Osteopenia and Stable Isotope Ratios in Bone Collagen of Nubian Female Mummies

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ABSTRACT Stable carbon and nitrogen isotopes were analysed on bone collagen of 43 Sudanese Nubians from the X-Group period to test dietary hypotheses for the high frequency of osteopenia in this population. Stable carbon isotope ratios indicate that both normal and osteopenic individuals consumed the same mixed diet of C3 and C4 sources, which are assumed to have been constituted by the grain staples wheat/barley and sorghum/millet respectively. Females with osteopenia, however, have significantly elevated δ15N values. The enrichment effect is greatest in the third and fifth decades of life, and is consistently patterned with microstructural and frequency differences previously reported by other researchers. It is suggested that δ15N is reflecting differences in urea excretion and the renal processing and clearance of calcium and phosphorus. The study not only alerts us to the susceptibility of stable nitrogen isotopes to non-dietary (i.e. physiological) factors, but also identifies nitrogen isotope ratios as a possible new marker for osteopenia. Am J Phys Anthropol 103:185–199, 1997.

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Abnormal bone loss or maintenance is a major modern health concern, which, like many modern chronic and degenerative conditions, has frequently been documented in ancient skeletal samples (Perzigian, 1973; Ortner, 1976; Erikson, 1976; Stout and Tietelbaum, 1976; Richman et al., 1979; Pfeiffer and King, 1982; Armelagos et al., 1984; Ruff, 1991). The growth, development and maintenance of bone may be related to both genetic endowment (Bell et al., 1995) and culturally determined factors such as diet and exercise (Villa, 1984). As such, bone maintenance and loss are considered to be measures of population adaptability, a reflection of health status and living conditions.

Osteoporosis is a metabolic bone disease in which poorly mineralized bone and micro-architectural changes create a reduction in bone mass which is abnormal for age and either results in, or predisposes to, fracture from relatively mild trauma (Mundy, 1995; Stini, 1995). Because the term osteoporosis presupposes fracture, and many people may have abnormally low bone mass but fracture has not yet occurred, its definition is often controversial (Mundy, 1995). To clarify the state of effect on bone, the term osteopenia may be more appropriately used to describe abnormal reduction in bone mass due to poor mineralization but where there are no fractures (Mundy, 1995). Preservation restrictions of ancient skeletal material often hinder researchers from making a differential diagnosis between osteoporosis and osteopenia. Therefore, in this study, we

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will adopt the more parsimonious term, osteopenia.

The complex etiology of osteoporosis and osteopenia continues to be the subject of intensive clinical investigation in modern populations. Categories of competing hypotheses include nutritional and disease stress, endocrine conditions, and biomechanical conditions (Birnbaum, 1992; Marcus, 1994; Ferretti et al., 1995). The determination of cause is particularly difficult for ancient populations as different causes are not necessarily manifest differently in gross morphology. Consequently, osteopenia continues to be classed as a nonspecific disease in most skeletal populations. Diet has been implicated in many of the archeological studies, but most dietary analyses have been based on indirect measures of food consumption, e.g. floral and faunal analyses, or ethnohistorical documentation. Stable isotopic ratios of carbon and nitrogen in human tissues now allow us to reconstruct diet more directly and provide a means of testing some of the dietary hypotheses for osteopenia in ancient peoples. In this paper, human remains from ancient Sudanese Nubia are analysed isotopically to examine the relationship between diet and osteopenia. The sample presents an ideal test opportunity because the high frequency of osteopenia in ancient Nubia (where it was originally referred to as “osteoporosis”) is among the most extensively studied in all of prehistory (Dewey et al., 1969; Armelagos et al., 1972, 1982, 1984; Huss-Ashmore, 1978; Martin and Armelagos, 1979, 1985; Martin et al., 1984).

MATERIALS AND METHODS

Diagnosis of osteopenia

Osteopenia was diagnosed macroscopically and microscopically in a sample comprising femora sectioned transversely below the lesser trochanter from 74 individuals (40 females, 34 males) from an X-Group period (AB 350–550) cemetery (NAX) located in the Wadi Halfa area of Sudanese Nubia. Both types of cross sectional data may be interpreted as an indication of ontogenetic processes. Macroscopic analysis done by Martin and Armelagos (1979) was based on calculation of the percent cortical area, a commonly used technique (Sedlin et al., 1963; Garn, 1970; Armelagos et al., 1972; Carlson et al., 1976). Females of all age groups have a markedly lower cortical thickness and lose twice as much cortical bone than males from the third to sixth decades. During the fourth decade, however, females show a slight gain in bone, which does not appear in males. The usefulness of using cortical area ratios to identify osteopenia has since been questioned (Ruff and Hayes, 1984). However, a microscopic analysis of the same material modified after methods by Wu et al. (1970), Ortna (1975), and Stout and Teitelbaum (1976) reinforces the pattern of bone loss indicated by percent cortical area, and further illuminates the processes resulting in loss (Martin, 1983; Martin and Armelagos, 1985). The frequency of intact or complete osteons, resorption spaces, and forming osteons is calculated for periosteal, middle, and endosteal locations in eight different radii circumlocuting the transverse section to determine bone turnover rates. The frequency of double zonal osteons which are indicators of growth disruption was added to this diagnostic array to assess bone quality. Females have significantly higher turnover rates in all age groups, but the highest degree of variability occurs in the third decade. Resorption and formation of bone decline sharply in 20- to 30-year-old females (indicating difficulty with maintenance of bone mass), and then increase markedly over age 30 (Martin and Armelagos, 1985). Peak ages for osteon formation are the fourth decade and the over-50 group. The same age groups also exhibit significantly fewer double zonal osteons in females, and there is a correlation between double zonal osteons and percent cortical area. Double zonal osteons increase in frequency as percent cortical area increases. The increase in double zonal osteons is taken as an indication of improved metabolic ability to recover from growth arrest. Osteopenic individuals used in this study were identified as having a lower percent cortical area than normal for age, decreased double zonal osteons, increased resorption, and fewer intact osteons.
Nubian diet

Based on the assumption that dietary and disease stresses are the most important factors involved in collagen synthesis and bone mineral formation in ancient Nubia, previous researchers (Armelagos et al., 1982; Martin et al., 1984; Martin and Armelagos, 1985) have proposed a nutritional cause for Nubian osteopenia, particularly for subadults and young adult females. These subgroups are often in the paradoxical position of having greater dietary demands than the population at large but lower social status and, therefore, reduced access to food resources. The source of nutritional deficit has been attributed to the cereal grain diet (previously thought to have been millet), which could produce protein-calorie malnutrition, or possibly a low calcium/phosphorus ratio (Martin et al., 1984).

Isotopic analysis of human bone, skin, muscle, and hair tissues (White and Schwarz, 1994) has recently been used to characterize the plant staple for these Early Intensive Phase agricultural populations. In the absence of postmortem chemical alteration (diagenesis), ancient human tissues reflect the isotopic composition of food consumed during life. The isotope ratios of carbon and nitrogen are most commonly used to reconstruct past diets because of their known sources of variation. The most basic level of isotopic variation is created by plants during the process of photosynthesis. Plants selectively incorporate $^{13}$C (the heavier isotope of carbon) from atmospheric carbon in three distinct ways. The most common plant type selectively discriminates against $^{13}$C (in favour of $^{12}$C) during photosynthesis, and therefore has the lightest $\delta$ values (or ratio of two ratios) averaging around $-26\%$e (Smith and Epstein, 1971; Van der Merwe and Vogel, 1978, O’Leary, 1988). These are called C3 plants and include wheat, barley, rice, most vegetables, fruits, and nuts. Plants which are more efficient at incorporating CO$_2$ are called C4, and have the heaviest values, averaging about $-12.5\%$e (Smith and Epstein, 1971; O’Leary, 1988). C4 plants are mainly tropical grasses including sorghum, millet, and maize. The $\delta^{13}$C values of these two plant types do not overlap. CAM (Crassulacean acid metabolism) plants are characteristically succulents which exhibit a wide range of $\delta^{13}$C values ($-12$ to $-27\%$e), overlapping the values of C3 and C4 plants. Because succulents seldom form a major portion of human diets, they are usually not considered to be a confounding dietary source.

The $\delta^{13}$C values cited above have been calculated for modern plants, but must be adjusted for preindustrial samples. The carbon used for photosynthesis comes from atmospheric CO$_2$. The burning of C3-based fossil fuels since the industrial era has depleted $^{13}$C in atmospheric CO$_2$, resulting in approximately a 1.5% reduction in the value of atmospheric $\delta^{13}$C (Keeling, 1961; Marino and McElroy, 1991). Therefore, modern plants will systematically have correspondingly more negative $\delta^{13}$C values.

The isotopic “signatures” created by the three types of photosynthesis are reflected in the tissues of consumers. The original ratios are altered only by the loss of some $^{14}$C during a secondary systematic discrimination or “fractionation” at each level in the food chain. For bone, the diet to collagen spacing is generally considered to be about 5%e (Van der Merwe and Vogel, 1978). As C3 and C4 foods are consumed, their proportions become registered in animal tissues. Thus an isotopic signature in the tissue of omnivores, such as humans, can also reflect the consumption of animals.

To help determine the degree and type of animal consumption, nitrogen isotope ratios are analyzed and reported as $\delta$ values in per mil (‰) using the following formula:

$$\delta^{15}N = \frac{^{15}N/^{14}N_{\text{sample}}}{^{15}N/^{14}N_{\text{standard}}} \times 1,000.$$  

These are theoretically used as indicators of both protein source (i.e. terrestrial vs. aquatic plants and animals, legumes vs. other plants), and level in the food chain (Schoeninger and DeNiro, 1984; Schoeninger, 1985, Ambrose and DeNiro, 1986). The $\delta^{15}$N value increases as it passes from one trophic level to the next, making the tissues of consumers 3 to 4% more enriched than the diet (DeNiro and Epstein, 1981; Minagawa and Wada, 1984; Schoeninger and DeNiro,
Aquatic and marine sources tend to exhibit the greatest enrichment, legumes the least (Delwiche et al., 1979; Wada, 1980; Virginia and Delwiche, 1982; Shearer et al., 1983; Shearer and Kohl, 1986). Unlike δ¹³C which is derived from a fairly constant and universal atmospheric, values of δ¹⁵N in food sources can vary because of context-specific conditions such as variability in ¹⁵N in the growth medium (Granhall, 1981; Virginia and Delwiche, 1982; Heaton, 1987; Keegan and DeNiro, 1988), the way that ¹⁵N is incorporated into plants (Delwiche et al., 1979; Farnsworth et al., 1985), and the effects of fertilizer (Turner et al., 1983; Tiessen et al., 1984; Peterson and Fry, 1987). Climate, e.g., the degree of rainfall (Heaton et al., 1986), also has an effect on the δ¹⁵N of animal tissue. Thus it is wise to keep geographic (Schoeninger, 1985) and temporal controls (White and Schwarz, 1994) on interpretations of δ¹⁵N. To further confound interpretations of δ¹⁵N at the tissue level, nitrogen values can also reflect physiological factors. For example, Ambrose and DeNiro (1986) have suggested a relationship between water stress, kidney function, and δ¹⁵N values. All animals lose nitrogen through urea excreted in urine, but water-stressed animals appear to lose more nitrogen and have highly concentrated urine. If urea is depleted in ¹⁵N, animal tissue will be enriched in ¹⁵N. Because water is required for the metabolism of protein and the excretion of nitrogen, if a high protein diet cannot be maintained under water stress, the result can be a nitrogen imbalance. Thus, when interpreting δ¹⁵N values, it is important to have some a priori knowledge of the physiological characteristics of the study population. In the case of the Nubians, who live in one of the most arid environments on earth, it is possible that water stress could enrich ¹⁵N because of endocrine function. Homeostasis in calcium and phosphorus is also affected by kidney function. For those Nubians who are osteopenic, δ¹⁵N values might be distinctive for physiological rather than dietary reasons.

Previous isotopic analysis of bone collagen, skin, and muscle (White and Schwarz, 1994) of the X-Group Nubians at Wadi Halfa indicates that the dominant foods consumed were C3 (wheat, barley, fruits, nuts, and vegetables, and possibly animals who consumed these plants) as opposed to the C4 foods (sorghum and millet, and consuming animals) which were not only common throughout Nubia in ancient times, but are also common in this area today. Analysis of the hair, a unique tissue which lays down its carbon atoms in a linear sequence, has revealed the practice of seasonal scheduling of C3 and C4 staples, where millet and sorghum are summer crops, and wheat and barley are winter crops (White, 1993). Although there is a regular and significant input of C4 plants, the bulk of the diet has come from the more productive winter plant complex. The animal protein component of the diet appears to derive from browsing animals, such as desert sheep and goats. Significantly, neither fish nor legumes seem to have been of primary importance.

**Etiological possibilities**

Of the multiple causes for osteoporosis, we are limited in the number that can be tested directly from ancient human remains, particularly in the absence of bone fractures. However, we can identify some risk factors which would have been specific to the Nubian environment and population by examining the patterning in frequency and microstructure of osteopenia by age and sex in relation to isotopic data which provide information on diet (δ¹³C and δ¹⁵N values) and physiology (δ¹⁸O).

**Diet.** High carbohydrate and high fibre diets can lead to loss of calcium and thus predispose to osteopenia (Binnbau, 1992; Yanagawa et al., 1992). Although the diet characterized isotopically by White and Schwarz (1994) would have been high in carbohydrates, there is no difference between the carbohydrate content of wheat/barley species (n = 5, mean = 71.98 ± 2.85 g/100 g edible portion) and that of sorghum/millet species (n = 15, mean = 72.37 ± 2.57 g/100 g edible portion) (Wu Leung et al., 1968). There is, however, a difference in the fibre content. On average, wheat and barley species contain significantly more fibre (3.38 ± 1.49 g) than sorghum and millet species (1.85 ± 0.57 g). If fibre consumption
is a main etiological factor in Nubian osteopenia, then pathological individuals might exhibit lighter $\delta^{13}C$ values i.e. be consumers of more C3 plants.

The amount of calcium available for bone formation might also depend on the ratio of calcium to phosphorus in the diet. High phosphorus, low calcium intake may cause a secondary hyperparathyroidism which leads to a corresponding imbalance in plasma levels, ultimately resulting in bone resorption (Avioli, 1993; Calvo, 1993). Although there is no consensus on this relationship (Mundy, 1995), it should be considered here in light of a previous suggestion that phytate phosphorus formed from the husks of the cereal grains in the Nubian diet might have interfered with the absorption of calcium (Armelagos et al., 1984). All of the staple grains which could have been consumed in this population are low in calcium and high in phosphorus (Wu Leung et al., 1968), and it is possible that they could all have low ratios that create a generalized condition favouring osteopenia. However, the two main cereal groups do differ in their ratios of calcium and phosphorus. Wheat and barley range from 4.1 to 9.47 (mean 6.38 ± 1.95), but sorghum and millet ratios are markedly higher, ranging from 13.28 to 31.77 (mean 19.26 ± 6.0). If there is a critical ratio of Ca/P in the cereal staple that is responsible for osteopenia, we could hypothesize that pathological individuals will exhibit heavier $\delta^{13}C$ values, i.e. they will be consuming more C4 plants.

Reduced magnesium absorption is also implicated in osteopenia (Schwartz, 1990). Staple grain diets can be magnesium deficient because the phytates in grain also chelate magnesium making it unavailable to the body. Although low magnesium values have been found in the hair of neighbouring mummies from Kulubnarti (Sandford et al., 1983), magnesium quantities, like carbohydrates, could not be determined isotopically. Other dietary factors not testable using the isotopic method include caffeine, sodium, and alcohol consumption (Loré, 1989; Birnbaum, 1992; Stini, 1995).

Clinical and cross-cultural studies suggest that high protein diets create a risk for osteopenia (Olson et al., 1981; Licada et al., 1981; Yuen et al., 1984; Yano et al., 1985; Einhorn, 1990; Abelow et al., 1992; Hu et al., 1993) because calcium reabsorption in the kidney is depressed, and more calcium is excreted (Heaney et al., 1982; Kerstetter and Lindsay, 1990). Hyperparathyroidism resulting in bone loss might also be created from the low Ca/P ratio of animal protein (Calvo et al., 1990; Calvo, 1993), but the effect of parathyroid hormone on bone maintenance is debated (Dempster et al., 1989). Furthermore, high protein diets do not always produce osteopenia (Spencer et al., 1983; Tesar et al., 1992; Grynpas et al., 1993; Cooper et al., 1996), and the source of protein (i.e. meat vs. soy) is also a determining factor (Arjmandi et al., 1996). The northern Sudan, both past and present, does not support the kind of substantial animal production which would sustain the degree of high protein consumption needed to create osteopenia (Culwick, 1951; Jack, 1954; Darby et al., 1977) such as that found among Inuit populations (Mazess and Mather, 1974). Just as a diet too high in protein might predispose to osteopenia, so might protein calorie malnutrition (Garn, 1970). Sufficient protein intake for premenopausal women appears to be a determinant of peak bone mass regardless of age, weight, and degree of exercise, but does not seem to have the same effect on postmenopausal women (Cooper et al., 1996). Modern ethnographic data suggest that protein-calorie malnutrition is more likely to characterize Nubian diets (Corkhill, 1948; May and McLennan, 1970), and the archeological evidence does not alter this picture for the past, as there are few faunal remains (terrestrial or aquatic) to indicate substantial protein consumption from zoological sources. Minimal meat consumption might also be indicated by endemic levels of iron-deficiency anemia during the X-Group period (Armelagos, 1968; Martin et al., 1984), although this pathology could also have been consequence of parasitic infection (schistosomiasis).

**Physiology and reproductive history.**

The highest frequency of osteopenia among the Nubians is found in females who also show distinctions by age and clinical manifestation (Martin et al., 1984). The pattern-
ing of frequency and microstructure suggests that their osteopenia may have had more than one cause. Young (age 20–29) females have the fastest turnover rate, exhibit less recovery from stress, “lose bone throughout the entire cortex,” and mineralize bone more poorly at a slower rate. The high turnover rate is a common feature of juvenile osteopenia (Mundy, 1995), which has been associated with protein-calorie malnutrition (Garn et al., 1966; Garn, 1970; Mundy, 1995) and primary health problems (Mundy, 1995) in modern populations. The amount of bone mass attained during growth and the timing of peak bone mass are important determinants of risk for osteoporosis later in life (Sabatier et al., 1996). Among Nubian females in their third decade, multiparity, lactation, and diet have been strongly implicated as causes of osteopenia (Armelagos et al., 1984). Although pregnant women can be subject to an idiopathic form of transient osteopenia (Smith et al., 1985; Mundy, 1995) other studies suggest that bone is actually deposited during pregnancy (Garn, 1970; Galloway, 1988; Murphy et al., 1994) through adaptive hormonal action which improves absorption (Norman, 1980). Furthermore, absorption is also more efficient during lactation (Norman, 1980), but prolonged lactation may cause bone resorption (Garn, 1970; Wardlaw and Pike, 1986) depriving mothers of up to 300 mg of calcium and 55 kcal per day. Lopez et al. (1996) suggest that mothers who nurse for longer than 6 months experience increased rates of bone turnover and loss. The balance of intake, absorption, and infant demand would need to be appropriate in order to avoid bone loss. The high turnover rates and low frequency of double zonal osteons do seem to indicate problems of balance and maintenance in this sample. Evidence for physiological and dietary stress also exists in Nubian females of the same age group from the neighbouring site of Kulubnarti where trace element analysis of bone indicates abnormally low levels of iron (Sheridan, 1993). Multiparity and prolonged lactation are likely typical of reproductive behaviour in ancient contexts such as this. Certainly lactation among the Nubians was prolonged until at least 2 years of age. The age of weaning in this sample has been reconstructed previously in two ways. Rudney (1983) found a peak in dental enamel disturbances occurring around age 2, a pattern typical of weaning stress. The weaning period is also indicated by trophic level differences in nitrogen isotope ratios comparing infants with adult females (White and Schwarz, 1994). Infants up to and including the age group 2 to 4 have higher δ15N values than adult females, suggesting that nursing may have continued up to age 4.

Of the oldest women in the sample (age 50+), bone loss appears to be a result of lack of replacement in the face of increasing resorption, combined with decreasing remodelling. Bone loss is most evident in the endosteal surface, a pattern typical of Type I or postmenopausal osteoporosis (Riggs and Melton, 1983). This pattern is etiologically distinct from the younger females, and may be reflecting the more normal tendency to lose bone mass during the period of menopause. However, not all menopausal women experience osteopenia in either this sample or modern populations. The acceleration of bone loss in this age group might also result from a cumulative effect of long, repeated periods of lactation in the reproductive years, previous and ongoing chronic calcium and protein deficiency, and other dietary and lifestyle factors.

Although some males also exhibit osteopenia, they did not lose bone mass to the same degree or show the same patterning of loss and turnover. Males typically have more bone mass than females to begin with; they do not experience the abrupt loss caused by menopause, are more likely to experience osteoporosis as a result of diet and lifestyle (smoking, alcohol) and as a secondary condition (Orwoll and Klein, 1994; Mundy, 1995). Osteopenia also occurs in juveniles in this population (Huss-Ashmore, 1978; Armelagos et al., 1984) and has been attributed mainly to dietary stress (protein calorie malnutrition) during the growing years.

Sample description

The sample was selected from two sites (North Argin and 2413) in the Wadi Halfa area which date to the X-Group period (AD 350–550). It consists of 27 normal adults.
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(female n = 13, male n = 14) and 16 adults identified by Martin (1983) as osteopenic (female n = 8, male n = 8). Three of the osteopenic individuals also have cribra orbitalia, a manifestation of iron deficiency anemia, which was common in these and neighbouring populations (Carlson et al., 1974; Sandford et al., 1983; Sheridan, 1993). Although cribra orbitalia could indicate dietary iron deficiency, microscopic analysis of soft tissue has revealed the presence of parasitic infection (Schistosomiasis), which could also be a cause of iron deficiency. The bodies are extremely well preserved due to natural desiccation caused by burial in hot desert sands. Fine preservation is confirmed by the presence of soft tissues, and our ability to extract high yields of collagen from the bones (12.39 ± 3.8%). Osteopenia in the Wadi Halfa area also exists in populations belonging to the previous Merotic period (350 BC–AD 350) and the following Christian period (AD 500–1400), and does not seem to have varied in its frequency over time (Martin, 1983; Martin and Armelagos, 1985). However, the sample is temporally restricted here to the X-Group in order to control for the possible influence of significant temporal shifts in diet measured isotopically in earlier research (White and Schwarcz, 1994).

Analytical procedures

Gelatin containing collagen was extracted from powdered cortical bone samples using an adaptation of the procedure developed by Longin (1971). The adaptation is a more gentle procedure which maximizes collagen yield by using a more dilute solution of HCL (0.25 N), as recommended by Chisholm et al. (1983), and by refluxing the gelatin at a lower temperature (58°C), as recommended by Brown et al. (1988). In comparing the modified Longin procedure with other demineralization methods (Sealy and van der Merwe, 1986; Tuross et al., 1988), Schwarcz and Schoeninger (1991) recommend the Longin method be used only for well preserved bone because it does not always produce an amino acid profile similar to that of collagen. Selective loss of amino acids can influence the isotopic composition of the sample (Hare et al., 1991). The fine degree of preservation in this sample is indicated by the presence of soft tissues as well as previous analyses (White, 1991; White and Schwarcz, 1994) which demonstrate that the gelatin produced with this technique provides amino acid profiles consistent with those in modern bone. The extracted gelatin was then oxidized with CuO in vacuum-sealed pyrex tubes at 550°C. Carbon and nitrogen gases created from this process were analysed on a SIRA mass spectrometer at McMaster University, against the NBS graphite standard for δ13C, and an interlaboratory glycine standard for δ15N. The analytical precision was ±0.1‰ for δ13C and ±0.2‰ for δ15N. Precision of analysis on six duplicate samples was 0.01 for δ13C and 0.07 for δ15N. The δ values were found to be unaffected by the presence of lipids or humics (White and Schwarcz, 1994). There is no correlation between “collagen” yield and δ13C or δ15N values for the samples used in this study (r = −0.15, N = 37). Good sample integrity was also demonstrated by a comparison of amino acid profiles, C/N ratios, and histomorphology (White and Schwarcz, 1994).

Osteopenic and normal individuals are compared to each other by their δ13C and δ15N values using Student’s t-tests. Sex differences between and within osteopenic and normal groups are then analysed statistically using the same tests as well as the nonparametric Mann-Whitney U test where sample sizes are too small or the assumptions of the t-test are not met. Age differences for each sex within both conditions are then compared, but the sample size of these groups is too small to analyse statistically.

RESULTS AND DISCUSSION

Collagen integrity

Martin and Armelagos (1985) have noted that the amount of extractable collagen decreases with age, probably as a function of cross-linking (after Ham and Cormack, 1979). This relationship is indeed demonstrated in a previous study which also includes Merotic and Christian Period material (ANOVA, F(6,80) = 4.03, P < 0.002, White and Schwarcz, 1994). In the same study, low gelatin yield (i.e. less than 5%) is also significantly associated with high δ13C values (r = −0.65, n = 140, P < 0.0001) where yield is less than 5%, and with high δ15N values
(r = -0.243, n = 127, P < 0.003). Thus, an attempt was made to select individuals with good yields for this study. There is only one individual (2413-9) with a low yield included here, but her δ¹³C and δ¹⁵N values are well within the range of the rest of the sample, and her C/N ratio does not indicate diagенesis.

The structure of bone collagen is also altered by the accelerated turnover that occurs in osteopenia (Teitelbaum and Bullough, 1979, cited in Martin and Armelagos, 1985). Although there is a correlation between age and gelatin yield in the population these samples were drawn from, yield from this X-Group subsample of Nubians does not differ statistically between normal and pathological individuals (Table 1). Similarly, there are no significant differences in the C/N ratios of normal vs. osteopenic individuals. The possibility that the microstructural difference created by osteopenia could still result in different collagen quality was tested by Baker (1992). Baker analysed the amino acid profiles of 12 of the normal individuals in this sample and six of the pathological individuals. She found no significant differences in the amino acids themselves or their breakdown products.

Isotopic data

There are no observable differences in δ¹³C values between normal and osteopenic groups (Table 1). Although the cereal grain diet might have generally predisposed people to osteopenia, those affected were not consuming greater quantities of either wheat/barley or sorghum/millet. Therefore, neither the greater fibre content in C3 plants nor the lower Ca/P ratios in C4 plants can be considered a major cause of osteopenia in this sample.

Osteopenic individuals do, however, demonstrate significantly higher δ¹⁵N values (Table 1, Student’s t = 3.39, P < 0.001). When the data are broken down by sex and analysed both parametrically and nonparametrically, it is apparent that this difference is mainly attributable to higher values in females (Student’s t-test δ¹⁵N normal vs. osteopenic females, t = 3.95, P < 0.001; Mann-Whitney U, U = 29, P < 0.05; Student’s t-test δ¹⁵N normal vs. osteopenic males, t = .538; Mann-Whitney U, U = 38, Table 2, Fig. 1). Osteopenic females also have significantly higher δ¹⁵N values than osteopenic males (Student’s t-test δ¹⁵N t = 1.74, P < 0.1 [approaching 0.05]; Mann-Whitney U, U = 22, P < 0.05, Table 1). These data are interesting in a number of respects. When the normal sample is examined separately, males have significantly higher values than females in both parametric and nonparametric tests (Student’s t-test, δ¹⁵N t = 2.5, P < 0.01; Mann-Whitney U, U = 62, P < 0.05). This phenomenon is also consistent throughout both earlier Meroitic and later Christian period samples (White and Schwarz, 1994) and could be attributed either to social factors which create greater male access to animal protein or to a physiological difference that is created by a higher level of urea excretion in males (White, 1991). Notably, however, for the osteopenic group, the pattern between osteopenic males and females is completely reversed. One way of interpreting these data is that some females are getting so much protein, they become osteopenic. This hypothesis seems unlikely, not only because protein could not have been generally abundant, but also differential access that favours females would be ethnographically most unusual.

The nitrogen data are consistent with the pattern of bone formation, which is also reversed between the sexes in the osteopenic group. Martin and Armelagos (1985) have found that female bone turnover rates are higher than those of males. Females also appear to have more difficulty with bone formation and mineralization, and their pat-

| TABLE 1. Comparison of mean isotopic ratios, collagen yield, and C/N ratios in normal and osteopenic individuals |
|-------------------------------------------------|----------------|----------------|
| Normal (n = 27) | Osteopenic (n = 16) |
| Collagen yield (%) | | |
| Mean | 12.31 | 12.29 |
| SD | 3.04 | 4.68 |
| Carbon/nitrogen ratio | | |
| Mean | 3.35 | 3.31 |
| SD | 0.19 | 0.17 |
| δ¹³C (%) | | |
| Mean | -16.9 | -16.3 |
| SD | 0.66 | 1.88 |
| δ¹⁵N (%) | | |
| Mean | 11.1 | 12.9 |
| SD | 1.15 | 0.86 |
TABLE 2. Comparison of normal and osteopenic individuals by sex

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<th>δ¹⁵N</th>
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| Osteopenic |     |       |      |      |            |     |       |      |      |
| NAX 623    | 29  | 12.8  | −16.7| 12.7 | NAX 652    | 48  | 6.2   | −19.4| 12.8 |
| NAX 629    | 29  | 10.7  | −16.0| 12.3 | NAX 654    | 29  | 10.6  | −17.8| 13.5 |
| NAX 637b   | 50  | 11.5  | −16.7| 10.8 | NAX 667b   | 37  | 14.1  | −15.8| 11.8 |
| NAX 640b   | 25  | 14.6  | −15.9| 12.5 | 24I3-8     | 27  | 9.5   | −13.3| 12.1 |
| NAX 648    | 33  | 15.5  | −16.4| 12.4 | 24I3-9     | 50  | 2.7   | −17.2| 11.6 |
| NAX 651a   | 48  | 7.0   | −16.2| 12.4 | 24I3-40    | 50  | 18.5  | −13.8| 13.0 |
| NAX 663a   | 30  | 11.7  | −15.9| 12.1 | 24I3-42    | 36  | 15.7  | −17.8| 12.4 |
| 24I3-29    | 26  | 13.3  | −17.1| 12.2 | 24I3-43    | 36  | 18.4  | −18.5| 13.2 |
| Mean       |     | 12.1  | −16.5| 11.8 | Mean       |     | 12.1  | −16.5| 11.8 |
| SD         |     | 2.61  | 0.39 | 1.03 | SD         |     | 5.73  | 2.68 | 0.66 |

1 Individual has both osteopenia and cribra orbitalia.

Fig. 1. Comparison of δ¹⁵N values (means and standard deviations) in osteopenic individuals by sex.

Arrest, and females exhibit less evidence of growth arrest. Martin and Armelagos (1985) suggest females are less able to recover from growth arrest because they “do not have the mineral stores to begin calcification of osteons which have ceased growing” (p. 532), a condition which could be created by multiparity and prolonged lactation in the context of a calcium and protein poor diet. The high δ¹⁵N values, therefore, might not be reflecting consumption of dietary protein, but rather a physiological phenomenon such as urea excretion, which is related to kidney function. Isotopic studies, in which elevated δ¹⁵N values in bone collagen are found, suggest water and protein intake as possible dependent variables (Schoeninger and DeNiro, 1984; Ambrose and DeNiro, 1986; Ambrose, 1991). Both are believed to alter urea excretion, but the effects are ambiguous across species. For example, although controlled diet and climate experiments on rats have failed to produce elevated ¹⁵N in the bone collagen of heat-, water-, or protein-
stressed rats (Ambrose, in press), high δ¹⁵N values in larger herbivores are associated with low rainfall areas, i.e. less than 400 mm per year (Heaton et al., 1986), like the Nubian environment. When rainfall is held constant, water-stressed herbivores also tend to have higher δ¹⁵N values than obligate drinkers (Ambrose and DeNiro, 1986; Ambrose, 1991).

Bentley et al. (1996) have suggested that lactating women in hot environments often experience dehydration even in the presence of an available water source. Thus a water stress phenomenon might have contributed to the elevated δ¹⁵N of nursing mothers in the extremely dry Nubian environment. In addition, lactation is also a cause of urea loss (Steele and Daniel, 1978). The total nitrogen in milk consists of 13% urea, of which 90 ± 2% is absorbed by the infant (Donovan et al., 1986). If the urea model proposed by Ambrose and DeNiro (1986) is correct, i.e. δ¹⁵N-depleted urea should be balanced by δ¹⁵N enrichment in the nitrogen pool within animals, then lactating women should have more enriched δ¹⁵N in their bone collagen. However, nonosteopenic X-Group women (and those from other time periods in the Wadi Halfa series) have significantly less elevated δ¹⁵N than men, even for the age group which should contain the greatest percentage of lactating women (18-40 years) (Table 4). Furthermore, the age group which is expected to contain the majority of lactating women does not differ in δ¹⁵N than the oldest female group. Therefore, if lactation does contribute to elevated δ¹⁵N in the osteopenic individuals in this sample, it is most likely to do so in the third decade under conditions of repeated and prolonged practice, conditions which are expected in this kind of cultural context and conducive to loss of bone mass.

It is well known that kidney function is also related to osteoporosis (Ott, 1994). For example, the kidneys produce the enzyme 1,25(OH)₂D (calcitriol in its synthetic form) which is needed to absorb calcium. Levels of this enzyme are low in those with osteoporosis (Riggs and Melton, 1983) and its activity can be modified by estrogen (Ott, 1994; van Hoof et al., 1994; Aloia et al., 1996; Dick et al., 1996), therefore women in menopause are less able to absorb calcium even when it is dietarily available. Because low levels of 1,25(OH)₂D are also associated with increased blood urea nitrogen in the incidence of osteoporotic fractures (Rudman et al., 1989), it is possible that δ¹⁵N values in the osteopenic Nubians are reflecting altered urea nitrogen excretion. Low levels of 1,25(OH)₂D and increased urea nitrogen might explain the sex difference in this sample, and although the sample size is small, this suggestion has some support in the consistent ability of δ¹⁵N values to reflect the subgroup frequency and microstructural patterning of osteopenia in this sample. This might make δ¹⁵N a biochemical marker for osteopenia in women, adding to conventional markers currently used, e.g. urinary hydroxyproline (Civitelli, 1993). Clinical trials on urinary δ¹⁵N would, however, be needed to test its usefulness in diagnosing osteopenia for modern populations.

With the sexes combined, normal and osteopenic samples demonstrate no statistically significant differences between 10 year adult age categories (Table 3). The greatest amount of dietary variation, however, appears to occur in the youngest group (third decade) of osteopenic individuals. A breakdown by both gender and age for normal and pathological individuals also fails to demonstrate any significant difference between age groups for males (Table 4, Fig. 2). Variation between groups is on the order of 0.3 to 0.6‰. However, when the osteopenic individuals are compared decade by decade with the normal individuals, an interesting pattern again emerges among the females (Fig. 3). Although osteopenic females in all

<table>
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<th>TABLE 3. Comparison of normal and osteopenic individuals by age</th>
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<tr>
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<tr>
<td>31-40</td>
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1 Years.
TABLE 4. Comparison of normal and osteopenic individuals by age and sex

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<td>−16.4 0.37</td>
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¹Years.
²One sample only.

Fig. 2. Comparison of δ¹⁵N values (means and standard deviations) in normal and osteopenic males by age.

Fig. 3. Comparison of δ¹⁵N values (means and standard deviations) in normal and osteopenic females by age.

Age groups are significantly enriched in δ¹⁵N, the greatest degree of enrichment occurs in the third and fifth decades. Both of these age groups exhibit δ¹⁵N values 2 to 2.5‰ greater than the corresponding groups in the normal population. This is a rather dramatic difference, given that it is even greater than the statistically significant difference found in females in the broader temporal context of the sample, i.e. the shift in δ¹⁵N values from the earlier Meroitic period to the later Christian period was only 1.5‰. Although the sample size precludes statistical testing, the isotopic pattern of distinction in the third and fifth decades is consistent with the microstructural differences found in these two age groups (Martin et al., 1984). It would also be consistent in an environment of chronic calcium and protein deficiency, and an expected pattern of early, repeated, and prolonged lactation. Calcium stores could be depleted in females particularly during the peak period of reproductive, and later predispose to postmenopausal (Type I) osteoporosis.

CONCLUSION

This study demonstrates the potential of using chemical dietary reconstruction to test etiological hypotheses for nonspecific diseases in ancient populations. Although it has long been assumed that the cereal grain staple was a major contributor to osteopenia in Sudanese Nubia, the isotopic data (δ¹³C) do not support any grain consumption differences that would relate to testable predisposing dietary factors, i.e. carbohydrates, fibre,
or Ca/P ratios. All members of the population consumed predominantly C3 foods, which we assumed were characterized by a core grain staple of wheat and barley.

There are, however, very marked differences in the $\delta^{15}N$ of bone collagen between normal and osteoporotic females. The patterning in $\delta^{15}N$ values not only reflects frequency patterning of osteopenia in the population at large, but also the observations made previously by Martin et al. (1984) on bone microstructural differences within the population. Osteopenic females are significantly more enriched in $\delta^{15}N$, particularly in their third and fifth decades of life. These are the population subgroups which morphologically also exhibit the highest frequencies of osteopenia. Because of the different microstructural characteristics between these two age groups, the etiology of osteopenia may have been distinct and age dependent, the premature form reflecting an imbalance between physiological demands placed on young females, and dietary sufficiency and the postmenopausal form primarily a result of menopause and chronic calcium deficiency. The isotopic analysis is unable, with its limitations, to demonstrate any real dietary associations. However, both young and menopausal women with osteopenia share high $\delta^{15}N$ values, which might reflect altered urea nitrogen excretion or problems in the renal processing and clearance of calcium and phosphorus. Because $\delta^{15}N$ values in body tissues can be affected by kidney function and urea excretion, the $\delta^{15}N$ enrichment in the osteopenic female Nubians may be signifying a physiological difference. This difference could be related in part to the renal production of the enzyme calcitriol (1,25(OH)$_2$D), which is needed to absorb calcium. Calcitriol is reduced in osteoporotic individuals, and is also associated with increased blood urea nitrogen. Because its production is modified by estrogen, this might explain the differential values of $\delta^{15}N$ between men and women, but this hypothesis requires further testing. Regardless, these data serve to alert paleodiet researchers to the susceptibility of $\delta^{15}N$ values in vivo physiological processes. It is also possible that $\delta^{15}N$ might be a useful marker for osteopenia in female skeletons, particularly when used in conjunction with other measures.

**ACKNOWLEDGMENTS**

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**LITERATURE CITED**


Osteopenia and Stable Isotopes


Keegan WF and DeNiro MJ (1988) Stable carbon and nitrogen isotope ratios of bone collagen used to study
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