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When toxic levels of fluoride are consumed, the pathological condition of fluorosis ensues. Skeletal fluorosis is an endemic problem in populations at risk of ingesting excess fluoride. Whether ingestion takes place as result of toxic levels of natural mineral content in water of from anthropogenic factors such as tea drinking or smoking, the results can be detrimental to an individual's health. Fluorosis has been identified in archaeological skeletons from Bahrain, Naples, and the United Arab Emirates. While a very serious concern in some parts of the world today, the United States continues to fluoridate municipal water supplies. Although the natural geologic environment in many regions of the United States (US) is conducive to fluorosis, this condition is largely ignored in the US. This may suggest an absence of the condition in this region of the world; however, the etiology and history of this metabolic bone disease is poorly understood therefore, it may often be misdiagnosed or overlooked clinically and perhaps archaeologically. In this paper, I review clinical and epidemiological literature discussing the prevalence of fluorosis. From this review, I conclude that fluorosis may in fact be a current public health issue in the United States. This is demonstrated by the frequency of fluorosis reported in both National Research Council and Center for Disease Control documents. Furthermore, although this condition is not yet described in archaeological collections from North America, I hypothesize it may be present in

the Illinois River Valley, due to the natural geologic conditions conducive to fluorosis. Skeletal signs of fluorosis, including ossification of tendinous and ligamentous attachments, an increased presence of fractures, and periosteal and sclerotic deposition, are documented in 8 individuals from the Ray Site.

POSSIBLE FLUORIDE TOXICITY IN NORTH AMERICA:

A PALEOPATHOLOGICAL ASSESSMENT AND

DISCUSSION OF MODERN OCCURENCE

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CHAPTER 1

INTRODUCTION

1.1 Background

The fluoride ion is derived from the element fluorine, which is commonly regarded as an essential trace element for mammals, including humans (Cerklewski, 1997). However, there is a growing debate about the role of fluoride in human health (Barbier et al., 2010). In part, this is related to the complex effects of fluoride on the human system and varied individual responses to fluoride. Fluoride function in the human body requires a delicate balance, as too little (<.5ppm) results in dental carries and poor mineralization of bone, and too much (>1.0ppm) results in compromised mineralization of bone and excessive bone formation with ossification of associated soft tissues (Littleton, 1999; Petrone et al., 2013; Freni, 1994).

Fluorosis is a condition in which ingestion of high levels of fluoride leads to compromised functioning of the endocrine, reproductive, neurological, cardiovascular, gastrointestinal, and renal systems (Freni 1994; Reddy, 2009; Waldbott, 1976). Chronic consumption of fluoride will result in structural changes to the musculoskeletal system in the form of ossification of ligamentous and tendionous attachments and compromised mineralization of bone. Fluoride toxicity is considered an environmentally influenced condition, dependent on high concentrations of fluoride in the soil, water, or air (Littleton, 1999; Petrone et al., 2013; Teotia et al., 1977).

1.2 Geography of Skeletal Fluorosis

Fluorosis is a globally occurring condition which is most prevalent in locations high in natural fluoride such as Naples, Bahrain, India, and China (Figure 1) (Petrone et al., 2011; Petrone et al., 2013; Littleton, 1999). These locations present both modern and ancient evidence of fluoride toxicity (Petrone et al., 2013; Teotia et al., 1977; Dai et al., 2004). Modern examples are represented through clinical symptoms and skeletal signs, yet, paleopathological evidence exists as presentation of skeletal signs suggestive of fluorosis.

Figure 1. Map of countries with endemic fluorosis due to excess fluoride in drinking water . Modeled after WHO reports on fluorosis.

Regions of North America are naturally high in fluoride (Figure 2), yet this is not represented accurately on maps provided by the Center for Disease Control or the World Health Organization (Figures 1). Furthermore, little discussion of this condition occurs in clinical literature. This may illustrate a lack of fluoride related

maladies in North America, or, perhaps, the result of failing to consider environmental, dietary, epidemiological, and physiological factors when developing a differential diagnosis. Considering that symptoms of fluorosis are commonly shared with several other illnesses, misdiagnosis is very possible (Goal, 2006; Waldbott, 1976) .

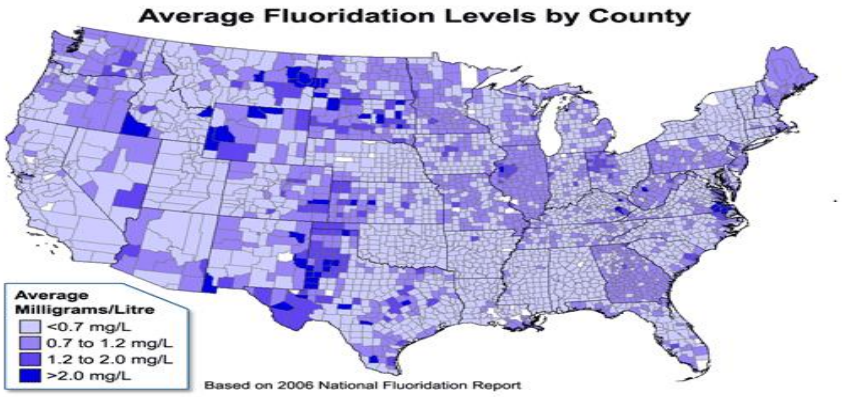

Figure 2. Map of estimated fluoridation levels by county in the USA based on the 2006 National Fluoridation Reports. Provided by the CDC at cdc.gov.

1.3 Research Focus

I hypothesize that fluoride is a widely overlooked toxic factor affecting the health of many North American populations, both presently and before fluoridation of municipal water. Furthermore, I hypothesize skeletal fluorosis has been overlooked in paleopathological assessments of the North American archaeological record. In order to investigate the presence of fluorosis in modern North America, I have thoroughly reviewed clinical literature and epidemiological data as provided by the WHO, CDC, US Department of Health, National Research Council, and the University of North Texas Health Science Center library database. Literature and data review of modern cases of fluorosis occurring in the United States of America

was performed in conjunction with analysis of an archaeological population from a region of North America with natural levels of fluoride conducive to toxicity: west-central Illinois (Omueti and Jones, 1977). The population examined is from the Ray Site, a Middle Woodland site dated to 50_{BC} to $_{AD}400$ (Flotow, 1983; Nelson et al., 2015; Nelson et al., in prep).

CHAPTER 2

SKELETAL FLUOROSIS: BIOMEDICAL AND EPIDEMIOLOGICAL INSIGHTS

Interpretation of abnormal skeletal changes requires knowledge of bone physiology, etiology, and various causative factors of pathological bone formation (Klaus 2014; Ragsdale and Lehmer 2012). Through pathophysiological research we gain a deeper understanding of lesion formation, thus enabling recognition of early and later stages of pathological changes associated with specific conditions. Clinical data, incorporated with pathophysiological research, illuminates the spectrum of changes expected for each condition, which is frequently broad and varied (Nelson et al, 2015a). The effects of dietary toxicity are often gradual and accumulative. Therefore, investigations of fluorosis must take into account the full spectrum of progressive changes and inferred symptoms for fluorosis (Petrone et al. 2013; Littleton 1999; Teotia et al. 1988).

2.1 Pathophysiology of Fluoride Toxicity

As fluoride is ingested, it is first absorbed in the stomach, a process facilitated by stomach acid, which converts fluoride to hydrogen fluoride (Dhar and Bhatnagar, 2009). Forty - fifty percent (40-50%) of fluoride is absorbed in the stomach, while absorption in the small intestine accounts for roughly 45%, and is accomplished through facilitated diffusion (Barbier et al., 2010). With a permeability coefficient close to that of water, hydrogen fluoride can easily permeate cells, unlike dissociated fluoride (Barbier et al., 2010; Goal, 2006).

Fluoride is easily taken up in the body due to multiple co-transport mechanisms (Barbier et al., 2010).

Unabsorbed fluoride is primarily removed by the kidneys and released in urine (Goal, 2006; Barbier et al., 2010; Santoyo-Sanchez et al., 2013). Individuals with renal pathologies are therefore more susceptible to the accumulation of fluoride (Goal, 2006). Once absorbed, fluoride circulates in the blood stream and is readily distributed throughout the body, facilitating physiological functions. Due to fluoride's affinity to calcium, fluoride will naturally concentrate in calcium-rich tissues like bone and teeth (Goal, 2006; Petrone et al., 2013; Barbier et al., 2010). It is estimated that 99% of fluoride existing in the body is stored within calcified tissues (Goal, 2006).

Toxic fluoride consumption leads to altered biochemical and physiological processes in the renal, cardiovascular, reproductive, neurological, endrocrine, gastrointestinal, and musculoskeletal systems (Carton, 2006; Petrone et al., 2011; Walbott, 1976). There is no absolute threshold for fluoride toxicity due to individual variation and environmental factors; in general, chronic consumption of levels greater than 1mg/L is considered toxic (Carton, 2006; Littleton, 1999; Petrone et al., 2011). Individuals may experience symptoms within the first two weeks of continuous consumption of toxic levels of fluoride. These include headache, nausea, constipation, and body aches (Waldbott, 1976). Over time, continued ingestion of excess fluoride will cause an increased stiffness of vascular structures, joint degeneration, muscle stiffness, difficulty or inability to reproduce, decreased thyroid function, impaired glucose tolerance (Type II diabetes), early sexual maturation, and

neurological complications including muscle spasticity or lack of response (Carton, 2006; Petrone et al., 2013; Reddy, 2009; Waldbott, 1976).

Chronic ingestion of toxic levels of fluoride leads to compositional change of the organic matrix and mineral of bone. Fluoride replaces the hydroxyl component of bone and mineralizes bone as fluoroapatite, a more stable mineral complex than hydroxyapatite (Petrone et al., 2013; Goal, 2006). Microstructural changes in bone have deleterious effects as fluoride accumulates, resulting in excessive ossification of bone and associated soft tissues, as well as densification of existing bone (Boivin et al., 1998; Petrone et al., 2013; Littleton, 1999). This results in decreased bone strength and compliance, leading to an increased incidence of fractures (Littleton 1999; Petrone et al. 2013; Fabiani et al., 1999). If the level of consumption remains unchanged, immobilization will ultimately result due to joint fusion (Teotia et al., 1971).

Histological characteristics of fluorotic bone include cancellous linear formation defects, mottled bone, and an increase in the cancellous osteoid perimeter (Boivin et al., 1998). The bone cell response to fluoride observed in histological samples of bone is not exclusive to fluorosis but mimics osteoblastic lesions, Paget's disease, and osteopetrosis (De Boer et al., 2013). When observed, these changes can, however, provide supportive evidence for a suite of diagnostic changes caused by fluoride toxicity.

To consider genetic factors for fluorotic bone development, we must also understand genetic influences upon mineral absorption, mineral transporters/receptors, and mineral metabolism (Kobayashi et al., 2014; Mounsy et

al., 2006; Lee, 2004; Sakaeda et al., 2004). Although fluoride has not been known to act as an epigenetic agent in DNA methyltransferase and microRNAs, fluoride may serve as a modulator for multiple cell types including normal pathological cells *in vitro* and *in vivo* (Barbier et al., 2010). For example, fluoride influences the expression of genes involved in bone modeling and tissue formation, which impairs the formation of the intracellular matrix leading to abnormal bone and dentin formation (Barbier et al., 2010). Dentin malformation occurs primarily through the response of ameloblasts to fluoride in which cells down-regulate transcription through the JNK/c-Jun signaling pathway (Barbier et al., 2010; Zhang et al., 2007). Numerous genes are up-regulated or down-regulated in response to fluoride. Increased fluoride consumption results in an up-regulation of NF-κB RNA levels, known to influence tumor cell growth, induce early response of inflammatory responses, and regulate miRNA (Barbier et al., 2010). Recent studies from a Mexican population experiencing endemic fluoride toxicity revealed modified expression of apoptic genes in peripheral blood mononuclear cells (PBMC) along with down-regulation of several immunological and protein encoding genes including LT-b, CD40L, HVEM, capase-6, TRAF-2, and TRAF-5 (Salgado-Bustamente et al., 2015). This down-regulation ultimately compromises immune system function (Barbier et al., 2010; Petrone et al., 2013).

Research has identified the stimulatory influence of fluoride on receptor activators of nuclear factor kappa-B ligand (RANKL) to induce osteoblast formation (Pei et al., 2012). Pei and colleagues found that fluoride significantly reduces osteoblast bone resorption at levels as low at 0.5mg/L (0.5 ppm) (2012). Studies

show fluorotic patients have a greater number of osteoblasts (Boivin et al. 1998); whether this is an effect of fluoride toxicity on cells or a genetically predetermined factor is, as of yet, unknown. This increased osteoblastic presence is the apparent primary contributor to the dense, osteosclerotic bone formation characterizing skeletal fluorosis.

2.3 Environmental and Dietary Influences

Fluorosis emphasizes disease-environment synergism, as the development of this condition is dependent upon the existence of fluoride in the environment and diet. Although the mineral fluoride is found globally, a number of environmental factors influence the uptake and absorption of fluoride. These include geologic weathering, temperature, and the natural mineral composition of rocks and soil (Barbier et al., 2010; Goal, 2006). Fluoride-binding minerals in the groundwater facilitate leaching of fluoride from surrounding geologic features and increase fluoride concentration within the water (Barbier et al., 2010; Petrone et al., 2011). The same process can be inferred for the storage of water in containers made from fluoride-rich soils (Littleton, 1999).

Consuming fluoride-rich water is the most common means of ingestion (Goal, 2006; Barbier et al., 2010; Littleton, 1999; Lund et al., 1997). Hot, humid climates increase the concentration of the mineral within standing or stored waters (Littleton, 1999). These environments also contribute to high fluoride ingestion with increased water intake to maintain hydration in the hot weather (Littleton, 1999).

Skeletal expression of the disease has been reported in exposure levels as low as 0.7 ppm, or 0.699mg/L (Jolly et al. 1973). Generally, it is accepted that adverse effects may occur at levels greater than 1mg/L (1.002 ppm)(Littleton 1999; Petrone et al. 2011). It remains unclear, however, how long high levels of fluoride must be ingested before an individual begins to exhibit signs of skeletal fluorosis. Though symptoms may occur with acute exposure, chronic exposure of ingestion at levels higher than the rate of renal clearance are required to produce skeletal changes (Goal, 2006). Skeletal signs have been reported with prolonged intake in as little as 1-4 years of exposure (Siddiqui, 1955).

Absorption of fluoride may be influenced by other dietary factors. The agastric absorption of fluoride can be reduced, even when the bioavailability of fluoride is high, through the ingestion of fluoride-binding minerals (i.e., calcium, magnesium, aluminum) to create insoluble complexes (Barbier et al., 2010; Goal, 2006). A diet with adequate calcium, magnesium, and aluminum will therefore result in little to no response to a moderately toxic level of fluoride (Littleton, 1999; Goal, 2006; Petrone et al., 2013). Calcium plays a role in buffering the effects of fluoride during digestion and metabolism. Once absorbed, in the presence of adequate extracellular calcium, fluoride will bind to calcium and its effects will be limited by calcium channel blocking agents (Barbier et al., 2010). A diet low in fluoride-binding minerals, particularly calcium, will enhance the effects of increased ingestion of fluoride.

2.4 Current Epidemiological Information

According to current epidemiological data, fluorosis is a significant problem in China, India, southern Italy, the Middle East, and various parts of Africa (Dai et al., 2004; Teotia et al., 1971; Petrone et al., 2013; Littleton, 1999; Yoder et al., 1998). In areas such as southern Italy, the naturally high level of fluoride due to volcanic activity increases the risk of fluorosis (Petrone et al., 2013). However, populations living in areas not naturally conducive to fluoride toxicity may still develop fluorosis due to social and cultural factors such as tea drinking, smoking, water storage methods, and industrial byproducts (Braun et al., 1984; Hodge and Smith, 1977; Cao et al., 1996; Dai et al., 2004; Littleton, 1999; Watanabe et al., 2000; Whyte et al., 2008).

According to the Center for Disease Control (CDC), minimal to moderate fluoride toxicity occurs today in some regions of the United States, including Illinois, Oregon, Texas, and Washington (Center for Disease Control, 2005; Freni, 1994). An increase in the prevalence of fluorosis in the United States has been identified in both fluoridated and non-fluoridated communities (Clark, 1994; Szpunar and Burt, 1998; Brunelle 1989; Driscoll et al. 1986; Ismail et al. 1990). The Public Health Service (PHS) (1991) and National Research Council (NRC) (1993) report only five cases of severe, "crippling" skeletal fluorosis occurring in the United States from 1972-2005. These occurred in areas of water fluoride levels ranging between 2.4-7.8 ppm. The known incidence of mild to moderate fluorosis in the United States ranges between 35-60% of individuals living in fluoridated communities and 20-45% in non-fluoridated communities (Clark, 1994). This prevalence of fluoride

toxicity, although noted by the CDC and NRC, is largely overlooked on public health websites and resources. This includes the CDC and WHO maps illustrating fluoridation levels and regions of fluorosis (Figures 1).

Skeletal fluorosis appears to occur more frequently in males than females, and in older individuals, though this varies between populations (Ortner 2003; Littleton 1999). Factors unique to the individual, such as activity patterns and genetic predisposition, can result in variation across a population (Littleton 1999; Mousny et al. 2006; Mousny et al. 2008). Individuals with relatively high levels of physical activity tend to show increased expression of fluorotic skeletal changes (Brickley and Ives 2010; Littleton 1999). It is argued this is a response to both increased fluoride intake due to hydration demands of physical activity and bone remodeling in response to stress and loading (Littleton 1999).

Epidemiological research reveals there are human "non-responder" populations (Dequeker and Declerck, 1993; Duursma et al., 1987) as well as populations that are sensitive to increased fluoride ingestion (Russell, 1962; Butler et al., 1985; Yoder et al., 1998; Chuobisa et al., 2001). These results are evidenced by a number of studies demonstrating that sex, age, activity, and ancestry all play a role in the response to fluoride (Littleton, 1999; Petrone et al., 2013; Everett, 2011; Durusma et al., 1987; Rich et al., 1964). These data suggest a genetic basis for fluoride in osteogenesis and have led to increased molecular research concerning fluorosis (Mousny et al., 2006; Everett, 2011; Dequeker and Declerck, 1993; Louw et al., 2002; Russell, 1962; NRC, 1993; Yoder, 1998; Choubisa et al., 2001). Challenged by the complex interactions of fluoride within the body, molecular research on the

human genome and fluoride toxicity continues to investigate the identity of genes associated with fluorotic bone development (Everett 2011; Mousny et al. 2006). Evidence of the existence of such genetic factors has been uncovered in murine and nematode research (Mousny et al. 2006; Everett 2011). These studies reveal novel fluoride resistant genes *flr1, flr3, and flr4* (Everett 2011), which decrease the susceptibility to skeletal response associated with fluoride toxicity.

The influence of fluoride on genetic mechanisms and subsequent diverse effects of fluoride on physiological processes illustrate the complex influence of fluoride on human health. Continued research in the related fields of epidemiology, anthropology, and environmental and biomedical sciences will help us to identify susceptibility to fluorosis and improve preventative measures.

CHAPTER 3

EVIDENCE OF FLUORIDE TOXICITY IN THE NORTH AMERICAN PALEOPOPULATION

3.1 Paleopathology

Paleopathology is foundational in the study of ancient health. Through paleopathological analyses we not only gain evolutionary understanding of disease, but we may also gain insights about the social construction of pathological conditions. Another important goal of paleopathology should be to provide information of relevance to present day populations (Nelson et al., 2015a). For example, dietary reconstructions and environmental studies reveal the impact of resource exploitation on health, often illuminating environmental influences of disease. Through understanding environmental and social influences of disease, preventative measures can be improved.

The study of disease in past populations requires the researcher to make several assumptions and to voice several caveats. We must make the uniformitarian assumption that disease processes and host responses in the past were similar to those today. This assumption is, of course, less justified as we move back in time to extinct species with no living representatives. Paleopathological studies are inherently limited by the fact that many diseases are simply invisible archaeologically, leaving no skeletal or desiccated tissue evidence. Further, researchers must appreciate the full spectrum of possible disease changes across sex and age groups, keeping in mind that many canonical resources (Aufderheide

and Rodriguez-Martin, 1998; Ortner, 2003) typically present exemplar, extreme cases to illustrate a given condition.

Paleopathology is by definition an interdisciplinary endeavor, at its center uniting the biomedical and archaeological sciences. Though time consuming and often expensive, the application of new technological methods, such as next generation DNA approaches, may strengthen differential diagnoses. In addition, theories and contextual information drawn from archival sources brings the social sciences and humanities, writ large, to the study of ancient health (Buikstra, in prep).

Evaluating health in ancient communities requires considering the synergistic relationship between diet and disease. Most studies of ancient disease-diet relationships have focused on menu reconstructions and inferred nutritional quality of the diet. The environment, however, may also affect health by introducing toxic levels of substances or deficiencies into the diet. For example, diets deficient in iodine commonly lead to goiter (Fuge, 2013). The impact of toxic levels of lead is also well known (Flora et al., 2012). Here, I develop a paleopathological analysis of a less well-known condition in paleopathology, fluorosis, wherein an environmental abundance may lead to toxic diets and ensuing poor health. In so doing, I illustrate by example the importance of rigorous evidence-based differential diagnoses in paleopathology.

3.2 Materials: the Ray Site

The Ray Site (11Br-104) is a Middle Woodland (50 BC to AD 450) burial site located on a steep, narrow ridge near the confluence of the Illinois and La Moine Rivers (Figure 3)(Flotow, 1983). The site contained no superstructure typical of Middle Woodland mortuary sites, comprising an apparent accretional cemetery. At least 117 individuals had been interred, aligned northwest to southeast, along the ridge crest. Most individuals had been interred in a linear pattern, with four apparent clusters of three or more individuals (Nelson et al., 2015a; Nelson et al., 2015b). There was also a concentration of nine burials isolated from the others in the southeast area of the site (Flotow, 1983). The burials were excavated as part of a rescue excavation conducted by trained, avocational archaeologists Mary and Glen Hanning between 1975 and 1980 (Flotow, 1983).

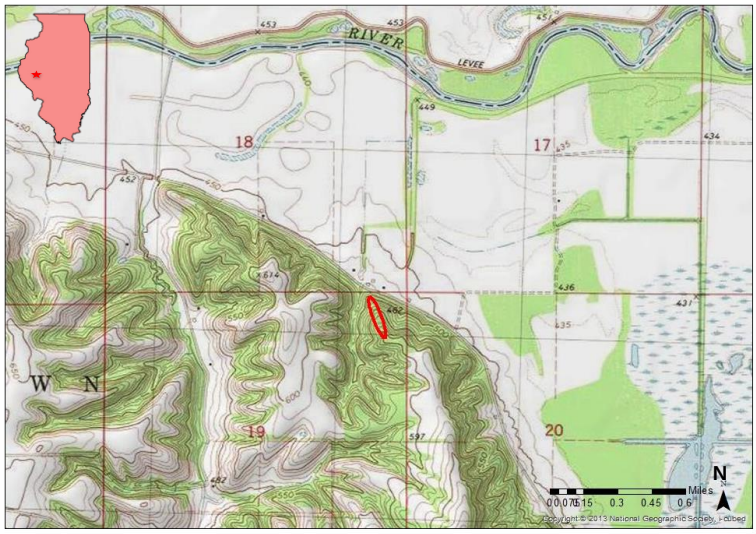

Figure 3. Map of Ray Site location within Illinois. The La Moine Rive to the north, Illinois River (not shown) to the east of location. Ray site indicated with red circle.

3.3 Methods: Data Collection

I estimated the age at death and biological sex of each individual using standard data collection protocols as outlined by Buikstra and Ubelaker (1994). These methods employ morphological observation techniques developed using specimens of known age at death and biological sex (Brooks and Suchey, 1990; Meindl ad Lovejoy, 1989; Phenice, 1969; Walker, 2005). Specifically, age at death assessments use expected morphological skeletal changes of development and degeneration to estimate, within a given time range, at what age the individual being analyzed died.

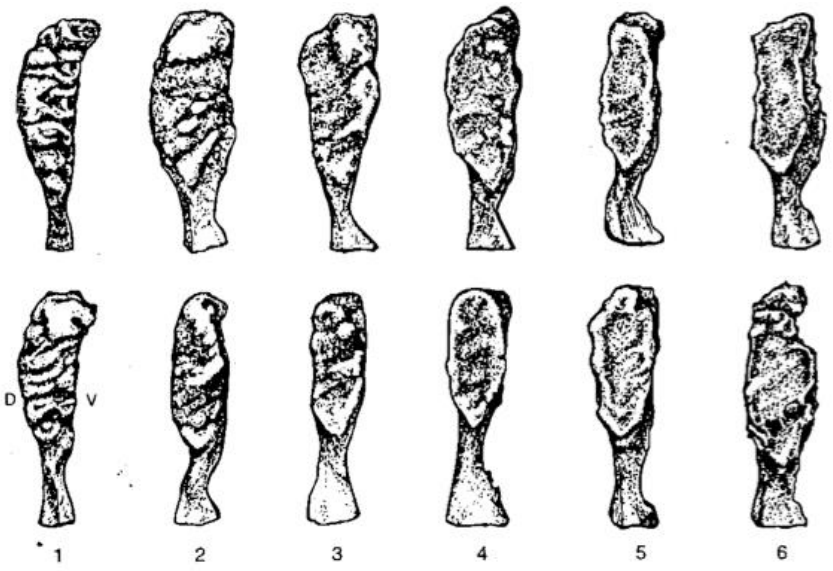

Figure 4. Suchey-Brooks illustrations for stages of morphological changes associated with age in female specimens. From Brooks and Suchey, 1990; as provided by Buikstra and Ubelaker, 1994.

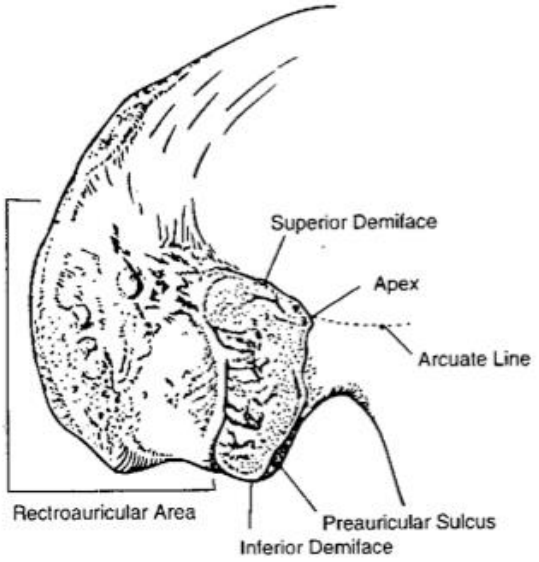

Figure 5. Posterior aspect of the Ilium presenting auricular and retro auricular areas. Morphological changes in these regions are associated with aging. Drawing by Zbigniew Jastrzebski after Meindl and Lovejoy, 1989; as provided by Buikstra and Ubelaker, 1994.

To estimate age at death, I focused on developmental stages of the dentition and epiphyseal fusion for adolescents, and degenerative changes of the symphyseal face of the pubis (Figure 4) and auricular surface of the ilium (Figure 5) for adults (Brooks and Suchey, 1990; Meindl and Lovejoy, 1989; Ascadi and Nemeskeri, 1970; Walker, 2005). I also observed rates of cranial suture closure for adults, particularly the lateral, anterior aspect. It should be noted that this method is not as reliable as methods involving the os coxa (Brooks, 1955; Herschkovitz et al., 1997) and, therefore, was limited in its consideration. The accuracy of these estimations for this population is unknown given that timing of morphological changes varies somewhat between populations (Todd, 1921; Katz and Suchey, 1989).

Sexual dimorphism is also subject to variation between populations and individuals; estimations of biological sex of individuals are therefore performed using a 1-3 (Phenice method) or 1-5 (skull and greater sciatic notch assessments) scale in which one (1) is considered female, two (2) probable female, three (3) ambiguous, four (4) probable male, and five (5) male. These assessments are

performed through morphological observations of features of the os coxa (sub-pubic aspect, greater sciatic notch) (Walker, 2005) and skull, both cranium and mandible (glabella, supraorbital margins, nuchal region, mastoid process, and mental process) (Ascadi and Nemereski, 1970). The Phenice method (1969), which involves morphological observations of the subpubic region, is considerably more reliable, with 95% observer accuracy; when considering the suite of sexually dimorphic features, I therefore had more confidence in results from the subpubic region assessments.

I observed pathology macroscopically using a 10x, hand-held loupe. In this assessment I described lesion appearance, location, condition at time of death (active or healing), severity, and distribution. Using the observed changes, I developed a broad differential diagnosis by generating comparative tables incorporating epidemiological information on predilection of conditions to biological sex, populations, and clinical information of etiology and biological and/or environmental influence.

3.4 Results

Age and Sex

The inventory of the Ray Site burials records at least 117 individuals. The age-at-death profile is estimated to include 30 adult males, 29 adult females, 37 juveniles, and 21 unassigned individuals. The large number of skeletal remains of indeterminate sex and age is a result of extended Middle Woodland burial programs that produce incomplete and highly fragmented skeletal remains (Nelson et al.,

2015a). Also, because many of the burials at the Ray Site were shallow (near the surface) some skeletons fell victim to postmortem damage related to modern use of the land as cattle pasture and intentional burning for land management (Flotow, 1983).

Pathology

Eight adult individuals (7%) displayed a similar, unusual pattern of bony changes (Table 1). These related changes involved signs of excessive ossification including enthesophytes on the ossa coxae, femora, ribs, tibiae, vertebrae, and patellae, often accompanied by periosteal deposition, and enlargement of ribs due to ossification at the intercostal margin. Five of these individuals displayed well-healed fractures, primarily on the ribs. It should be noted, no other individuals in the Ray Site population display signs of fractures.

Table 1. Demographic profile of individuals displaying similar skeletal pathology.

	Biological Sex	**Age at Death**
Ray 2-69	Male	40-49 years
Ray 2-77	Male	40-50+ years
Ray 3-18	Female	40-50+ years
Ray 3-19	Male	40-49 years
Ray 3-36	Female	50+ years
Ray 3-38	Female	35-45 years
Ray 3-71	Male	35-50+ years
Ray 4-66	Male	25-35 years

This syndrome affected five males and three females. Males present a more extreme expression of the disease, with an increased presence of enthesophytes, osteophytes, and periosteal deposition. Enlarged ribs appeared only in males (Table

1 and Table 2). The pattern of bony changes varied slightly between individuals (Table 1 and Table 2), although generally the axial skeleton was more affected than the appendicular skeleton (Figure 6). The iliac crest and ishiopubic rami of the ossa coxae frequently displayed enthesophyte development surrounding the acetabulum. The vertebral bodies and zygapophyses presented osteophyte development. Ossified spinal ligaments, primarily the ligamenta flava and supraspinous ligaments, left bony spicules at the margin of the laminae and the most posterior aspect of the spinous processes. Within the appendicular skeleton, the lower limbs were most commonly affected, including osseous deposition on the femoral trochanters, as well as enthesophyte development along the linea aspera (Figure 7). Two males displayed a bilateral, expansive, irregular bony surface at the distal end of the femoral diaphysis. The tibiae and fibulae often displayed periosteal deposition, both healed and active, along with enthesophyte development at the tibial tuberosity (Figure 8), soleal line, and the interosseous crest.

Table 2. Pathological features present on the eight individuals the Ray Site. (M= male, F= female).

Individual	Sclerotic activity: Cranium	Enthesophyte formation: Long Bones	Periosteal Bone formation	Rib Enlargement	Sclerotic activity: Vertebra/Os Coxa	Vertebral Enlargement	Enamel Defects	Osteoarthritis	Fractures
Ray 2-69 (M) (40-49 yrs)	◆	◆	◆	◆	◆		◆	◆	◆
Ray 2-77 (M) (35-50 yrs)	◆	◆	◆	◆	◆	◆		◆	◆
Ray 3-18 (F) (40-50+)		◆	◆		◆	◆		◆	
Ray 3-19 (M) (40-49 yrs)	◆	◆	◆	◆	◆	◆	◆	◆	◆
Ray 3-36 (F) (50+ yrs)	◆	◆	◆		◆	◆	◆	◆	
Ray 3-38 (F) (35-45 yrs)	◆	◆	◆		◆	◆	◆		
Ray 3-71 (M) (35-50+ yrs)	◆	◆	◆	◆	◆	◆		◆	◆
Ray 4-66 (M) (20-35 yrs)		◆	◆	◆	◆	◆	◆	◆	◆

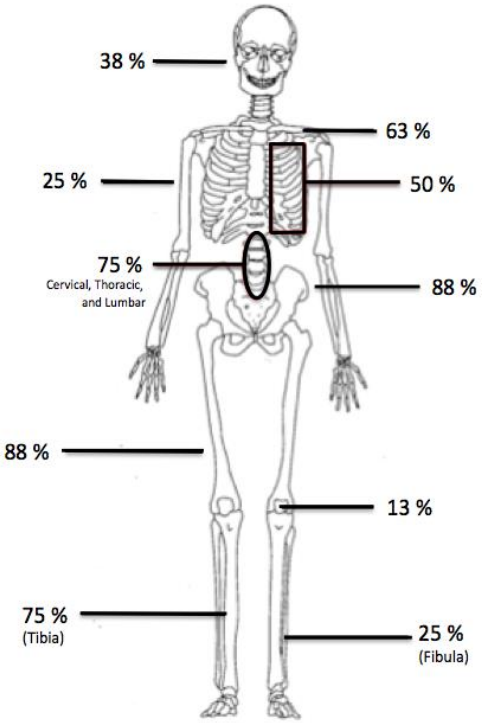

Figure 6. Frequency and distribution of pathological skeletal involvement for the eight individuals from the Ray Site (4-66, 3-71, 2-69, 2-77, 3-18, 3-19, and 3-38).

Figure 7. Ray 3-71, enthesophytes present on linea aspera of right femur.

Figure 8. Ray 2-69, right tibia displays dense sclerotic deposition with ossification of the patellar tendon at the site insertion

Signs of osteoarthritis, especially at the knee joint and vertebral zygapophyses, accompany sclerotic bone deposition; bony spicules and abnormal bone deposition surround articular surfaces displaying porosity and lipping.

Individuals presented various stages of joint degeneration; only one displayed eburnation (Ray 3-19). Five individuals present a total of nine fractures, two with multiple fractures (Ray 2-77 and Ray 3-19). All fractures of long bones are well healed, but three were not in anatomical alignment. There are no indications of disuse atrophy in any of the affected limbs.

Differential Diagnosis

The features that characterize these remains may be found in nine present-day conditions. These include ankylosing spondylitis, diffuse idiopathic skeletal hyperostosis (DISH), hematogenous osteomyelitis, hypoparathyroidism, myositis ossificans (*fibrodysplasia ossificans progressiva*), osteopetrosis, treponemal infection, Paget's disease of bone, and skeletal fluorosis (Tables 4 and 5).

Most clinical descriptions of these conditions derive from individuals in advanced stages of disease, and they therefore report extreme bony changes. In this example, the abnormal bony changes are generally not so extreme. This leads to the development of a broad differential diagnosis, as many of the suggested pathologies may produce similar signs in early stages of development. As illustrated in Table 2 and Figure 6, skeletal involvement frequently includes lower limbs, ossa coxae, and vertebrae. However, these examples do not present the bony ankylosis observed in clinical descriptions of such diseases as ankylosing spondylitis, DISH, myositis ossificans, and skeletal fluorosis. I include these conditions in this differential diagnosis, inferring that these individuals may have died prior to reaching an advanced stage, which could have included ankylosis.

Hematogenous osteomyelitis is a globally occurring condition caused by several species of bacteria (Aufderheide and Rodriguez-Martin, 1998; Conrad, 2010; Flensborg et al., 2013; Lew and Waldvogel, 2004; Ortner, 2003). This condition is associated with abnormal formation of multiple parts of the bone including the medullary cavity, periosteum, and cortical bone, as well as lytic areas of bone (Flensborg et al., 2013; Ortner, 2003). In juveniles, the appendicular skeleton, specifically the femur, is the most frequent site of infection, specifically the metaphyseal region (Conrad, 2010). Although this condition is rare in adults, involvement includes vertebrae, sternoclavicular joints, and sacroiliac joints along with long bone involvement of the metaphyseal and diaphyseal regions (Aufderheide and Rodriguez-Martin, 1998; Flensborg et al., 2013; Lew and Waldvogel, 2004). Vertebral involvement is most significant in the lumbar vertebrae, but inclusion of vertebral segments decreases in likelihood with progression to cervical vertebrae (Kumar, 2013). The absence of involucra, distribution of periosteal deposition, and the anatomical distribution of abnormal bone formation of the individuals from the Ray Site is inconsistent with typical presentation of hematogenous osteomyelitis and thus removes it as a possible diagnosis.

Hypoparathyroidism is a rare disorder resulting in decreased osteoclastic activity and subsequent increased calcium concentration within the bony structure, causing increased bone density (Bilezikian et al., 2011; Resnick and Niwayama, 1988). Cases of individuals with hypoparathyroidism report calcification of

ligaments, particularly those of the spine (Chaykin et al., 1969). Scarce discussion of this condition provides limited data on fracture rates; Rubin et al. (2008) report no fractures due to fragility, but fractures only of the bones of the hands and feet in 24% of their subjects. Dental defects include weak enamel and thin, misshapen roots (Chaykin et al., 1969). Currently, one person per million people is affected by hypoparathyroidism. This condition occurs in adults and adolescents alike; there is no predilection of biological sex discussed in literature (Bilezikian et al., 2011). The rare nature of the disease, along with the absence of dental defects such as abnormal, frail roots suggests hypoparathyroidism is an unlikely diagnosis for the 8 individuals from the Ray Site.

Myostitis ossificans progressiva, also known as fibrodisplasia ossificans progressiva, is characterized by extensive ossification of tendons, ligaments, and muscles (Thakker et al., 2013). Early stages present bony spicules and enthesophyte formation. Advanced stages display soft tissue involvement including muscles, joint capsules, and skin. Those with the condition are born appearing normal, apart from congenital malformation of the great toes, described as shorter and smaller (Aufderheide and Rodriguez-Martin, 1998; Pignolo et al., 2011; Thakker et al., 2013). The disease progresses in a specific anatomical pattern: first forming in the posterior region of the axial skeleton and secondly in the anterior region and appendages (Pignolo et al., 2011). Identification of the disease involves soft tissue ossification and hallux malformation (Aufderheide and Rodriguez-Martin, 1998; Pignolo et al., 2011). The eight individuals from the Ray Site do not display the characteristic, symmetrical, congenital malformations of the great toes, nor is the

characteristic pattern from posterior region of axial skeleton to anterior region present. Consequently, myositis ossificans is not an appropriate diagnosis.

Osteopetrosis, also known as Albers-Schönberg disease, now includes a group of conditions varying in symptom severity and genetic causative factors (Aggarwal, 2013; Sobacchi et al., 2013). The two primary groups are divided into autosomal recessive (ARO) and autosomal dominant (ADO) (Sobacchi et al., 2013). ARO is considered to be "malignant" osteopetrosis and is characterized by increased bone density, increased incidence of fractures, and impaired longitudinal growth of bones (Stark and Savariryan, 2009). Affected individuals typically present short stature, an abnormally small thorax, frontal bossing, and macrocephaly (Sobacchi et al., 2013; Stark and Savariryan, 2009). ADO, classically considered Albers-Schönberg disease, results in osteosclerosis and increased fracture incidence, but is considered to be a less severe form. Characteristic disease presentation includes scoliosis, osteoarthritis of the hip, osteomyelitis of the jaw, and increased incidence of abscesses and dental caries (Bollerslev et al., 2013; Stark and Savariryan, 2009). The eight affected Ray Site individuals do not display macrocephaly, smaller thoraces, or shorter statures compared to the site population, nor is there an increased incidence of abscesses or dental caries among them. Osteopetrosis is therefore rejected as an appropriate diagnosis.

Non-venereal treponemal infection has a substantial presence in the North American archaeological record and has been recorded in sites from Illinois (Cook and Powell, 2005; Mosher et al., 2013; Powell and Cook, 2005). Treponemal infections commonly affect the cranial vault and appendicular skeleton, specifically

the forearm and leg (Ortner, 2003; 2008). Skeletal lesions are more common in yaws as compared to syphilis or bejel; pinta does not affect skeletal tissue (Ortner, 2003). The woven periosteal deposition and well-integrated periosteal deposition displayed on the individuals from the Ray Site may be suggestive of treponemal infection. The affected individuals also display elevated bony surfaces and are heavy in comparison to other individuals from the site. These features are consistent with non-diagnostic periosteal lesions of treponemal infection (Ortner, 2003). The diagnostic features of lytic foci, *carries sicca,* and gummatuos lesions are absent (Cook and Powell, 2005; Hackett, 1957; Ortner, 2003). The pathology present at the Ray Site displays prominent, osseous development on sites of ligamentous and tendinous attachments as well as sclerotic activity of the cranium. The periosteal deposition is sclerotic and dense, while superficial cavitation and lytic lesions are absent. In light of these pathological features, I conclude treponemal infection is unlikely.

Paget's disease of bone is of unknown etiology, though many have suggested a viral origin; it is clear genetic factors play a role in both rare forms and classic Paget's disease of bone (Aufderheide and Rodriguez-Martin, 1998; Mays, 2008; Ralston, 2013). Paget's is considered a "European disease" occurring mostly in Caucasians of Europe, North America, Australia and New Zealand, with few accounts in Africa and Asia (Brickley and Ives, 2011; Ralston, 2013; Roberts and Manchester, 2007). The presence of Paget's disease in the archaeological record is limited and significantly localized to Europe (Ortner, 2003).

Paget's disease is characterized by abnormal bone morphology and osteosclerotic activity. The characteristic deformed appearance is due to the dense, sclerotic, irregular deposition of bone on the cranium and post-crania (Ortner, 2003; Ralston, 2013). The compromised structure results in an increased incidence of vertebral fractures and "bowing" of the lower limbs, particularly the femora (Brickley and Ives, 2010). Skeletal elements most often affected by Paget's are the pelvis, femur, lumbar spine, skull, and tibia (Ralston, 2013). There is a progressive patterning from the sacrum to the cervical spine and expansion of the diploë resulting in a physically deformed appearance of the skull (Ortner, 2003). The disease is reported to affect males more than females and commonly occurs in individuals over 55 (Ortner, 2003; Ralston, 2013).

The affected individuals from the Ray Site display sclerotic deposition of bone throughout the skeleton. However, the diploë do not appear to be actively expanding at time of death as expected in Paget's disease. There is also no progressive engagement extending cranially from the sacrum to the cervical spine and no facial deformity characteristic of Paget's disease of bone. This suggests that Paget's disease of bone is inappropriate as a diagnosis.

Skeletal fluorosis is a bone forming, pathological condition caused by ingestion of high levels of fluoride (Littleton, 1999; Petrone et al., 2011). Chronic fluoride exposure and consumption causes changes in the skeleton and the dentition (Littleton, 1999). The condition is believed to affect men more commonly than women, yet this may vary between populations (Littleton, 1999; Ortner, 2003). Skeletal fluorosis more frequently develops in middle to older adults but it may

occur as early as adolescence (Jolly et al., 1969; Littleton, 1999). Characteristic signs of skeletal fluorosis include the presence of enthesophytes and osteophytes along with dense periosteal deposition, enlargement of ribs, increased incidence of fractures, and osteosclerotic development on vertebral bodies (Brickley and Ives, 2010; Greenfield, 1990; Littleton 1999). Early signs of skeletal involvement include coarsening of trabeculae along with irregular bony outgrowths at locations of ligament and tendon attachment, as well as joint surfaces (Izuora et al., 2013; Littleton, 1999). More advanced stages display excessive production of new bone, resulting in ankylosis of joints and subsequent immobility (Izuora et al., 2013; Littleton, 1999; Ortner, 2003; Petrone et al., 2011). Cranial changes include increased density of diploë along with general osteosclerotic activity, primarily at the base of the cranium (Brickley and Ives, 2010; Littleton, 1999). Dental indicators of fluorosis include hypoplastic defects, enamel staining, and pitting (Littleton, 1999).

Related skeletal changes displayed by the 8 affected Ray Site individuals are consistent with skeletal fluorosis. The presence of signs such as dental pitting and mottling, dense periosteal deposition, calcification of ligamentous and tendinous attachments occurring predominantly in older male individuals, along with an increased incidence of fractures supports a diagnosis of skeletal fluorosis. There are no individuals who display fused joints or complete ankylosis of the vertebrae, which would be typical of advanced stages. However, the presence of these bony changes consistent with skeletal fluorosis in conjunction with the natural environment of the Illinois River Valley support the hypothesis of fluoride toxicity.

Table 3. Differential diagnoses of pathological signs present on eight individuals from the Ray Site.

Pathological Feature:	**Ankylosing Spondylitis**	**DISH**	**Hematogenous Osteomyelitis**	**Hypopara-thyroid**	**Myositis Ossificans**	**Osteopetrosis**	**Paget's Disease of Bone**	**Skeletal Fluorosis**	**Treponemal Infection**
Osteosclerosis of cranium	-	◆	-	◆	◆	◆	◆	◆	-
Enthesophyte formation	◆	◆	-	-	◆	-	-	◆	-
Periosteal deposition	◆		◆	◆	-	~	-	◆	◆
Increased incidence of fractures	-	◆	-	~	~	◆	◆	◆	-
Rib: Enlargement Expanded/irregular inferior margins	 - -	 - -	 - -	 ◆ -	 ◆ ◆	 ◆ ◆	 ◆ -	 ◆ ◆	 - -
Vertebra: Enlargement Osteophyte/Entheso phyte formation	◆	◆	◆	 ◆ ◆	◆	◆	 ◆ ◆	 ◆ ◆	 - ◆
Dental enamel defects	-	-	-	◆	-	◆	-	◆	-
Associated osteoarthritis	◆	◆	~	~	-	◆	◆	◆	◆
Characteristics:									
Affecting primarily older individuals	-	◆	-	-	-	-	◆	◆	-
Affects men more than women	◆	◆	◆	-	-	-	◆	◆	-
Bilateral/Systemic	◆	◆	-	◆	-	◆	◆	◆	◆
Displayed in mostly in lower limbs	-	-	◆	-	-	◆	-	◆	-
Abnormally thick/heavy bones	-	-	◆	◆	◆	◆	◆	◆	◆

Key: ◆ = present, - = absent, ~ = data unclear.

Table 4. Differential diagnosis with skeletal description and epidemiology

Pathology	Skeletal Description	Epidemiology
Ankylosing Spondylitis[1]	Primarily affects sacroiliac joint and vertebrae. Peripheral enthesophyte and osteophyte formation observed and in advanced stages ossification/ankylosis of elements occurs. Ankylosis proceeds from sacroiliac joint superiorly to lumbar, thoracic, and the cervical region in severe cases. Though extra-vertebral involvement is not as common, enthesophyte development has been noted at ankles, hips, knees, shoulders, and sternoclavicular joints. Some associated osteopenia is common.	A highly heritable disease affecting .55% of European population and .23% of Chinese but is rare in African and Japanese populations. Two to three times more prevalent in males than females. Symptoms usually begin in second or third decade of life. Pathological changes increase with age.
DISH (Diffuse Idiopathic Skeletal Hyperostosis)[2]	Spine is primary site of involvement with thoracic vertebrae most commonly affected. Bony changes include large syndesmophytes forming a "dripping candle wax" appearance. In thoracic region changes are most often observed on the right side of the anterior aspect of thoracic vertebral bodies. Lumbar spine shows similar involvement displayed equally on both sides of anterior aspect. Cervical involvement is rare, as is posterior vertebral involvement. Increased incidence of fractures.	Unknown etiology. Said to occur in 28% of individuals 45-85 years old. Occurs in males more than females. More common in individuals of European descent; also found in Asian (Korean) individuals.
Fluorosis[3]	Excessive osteosclerotic deposition. Periosteal deposition, enthesophyte formation, and ultimately fusion of joints. Sclerotic activity most significant on lower limbs, os coxae, vertebrae, ribs, and crania. Dental defects include pitting and mottling. Increased incidence of fractures.	Affects men more than women, commonly older adults. Cases reported globally: Middle East, Africa, Europe, North America, and Asia. Disease conditional to ingestion of fluoride, diet, and genetic factors
Hematogenous Osteomyelitis[4]	May involve multiple parts of bone including medullary cavity, periosteum, and cortical bone. Long bones most commonly affected. Vertebral involvement in adults. Abnormal periosteal bone formation. Pathology involves abscesses, involucra, and sequestra. Abnormal bone	One in 5,000 children affected; one in 450,000 adults affected. Occurs globally and is the most common type of osteomyelitis. Skeletal involvement is more marked in children than adults but it can occur in both children and adults. Males are more commonly affected than

	formation near joints and surrounding joint capsules.	females.
Hypoparathyroidism[5]	Osseous build up does not display a distinct diagnostic pattern. Excessive endosteal and periosteal deposition. Lesions include calcification of ligaments, particularly those of the spine. Cortical and trabecular bone increases in density. Dental defects include weak enamel and misshapen roots.	One person per million is affected. Most prevalent in Finns, Sardinians, and Iranian Jews. Occurs in adults and children alike. No predilection for biological sex.
Myositis ossificans progressiva[6]	Characterized by excessive ossification of tendons, ligaments, and muscles. Early stages display bony spicules and enthesophytes while advanced stages present significant soft tissue involvement (muscles, joint capsules, and skin). May lead to fusion of joints. Individuals display congenital malformation of the great toes appearing abnormally small. Progresses posterior to anterior and axial to appendicular.	One in two million are affected. Considered a childhood syndrome, may continue into adulthood often in response to trauma or stress. No geographic or ethnic predispositions observed though the condition is genetically hereditary. Fatal near the second decade of life.
Osteopetrosis (Albers-Schönberg disease)[7]	Osteosclerotic activity affecting skull, spine, pelvis, and appendicular bones. Defects are most significant on metaphyses of long bones causing an "Erlynmeyer flask" appearance. Increased incidence of fractures along with increased density and "hardness" of bones. Increased bone mass leads to altered craniofacial morphology involving increased frontal bossing, macrocephally, loss of mandibular angle, and prognathism. Dentally this disease results in tooth eruption defects and dental caries. Longitudinal growth may be impaired resulting in short stature. The skeletal defects often lead to osteomyelitis and osteoarthritis.	Several variants of this condition exist, all displaying similar conditions but originating from failure of osteoclastic development or mutations on at least one of 10 genes. Genetically recognized as two types: autosomal recessive (AR) and autosomal dominant (AD). Incidence varies between types with AR occurring in 1 of every 250,000 and AD 5 of every 100,000. Onset usually takes place in childhood though it may be perinatal or late adolescence. AR is usually fatal in infancy but AD has a normal life expectancy. Affects both men and women equally.
Paget's disease of bone[8]	Compromised mineralization of bone leading to dense, sclerotic, irregular deposition of bone. Affects the cranium and post-crania. Skeletal elements most affected are the cranium, sacrum,	Known to occur primarily in Europe, in those of European descent in North America, Australia, and New Zealand with few accounts in Africa and Asia. More common in males than females.

	spine and femora. Affected crania often exhibit expansive, dense diploë. Characteristic coarsening of trabeculae and mosaic pattern assist in histological identification.	Prevalence increases with age groups.
Treponematonematosis[9]	Resorptive, lytic, "gummatous" lesions followed by bone regeneration. Periosteal deposition may be localized and may present some bony exostoses at sites of major overlying muscle. Cortical thickening and expansion of the endosteal surface may occur. Affects the cranial vault and appendicular skeleton. Characteristic lesions include *caries sicca* of the cranial vault and *saber tibia*.	Globally occurring. 4 diseases caused by two species of *Treponema:* Pinta located in Central America, yaws is found in tropical environments near the equator, bejel is found in northern and southern hemispheres, and venereal syphilis is currently global.

[1]·Chaykin et al. (1969), Lin et al. (2012), Meirelles et al. (1999), Olivieri et al. (2009), Thakker et al. (2013) .
[2]. Belanger et al. (2001), Mader et al. (2009), Olivieri et al. (2009).
[3]. Barbier et al. (2010), Brickley and Ives (2010), Littleton (1999), Petrone et al. (2011), Petrone et al. (2013), Ortner (2003), Jolly et al. (1969), Reddy (2009), Teotia et al. (1971), Teotia et al. (1986).
[4]. Aufderheide and Rodriguez-Martin (1998), Conrad (2010), Flensborg (2013), Jaffe (1972), Kumar (2013), Lew and Waldvogel (2004), Ortner (2003).
[5]. Anderson et al. (1992), Bilezikian et al. (2011), Chaykin et al. (1969), Resnick and Niwayama (1988).
[6]. Auderheide and Rodriguez-Martin (1998), DiMiao and Francis (2001), Pignolo et al. (2011), Thakker et al. (2013).
[7]. Aggarwal (2013), Bollerslev et al. (2013), Henriksen et al. (2010), Reddy (2011), Sobacchi et al. (2013).
[8]. Aufderheide and Rodriguez-Martin (1998), Brickley and Ives (2010), Martin (1998), Mays (2008), Ortner (2003), Ralston (2013), Seitz et al. (2009), Tan and Ralston (2014).
[9]. Cook and Powell (2005), Fenton (2008), Hackett (1975), Kent and Romanelli (2008), Mosher et al. (2013), Ortner (2003

Environmental Context

The proposed diagnosis of skeletal fluorosis is further supported by the environmental context in which these individuals lived. The Ray Site's location (Figure 9) near the La Moine and Illinois rivers is reported to have fluoride levels of 2.2 – 3.0 mg/L by a national study (US Dept of Health, 1969). Although this is considered a toxic level of fluoride, it is a relatively moderate level in comparison with the eastern area of Illinois where levels peak at 5.5 mg/L. The water tested for this modern analysis had not yet been subjected to fluoridation or filtration. Surface analysis of Illinois soils in the area yielded fluoride content ranging from 275 mg/kg to 303 mg/kg (Omueti et al., 1977). While these modern values of surface soil fluoride content may be influenced by anthropogenic factors, such as farming practices, they still reflect expectations for the Middle Woodland period.

Figure 9. Location of the Ray Site in west-central Illinois, USA. Shown here indicated by the red circle, near the confluence of the La Moine River to the north and the Illinois River to the east.

It should be noted that high levels of fluoride do not necessarily result in fluorosis, since a variety of factors influence dietary uptake of fluoride.

Environmental factors such as weathering, temperature, and the natural geological composition of the rocks and soil all affect the presence of available fluoride (Barbier et al., 2010; Petrone et al., 2011). Fluoride-bearing minerals in the groundwater facilitate the movement of the ionic form of the fluoride from the surrounding rocks. Consequently, fluoride is found in soil, water, plants, and marine resources (Littleton, 1999; Omueti and Jones, 1977; Petrone et al., 2011). Inhalation of fluoride, from thermal gases or anthropogentic factors, is possible and has been linked to respiratory disease (Barbier et al., 2010). Also, water storage methods may increase the amount of fluoride to water molecules through evaporation or leaching of the mineral from the material used for storage (Littleon, 1999).

It is probable, due to the naturally high level of fluoride of the environment, that the individuals of the Ray Site experienced symptoms of fluoride toxicity. Communities exploiting the resources of the Illinois River Valley area would have been in continuous contact with fluoride through plant foods, fresh-water resources such as mollusks and fish, and most significantly, groundwater. This interaction with the environment would have encouraged fluoride ingestion.

Burial Pattern

Clusters of individuals on the eastern and western peripheries of the burial complex consist primarily of juvenile remains with only a few adults, both male and female (Nelson et al., 2015b). Individuals in these burial groups were discovered in both flexed and extended positions (Flotow, 1983). In contrast, the two central clusters were predominantly adults, with only a few infant remains and one child

(Nelson et al., 2015b). This may reflect the influence of the age at death as a determining factor for burial locus.

Biological sex, and by inference gender, does not appear to have affected choice of burial location since both males and females were found throughout the burial complex. Orientation of the body varied without sex-specific patterning. Five paired burials included a pair of males, a pair of females, and three others included a male and a female (Flotow, 1983).

Interestingly, the remains displaying osteosclerotic changes suggestive of fluorosis are primarily located in the southeast portion of the site (Figure 10). The artifacts associated with these burials did not distinguish them from others, nor were there any other unique attributes of the skeletons (Flotow, 1983).

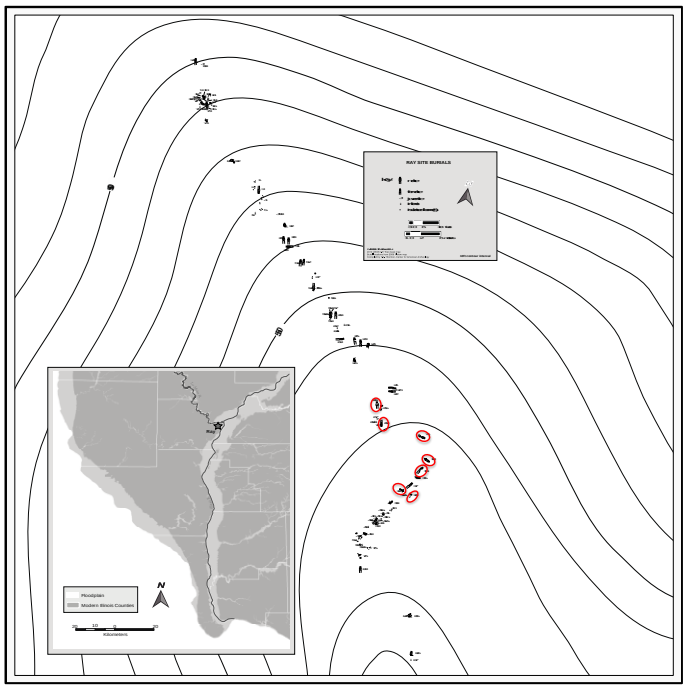

Figure 10. Ray Site burial map. The eight individuals showing similar bony changes are indicated in red. Ray 2-77 is not shown on the map but was located with a cluster of individuals interred south-east from the site. Adapted from a map created by Jason King and Taylor Thornton.

3.5 Discussion: Implications for Community Health

A variety of factors influence the incidence of fluorosis and may offer some insight concerning the lives of these eight individuals, including activity patterns, period of exposure, and genetic susceptibility (Littleton, 1999; Ozsvath, 2008; Petrone et al., 2011). The correlation between physical activity and expression of skeletal fluorosis could suggest those with relatively high physical activity within the population were those who presented an expression of bony changes (Brickley and Ives, 2010; Littleton, 1999). Diet is also known to affect fluoride absorption, and therefore perhaps variability in diet may explain the manifestation of the condition in these eight individuals. Also, a possible genetic factor influencing the uptake of fluoride and mineralization of bone may increase susceptibility to fluorotic bone development (Mousny et al., 2008).

Skeletal fluorosis is a progressive disease. The pathological changes of osseous material require chronic ingestion of excessive levels of fluoride over time. When skeletal fluorosis is observed, it can therefore be inferred that toxic effects have long been at work (Littleton, 1999). In environments with high fluoride levels, it is possible individuals experienced some sort of physiological and soft tissue effects without displaying pathological skeletal changes. With fluoride levels in ground water near the Ray Site reaching 3.0 mg/L, it is likely the inhabitants of the Ray Site experienced symptoms.

Fluoride is known to inhibit antibody formation and therefore suppress the immune system, leading to an increased incidence of infection (Barbier et al., 2010; Gibson, 1992; Taylor and Taylor, 1965). Symptoms experienced in the presence of

fluoride toxicity include nausea, constipation, headache, and body aches, along with possible cardiovascular, neurological, renal, and endocrine maladies (Reddy, 2009; Taylor and Taylor, 1965; Varol, 2012). The reproductive system, specifically male, is negatively affected by fluoride toxicity leading to inability to reproduce (Freni, 1994).

If the community that buried at the Ray site was experiencing fluoride toxicity, there should also have been an increase in infections, an increase in neurological deficits, and a decrease in birth rate (Petrone et al., 2013; Littleton, 1999; Goal, 2006). Fatality due to toxic fluoride ingestion has been reported, though this is not common (Waldbott, 1976).

CHAPTER 4

IMPLICATIONS FOR MODERN POPULATIONS AND PALEOPAHOLOGICAL ASSESSMENTS

For affected individuals, fluoride toxicity is an increasingly debilitating disease. Although not always evident on skeletal elements, toxic ingestion of fluoride disrupts necessary physiological processes, negatively impacting an individual's health and well-being. Currently, health care access in much of the world allows for early detection, resulting in a decrease of skeletal involvement in clinical presentations of fluoride toxicity.

4.1 Concerns for Modern Population Health

Science is still developing its understanding of the influence of fluoride on human health; subsequently, population health studies appear to poorly illustrate the prevalence of fluorosis in North America. This is reflected in the maps provided by popular population health information resources such as the World Health Organization and the Center for Disease Control (Figure 1).

So great is the concern of fluoride toxicity that many researchers have begun rigorous examination of global fluoride levels for the development of preventative measures (Amini et al., 2008; Ahmari et al., 2013). For example, Amini and colleagues recently developed statistical probability modeling of fluoride concentrations and mapping of fluoride toxicity (2008). They did this using an ArcGIS global database of over 60,000 measures of fluoride concentrations in over

25 countries, including the US. They found the United States is among the countries considered "greatly affected" by dangerous fluoride concentrations (Amini et al., 2008).

Through review of clinical and epidemiological literature, I demonstrate that although popular national and international health resources largely overlook this condition in the US, there is in fact a significant prevalence of fluorosis in North America. The lack of discussion concerning this issue may be alarming when considering the many symptoms and signs associated with this condition.

4.2 Considerations for Paleopopulation Health Studies

Skeletal fluorosis has not thus far been identified in North American archaeological samples, despite favorable environmental conditions. The increased prevalence of fluorosis may suggest this is a recently developing issue. However, the Ray Site example suggests fluorosis has previously been overlooked archaeologically.

Skeletal fluorosis has only recently been included in differential diagnoses for paleopopulations (Littleton, 1999; Ortner, 2003). Rather than evidence of absence, I argue that this condition may have been more frequent than previously recognized, particularly in groups wherein only minimal to moderate expression is present. Although the individuals from the Ray Site do not display severe signs of skeletal fluorosis, the pathological features consistent with skeletal fluorosis and the environmental conditions conducive to such a disorder both support the diagnosis of skeletal fluorosis for the eight individuals at the Ray Site.

A challenge of past population health studies is the limited number of conditions which leave skeletal signs (Zuckerman et al., 2012). It is important to recall that skeletal changes occur after chronic toxicity, implying the individuals who did not display these skeletal changes may have also been experiencing symptoms associated with fluorosis.

This study emphasizes the importance of consideration of environmental influences of health while also highlighting the clinical considerations of conditions. In the study of paleopathology, pathological processes must be considered at all stages of development and progression in relationship to the environment. Through this practice, biocultural changes through space and time may be realized. Acquiring non-skeletal, clinical information of a condition represented by skeletal remains is challenging. Although extreme skeletal changes are often indicative of certain advanced conditions, focusing exclusively on such manifestations may narrow the scope and remove possible alternatives or co-morbidities. In order to identify all possibilities, consideration of influencing environmental and lifestyle factors, along with a suite of indicators of pathological processes, offers the best reference for any condition.

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