (MNI = 457, ca. AD 1500) ossuaries show high lesion frequencies, suggesting that nearly all people suffered from tuberculosis, based on modern probabilities of osseous tissue involvement. Bone tissue from Uxbridge has yielded DNA consistent with *Mycobacterium tuberculosis* (Braun, Collins Cook, and Pfeiffer 1998).

The Uxbridge remains show osseous signs of tuberculosis in very young children as well as adults (Pfeiffer 1984). Vertebral tissue modifications akin to Pott's kyphosis are seen in one child aged 3–5 years; a minimum of eight of the 145 immature individuals show vertebral lesions consistent with tuberculosis. At the smaller Moatfield ossuary (Pfeiffer 2003), there were no diagnostic vertebral lesions among juveniles, but there were three crania (infant to 3–5 years) showing resorptive, endocranial lesions that may have been tuberculous meningitis (cf. Schultz 1999) but were not scrutinized for those specific characteristics before their reinterment. Information on *cribra orbitalia* is not available for Uxbridge; at Moatfield the frequency (28%) is comparable to that from other Iroquoian ossuaries, but the proportion of affected juveniles (56.5%) is the highest documented for this region. Lesions on the pleural aspect of adult ribs are present in both groups; they represent a higher proportion of the sample at Uxbridge (Pfeiffer 1991) than at Moatfield.

How does Iroquoian dietary information fit with this picture? The presence of lesions among all age groups suggests that they fall into category 1, that of people with dietarily adequate protein and iron. This does not appear to have been the case. Adults from Uxbridge as well as the Kleinburg ossuary (MNI = 561, AD 1600) have been radiographically shown to have low bone mass, suggesting dietary inadequacy relating to a heavy reliance on maize (Pfeiffer and King 1983). Regional analyses demonstrate strong reliance on maize by

AD 1300 (Schwarcz et al. 1985). Stable isotopes in teeth from the Moatfield sample show this pattern (van der Merwe et al. 2003). The spacing of  $\delta^{13}\text{C}$  from collagen and enamel (7‰) indicates an herbivorous diet. Nitrogen isotopes indicate that Moatfield people were getting protein from eating fish, especially large-bodied carnivorous fish. Some communities appear to have focused on fish (lacustrine/riverine protein), and others on deer (terrestrial protein) during this period (MacDonald 2002). Caries rates and dental health are consistent with this picture. Caries rates for all teeth range from around 25% to over 40%, but this understates the effect of caries because of the high levels of antemortem tooth loss through abscessing. Moatfield mandibles showed 32% antemortem loss and 39% caries among the remaining teeth (Crinnion, Merrett, and Pfeiffer 2003). Dietary energy was apparently adequate and evidence for nutritional diseases is equivocal, but both protein and iron seem to have been in short supply. This places Iroquoians in category 4.

As noted by Wilbur et al., diet is not the only factor influencing infectious disease patterns. Among Iroquoians, two exacerbating factors deserve particular attention. On the plus side, they may have been ingesting fish oil from species that provide significant health benefits. The nitrogen isotope ratios are consistent with types of fish that are not common in faunal remains from the middens, including burbot (a type of cod). The absence of burbot bones may indicate that the oil was a commodity that was traded, as was observed historically (Fox 2000). Cod liver oil is an excellent source of fat soluble vitamins and omega-3 fats, benefitting the immune system and bone tissue. Even a small amount of this type of fish oil could counterbalance some of the less beneficial aspects of their diet. On the negative side of the health equation, Iroquoians lived in smoky, crowded longhouses. Maxillary sinusitis is ubiquitous by adulthood at Uxbridge and Moatfield (Merrett and Pfeiffer 2000; Merrett 2003). The indoor combustion of biomass fuels (e.g., wood smoke in this case) is associated with heightened respiratory infections and suppressed immune response (Roberts 2007). Poor air quality could have sped or amplified the course of tuberculosis.

The combination of reliance on an imperfect carbohydrate dietary staple, a climate that is cold, damp, and cloudy for much of the year, and life in crowded, smoky longhouses allowed tuberculosis to spread readily. The mystery is how Iroquoian people survived long enough to show skeletal lesions. Low dietary iron intake could have slowed disease progress. Fish oil in the diet may have also had a salutary effect. It would be interesting to study patterns of tuberculosis from ossuaries representing groups with different protein sources (fish versus deer). While intrigued, I am not sure whether we will be able to differentiate categories 1 and 4 on osteological evidence alone. Nevertheless, their work certainly stimulates thought.

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**Nancy Tayles and Judith Littleton**

Department of Anatomy and Structural Biology, Otago School of Medical Sciences, University of Otago, P.O. Box 913, Dunedin, New Zealand (nancy.tayles@otago.ac.nz)/ Department of Anthropology, University of Auckland, Private Mail Bag 92019, Auckland, New Zealand. 25 VIII 08

We applaud the approach taken by Wilbur et al. to the challenging task of attempting to determine the prevalence and significance of TB in prehistory. It is refreshing to see a move beyond the traditional interpretation of the lesions to one drawing in epidemiological and immunological knowledge and incorporating this with nonspecific skeletal markers of malnutrition, isotopic and archaeological evidence of dietary composition, and archaeological evidence of subsistence and habitat to produce a model of TB prevalence and distribution.

We do, however, have some issues with the development of the model. Wilbur and coauthors point to the interaction between nutrition and infection and highlight some of the specific linkages that are hypothesized to exist between TB disease and iron deficiency or protein undernutrition. These are seen acting in one direction, yet interactions between TB infection and disease and nutritional status are two-way. For example, a characteristic symptom of TB disease is weight loss and a recent review suggests that the anemia seen in TB is most frequently the anemia of chronic infection (van Lettow et al. 2003).

The authors justify the emphasis on protein and iron on the basis that these have the best-understood effects on mycobacterial infection. However, there are long-recognized linkages between TB and other nutrients, particularly vitamin D inadequacy (e.g., Roberts and Buikstra 2003, 54–55; van Lettow et al. 2003). The model simplifies a set of complex relationships between nutrition and TB.

Beyond this, their identification of protein deficiency in human remains relies on two assumptions, first, that high caries rates are indicative of high carbohydrate consumption, and second, that a high carbohydrate diet is of necessity low in protein. On the first point, although some carbohydrates (notably maize in the New World) are cariogenic, not all are equally so (Lubell et al. 1994; Oxenham et al. 2006). On the second point, the inclusion of agriculture as a contributor to subsistence does not necessarily equate to inadequate protein.

The model also privileges nutrition over the other characters that influence susceptibility to TB—in particular, past population history of TB, the age structure of the population, other infections and immunosuppressing conditions. It could be argued that the specific case studies examined in this paper share a population history of tuberculosis but globally that varies substantially. The population ratio between pulmonary and extrapulmonary TB seems to be, at least partially, a function of population and individual life history. Populations with a long history of TB are more likely to experience higher

rates of extrapulmonary disease than pulmonary (Farer et al. 1979; Verver and Veen 2006, 881–2).

Similarly age structure reflects TB mortality as well as affecting TB transmission and disease location (Roberts and Buikstra 2003, 48–50). For example, children who are susceptible to tuberculous meningitis are also the sentinel indicator of active TB transmission because until they can have a productive cough, they are not agents of transmission (Howie et al. 2005). In this instance, the age structure of the population and the distribution of active and inactive lesions become important variables.

The further complicating point is the increasing recognition of the synergistic relationship between tuberculosis and other conditions (infectious and noninfectious). The chronicity of TB means that it is a prime candidate for syndemic (Milstein 2002) interactions; for example, other zoonoses can influence the presence and distribution of skeletal signs within populations.

Such studies require a detailed analysis of context, and while the model proposed here may be a heuristic device, the lack of consideration of the composition and context of the samples discussed limits its interpretive power. The authors have shown us how understanding of, for example, the immune system, can improve interpretation. However, we also need to accept that the interactions are complex, and despite the appeal of a simple model, human biology is rarely that easily codified.

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**Vera Tiesler**

Facultad de Ciencias Antropológicas/Autonomous University of Yucatan, Carr. Mérida Tizimín, km 1; CP 97305, Mérida, Yucatán 97305, Mexico (vtiesler@uady.mx). 3 IX 08

Only recently have we started to understand some of the specific mechanics involved in mycobacterial exposure, disease development, and skeletal involvement of tuberculosis in world prehistory. A laudable effort in this direction is the paper by Alicia Wilbur and her colleagues. Building on knowledge of host-pathogen interaction, it explores the role of dietary protein and iron in the deadly spread of tuberculosis through the Americas. To test their model, the authors discuss the presence versus absence of skeletal indicators of tuberculosis in four large cultural settings with different dietary patterns. This “cross-cultural” comparison is intended to provide viable answers on the possible role of nutrition in the spread of the disease.

While this paper benefits from a rigorous research design that builds on a set of hypothetical predictions and observed patterns, I think it does not escape the shortcomings that are inherent in this type of research. One of my main concerns in this regard is the use of nonspecific stress markers (caries, porotic hyperostosis, dental enamel hypoplasias), which all can have different etiologies, in order to make inferences spe-

cifically about carbohydrate intake or iron deficiency, a shortcoming that the authors themselves are probably well aware of. Also their decision to employ a unifactorial approach (i.e., diet) to examine the decisively multifactorial mechanisms involved in mycobacterial diseases naturally comes to limit any affirmative statement or causal explanation regarding the relationship between dietary patterns and tuberculosis. I agree therefore with the authors when they conclude that the purpose of their study has a heuristic quality, to elucidate areas of future research and, more specifically, to incite new questions regarding the relatively late outbreak of the disease in the Americas.

I will take up this last issue here, since it is only marginally discussed in the paper. Why have no convincing cases of probable tuberculosis been traced before AD 300, and why is the bulk of suggestive specimens dated to the second millennium AD? This comes as a surprise especially when considering that at least two of the examined areas (parts of the Andes and Mesoamerica) look back on a long-standing millenary trajectory of relatively unchanged subsistence patterns, with eras of centralized geopolitical systems and high population densities, which would have made feasible environs for earlier tuberculosis outbreaks. Well known are the crowded living conditions in the many large pre-AD 1000 urbanized centers such as Teotihuacan in the highlands of Mexico or Tiwanaku in the south central Andean highlands. It follows from this that the critical bacterial thresholds for tuberculosis dissemination had not been reached in the early times and/or that the transmission mechanisms that did lead to the initial outbreaks of the disease might have been different from those operating during the second millennium, a time when the disease had already spread over large parts of the New World. A stronger diachronic emphasis in the research design would surely have enriched the discussion. Alternatively, stricter control on coetaneous populations when selecting the cohorts to be assessed, preferably post-AD 1000, would have been convenient when assessing the outcomes of the mycobacterial coevolution with humans.

As it stands, the selection of Maya skeletal series in particular is inconvenient in this respect, since the great majority derives from precollapse populations (pre-AD 800), and some even clearly predate the Classic period (pre-AD 150). Considering that all datable Mesoamerican specimens suggestive of tuberculosis have been traced to the second millennium AD, the Classic Maya world appears to be an unlikely setting for initial outbreaks of tuberculosis that were sufficiently massive enough to leave their traces in the minute fraction of preserved and recovered remains that have been examined by specialized personnel. Here, instead of linking the disease's presence or absence to nutritional patterns, which have analogs in most other parts of the pan-Mesoamerican sphere, I would argue that factors found in the Maya Lowlands' dense vegetation and its particular geography should have worked as natural barriers against disease spread. Also, the year-round warm climate, the design of ancient Maya living spaces, and

an outdoor lifestyle all make the Maya Lowlands an improbable milieu for early outbreaks and along with the local geography should account for the absence of clear diagnostic lesions in the Maya skeletal series cited by the authors.

While these thoughts potentially dismiss the relevance of Classic Maya nutritional patterns in the development or absence of tuberculosis, they trace promising future lines of research, at least in this area. Postclassic inland populations that settled in the karstic plains of Yucatan during the second millennium AD could constitute new target populations for researchers, since the skeletal collections from this part of the Maya world have been only partly scrutinized and are steadily growing. Also the systematic examination for diagnostic features of the large and relatively well-preserved Classic and Postclassic skeletal populations of the coastal fringes should make promising future research objects. Most of the skeletal indicators from these populations are consistent with category 5 (sufficient dietary protein and iron but high load of infections), thus allowing a possible interpretation of tuberculosis outbreaks and detectable skeletal signatures, to keep with the reasoning of the authors. It is precisely this set of ideas and agendas that the present paper encourages, and my colleagues should be congratulated for it.

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## Reply

We thank the commentators for an excellent set of suggestions and criticisms aimed at improving our model. Before addressing the comments, we think it important to reiterate the original purpose of this paper: to better inform our understanding of disease in the ancient past by developing models based upon information available that was independent of the disease in question. Our interest was in the interplay of diet and the immune system and how these affected the development and course of tuberculosis in humans. Examining the published epidemiological and experimental literature, we first determined the ways in which two nutrients, protein and iron, could affect the immune response to tuberculosis. Five dietary categories were constructed based on coarsely divided levels of these two nutrients. Population level expectations for disease course—and how that might or might not be seen in the skeleton—were then posited for each category.

Following development of this model, we compiled information on the diets of several ancient New World peoples. The dietary information was obtained from the archaeological record as well as being inferred from dental and osteological nonspecific indicators of stress. We then assigned each population group to a dietary category and finally compared our observations of skeletal tuberculosis indicators (i.e., rib and vertebral lesions) to our expectations for indicators based on the dietary category previously assigned. We do not expect

the model to be useful as a predictor of diet from presence or absence of tuberculosis lesions.

In general, our idea for development of such models seems well received. Most commentators suggested potential inclusion of other variables to enhance our diet and immunity model or inclusion of other types of information that could inform our interpretations of the paleopathological record. Bernardo Arriaza, for example, suggested the addition of epidemiologically relevant social, demographic, and cultural variables that were probably as important in ancient societies as they are today. Ekaterina Pechenkina raises an excellent issue with our neglect of the effects of stress on the immune system and how various emotional, physiological, and environmental stressors can impact morbidity and mortality. Pechenkina's point that a variety of stressors can negatively impact health is well taken. Of the stressors Pechenkina lists as examples, however, many are interlinked: poor nutrition often goes hand in hand with war, deprivation, migration, and natural disaster. Despite being interlinked, these social and physiological stressors may act in physiologically and biochemically disparate ways; separating them—or even clearly distinguishing them in prehistoric populations—may prove extraordinarily difficult but worthwhile to attempt.

Susan Pfeiffer, Nancy Tayles, and Judith Littleton all emphasize other nutrients that historic, anecdotal, and modern clinical and experimental literature suggest to be of importance in immunity to tuberculosis, in particular vitamin D. However, the intricacies of this compound's role in tuberculosis immunology render it ill-suited for the relatively simple model we aimed to generate here. While vitamin D inadequacy has long been linked to active tuberculosis, the "massive" amounts of vitamin D's active form, calcitriol, produced in tuberculosis patients (Rook 1988, 769) are strongly implicated in immunopathology favoring bacterial dissemination and tissue destruction. Production of calcitriol in granulomas downregulates the signaling molecule interleukin-12 (Rook and Hernandez-Pando 1996), the consequences of which are likely to include reduced induction of the Th1 response, reduced activity of bactericidal cells, and promotion of activated T-cell death (Gately et al. 1998), leading to immune hyporesponsiveness to tuberculosis bacteria (Hirsch et al. 1999) and probable failure to contain the bacteria at the initial point of infection. In cells exposed to *Mycobacterium tuberculosis*, calcitriol also upregulates the proinflammatory tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ), a signaling molecule required for organized granuloma formation in mice (Flynn and Chan 2001) but also associated with fever, weight loss, and tissue destruction, as well as liquefaction and necrosis of pulmonary tuberculosis lesions (Rook et al. 1987).

More disturbingly for those of us attempting to link vitamin D status with tuberculosis skeletal pathology, calcitriol can have both mediated and direct effects on the skeleton. The TNF- $\alpha$ , upregulated by the calcitriol produced at the granuloma, is shown in some systems to be a paracrine suppressor of osteoblast activity (Evans et al. 1998; Qi et al. 1999; Silvestri

et al. 2004; Vermes et al. 2004) and stimulator of osteoclast differentiation and activity (Evans et al. 1998; Qi et al. 1999). Although these effects were countered in one study (Vermes et al. 2004) by addition of calcitriol itself, the rescue was only partial. Furthermore, calcitriol has been shown to induce osteoclast-like differentiation of progenitor cells (e.g., Clohisy et al. 1987; Qi et al. 1999). It is further established that circulating calcitriol upregulates osteoclast activity, resulting in calcium withdrawal from the bones. It is not unlikely that this effect may also function locally in skeletal tuberculosis, with calcitriol overspill at the margins of bone-adjacent granulomas upregulating osteoclastogenesis and bone resorption during tuberculosis infection. Because of these complications, for the purposes of this paper we settled on protein and iron as nutrients better understood with respect to tuberculosis immunology; however, we continue to more closely examine the role of vitamin D.

Particularly inspiring to us was Vera Tiesler's venture into the issue of tuberculosis cases in ancient American societies and why the vast majority of convincing cases of skeletal TB do not show up until after AD 1000. She advocates tighter temporal control of samples as well as suggesting that research among the ancient Maya remains might be better informed by climate and environment, including habitation style and construction, rather than diet. However, she also suggests newly analyzed ancient Maya collections from contrasting environments for such a modeling study, and it is this type of exploration that we hope to stimulate with our paper.

Deborah Blom and Piers Mitchell both requested more quantification in our case studies, and we agree that this would enable statistical comparisons that could be illuminating in future studies. For the purpose of this paper, our aim was to use data that were available in the published literature with the intent of demonstrating how modeling of diet and nutrition information from the archaeological record can guide expectations and generation of future hypotheses, rather than to actually "test" a hypothesis or model in this case. This issue does, however, highlight the need for presentation of data in published literature so that independent testing and verification of results can be achieved by other groups, which is one of the principle tenets of science. Indeed, some sort of osteological database similar to Genbank would be a dream come true for many researchers, although the feasibility of such a project is debatable.

On the subject of databases, for now we have to disagree with Arriaza's suggestion of the creation of an ancient tuberculosis sample collection center. As we discuss in our introduction, a number of serious challenges with ancient DNA studies of disease remain. Most researchers have now adopted the strict quality control measures necessary for ancient DNA extraction and amplification, but limitations of template size and preservation necessitate analysis of very small, often non-specific sequences whose information has been further compromised by the incorporation of amplification errors. Attempts to identify these errors are met with the same lack of

phylogenetic information that error-free small fragments would confront: what are our expectations for a 1,500-year-old *M. tuberculosis* fragment, for example? To develop such expectations, it is necessary to understand the temporal and geographical distribution of not only the *M. tuberculosis* complex organisms of interest, but also of the closely related, genetically similar, and environmentally ubiquitous mycobacterial species that are most certainly present in both the ancient and modern burial context of the sample.

There are insufficient analyses even from modern mycobacterial strains to inform identification of ancient fragments recovered by researchers, and this negatively impacts the formation of an ancient tuberculosis sample collection. That is, fragments must be securely placed phylogenetically before they can be considered for inclusion in the collection Arriaza suggests. What might be an excellent starting point along the road to an ancient disease DNA database would be development of a database with accurate genome information from modern disease organisms and closely related species. Current sequencing efforts by many groups are rapidly expanding the genetic information publicly available through such sites as Genbank, although genetic studies of disease organisms tend to be biased in favor of economically significant organisms and strains.

To conclude, we were delighted to see that our model was well received and that it generated discussion of future research avenues. We recognize, as did several commentators, that such an approach is always limited, in the sense that it must be general enough to be broadly applicable to a wide set of situations and yet specific enough to capture the nuances of each individual case examined. Our hope is that our general approach may be useful to researchers who can add other parameters relevant to their study areas to stimulate further hypothesis formation and testing.

—A. K. Wilbur

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