Stress and Disease in Stable Isotopes

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Introduction

The identification of disease and nutritional stress in ancient populations relies heavily on osteological analyses to classify skeletal markers such as dental hypoplasia, cribra orbitalia, and osteopenia[3][13]. However, not all pathological disorders or diseases leave physical tracers on the bone. Likewise, physical markers depend on the individual surviving long enough for the disease to spread to the bone, and have multiple factors in the development of disease [3][11] (see Figure 1). Because diet and pathology are intrinsically related, whether or not these pathological conditions affect stable isotope values is of interest in more recent years.

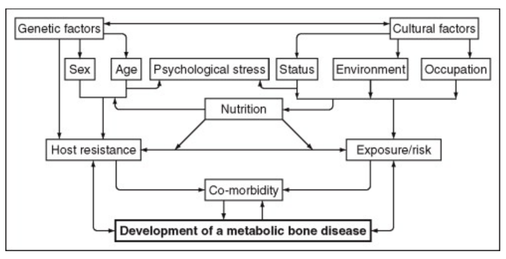

Figure 1. Key factors that may impact the development of metabolic bone diseases. Location of factors does not equate importance. Adapted from *The bioarchaeology of metabolic bone disease* by Brickley, M., & Ives, R. (2008) Academic Press, page 8. Copyright 2008 Elsevier Ltd.

Background

Stable isotope analysis uses the ratios of stable isotopes from elements that have been preserved in bone and teeth as chemical signatures, which can then be examined to infer the diet of individuals, the location of residence or migration, and the climatic condition when their tissues formed[12]. As many pathological conditions are influenced by dietary health or changes in the diets of individuals, stable isotope analysis presents a unique case where researchers can indirectly investigate ancient health and disease.

Early work[6] demonstrated that bones with different pathologies presented variable isotopic ratios between normal and pathological bones of the same individual (see Figure 2). However, these early studies were unable to determine any methods of pathological identification using stable isotope analysis due to the inherent limitations of the isotopic data produced[11].

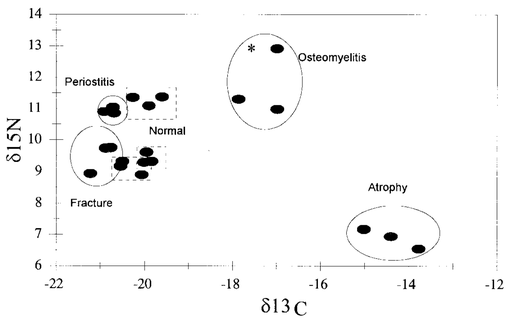

Figure 2. Graph of $\delta^{15}N$ and $\delta^{13}C$ for normal and pathological bone samples; normal bones are represented by dashed outline. Pathological bone is represented by ellipses. The asterisk (*) indicates the affected segment in comparison to unaffected samples. All segments were similarly affected in the case of atrophy. The affected segment for the fracture is the lowest point ($\delta^{15}N$ = 8.9‰). Adapted from "Stable isotope variation in pathological bone by Katzenberg, M. A., & Lovell, N. C. (1999). *International Journal of Osteoarchaeology, 9*(5), 321. Copyright © 1999 John Wiley & Sons, Ltd.

Famine

Skeletal manifestations of dietary stress such as dental hypoplasia (see Figure 3) and harris lines on long bones indicate growth interruption due to generalized stress. These are often attributed to insufficient dietary intake, though there are many other factors influencing growth patterns, such as psychosocial stress[3].

Isotopic manifestations of dietary stress such as famine can present specific patterns indicated by a sizable decrease in $\delta^{15}N$ values and a smaller decrease in $\delta^{13}C$ values as shown by Beaumont and Montegomery's (2016) study (see Figure 4). These results are supported through historical accounts, and can pinpoint the time at which maize was introduced as a relief food as indicated by steadily increasing δC^{13} values[1].

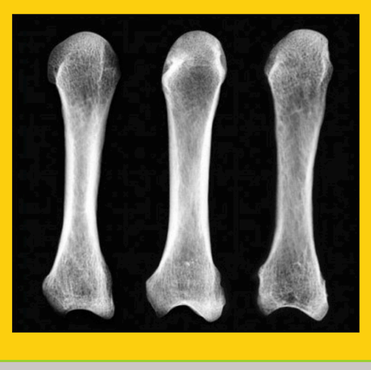

Figure 5. Thinning of cortical bone and loss of trabeculae in the adult second metacarpal with increased age. Left most metacarpal represents a young adult woman, middle metacarpal represents a middle age woman, right-most metacarpal represent a woman in advanced age. Adapted from *The bioarchaeology of metabolic bone disease* by Brickley, M., & Ives, R. (2008) Academic Press, page 160. Copyright 2008 Elsevier Ltd.

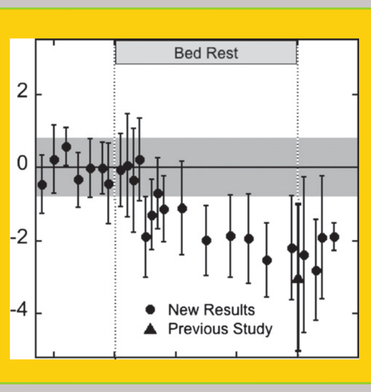

Figure 6. Variation in $\delta^{44/42}Ca$ vs. average baseline value (x10,000) before, during, and after an extended period of bed rest. Adapted from "Rapidly assessing changes in bone mineral balance using natural stable calcium isotopes" by Morgan, J., Skulan, J., Gordon, G., Romaniello, S., Smith, S., & Anbar, A. (2012). *Proceedings of the National Academy of Sciences, 109*(25), 9990. Copyright 2012 Morgan, Skulan, Gordon, Romaniello, Smith, & Anbar.

Figure 7. Cribra orbitalia along the inside of the ocular fossa. A skeletal manifestation of iron deficiency due to non-specific disease. Adapted from *Health and Disease in Byzantine Crete (7th – 12th Centuries AD), by* Bourbou, C. (2010).Farnham, Surrey: Routledge. Figure 3.6. Copyright 2010 Chryssi Bourbou.

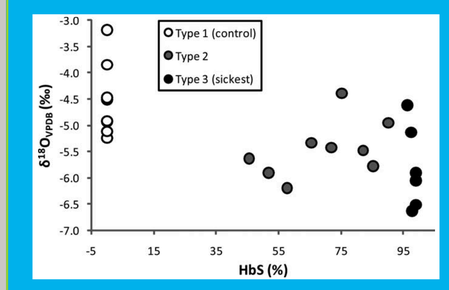

Figure 8. Scatterplot of mice with percent HbS hemoglobin measured in blood upon sacrifice plotted against $\delta^{18}O$ ratios. Adapted from "Brief communication: Oxygen isotopes as a biomarker for sickle-cell disease? Results from transgenic mice expressing human hemoglobin S genes" by Reitsema, L. J., & Crews, D. E. (2011). *American journal of physical anthropology, 145*(3), 497. Copyright 2011 WILEY-LISS, INC.

Figure 3. Teeth presenting evidence of severe enamel hypoplasia indicated by the white arrows on an individual from Bahrain. Black arrows indicate staining on teeth most likely related to fluorosis. Adapted from *The bioarchaeology of metabolic bone disease* by Brickley, M., & Ives, R. (2008) Academic Press, page 248. Copyright 2008 Elsevier Ltd.

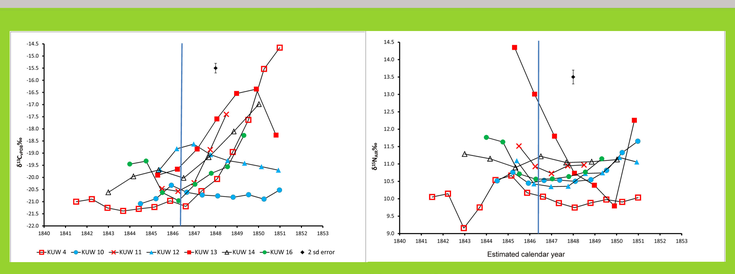

Figure 4. Incremental dentine isotope ratios of $\delta^{13}C$ and $\delta^{15}N$ of juveniles from Kilkenny Union Workhouse aligned with the estimated calendar year of life. The blue line denotes the introduction of maize. Adapted from Beaumont, J., & Montgomery, J. (2016). "The Great Irish Famine: identifying starvation in the tissues of victims using stable isotope analysis of bone and incremental dentine collagen". *PLoS One, 11*(8), 14. Copyright 2016 Beaumont and Montgomery.

Osteoporosis

Osteoporosis is the reduction in total bone volume accompanied by thinning and loss of trabeculae, and an increase in bone porosity that result in fractures[4]. Physical manifestations usually show thinning of cortical bone and loss of trabeculae through x-rays (see Figure 5). There are two types of osteoporosis; Type I osteoporosis is seen mostly in post-menopausal women, while Type II is correlated with age and is seen in both men and women[14].

Stable isotopes of calcium have the potential to produce intriguing results in the identification of osteoporosis in past populations. Jennifer Morgan et al.'s, (2012) study demonstrated that under conditions of chronic bone resorption, light isotopes that are usually fixed in bone mineral are preferentially excreted though the urine during extended periods of bed rest, causing a decrease in $\delta^{44/42}Ca$ values[8] (see Figure 6). However, since these values focus on stable calcium isotopes of urine, the application to skeletal material is yet unknown.

Anemia

Anemia results from a variety of disease processes, and is most often expressed as low hemoglobin concentration below the normal values associated with sex and age[13]. Vitamin deficiencies, or hereditary anemias can result in skeletal manifestations such as porotic hyperostosis or cribra orbitalia when severe anemic conditions are maintained over a long period[15] (see Figure 7).

Recent research has shown that $\delta^{18}O$ isotope fractionation of respired CO_2 varies between individuals with anemia and individuals without[5]. Subsequent research building on this foundation investigated the $\delta^{18}O$ values of mice with sickle-cell hemoglobin compared to mice with normal hemoglobin[10]. Sickle-cell mice exhibited significantly lower bone $\delta^{18}O$ values than the control population, indicating that sickle-cell anemia in human populations may be identifiable from stable isotope analysis (see Figure 8).

Discussion

While stable isotope analysis can provide exciting opportunities to approach palaeopathological questions through different scientific means, there are some inherent limitations to dietary applications to the identification of pathological conditions.

Problems in Disease Identification using Dietary Applications of Stable Isotopes[6][7][9].

- Issues with bone turnover rates
- Comparison-based analyses
- Utilized dietary information as indirect evidence
- Failing to consider other factors in disease process

The successful applications of stable isotope analysis in the field of pathology as previously shown have focused on targeted analyses and utilized selective sampling to identify specific physiological changes in the body due to the presence of pathological disease or stress.

Future researchers interested in identifying pathological conditions from stable isotope values should focus on:

- Targeted applications
- Metabolic diseases that affect bone
- Analysis of childhood diet
- Microsampling of tooth enamel
- Physiological processes of tissue formation

In addition to the above, future work should focus on understanding the multiple key factors that impact the development of metabolic disease (see Figure 1).

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