**Supplemental Text 2: Preservation Determination Methods**

Several different methods have been employed for extracting collagen from archaeological bones. One of the least expensive and most rapid methods for extracting collagen from well-preserved specimens involves cleaning specimens with an acid and base wash. The extracted substance can be tested for quality of preservation and characterized as collagen using infrared spectroscopy and by measurement of collagen yield in wt.%. Only well-preserved specimens were used for isotopic analysis (Reed 1998), as described below.

*Infrared Spectroscopy*

IR analysis measures physical characteristics of samples (DeNiro and Weiner 1988). Infrared spectra show C=O stretching (1650 cm–1) and N–H bending vibrations in absorption spectra between 1500 and 1800 cm–1 (Ramachandran 1967:142-143). Spectral peaks for modern collagen are strong for amide I (ca. 1700 wavenumbers), amide II (1540 wavenumbers), and proline (1450 wavenumbers), and polysaccharids display a broad band at 1050 wavenumbers (Goldberg 1993:92; Ramachandran 1967). Poorly preserved collagen specimens do not display the infrared peak structure of modern, fresh samples, but tend to have a single strong spectral peak around 1100 wavenumbers.

A Perkin–Elmer 283B Infrared Spectrophotometer was used. After setting the infrared instrument to 6 scan minutes, with an abscissa expansion of 1, and ordinate settings of absorption mode, expansion of 1, and a response of 1, each specimen was scanned between wavenumbers 1800 and 800.

*Collagen Yield*

Specimens in which original isotopic compositions are well preserved generally have a concentration or dry weight percentage of collagen greater than 2% (DeNiro and Weiner 1988). The transition from well-preserved to poorly preserved bone collagen occurs between 1.2 and 1.8 dry weight percent (Ambrose 1990:440).

*Dinitrogen Gas Collection*

An ampule for N2 collection contained 50 mg of 6–16 mesh size “Tel–Tale” brand blue silica gel desiccant (W. R. Grace and Co. Davison Chemical Division purchased from Fisher Scientiﬁc). Mariotti (1983) showed an isotopic fractionation factor of 1.00028 ± 0.00009 when greater than 90% of the nitrogen is adsorbed and desorbed on silica gel. For adsorption, a liquid nitrogen trap was used. The collected N2 was released at room temperature while attached to the mass spectrometer. After each desorption, the ampule was heated at 177 °C (350 °F) for 15 minutes to drive any residual N2 off the silica gel.

*Carbon to Nitrogen Ratio*

Carbon to nitrogen ratio (C:N) of the bone collagen preparation can be made manometrically during the CO2 to N2 gas distillation stage. However, performing more than one adsorption and desorption set was observed to cause fractionation. Therefore, manometric C:N measurements were deemed impossible during the course of the measurements reported here.

The C/N ratio of collagen can also be measured using a carbon (C), hydrogen (H), nitrogen (N), sulfur (S), and oxygen (O) (CHN(O)S) elemental analyzer. At the time of these analyses, however, a CHN(O)S elemental analyzer was not available.

*Combustion Procedure*

After collagen was extracted from a bone specimen it was combusted in an evacuated and sealed Vycor or quartz tube (DeNiro and Epstein 1978). One gram each of granular copper (Cu) and black wire cupric oxide (CuO) were placed into the combustion tube along with less than 10 mg of collagen sample. Cupric oxide attains pyrolysis at temperatures above 500 °C, thus providing O2 for oxidation of organic carbon to CO2 and the formation of H2O (Boutton 1991:164; Hachey et al. 1987:300). Copper acts as a catalyst in converting carbon monoxide (CO) to carbon dioxide (CO2), nitric oxides (NO) to dinitrogen (N2), and sulfur oxides (SOx) to CuSO4 (Boutton 1991:165; Hachey et al. 1987:300). For production of a mixture of carbon dioxide and dinitrogen gases, combustion was undertaken between 900 and 1000 °C for 3 hours to insure complete combustion (Boutton 1991:167). The addition of 50 mg silver wool or 9 mm2 silver foil to the combustion mix served to convert the nitrogen oxides to N2 at 450 °C, remove halogens (fluorine, chlorine, bromine, iodine, or astatine), and convert sulfur oxides to silver sulfate (Scholten 1991; Hachey et al. 1987:300). Combusted samples were then processed within five days, otherwise isotopic fractionation is known to occur within the sealed tubes (Boutton 1991:165).

*Separation Process*

The resultant gas mixture must be separated before *δ*13C and *δ*15N can be measured. The separation process requires a vacuum system designed for cryogenic distillation. Separation involves placing the sample tubes of combusted collagen into crackers that break the tubes under sealed conditions and release the gas mixture into an evacuated system. A chloroform and liquid nitrogen slush trap at –61 °C (Coyne 1992:264) was used to freeze the water vapor in the system, while a liquid nitrogen trap (–196 °C) froze the CO2 gas and allowed the N2 gas to pass. The volume of N2 gas was collected in ampules designed to ﬁt the mass spectrometer. The CO2 was then thawed once the nitrogen trap was removed and was collected in another ampule for mass spectrometry.

*Mass Spectrometry*

The mass spectrometer used for carbon dioxide stable isotope ratio measurements (i.e., *δ*13C and *δ*18O) was built for the Pennsylvania State University’s Department of Geosciences Mass Spectroscopy of Minerals Laboratory by L. F. Herzog, R. N. Clayton, and T. J. Eskew (Deines 1967:138-141; 1970). It is a modiﬁed version of the instrumentation described by Nier (1947), with instrument control and data collection software written to meet the speciﬁcations described by Deines (1970). The mass spectrometer used for nitrogen stable isotope ratio measurements was a Finnagen MAT 251 controlled by Isodat version 4.1 software.

**References Cited**

Ambrose, Stanley H.

 1990 Preparation and Characterization of Bone and Tooth Collagen for Isotopic Analysis. *Journal of Archaeological Science* 17:431–451.

Bada, Jeffrey L., Margaret J. Schoeninger, and Arndt Schimmelmann

 1989 Isotopic Fractionation during Peptide Bond Hydrolysis. *Geochimica et Cosmochimica Acta* 53:3337–3341.

Boutton, Thomas W.

 1991 Stable Carbon Isotope Ratios of Natural Materials: I. Sample Preparation and Mass Spectrometric Analysis. In *Carbon Isotope Techniques*, edited by D. C. Coleman and B. Fry, pp. 155–171. Academic Press, San Diego, California.

Coyne, George S.

 1992 *The Laboratory Handbook of Materials, Equipment, and Technique*. Prentice–Hall, Englewood Cliffs, New Jersey.

Deines, Peter

 1967 *Stable Carbon and Oxygen Isotopes of Carbonatite Carbonates and Their Interpretation*. Ph.D. dissertation, The Pennsylvania State University, University Park.

 1970 Mass Spectrometer Correction Factors for the Determination of Small Isotopic Composition Variations of Carbon and Oxygen. *International Journal of Mass Spectrometry and Ion Physics* 4:283–295.

DeNiro, Michael J., and Samuel Epstein

 1981 Inﬂuence of Diet on the Distribution of Nitrogen Isotopes in Animals. *Geochimica et Cosmochimica Acta* 45:341–351.

DeNiro, Michael J., and Stephen Weiner

 1988 Chemical, Enzymatic and Spectroscopic Characterization of Collagen and Other Organic Fractions from Prehistoric Bones. *Geochimica et Cosmochimica Acta* 52:2197–2206.

Goldberg, Carol F.

 1993 *The Application of Stable Carbon and Nitrogen Isotope Analysis to Human Dietary Reconstruction in Prehistoric Southern California.* Ph. D. dissertation, University of California, Los Angeles.

Hachey, David L., Wong, William W., Boutton, Thomas W. and Klein, Peter D.

 1987 Isotope Ratio Measurements in Nutrition and Biomedical Research. *Mass Spectrometry Reviews* 6:289–328.

Mariotti, André

 1983 Atmospheric Nitrogen Is a Reliable Standard for Natural 15 N Abundance Measurements. *Nature* 303:685–687.

Masters, Patricia M.

 1987 Preferential Preservation of Noncollagenous Protein during Bone Diagenesis: Implications for Chronometric and Stable Isotopic Measurements. *Geochimica et Cosmochimica Acta* 51:3209–3214.

Nier, Alfred O.

 1947 A Mass Spectrometer for Isotope and Gas Analysis. *The Review of Scientiﬁc Instruments* 18(6):398–411.

Ramachandran, G. N.

 1967 Structure of Collagen at the Molecular Level. In *Treatise on Collagen, Volume 1, Chemistry of Collagen,* edited by G. N. Ramachandran, pp. 103–183. Academic Press, London.

Reed, David M.

 1998 *Ancient Maya Diet at Copán, Honduras*. Ph.D. dissertation, The Pennsylvania State University, University Park.

Scholten., S. O.

 1991 *The Distribution of Nitrogen Isotopes in Sediments*. No. 81, Geologica Ultraiectina. Mededelingen van de Faculteit Aardwetenschappen der Rijksuniversiteit Utrecht, Utrecht, Netherlands.