

Trophic Level and Macronutrient Shift Effects Associated With the Weaning Process in the Postclassic Maya

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ABSTRACT The weaning process was investigated at two Maya sites dominated by Postclassic remains: Marco Gonzalez (100 BC–AD 1350) and San Pedro (1400–AD 1650), Belize. Bone collagen and bioapatite were analyzed from 67 individuals ($n \leq 6$ years = 15, $n > 6$ years = 52). Five isotopic measures were used to reconstruct diet and weaning: stable nitrogen- and carbon-isotope ratios in collagen, stable carbon- and oxygen-isotope ratios in bioapatite, and the difference in stable carbon-isotope values of coexisting collagen and bioapatite. Nitrogen-isotope ratios in infant collagen from both sites are distinct from adult females, indicating a trophic level effect. Collagen-to-bioapatite differences in infant bone from both sites are distinct from adult females, indicating a shift in macronutrients. Oxygen-isotope ratios in infant bioapatite from

both sites are also distinct from adult females, indicating the consumption of breast milk. Among infants, carbon- and nitrogen-isotope ratios vary, indicating death during different stages in the weaning process. The ethnohistoric and paleopathological literature on the Maya indicate cessation of breast-feeding between ages 3–4 years. Isotopic data from Marco Gonzalez and San Pedro also indicate an average weaning age of 3–4 years. Based on various isotopic indicators, weaning likely began around age 12 months. This data set is not only important for understanding the weaning process during the Postclassic, but also demonstrates the use of collagen-to-bioapatite spacing as an indicator of macronutrient shifts associated with weaning. *Am J Phys Anthropol* 128:781–790, 2005.

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Weaning is a process where breast milk is gradually removed from an infant's diet and replaced by food staples characteristic of the environment (Dettwyler and Fishman, 1992). The age that breast milk is completely removed from infant diet, generally referred to as "weaning age," varies widely across cultures, as does the nutritional value and type of introduced foods. Breast milk is important in the first 6 months of life because it contains immunoglobulin, staphylococcus, and lymphocytes to provide protection from infection and disease (reviewed by Katzenberg et al., 1996). However, prolonged breast-feeding without the introduction of additional foods may impair proper brain growth and development (Hendricks and Badruddin, 1992; Lutter, 1992). Consequently, additional foods are often introduced by age 1 year (Lutter, 1992). In traditional societies, supplementary foods are often based on staple grains, which, depending on the environment, vary in their nutritional adequacy. Weaning age is inversely correlated to an infant's risk of morbidity and mortality. Environmental factors such as water quality also play an important role in the degree of risk (Knodel and Kintner, 1977). Additionally, breast-feeding is related to fertility; frequent nursing can prevent the return of ovulation, thereby increasing birth spacing (Kennedy et al., 1989; McNeilly et al., 1994). In both modern and archaeological populations, infant feeding practices and diet provide important information about fertility, population growth, infant health, mortality, and morbidity.

The nitrogen isotopic analysis of human bone collagen has been used to investigate weaning in archaeological

populations since the method was first introduced by Fogel et al. (1989; e.g., Dupras et al., 2001; Herring et al., 1998; Katzenberg et al., 1996; Katzenberg and Pfeiffer, 1995; Schurr, 1997, 1998; White et al., 2001). There is a stepwise enrichment in ¹⁵N between trophic levels (Minagawa and Wada, 1984; Schoeninger and DeNiro, 1984), which can be used to determine an individual's position in the foodweb. Since infants consume breast milk formed from the mother's tissues, the infant will be a trophic level higher. As such, the nitrogen-isotope ratio in infant bone collagen will be 2–4‰ enriched in ¹⁵N relative to the mother (Fogel et al., 1989).

White and Armelagos (1997) and Katzenberg and Lovell (1999) found evidence for elevated nitrogen-isotope ratios in individuals with osteopenia and wasting diseases. The stable isotope ratio of carbon was unaffected in individu-

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als from both of these studies. We generally do not know the cause of death, or whether disease processes affected the nitrogen-isotopic composition of infant bone. Consequently, investigations of the weaning process would be strengthened by combining nitrogen-isotope analyses with additional isotopic indicators that reflect different metabolic processes, components of bone, macronutrients, and/or liquid sources unaffected by disease processes.

Wright and Schwarcz (1998) investigated weaning age in a population from Kaminaljuyú, using the stable isotopes of oxygen and carbon in tooth enamel carbonate. These data indicate that breast milk was enriched in ^{18}O relative to the water consumed by the mother. Consequently, the stable oxygen-isotope ratios in bone bioapatite from breast-feeding infants were 0.5–0.7‰ enriched in ^{18}O relative to adults. The same effect was confirmed in oxygen-isotope ratios of enamel phosphate of the same population (White et al., 2000). Among Nubians, the stable oxygen-isotope ratios in bone phosphate from breast-feeding infants were 1.8‰ enriched in ^{18}O relative to adults (White et al., 2004). Also, there is generally an enrichment of 1‰ in the stable carbon-isotope ratio between trophic levels (DeNiro and Epstein, 1978). However, this does not appear to be consistent between breast-feeding infants and adults (Lovell et al., 1986; Wright and Schwarcz, 1998; but see Dupras et al., 2001; Richards et al., 2002; White et al., 2001) and may not be a reliable indicator for age of weaning.

This paper introduces an additional method to investigate weaning age, using the differences in $\delta^{13}\text{C}$ values between coexisting bioapatite and collagen ($\Delta^{13}\text{C}_{\text{ap-col}}$). The combination of the multiple indicators of $\Delta^{13}\text{C}_{\text{ap-col}}$, $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, and $\delta^{18}\text{O}$ may be useful to examine weaning, regardless of disease processes, since they reflect different: 1) components of bone, 2) aspects of diet, and 3) metabolic processes.

SITE AND SAMPLE

This sample was obtained from two ancient Maya sites: 1) Marco Gonzalez, which was occupied from the late Pre-classic to the late Postclassic (100 BC–AD 1350), with some evidence suggesting occupation until the early 16th century (Graham and Pendergast, 1989), and 2) San Pedro, which was occupied from the Terminal Postclassic to Historic times (AD 1400–1650) (Pendergast and Graham, 1991). Marco Gonzalez is located on the leeward side of the southernmost tip of Ambergris Cay, the northernmost island off the east coast of Belize, and San Pedro is located 8 km north of Marco Gonzalez along the windward side of Ambergris Cay (Fig. 1).

Excavations at Marco Gonzalez began in 1986 and continued until 1990 under the supervision of David Pendergast (formerly of the Royal Ontario Museum) and Elizabeth Graham (University College of London). The 6.6-ha site contains at least 49 structures (all comparatively low platforms ranging in height from 30 cm to 4.2 m), and appears to represent a permanent occupation (Graham and Pendergast, 1989). Artifactual evidence from Marco Gonzalez is suggestive of trade links with other Maya communities in Mesoamerica. This evidence includes: green obsidian from central Mexico, grey and black obsidian from highland Guatemala, slateware pottery from the Yucatan, plumbate ware, and a button face jar from Guatemala and/or El Salvador (Graham and Pendergast, 1987; Pendergast and Graham, 1990). In addition, there is extensive ceramic evidence for a connection to the major Maya cere-

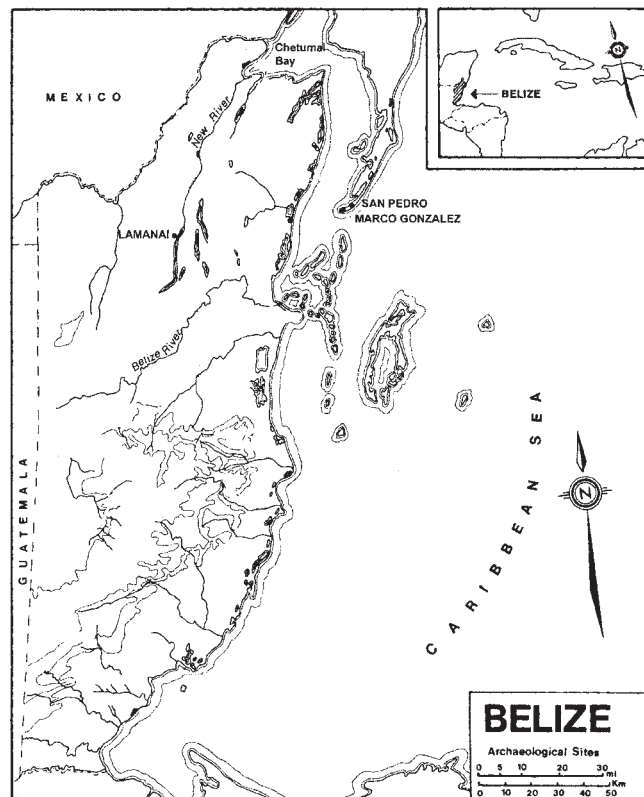


Fig. 1. Map of Ambergris Cay, showing location of Marco Gonzalez and San Pedro, Belize (adapted from Pendergast, 1993, p. 2).

monial center of Lamanai, which is located on mainland Belize, northwest of modern Belize City.

The excavations at San Pedro began in 1990 and continued until 1993; they revealed numerous human burials. Artifacts recovered from middens include: lithics, a basalt mano, a piece of jade, and 16th and 17th century Maya pottery similar to that found at Lamanai (Pendergast and Graham, 1991; Guderjan, 1995).

In total, 67 human bone samples, mostly ribs, were analyzed. Of these, there are 19 subadults and 48 adults with a similar ratio of males ($n = 20$, including four probable males) and females ($n = 22$, including five probable females), and six individuals of indeterminate sex. The individuals sampled from both Marco Gonzalez and San Pedro represent various stages of the Postclassic period that were assigned based on grave goods, construction fill, and stratigraphy. These data are summarized in Table 1.

MATERIALS AND METHODS

Bone collagen was analyzed for its stable nitrogen- and carbon-isotope compositions and prepared using a modified Longin method (Chisholm et al., 1983; DeNiro and Epstein, 1978; Longin, 1971). A 1.5–2.0-g sample was ground and placed in 0.25 M HCl solution until the mineral component of bone was dissolved. The sample was then soaked in 0.125 M NaOH to dissolve humic contaminants (Schoeninger and DeNiro, 1984). Following this, the collagen was solubilized for 20 hr in 0.001 M HCl in a 90°C oven. Collagen was analyzed directly by combustion using a Fisons elemental analyzer connected in continuous-flow mode to a triple-collecting VG Optima stable iso-

TABLE 1. Isotopic values and alteration indices by site, burial number, age, and sex

Burial	Sex	Age	$\delta^{13}\text{C}_{\text{col}} \text{‰}$	$\delta^{13}\text{C}_{\text{ap}} \text{‰}$	$\Delta^{13}\text{C}_{\text{ap-col}} \text{‰}$	$\delta^{15}\text{N} \text{‰}$	$\delta^{18}\text{O} \text{‰}$	C/N ratio ¹	Collagen yield % ²	CO ₂ gas yield % ³	CI ⁴	C/P ⁵
Marco Gonzalez												
14/1b		neonate	-6.0	-5.5	0.5	10.4	27.5	3.40	4.78	1.09	2.96	0.23
11/5		2-3 months	-7.8	-7.9	0.1	11.9	27.3	3.33	4.23	0.99	3.08	0.20
14/6		6 months	-6.1	-4.5	1.6	10.7	28.1	3.23	10.19	0.93	3.23	0.17
14/18		18 months	-6.0	-5.1	0.9	12.1	27.8	3.24	3.55	0.60	3.57	0.16
14/21c		12-36 months	-5.6	-4.4	1.2	11.8	26.1	3.19	7.38	0.82	3.16	0.20
11/4a		18-24 months	-6.2	-5.7	0.5	11.6	27.9	3.22	2.75	0.91	3.41	0.18
14/13c		6-36 months	-6.8	-5.5	1.3	11.6	27.9	3.25	8.82	0.80	3.60	0.18
12/5		4-5	-6.0	-4.5	1.5	7.1	28.1	3.29	4.73	1.10	3.17	0.23
204b		5	-7.0	-7.6	0.6	11.0	27.9	3.27	3.20	0.65	3.23	0.23
14/28a		5.5-6.5	-7.7	-6.4	1.3	9.2	27.8	3.61	1.35	0.77	3.32	0.16
14/15	F	30+	-7.1	-6.2	0.9	10.0	25.8	3.34	3.71	1.91	3.27	0.18
14/29a	F	30+	-9.2	-7.9	1.3	11.0	26.8	3.23	2.55	1.30	3.22	0.20
18/1a	M	30+	-6.7	-6.2	0.5	10.4	27.2	3.41	1.70	1.23	3.24	0.21
204d	F?	30+	-9.9	-6.6	3.3	9.7	25.9	3.48	4.01	1.44	2.88	0.51 ⁶
11/2a	F	35+	-8.2	-8.8	0.6	10.9	26.7	3.39	1.93	1.48	3.53	0.22
11/8	M	40+	-7.3	-7.5	0.2	11.5	26.5	3.33	1.81	0.90	2.93	0.28
12/2	F?	40+	-8.4	-5.5	2.9	10.0	27.3	3.19	7.88	1.01	3.19	0.20
12/6	F	40+	-6.9	-5.1	1.8	10.8	27.7	3.27	3.05	0.91	3.04	0.21
14/29b	F?	40+	-9.1	-7.8	1.3	11.2	26.6	3.22	2.86	1.49	3.01	0.22
14/10a	M	40+	-7.9	-5.3	2.6	11.0	26.2	4.20	0.94	0.63	3.16	0.15
14/11	M	40+	-7.7	-6.4	1.3	10.9	27.2	3.21	4.49	0.74	3.22	0.20
14/13a	F	40+	-7.9	-5.4	2.5	10.4	28.0	3.48	0.64	n/a	3.68	0.15
18/1b	F	Adult	-7.4	-5.3	2.1	8.2	28.1	3.40	6.38	1.23	3.29	0.19
San Pedro												
11/8-1		9-12 months	-6.0	-3.5	2.5	12.6	27.4	3.33	6.37	0.82	3.30	0.16
14/24a	M	20-30	-7.3	-6.6	0.7	10.9	26.9	3.32	2.32	0.69	3.28	0.21
205b	?	20+	-8.2	-6.8	1.4	10.0	26.2	3.61	4.07	1.55	3.33	0.21
6	M?	20+	-6.5	-4.8	1.7	9.8	27.4	3.30	2.52	0.87	3.46	0.18
23/91-R4	F	20+	-7.6	-4.3	3.3	9.1	26.6	3.23	5.11	1.17	3.07	0.28
14/1a	F	21-25	-6.6	-5.8	0.8	8.9	27.4	3.55	3.79	1.36	3.14	0.20
11/7	F	25-30	-7.1	-5.0	2.1	8.3	26.0	3.82	0.94	2.03	3.64	0.13
14/7c ⁷	M	25-30	-12.4	-6.6	5.8	8.1	24.3	4.71	0.26	0.69	3.84	0.14
11/2-5	F	25-30	-6.1	-5.3	0.9	9.4	27.1	3.27	3.02	0.97	3.26	0.21
7	F	25-30	-6.3	-3.5	2.6	9.7	27.1	3.25	3.10	0.84	3.06	0.26
14/5a	M	25-50	-6.6	-5.6	1.0	9.2	26.8	3.21	7.86	0.87	3.54	0.15
14/10b	?	25-50	-7.6	-4.7	2.9	10.5	26.5	3.29	4.87	0.73	3.31	0.15
14/26a	?	25-50	-6.7	-4.9	1.8	10.9	27.0	3.26	4.25	0.80	3.53	0.17
38	?	25-50	-5.7	-4.8	0.9	10.7	26.9	3.26	6.82	1.16	3.18	0.19
137	?	25+	-8.8	-7.4	1.4	11.0	27.0	3.35	2.02	0.71	4.15	0.17
14/27	M	30-40	-8.2	-6.5	1.7	11.3	27.1	3.35	2.30	0.81	3.18	0.22
14/17	M	30-40	-8.8	-6.9	1.9	11.2	27.0	3.25	3.57	1.66	3.42	0.20
4	M	30-40	-6.7	-4.4	2.3	9.8	26.7	3.24	6.42	0.91	3.11	0.23
R1	M	30-40	-9.5	-6.9	2.6	11.5	27.3	3.31	3.34	1.03	3.23	0.26
12/3	M	30-45	-9.8	-7.4	2.4	11.5	25.4	3.30	2.85	1.02	3.56	0.14
14/16	F	30+	-9.2	-6.7	2.5	10.2	27.5	3.24	3.02	0.83	3.67	0.16
14/23	F?	30+	-8.5	-7.2	1.3	10.3	26.2	3.52	1.00	1.94	3.45	0.17
17/6-4		24-36 months	-5.0	-5.0	0.0	13.0	26.5	3.26	6.41	0.92	3.20	0.21
2		4-5	-5.7	-4.8	0.9	10.3	26.6	3.24	5.81	0.97	3.07	0.25
11/2-3b		3-7	-6.8	-4.5	2.3	9.3	27.1	3.40	3.55	1.12	3.20	0.25
11/2-1		6	-6.2	-4.4	1.8	10.2	26.6	3.27	7.31	0.85	3.12	0.24
11/2-8a		6-7	-5.7	-5.9	0.2	8.8	27.3	3.23	4.90	1.00	2.91	0.30
11/2-2		9-10	-6.0	-3.8	2.2	9.0	27.0	3.34	2.19	0.61	3.42	0.15
17/6-3		9-10	-5.6	-3.9	1.7	9.3	26.4	3.33	5.26	0.90	3.26	0.23
11/2-7b		9-12	-5.4	-3.3	2.1	10.1	26.5	3.21	6.03	1.04	3.28	0.21
11-3/5	F	17-19	-5.8	-3.2	2.6	10.0	26.7	3.29	4.84	0.92	3.22	0.20
11/2-6	M?	18-20	-5.8	-4.5	1.3	9.3	26.7	3.33	2.91	1.00	3.10	0.27
R6	F	18+	-8.8	-5.5	3.3	9.4	26.8	3.40	4.04	1.11	3.04	0.28
11/3-1	F	20-25	-5.9	-2.6	3.4	9.7	26.7	3.24	3.65	1.24	3.18	0.25
11/2-3a	M?	35-40	-7.0	-5.2	1.7	9.3	27.0	3.36	1.85	1.10	3.10	0.27
17/6-1	M	35-39	-6.0	-2.8	3.2	9.7	26.7	3.29	5.36	1.15	3.03	0.24
17/6-5	M	39-44	-5.8	-2.0	3.8	9.8	27.2	3.35	3.37	1.65	3.03	0.25
R5	F	40+	-8.6	-6.2	2.4	9.5	26.9	3.33	5.11	1.10	3.27	0.20
11/2-4a	M?	40+	-6.0	-4.6	1.4	9.5	27.1	3.37	1.51	1.20	3.02	0.27
11/3-2	F	40+	-6.2	-3.2	3.0	10.5	27.2	3.34	1.79	0.79	3.41	0.19
11/3-4	F?	40+	-7.4	-4.0	3.4	9.4	26.6	3.30	4.80	1.02	3.10	0.24
1B	M	45-50	-7.0	-3.9	3.1	9.5	26.7	3.30	4.09	0.97	3.52	0.17
1A	M	50+	-7.6	-3.6	4.0	10.0	26.7	3.41	4.55	0.74	3.19	0.23
11/2-4b	?	Adult	-6.3	-3.8	2.4	9.9	26.9	3.26	6.59	0.84	3.14	0.24

¹ % carbon vs. % nitrogen in sample, measured by elemental analyzer.² mg finished material/mg starting material \times 100.³ In $\mu\text{moles/mg}$ bioapatite.⁴ Crystallinity index.⁵ Carbonate to phosphate ratio.⁶ Values in bold are outside accepted range for samples unaffected by postmortem alteration.⁷ Sample in italics was removed because of postmortem alteration.

tope ratio mass spectrometer. The relative abundances of stable isotopes were measured against a standard reference material, VPDB for carbon (see Coplen, 1994), and atmospheric N₂ for nitrogen. Both are expressed using the delta (δ) notation as per mil (‰). Precisions of replicate analyses for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were $\pm 0.1\text{‰}$ and $\pm 0.3\text{‰}$, respectively. Two laboratory reference materials, calibrated to VPDB via NSB-19, were included in each analytical session as a check on the accuracy of the carbon-isotope measurements. Laboratory calcite had an average $\delta^{13}\text{C}$ value of 0.90‰ (accepted value = 0.89‰). Laboratory CO₂ gas had an average $\delta^{13}\text{C}$ value of -43.9‰ (accepted value = -43.8‰).

Bone bioapatite was prepared for stable carbon- and oxygen-isotope analysis of its structural carbonate (bone phosphate was not analyzed in this study) following Lee-Thorp and van der Merwe (1991). A 0.5-g sample of bone was ground, to which 4% sodium hypochlorite was then added and left for 24 hr to remove collagen and other organic materials. Following this, the sample was soaked in 1 M acetic acid for 1 hr to remove secondary carbonates (but see Zazzo et al., 2004). Bone bioapatite was then reacted under a vacuum with orthophosphoric acid for 72 hrs at 25°C. The CO₂ gas produced by this reaction was collected by cryogenic distillation. The stable carbon- and oxygen-isotope ratios of bone bioapatite CO₂ were measured using either a VG Prism or a VG Optima dual-inlet, triple-collecting stable isotope ratio mass spectrometer and are presented relative to VPDB and VSMOW, respectively (Coplen, 1994). Precision of replicate analyses for $\delta^{13}\text{C}_{\text{ap}}$ was $\pm 0.3\text{‰}$, and for $\delta^{18}\text{O}$, $\pm 0.4\text{‰}$.

The preservation of bone collagen was assessed using the carbon/nitrogen (C/N) ratio and collagen yield. The acceptable range of C/N ratios for archaeological bone is 2.9–3.6 (DeNiro, 1985). The mean C/N ratio for samples in this study was 3.3 ± 0.2 , with a range from 2.9 ± 4.7 . The minimum acceptable collagen yield is 1% (van Klinken, 1999). The mean collagen yield for samples in this study was $4.0 \pm 2.0\%$, with a range from 0.3–10.2%. The preservation of bone bioapatite was assessed using its CO₂ yield, crystallinity index (CI), and C/P ratio. The acceptable range of CO₂ yields for unaltered samples is $>0.6\%$ – $\leq 1.3\%$ (Ambrose, 1993). The mean CO₂ yield of samples in this study was $1.0 \pm 0.3\%$, with a range from 0.6–2.3%. The acceptable range for CI of modern bone is 2.8–4.0 (Wright and Schwarcz, 1996). The mean CI of samples in this study was 3.2 ± 0.2 , with a range from 2.4–4.2. The acceptable range of carbonate/phosphate (C/P) ratios for unaltered samples is 0.15–0.30 (Wright and Schwarcz, 1996). The mean C/P ratio of samples in this study was 0.2 ± 0.1 , with a range from 0.1–0.5.

The values from various tests for postmortem alteration were plotted against the respective isotopic signature to test the established ranges of these indicators further, and to ensure the recognition of samples whose isotopic signature may have been altered by diagenesis (Williams, 2000). If a statistically significant correlation ($P < 0.05$) existed between the diagenetic indicator and the isotopic signature, the samples that created the correlation were excluded. Based on these tests for correlation, and unacceptable values for three out of five diagenetic indicators, one sample (MG 14/7c) was eliminated (Table 1). There were other samples (shown in bold in Table 1) whose values for one of the five diagenetic indicators were outside the acceptable range. These data were retained.

Age-at-death was assigned to individual skeletal remains from both San Pedro and Marco Gonzalez using

the standards outlined in Buikstra and Ubelaker (1994). The subadult ages used for this study represent the midpoint of estimated age ranges (following Herring et al., 1998). The statistical package SPSS was used for all statistical analyses, and significant values are reported at $P < 0.05$ unless otherwise indicated. When the data set was normally distributed, we used Student's *t* (*t*) or ANOVA (*F*) to compare means between groups, and Pearson's *r* to test for correlations between groups. When the data set was non-normally distributed, we used Mann-Whitney *U* (reported as a *z* score) to compare means.

Theoretical background

In addition to vitamins and minerals, foods contain three macronutrients (lipids, carbohydrates, and protein) which are preferentially routed to different body tissues. Collagen, the organic component of bone, is a protein composed of both essential and nonessential amino acids (Hare, 1980). Essential amino acids cannot be synthesized from the body and must be ingested either whole in animal proteins or in complementary sequences from vegetable proteins. Consequently, dietary protein is preferentially routed to bone collagen for tissue maintenance and growth (Ambrose and Norr, 1993; Jim et al., 2004; Tieszen and Fagre, 1993; Schwarcz, 2000). Since the synthesis of amino acids from nonamino-acid precursors (carbohydrate and lipid) often incurs significant energy costs, it can be assumed that amino acids will be preferentially obtained from dietary sources when possible (Ambrose et al., 1997). Unlike collagen, carbonate in bone bioapatite is formed by precipitation of blood bicarbonate. The unused carbon from all macronutrients is respired as CO₂, which is carried by blood to the lungs. Consequently, the isotopic composition of bone bioapatite carbonate will primarily reflect carbon from carbohydrates and lipids, with the amount of carbon from protein depending on the level of protein in the diet (Ambrose, 1993; Jim et al., 2004; Parkington, 1991).

Breast-feeding infants consume a single dietary source of carbon, i.e., breast milk, which is carbohydrate-rich (in the form of lactose), lipid-rich, and (by comparison) protein-poor (Whitney and Rolfes, 2002). Over time, the protein and lipid content of breast milk decreases, while the carbohydrate content increases (Whitney and Rolfes, 2002). The bone bioapatite of breast-feeding infants will primarily reflect the carbohydrate lactose (with some carbon from lipids), and bone collagen should reflect the protein component of breast milk as long as the diet is no less than 5% protein (Ambrose, 1993).

The Maya used maize, a C₄ grain, as the primary weaning food (Tozzer, 1941). Like breast milk, the most abundant dietary macronutrient of maize is also carbohydrate, but unlike breast milk, maize has virtually no fat and little protein (Whitney and Rolfes, 2002). However, the overall carbon-isotope composition of each should be similar, since fats are depleted of ¹³C and proteins are enriched in ¹³C relative to carbohydrates (DeNiro and Epstein, 1978; Krueger and Sullivan, 1984). The isotopic composition of human breast milk has not been investigated, but analyses of animal breast milk indicate that its isotopic composition reflects the diet of the mother (Jenkins et al., 2001; Nelson et al., 1998; Polischuk et al., 2001). Since the total available carbon in breast milk and the weaning food is isotopically similar, the $\delta^{13}\text{C}$ values of bone bioapatite should change very little with weaning.

Breast milk contains more protein than does maize. Because protein is the primary source of carbon for bone

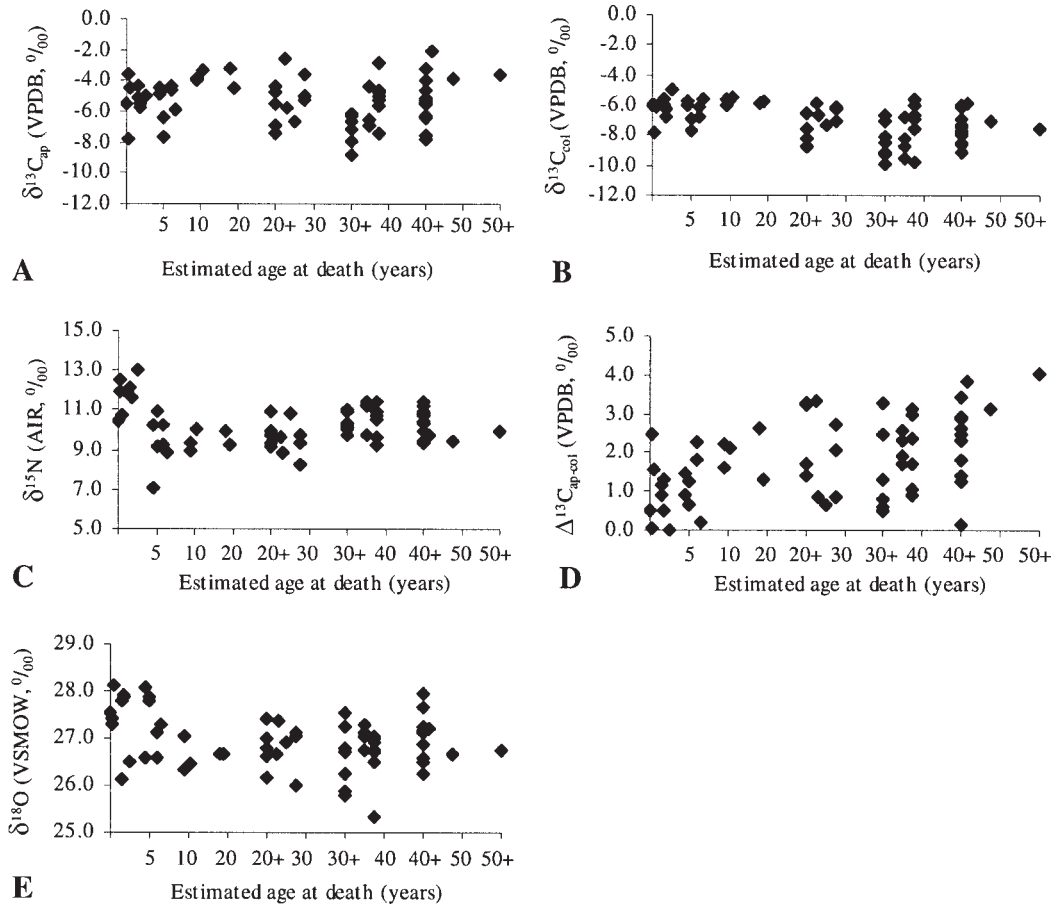


Fig. 2. Stable isotope values vs. age for all individuals of known age from San Pedro and Marco Gonzalez ($N = 64$). **A:** $\delta^{13}\text{C}_{\text{Cap}}$. **B:** $\delta^{13}\text{C}_{\text{Col}}$. **C:** $\delta^{15}\text{N}$. **D:** $\Delta^{13}\text{C}_{\text{Cap-col}}$. **E:** $\delta^{18}\text{O}$.

collagen synthesis, $\delta^{13}\text{C}_{\text{col}}$ values will change with weaning. Since protein is enriched in ^{13}C relative to lipids and carbohydrates, we predict that the $\delta^{13}\text{C}$ values of infant bone collagen will become more negative with weaning. Since the level of protein in the diet is decreasing, and the isotopic composition of bone collagen is changing, we would expect $\Delta^{13}\text{C}_{\text{ap-col}}$ values to increase with weaning. Based on tested relationships between different tissues and the relative isotopic composition of various macronutrients, we can predict how the isotopic signatures of subadult bone will change during weaning:

1. Because the total available carbon in breast milk and the weaning food is isotopically similar, the carbon isotopic composition of bone bioapatite will not change with weaning.
2. Because the weaning pap has less protein and is depleted of ^{13}C relative to breast milk, the $\delta^{13}\text{C}$ value of bone collagen will decrease with weaning.
3. Because the amount of breast milk in the diet decreases during weaning and infants are therefore consuming less protein, we would expect an increase in $\Delta^{13}\text{C}_{\text{ap-col}}$ values.
4. Because the amount of breast milk in the diet decreases during weaning, the individual is relying less heavily on the tissues of the mother. As such, the $\delta^{15}\text{N}$ values for weaned infants should decrease to reflect a trophic level similar to or lower than adults.

5. Based on Wright and Schwarcz (1998), we predict that the $\delta^{18}\text{O}$ values of infant bone bioapatite carbonate will decrease with weaning as infants consume more environmental water than mother's milk.

RESULTS AND DISCUSSION

Five isotopic measures were obtained for each individual: $\delta^{13}\text{C}_{\text{col}}$, $\delta^{13}\text{C}_{\text{ap}}$, $\Delta^{13}\text{C}_{\text{ap-col}}$, $\delta^{15}\text{N}$, and $\delta^{18}\text{O}$ (Table 1). Trends with age were most pronounced for $\delta^{13}\text{C}_{\text{col}}$, $\Delta^{13}\text{C}_{\text{ap-col}}$, and $\delta^{18}\text{O}$ values (Fig. 2).

Age-related differences in isotopic composition: background

The young children from these sites would have been at various stages of weaning. This can be examined more closely by using smaller age categories and predicting possible equilibrium and bone remodeling rates for subadults. Equilibrium rate is the time required for the total body protein pool to gradually become modified to reflect the isotopic composition of the new diet. This is affected by the length of time required for the body to break down and resorb body-tissue proteins that were synthesized prior to dietary change. This process generally takes 5 months, but some proteins (such as bone collagen) take much longer to resorb (Tieszen et al., 1983). Fogel et al. (1989)

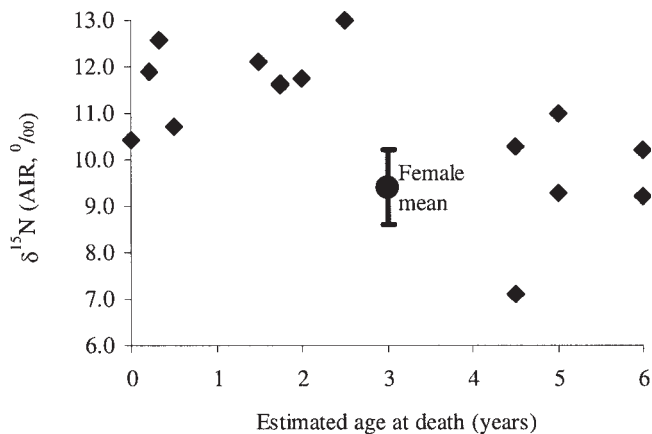


Fig. 3. $\delta^{15}\text{N}$ values for all children compared to the adult female mean (indicated by circle and error bars).

found that the nitrogen-isotopic composition of infant nails equilibrated with diet in 3–5 months. Based on measurements of adult human hair, O'Connell and Hedges (1999) estimated 7–12 months for the $\delta^{13}\text{C}$ values of the body to equilibrate isotopically to a new diet. In steers aged 9–10 months, readjustment of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ to reflect a new diet took 8 months (Balasse et al., 2001, p. 244). In a similar study using human infants, Herrscher (2003) found isotopic differences between bone and tooth dentin from three locations (representing diet at different periods), suggesting that equilibration of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values with the weaning diet takes longer than 3 months but less than 8 months (Herrscher, 2003). Fogel et al. (1989, p. 116) found that “the $\delta^{15}\text{N}$ [values] of the newborns was variable but, on average, was almost identical with that of the adults. These children were probably too young to have expressed the extra utero nursing pattern.” In contrast, Richards et al. (2002, p. 207) found that the $\delta^{15}\text{N}$ values of newborns were enriched in ^{15}N relative to adults.

Once the body has equilibrated isotopically to dietary change, newly formed tissues will reflect the isotopic composition of the new diet. However, the unpredictable and reversible nature of feeding practices during the weaning process does complicate this model (e.g., Marquis et al., 1998). The rates of bone modeling and remodeling (following Frost, 1973a,b) will affect how quickly this newly formed tissue can be detected isotopically. For adults, bone turnover rates are estimated between 10–25 years (Libby et al., 1964; Stenhouse and Baxter, 1979; Manolagas, 2000; Parfitt, 1983). In general, both cortical and cancellous bone of the axial skeleton turns over more rapidly than in the appendicular skeleton (Parfitt, 2002, p. 808). There is very little information on bone formation rates for postpartum infants and children; however, data from Parfitt et al. (2000) and Fazzalari et al. (1997) indicate that modeling is very rapid. Once the body has equilibrated to the new diet (likely between 3–8 months), there will be a gradual increase in the $\delta^{15}\text{N}$ value (if the infant is breast-feeding), with neonates showing little to no enrichment in ^{15}N relative to adults. We would expect to find the 2–4‰ enrichment in ^{15}N that characterizes breastfed infants after the following two conditions are met: 1) the body equilibrates isotopically with breast milk, and 2) newly modeled bone forms a larger portion of the overall bone than tissues formed in utero. Individuals

TABLE 2. Isotopic values for age categories of all children younger than age 6 years, with data for females older than 18 years listed at end for comparison

Individual	Age at death ¹	$\delta^{13}\text{C}_{\text{col}}$ ‰	$\delta^{13}\text{C}_{\text{ap}}$ ‰	$\Delta^{13}\text{C}_{\text{ap-col}}$ ‰	$\delta^{15}\text{N}$ ‰	$\delta^{18}\text{O}$ ‰
<1 year						
MG 14/1b	Neonate	−6.0	−5.5	0.5	10.4	27.5
MG 11/5	2.5	−7.8	−7.9	0.1	11.9	27.3
SP 11/8-1	10.5	−6.0	−3.5	2.5	12.6	27.4
MG 14/6	6	−6.1	−4.5	1.6	10.7	28.1
<i>Mean</i>		−6.5	−5.4	1.2	11.4	27.6
<i>SD</i>		0.9	1.9	1.1	1.0	0.4
1–3 years						
MG 14/18	18	−6.0	−5.1	0.9	12.1	27.8
MG 14/21c	19	−5.6	−4.4	1.2	11.8	26.1
MG 11/4a	21	−6.2	−5.7	0.5	11.6	27.9
MG 14/13c	21	−6.8	−5.5	1.3	11.6	27.9
SP 17/6-4	30	−5.0	−5.0	0.0	13.0	26.5
<i>Mean</i>		−5.9	−5.2	0.8	12.0	27.2
<i>SD</i>		0.7	0.5	0.5	0.6	0.9
4–6 years²						
SP 2	54	−5.7	−4.8	0.9	10.3	26.6
MG 12/5	54	−6.0	−4.5	1.5	7.1	28.1
MG 204b	60	−7.0	−7.6	0.7	11.0	27.9
SP 11/2-3b	60	−6.8	−4.5	2.3	9.3	27.1
MG 14/28a	72	−7.7	−6.4	1.3	9.2	27.8
SP 11/2-1	72	−6.2	−4.4	1.8	10.2	26.6
<i>Mean</i>		−6.5	−5.4	1.4	9.5	27.3
<i>SD</i>		0.7	1.3	0.6	1.4	0.7
Females						
<i>Mean</i>		−7.6	−5.5	2.2	9.4	26.9
<i>SD</i>		1.2	1.6	0.9	0.8	0.6

¹ Ages are in months and represent the midpoints of estimated age ranges.

² There are no individuals 3–4 years of age.

aged 4–6 years are old enough for the body to have equilibrated with the weaning diet and for new bone formed during weaning to dominate earlier bone formed during breast-feeding. Consequently, the isotopic composition of bone from these individuals should approach the adult female mean. The bodies of individuals aged 18 years or older are in equilibrium with the “adult” diet. Because enough time has elapsed for bone formed from nutrients in the adult diet to replace or dominate bone formed from nutrients in the childhood diet, these individuals typify the adult diet.

High-protein diets may increase the rate of resorption of body proteins and bone turnover (Ambrose, 1993; Parkington, 1991). Because Marco Gonzalez and San Pedro diets contained large amounts of marine protein (Williams, 2000), turnover and resorption rates at these sites may have been faster than those found by other researchers (Balasse et al., 2001; Fogel et al., 1989; Herrscher, 2003). There do not appear to be significant differences in the rates of turnover for collagen and bioapatite in adults (Hedges, 2003), but it is unclear whether this is also true for children.

$\delta^{15}\text{N}$ values of bone collagen

The average $\delta^{15}\text{N}$ values for children are given in Figure 3 and Table 2. When the three age categories (<1 year, 1–3 years, and 4–6 years) are compared, there are statistically significant differences in $\delta^{15}\text{N}$ values ($F = 8.356$, $P < 0.005$). Children aged 1–3 years have the highest $\delta^{15}\text{N}$ values; all are at least 2.2‰ enriched in ^{15}N relative to the

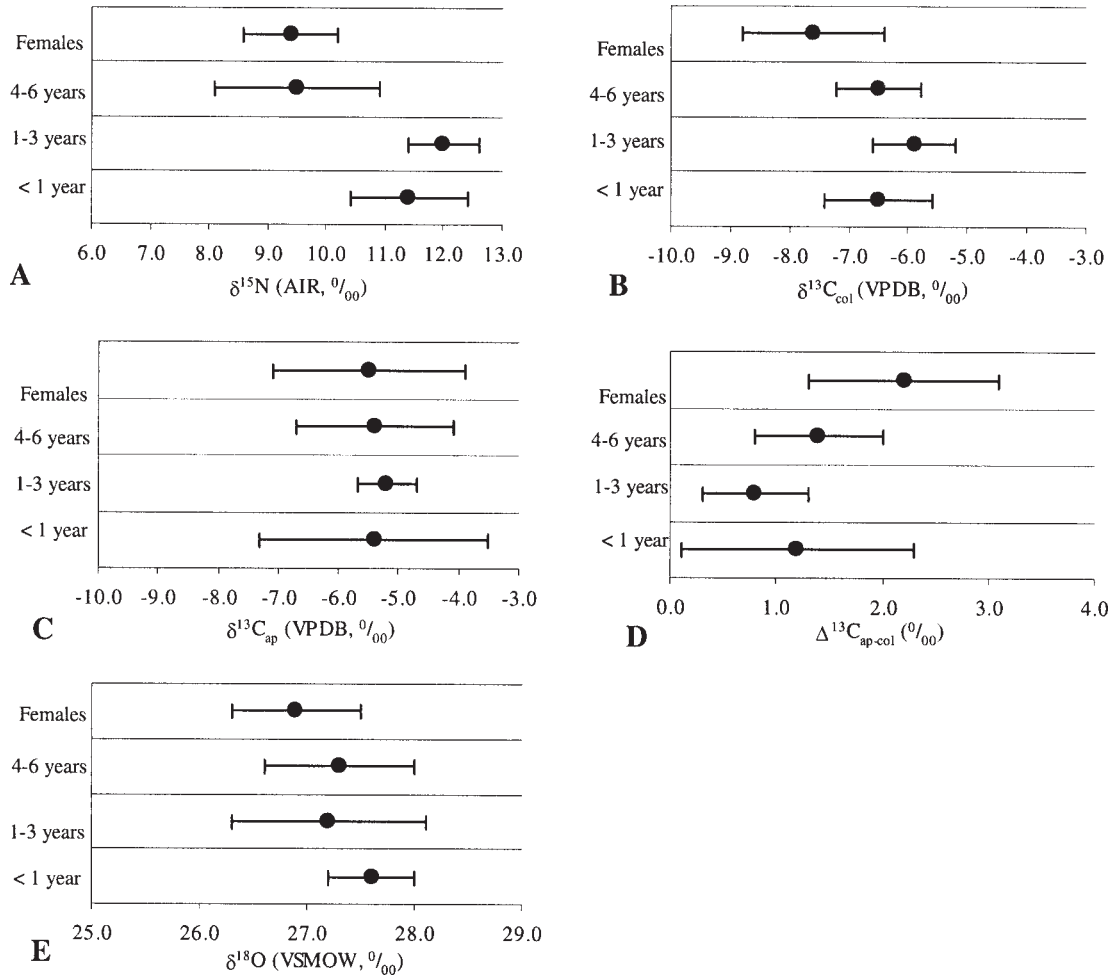


Fig. 4. Average isotopic values for children aged less than 1 year ($N = 4$), 1–3 years ($N = 5$), 4–6 years ($N = 6$), and adult females ($N = 22$). **A:** $\delta^{15}\text{N}$. **B:** $\delta^{13}\text{C}_{\text{col}}$. **C:** $\delta^{13}\text{C}_{\text{ap}}$. **D:** $\Delta^{13}\text{C}_{\text{ap-col}}$. **E:** $\delta^{18}\text{O}$.

female mean (Fig. 4A). This difference suggests that the body has equilibrated with breast milk, extra utero bone dominates in utero tissues, and all individuals were breast-feeding before death. For two individuals (MG 14/18, age = 18 months; SP 17/6-4, age = 30 months), enrichments in ^{15}N of 2.7‰ and 3.6‰ relative to the female mean indicate that weaning either had not begun or was started only shortly before death (Tab. 2). The remaining three individuals had slightly lower $\delta^{15}\text{N}$ values, suggesting that weaning had begun prior to their death.

For individuals younger than 1 year of age, there is one individual (SP 11/8-1) whose $\delta^{15}\text{N}$ value is enriched by 3.2‰ relative to the female mean, indicating breast-feeding at time of death (Fig. 3). This individual was 9–12 months of age, and would have been old enough for its body to equilibrate with breast milk and for bone formed extra utero to dominate tissues formed in utero. This is supported by the observation that, as a group, individuals younger than 1 year of age are enriched by 2‰ relative to the female mean (Fig. 4A). By comparison, MG 11/5, aged 2–3 months, is enriched in ^{15}N by 2.5‰ relative to the female mean. The elevated $\delta^{15}\text{N}$ value may reflect the incorporation of bone formed from nutrients in breast milk. However, equilibration of the body with breast milk before 2–3 months of age seems unlikely. Other explanations are: 1) a problem with the assigned age for this indi-

vidual, 2) an enrichment in ^{15}N related to pathology (Katzenberg and Lovell, 1999; White and Armelagos, 1997), or 3) the mother of MG 11/5 had an above-average $\delta^{15}\text{N}$ value. The latter explanation is possible, since the maximum $\delta^{15}\text{N}$ value for a female from Marco Gonzalez is 11.2‰. The neonate (MG 14/1b) and the 6-month-old (MG 14/6) are enriched in ^{15}N relative to the female mean. However, this enrichment does not meet the 2‰ that is expected for breast-feeding infants; this likely reflects the fact that they are not old enough for extra utero bone to be dominant in utero tissues.

As a group, the $\delta^{15}\text{N}$ values for individuals aged 4–6 years are virtually identical to the female mean (Fig. 4A). The $\delta^{15}\text{N}$ values for three individuals (MG 12/5, MG 14/28a, and SP 11/2-3b) aged 4–6 years (Fig. 3) are lower than the female mean, suggesting that they were completely weaned in the year(s) before death and their diet incorporated more C_4 plants (generally depleted of ^{15}N relative to marine resources) and/or less ^{15}N -enriched marine resources than adults. The $\delta^{15}\text{N}$ values of the other three individuals (SP 2, SP 11/2-1, and MG 204b) are enriched in ^{15}N relative to the female mean, suggesting that they were not completely weaned in the year(s) prior to death. Breast milk provided a significant portion of the diet until at least 2 years of age for the majority of children. The $\delta^{15}\text{N}$ values for the various age categories

between birth to 6 years indicate that weaning was probably a lengthy process beginning after the first year of life and ending in the third or fourth year.

$\delta^{13}\text{C}$ values of bone collagen

There are no significant differences in $\delta^{13}\text{C}_{\text{col}}$ values among the three age categories for children, but the means are all enriched in ^{13}C relative to the female mean (Fig. 4B, Table 2). As a group, children aged 1–3 years are the most enriched in ^{13}C , which is consistent with the nitrogen isotope data.

Ten of the 15 children are enriched in ^{13}C by at least 1.0‰ relative to the female mean; this corresponds to the 1.0‰ trophic level effect reported by others (Dupras et al., 2001; Richards et al., 2002; White et al., 2001). However, the carbon (collagen) isotope data do not correspond to the nitrogen isotope data for all children. There is no significant correlation between $\delta^{13}\text{C}_{\text{col}}$ and $\delta^{15}\text{N}$ values in children (Pearson's $r = 0.232$, $P = 0.405$, $N = 15$). However, for children aged 1–3 years, the correlation between $\delta^{13}\text{C}_{\text{col}}$ and $\delta^{15}\text{N}$ values approaches significance (Pearson's $r = -0.304$, $P = 0.075$, $N = 5$). This behavior suggests that for breast-feeding children who are old enough to have equilibrated to this diet, the carbon and nitrogen isotope values reflect trophic level effects. The weakest correlation between $\delta^{13}\text{C}_{\text{col}}$ and $\delta^{15}\text{N}$ values (Pearson's $r = -0.134$, $P = 0.801$, $N = 6$) is for 4–6-year-olds, who also exhibit the greatest variation in $\delta^{13}\text{C}_{\text{col}}$ values (–7.7 to –5.7‰). Both the discordance and variation are likely related to supplementation and weaning. Three of the four infants younger than 1 year old are at least 1.5‰ enriched in ^{13}C relative to adult females. The neonate has a $\delta^{13}\text{C}_{\text{col}}$ value that is 1.6‰ enriched in ^{13}C relative to the female mean, consistent with a trophic level effect. This, and the elevated $\delta^{15}\text{N}$ value, suggest consumption of breast milk by the neonate and a very fast equilibrium rate, as proposed by Richards et al. (2002).

$\delta^{13}\text{C}$ values of bone bioapatite

All children's age groups have $\delta^{13}\text{C}_{\text{ap}}$ values within 0.3–0.1‰ of the female mean and do not vary significantly from one another (Fig. 4C, Table 2). This supports the prediction that there should be very little change in $\delta^{13}\text{C}_{\text{ap}}$ values during the weaning process. The smallest variation in $\delta^{13}\text{C}_{\text{ap}}$ values is seen within the 1–3-year age category, indicating that these individuals were breast-feeding at death.

Differences in $\delta^{13}\text{C}$ values between bone bioapatite and bone collagen

There are no significant differences in mean $\Delta^{13}\text{C}_{\text{ap-col}}$ values among the three age categories for children (Fig. 4D, Table 2), and all are smaller than the female mean. This suggests a higher level of carnivory in the children, which is consistent with breast-feeding. There is a significant correlation between $\Delta^{13}\text{C}_{\text{ap-col}}$ and $\delta^{15}\text{N}$ values (Pearson's $r = -0.427$, $P = 0.008$, $N = 37$), which suggests that the two measures track similar variables (carnivory or a trophic level effect). The smallest $\Delta^{13}\text{C}_{\text{ap-col}}$ values were obtained for the 1–3-year-olds. This is suggestive of breast-feeding, which is consistent with the nitrogen and carbon (collagen) isotope data for this group. The greatest variation in $\Delta^{13}\text{C}_{\text{ap-col}}$ values occurs within infants younger than 1 year, but is largely due to one individual (SP 11/8-1) whose $\Delta^{13}\text{C}_{\text{ap-col}}$ value is 2.5‰, the largest of

all children under 18 years. The highest mean $\Delta^{13}\text{C}_{\text{ap-col}}$ value was for the 4–6-year-olds. This was expected: these individuals consume very little, if any, breast milk, and their values should more closely approximate the females' spacing.

$\delta^{18}\text{O}$ values of bone bioapatite

There are no significant differences in mean oxygen isotopic composition among the three age categories of children, but the means are all enriched in ^{18}O relative to the female mean (Fig. 4E, Table 2). There is no correlation between $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$ values in children (Pearson's $r = -0.281$, $P = 0.331$, $N = 15$). Therefore, the stable isotopes of oxygen do not record breast-feeding and weaning in the same way. Although both measures reflect trophic levels, $\delta^{15}\text{N}$ values reflect maternal protein resources, whereas $\delta^{18}\text{O}$ values reflect maternal water sources. Liquid supplementation during infancy (that may or may not be related to weaning) with environmental water would substantially reduce the trophic level effect in $\delta^{18}\text{O}$ values without affecting the $\delta^{15}\text{N}$ values.

CONCLUSIONS

This analysis used multiple lines of isotopic evidence from bone collagen and bone bioapatite to investigate weaning in two Postclassic Maya sites. There are significant differences in isotopic composition of bone between pre- and post-weaning age individuals. These differences reflect trophic level effects ($\delta^{15}\text{N}$, $\delta^{13}\text{C}_{\text{col}}$, $\delta^{18}\text{O}$); differences in macronutrient consumption with the introduction of a weaning food ($\delta^{13}\text{C}_{\text{col}}$, $\Delta^{13}\text{C}_{\text{ap-col}}$); and differences in water sources ($\delta^{18}\text{O}$). There is no significant difference in the $\delta^{13}\text{C}_{\text{ap}}$ values of children and adults because the total isotopic composition of carbon from breast milk is similar to the weaning food (maize). The 1–3-year age category exhibits the smallest variation for all isotopic measures except $\delta^{18}\text{O}$ values. Similarly, the mean values within the 1–3-year age categories most closely follow the predicted values for breast-feeding infants; 1) enrichment of ^{15}N values by 2–4‰ relative to adult females (Fogel et al., 1989); 2) enrichment of ^{13}C by 1.0‰ relative to adult females (DeNiro and Epstein, 1978); 3) $\Delta^{13}\text{C}_{\text{ap-col}}$ values that are smaller relative to adult females; 4) $\delta^{13}\text{C}_{\text{ap}}$ values that are similar to adult females, with very little variability; and 5) $\delta^{18}\text{O}$ values that are higher than those of adult females (Wright and Schwarcz, 1998). For children under 1 year of age and between 4–6 years, there is greater variation between individuals and less conformity with the isotopic compositions anticipated from theoretical considerations. This variability is related to individual differences in physiology and infant feeding behavior. The isotopic data indicate that breast milk, in addition to supplementary foods, was consumed until age 4 years by some individuals. Based on the 1–3-year age category, weaning appears to begin around 1 year of age and, based on the 4–6-year age category, is completed between 3–4 years of age. This conforms well to the ethnohistoric evidence for a weaning age of 3–4 years (Tozzer, 1941).

Perhaps the most significant finding of this study, however, is the usefulness of multiple isotopic measures to expand and refine our understanding of infant feeding behaviors. Data from both the organic and inorganic components of bone can be used to augment the timing of trophic level shifts by reconstructing changes in macronutrient (and possibly water) composition. This in turn pro-

vides greater detail about the weaning process that has broad applications in physical anthropology for understanding relationships between culture, health and morbidity patterns, growth and development, and environment.

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