Technical Note: Interpreting Stable Carbon Isotopes in Human Tooth Enamel: An Examination of Tissue Spacings From South Africa

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KEY WORDS paleodiet; diet reconstruction; bone apatite; collagen; Later Stone Age; coastal archeology

ABSTRACT Stable isotope analysis of skeletal tissues is widely used in archeology and paleoanthropology to reconstruct diet. In material that is poorly preserved or very old, the tissue of choice is frequently tooth enamel, since this is less susceptible to diagenesis. The relationships between carbon isotope ratios in tooth enamel ($\delta^{13}C_{enamel}$), bone collagen ($\delta^{13}C_{collagen}$), and bone apatite ($\delta^{13}C_{bone\ apatite}$) are, however, not well understood. To elucidate these, we have measured all three indicators in archeological humans from the western and southern Cape coastal regions of South Africa. The correlation between $\delta^{13}C_{enamel}$ and $\delta^{13}C_{collagen}$ is good (R^2 = 0.71 if two outliers are excluded, n = 79). The correlation between $\delta^{13}C_{enamel}$ and $\delta^{13}C_{bone\ apatite}$ is weaker (R^2= 0.37, n = 33) possibly due to bone diagenesis. No systematic offset between $\delta^{13}C_{bone\ apatite}$ and $\delta^{13}C_{enamel}$ was observed in this sample of archeological humans. Intertooth comparisons of $\delta^{13}C_{enamel}$ in three individuals showed little variation, despite the different ages of crown formation. Carbon isotope ratios in both enamel and bone collagen are good proxies for $\delta^{13}C_{diet}$. Am J Phys Anthropol 147:499–507, 2012.

Stable isotope analysis of mineralized human tissues (bones and teeth) is an important method of determining ancient diet. Recent studies have relied upon animal feeding experiments to assess the relationships between the carbon isotope values of bone collagen, bone apatite, and enamel apatite. $\delta^{13}C_{collagen}$ and $\delta^{13}C_{bone\ apatite}$ track different components of diet and so can profitably be used in conjunction with each other to reconstruct diet in more detail (Ambrose and Norr, 1993; Howland et al., 2003; Kellner and Schoeninger, 2007; Warinner and Tuross, 2009; Froehle et al., 2010). Unfortunately, bone apatite in archeological and paleontological specimens is frequently poorly preserved, particularly in the tropics and mid-latitudes; enamel apatite is therefore generally preferred for analysis (Koch et al., 1997; Krigbaum, 2003; Lee-Thorp and Sponheimer, 2003). There has, however, been little explicit discussion in the literature of the relationship between $\delta^{13}C_{enamel}$ and $\delta^{13}C_{bone\ apatite}$. It appears that most researchers have considered them to be equivalent since both are formed from carbonates and bicarbonates dissolved in the blood. Warinner and Tuross (2009) recently reported a significant isotopic difference between the two tissues in animal subjects, raising the question of whether this applies in humans. As enamel apatite is considerably more durable than bone apatite, it is necessary to explore more fully the relationships between $\delta^{13}C_{diet}$, $\delta^{13}C_{enamel}$, $\delta^{13}C_{collagen}$, and $\delta^{13}C_{bone\ apatite}$ if stable isotope analysis of archeological human populations is to be reliably extended into the more distant past. Understanding these relationships is also important in comparing isotope values from different body tissues to reconstruct dietary life histories. In this study, $\delta^{13}C_{enamel}$ values of archeological human skeletons from the southern and western Cape coastal regions of South Africa are compared with previously published $\delta^{13}C_{collagen}$ and $\delta^{13}C_{bone\ apatite}$ of the same individuals (Sealy, 1986, 1997, 2006, 2010; Sealy and van der Merwe, 1986, 1988; Lee-Thorp et al., 1989; Sealy et al., 1992; Sealy and Pfeiffer, 2000), to explore the relationships between these different signals.

ISOTOPIC PATTERNING OF THE STUDY AREA

In the geographical area covered in this article, patterning in terrestrial vegetation is complex. C_3 plants dominate the south-western Cape coast from the Namibian border to Cape Town (Fig. 1). These plants have very negative $\delta^{13}C$ values, averaging about −27‰ (O' Leary, 1988; Cerling et al., 1997), that are clearly differentiated from the enriched $\delta^{13}C$ values of marine foods, thus allowing the easy discernment of marine- versus terrestrial-based diets (Tauber, 1981; Schoeninger et al., 1983; Schoeninger and DeNiro, 1984; Sealy and van der Merwe, 1985, 1986, 1988). The southern Cape has more complex vegetation patterning, with the region between Cape Town and Mossel Bay having significant amounts of C_4 grass, while the area to the east, between Wilderness and the Tsitsikamma National Park, is dominated by C_3 vegetation (Mucina and Rutherford, 2006; Sealy,

Grant sponsor: National Research Foundation of South Africa; University of Cape Town.

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Received 4 March 2011; accepted 6 December 2011

DOI 10.1002/ajpa.22012

Published online 23 January 2012 in Wiley Online Library (wileyonlinelibrary.com).

Fig. 1. Map of the region with places mentioned in the text.

2010). Further east still, approaching Port Elizabeth and beyond, C_4 grass is an increasingly significant component of the vegetation. The carbon isotope signal of C_4 plants averages around −13‰ (O' Leary, 1988; Cerling et al., 1997), significantly more enriched than C_3 plants and closer to the value for marine foods. In regions with significant quantities of C_4 grass, $\delta^{15}N$ measurements of human skeletons are preferred over $\delta^{13}C$ as a means of differentiating between terrestrial and marine food sources (Schoeninger and DeNiro, 1984; Sealy 2006). Analysis of $\delta^{13}C$ and $\delta^{15}N$ in individual amino acids is a promising line of investigation (Corr et al., 2005, Styring et al., 2010) but requires further development.

Tooth enamel and bone formation

Tooth enamel is formed during only a limited part of an organisms' life, and thus the isotopic signal captured is that of the diet during the period of enamel formation. Mineralization of the permanent first molar begins at birth, while that of the third molar begins between 7 and 10 years of age and is completed between 12 and 16 years (Hillson, 1986). Because third molars are the last tooth to form, their isotopic signal best approximates that of adult diet. Bone, on the other hand, "turns over" so its isotopic values integrate diet over more of the individual's lifespan. Postadolescent bone collagen turnover has, however, recently been shown to be considerably slower than previously estimated (Libby et al., 1964; Hedges et al., 2007). For the present study, this is important because it means that enamel from the third molar and bone collagen (at least from weight-bearing long-bones) are both likely to include material laid down during adolescence, recording diet at approximately the same time.

Dietary macronutrients in collagen and apatite

There have been a number of studies of $\delta^{13}C$ and $\delta^{15}N$ in bone collagen and bone apatite among Holocene coastal populations from South Africa (Sealy and van der Merwe, 1985, 1986, 1988; Lee-Thorp et al., 1989; Sealy, 1997, 2006, 2010). The interpretation of $\delta^{13}C$ values is, however, complicated by poorly understood metabolic factors. Marine foods are normally rich in protein and in ^{13}C, while in this environment terrestrial plant foods are mostly C_3 and therefore depleted in ^{13}C. If bone collagen is synthesized primarily from dietary protein, as proposed by Krueger and Sullivan (1984), very positive $\delta^{13}C_{collagen}$ values in coastal human skeletons may derive mainly from the marine component of the diet, at the expense of carbohydrate-rich foods such as corms and tubers (Lee-Thorp et al., 1989; Parkington, 1991). Carbohydrates are more likely to be metabolized for energy, via the glycolysis cycle, and the carbon converted into carbonate and bicarbonate in the blood. This is the raw material from which bone and tooth enamel apatite is synthesized. Several controlled feeding experiments have tested these proposed relationships and confirmed the preferential (but not exclusive) routing of dietary protein to bone collagen, while apatite carbonate appears to be a better reflection of whole diet (Ambrose and Norr, 1993; Tieszen and Fagre, 1993; Howland et al., 2003; Jim et al., 2004).

Additional information can be obtained by analyzing both collagen and bone apatite, and by considering the collagen-apatite spacing ($\Delta^{13}C_{collagen\text{-}bone\ apatite}$). Lee-Thorp et al. (1989) interpreted patterning in the $\Delta^{13}C_{collagen\text{-}bone\ apatite}$ values as relating to trophic level differences and reported $\Delta^{13}C_{collagen\text{-}bone\ apatite}$ values of 6.8‰ ± 1.4‰ for herbivores and 4.3‰ ± 1.0‰ for carnivores, with omnivores falling in between at 5.2‰ ± 0.8‰. For humans from the south-western Cape coast, the spacing was unexpectedly small, at 2.6‰ ± 0.97‰, and varied according to the nature of the diet. Extremely low $\Delta^{13}C_{collagen\text{-}bone\ apatite}$ occurred most often in individuals with positive $\delta^{13}C_{collagen}$. This was interpreted as indicating the consumption of energy-rich foods with depleted $\delta^{13}C$ values such as C_3 corms, tubers and marine mammal fats, in combination with ^{13}C enriched marine protein foods. The correlation was, however, not very strong: in a plot of $\Delta^{13}C_{collagen\text{-}bone\ apatite}$ against $\delta^{13}C_{collagen}$, R^2 was 0.39. There is, therefore, considerable complexity here. A subsequent study (Sealy, 1997) on the southern coast of South Africa reported a larger $\Delta^{13}C_{collagen\text{-}bone\ apatite}$ for humans: 3.7‰ ± 1.0‰.

$\Delta^{13}C_{collagen\text{-}bone\ apatite}$ is used to reveal dietary components that are either under- or overrepresented in only one tissue. One model suggests that if $\Delta^{13}C_{collagen\text{-}bone\ apatite}$ is less than 4.4‰ then dietary protein is enriched in ^{13}C compared with the whole diet, e.g., a diet of marine protein and C_3 carbohydrates. If $\Delta^{13}C_{collagen\text{-}bone\ apatite}$ is greater than 4.4‰ then the diet is likely to have included C_4 carbohydrates and C_3 protein (Ambrose et al., 1997). $\Delta^{13}C_{collagen\text{-}bone\ apatite}$ has also been used to assess the individual combinations of C_3/C_4 foods in the diet and the relative contributions of the protein and "energy" components of the diet to different body tissues (Harrison and Katzenberg, 2003).

Kellner and Schoeninger (2007) used data from four animal feeding studies (Ambrose and Norr, 1993; Tieszen and Fagre, 1993; Howland et al., 2003; Jim et al., 2004—the first two based on rodents) to assess the relationships between $\delta^{13}C_{collagen}$, $\delta^{13}C_{\ bone\ apatite}$, $\Delta^{13}C_{collagen\text{-}bone\ apatite}$ and diet. They argue that the nature of the protein source (C_3 or C_4), rather than factors such as trophic position or body size, drives patterning between the variables. However, conclusions drawn from

experimental feeding studies are limited by a number of factors including metabolic differences between animals and humans and the dietary sources of the macronutrients in the animal feed (Warinner and Tuross, 2009; Froehle et al., 2010). An experimental feeding study of pigs found that $\delta^{13}C_{collagen}$, $\delta^{13}C_{bone\ apatite}$ and $\delta^{13}C_{enamel}$ all correlate well with $\delta^{13}C_{diet}$, suggesting that any of these values can be used in dietary reconstruction (Warinner and Tuross, 2009). This study also found $\delta^{13}C_{enamel}$ to be more positive than $\delta^{13}C_{bone\ apatite}$ by (on average) 2.3‰. However, Clementz et al. (2007) found a greater range of $\Delta^{13}C_{enamel\text{-}bone\ apatite}$ among marine mammals, with some species having consistently positive $\Delta^{13}C_{enamel\text{-}bone\ apatite}$ (sea otters, sirenians, harbor porpoises) while seals had consistently negative $\Delta^{13}C_{enamel\text{-}bone\ apatite}$. Discrepancies were attributed to differences in seasonality of diet and ages of tooth formation in different species.

Although Warinner and Tuross suggest that pigs compare well with humans, there are to our knowledge no data on $\Delta^{13}C_{enamel\text{-}bone\ apatite}$ in humans and it is thus unclear whether research on the relationship between diet and bone apatite will hold true for enamel apatite. To investigate relationships between $\delta^{13}C_{enamel}$, $\delta^{13}C_{collagen}$ and $\delta^{13}C_{bone\ apatite}$ in humans, $\delta^{13}C_{enamel}$ was measured for a number of skeletons from coastal regions of South Africa for which $\delta^{13}C_{collagen}$ and, in some cases, $\delta^{13}C_{bone\ apatite}$ values were already available. In this region, food production in the form of herding of domesticated animals began only c. 2000 BP. Skeletons older than 2000 BP are, therefore, the remains of hunter–gatherers, while more recent individuals may have been hunter–gatherers or herders. Crop-farming was practiced only after European settlement in the 17th century C.E., which postdates all the skeletons studied here. Coastal hunters and herders ate mixed diets incorporating seafood and terrestrial animal and plant foods.

MATERIALS AND METHODS

Sampling strategy

Eighty-one adult archeological skeletons from the western and southern coasts of South Africa were sampled for this study. Forty are curated at the University of Cape Town Medical School and 30 at Iziko/South African Museum. The remaining 11 are recently recovered skeletons currently housed in the Department of Archeology at the University of Cape Town. Third molars were sampled, if available, failing which a second, or in two cases, a first molar was sampled instead. Mandibular teeth were preferred. For three individuals, samples were taken from all eight teeth on one side of a mandible (I1—M3) to assess intraindividual variation.

Enamel was sampled from either the buccal or lingual surface of the tooth. To obtain an average of diet over several years, each tooth was drilled along a vertical line from the neck to the occlusal surface, using a mounted Dremel variable speed drill with a 0.5 mm diamond tip. The resultant enamel powder was collected on clean laboratory weighing paper, and transferred into a microcentrifuge tube. On some specimens it was difficult to obtain a sample by drilling, either because the teeth were very brittle, or because vibrations from the drill dislodged sand and other contaminants that could not be separated from the enamel powder. In these cases, a piece of enamel was broken off and manually ground using a carbonate-free agate pestle and mortar. A minimum of 5 mg of enamel was drilled from each tooth, to ensure that 2 mg of pretreated powder would be available for analysis. Care was taken to ensure the sample was not contaminated by sand, glue, varnish, plaque, or dentine.

TABLE 1. $\delta^{13}C_{enamel}$ for half a dental arcade in three individuals

UCT no.	Tooth sampled	$\delta^{13}C_{enamel}$
Swartriet, Vredenburg Peninsula (−11.87)		
11361	RI_1	−11.3
11362	RI_2	−12.2
11363	RC	−11.3
11364	RPM_1	−12.1
11365	RPM_2	−12.1
13437	RM_1	−12.5
13438	RM_2	−11.9
11368	RM_3	−11.6
Stompneusbaai (−8.69)		
13372	RM_3	−8.1
13439	RI_1	−8.9
13440	RI_2	−9.7
13441	RC	−8.7
13442	RPM_1	−8.5
13443	RPM_2	−8.2
13444	RM_1	−9.1
13445	RM_2	−8.5
13446	RM_3	−8.2
Atlantis Rd burial 1 (−11.31)		
13374	RM_3	−11.8
13447	LI_1	−11.0
13448	LI_2	−11.2
13449	LC	−10.8
13450	LPM_1	−11.5
13451	LPM_2	−11.9
13452	LM_1	−11.2
13453	LM_2	−11.1
13454	LM_3	−11.6

Values in brackets are averages for each individual. These skeletons are recent finds that do not yet have collection accession numbers.

Laboratory methods

Enamel powders were prepared according to the method of Lee-Thorp et al. (1997), with some modifications. Powders were placed in a 1.5 ml microcentrifuge tube and reacted in a 1:1 mixture of 3.5% sodium hypochlorite and distilled water for 45–60 min to remove organics. The samples were then washed to a neutral pH by rinsing three times with distilled water, and then reacted with 0.1M acetic acid for 15 min, to remove soluble diagenetic carbonates. They were again rinsed three times and then freeze dried.

Two milligrams of pretreated powder were weighed into a 12 ml borosilicate glass tube which had previously been thoroughly cleaned with phosphoric acid, rinsed several times with distilled water, dried, and then flushed with helium. Approximately 0.2 ml of 100% phosphoric acid was added manually through the septum via a syringe, and allowed to react with the powder at a constant temperature of 72°C for a minimum of 3 h in a Thermo Finnigan (Germany) model II gasbench. The CO_2 gas evolved was passed through a Nafion "Poraplot Q" GC column, and the carbon and oxygen isotope ratios measured in a Finnigan Mat 252 isotope ratio mass spectrometer using Isodat software. The laboratory reference gas was commercially available CO_2 (99.995% purity). The results were calibrated using Carrara-

TABLE 2. $\delta^{13}C_{enamel}$, $\delta^{13}C_{collagen}$, and $\delta^{13}C_{bone\ apatite}$ for all skeletons in this study, with radiocarbon date and tooth sampled

Accession no.	UCT no.	Locality	Radiocarbon date (BP)	Tooth sampled	$\delta^{13}C_{enamel}$	$\delta^{13}C_{collagen}$	$\delta^{13}C_{bone\ apatite}$
Western Cape coast—Cape Town to Namibian border							
					(−10.1)	(−14.0)	(−11.4)
SAM-AP 6020	13659	Saldanha[1,2,3,5,7,15]	Pta-4189 620 ± 30	LM_3	−12.4	−15.4	−13.1
SAM-AP 1863	13634	Cape Point[3,5,7,15]	Pta-4708 800 ± 50	LM_3	−8.3	−10.9	−7.7
UCT 60	13378	Saldanha[3,4,5,7,15]	Pta-2005 955 ± 50	RM^3	−10.5	−14.6	
UCT 230	13391	Melkbosch[3,5,15]	Pta-4736 1110 ± 50	RM_3	−12.0	−16.6	
SAM-AP 6063	13643	Saldanha[3,5,7,15]	Pta-4279 1170 ± 30	RM_3	−10.4	−12.9	−10.6
SAM-AP 1247a	13632	Blouberg[1,2,3,5,7,15]	Pta-4281 1180 ± 50	LM_3	−11.2	−15.2	−12.6
UCT 579	13375	Somnaas[14]	GX-32527 1250 ± 70	LM_3	−8.2	−12.8	
SAM-AP 6075	13657	Saldanha[1,2,3,5,7,15]	Pta-4186 1330 ± 40	RM_3	−13.0	−15.0	−10.9
SAM-AP 5034	13645	Hout Bay[1,2,3,5,7,15]	Pta-4771 1390 ± 40	RM_3	−10.3	−16.0	−12.3
UCT 97	13379	Kommetjie[3,4,5,15]	Pta-4828 1560 ± 40	LM_3	−9.2	−11.8	
UCT 55	13377	Bloubergstrand[1,2]	GrA-23075 1680 ± 40	LM_3	−12.4	−17.2	
SAM-AP 4630	13651	Sandy Bay[1,2,3,5,7,15]	GX13178 1775 ± 80	LM_3	−10.2	−15.4	−12.3
UCT 429	13396	Elandsbaai[1,2,5]	Pta-8814 1870 ± 35	RM_2	−11.6	−15.0	
UCT 120	13382	Llandudno[4,5]	Pta-5677 1960 ± 50	RM_3	−10.3	−13.4	
SAM-AP 6083	13644	Milnerton[3,5,7,15]	Pta-4358 2000 ± 50	LM_2	−11.6	−13.4	−10.9
SAM-AP 5041	13649	Melkbosch[1,2,3,5,7,15]	Pta-4376 2010 ± 50	RM_3	−13.4	−17.9	−13.5
UCT 387	13394	Faraoskop[6]	GrA-23218 2055 ± 40	LM_3	−13.3	−18.7	
UCT 220	13386	Bloubergstrand[1,2,3,5]	Pta-5678 2100 ± 21	RM_2	−8.8	−11.5	
SAM-AP 4636	13648	Blouberg[1,2,3,5,7,15]	Pta-4379 2130 ± 45	RM_3	−9.1	−13.5	−11.4
SAM-AP 4813	13646	Darling[3,5,7,15]	Pta-4204 2140 ± 45	RM_3	−10.7	−14.9	−11.6
SAM-AP 5082	13654	Hout Bay[1,2,3,5,7,15]	Pta-4199 2150 ± 60	RM_2	−6.1	−11.6	−9.9
SAM-AP 1441	13633	Melkbosch[1,2,3,5,7,15]	Pta-4201 2170 ± 60	LM_3	−10.7	−13.0	−11.9
	13368	Swartriet[a]	GX-32517 2180 ± 60	RM_3	−11.6	−16.0	
UCT 134	13383	Llandudno[1,2]	GrA-23226 2210 ± 40	LM^3	−8.5	−12.1	
UCT 436	13397	Langebaan[1,2]	Pta-8751 2240 ± 60	RM_3	−13.0	−17.4	
UCT 164	13384/13385	Kleinzee[14]	Pta-8750 2360 ± 30	L/RM_3	−8.1	−12.6	
UCT 224	13387	Elandsbaai[3,5,15,16]	OxA-455 2400 ± 100	LM^3	−9.6	−13.9	
SAM-AP 1157	13631	Blouberg[3,5,7,15]	Pta-4217 2420 ± 60	LM_3	−9.6	−13.8	−11.7
SAM-AP 4899	13650	Saldanha[1,2,3,5,7,15]	Pta-4149 2440 ± 60	RM_1	−10.7	−14.2	−11.6
SAM-AP 4935	13647	Stompneusbaai[3,5,7,15]	Pta-4275 2540 ± 50	LM_3	−7.5	−12.7	−10.9
	13360	Hanna's Baai[b]	GX-32520 2570 ± 70	RM_3	−8.1	−12.3	
SAM-AP 5095	13653	Saldanha[1,2,3,5,7,15]	Pta-4674 2660 ± 70	LM_3	−9.8	−13.2	−10.8
UCT 445	13398	Groenrivier[8,11,14]	Pta-5617 2720 ± 60	LM_3	−10.2	−14.8	
UCT 248a	13422	Noordhoek[3,5,15]	GX-13185 2730 ± 95	M3?	−11.6	−14.2	
UCT 421	13395	Darling[1,2]	GrA-23217 2895 ± 45	LM_3	−9.2	−12.8	
UCT 343	13392	Simonstown[1,2]	GrA-23221 2985 ± 45	LM^2	−12.6	−14.7	
UCT 229	13390	Melkbosch[3,5,7,15]	Pta-928 3220 ± 54	RM_3	−6.7	−12.1	−9.0
UCT 373	13393	Elandsbaai[3,15]	Pta-1754 3835 ± 50	RM_3	−10.1	−14.0	
UCT 112	13380	Darling[3,4,5,15]	Pta-2003 4445 ± 50	RM_3	−8.6	−11.2	
SAM-AP 5068	13658	Ysterfontein[3,5,7,15]	Pta-4370 5680 ± 70	RM_2	−9.7	−14.6	−11.8
SAM-AP 37	13630	Blouberg[3,5,7,15]	Pta-4353 6120 ± 70	M3?	−10.2	−14.9	−11.5
UCT 113	13381	Darling[3,4,5,15]	no date	RM^3	−9.0	−13.5	
	13359	Bloubergstrand[c]	no date	LM_3	−8.1	−12.7	
	13371	Bloubergstrand[d]	no date	RM_3	−10.8	−13.8	
	13372/13446	Stompneusbaai[e]	no date	RM_3	−8.2	−12.1	
	13373	Melkbosch[f]	no date	LM_3	−8.4	−12.9	
	13374/13454	Atlantis Rd[g]	no date	RM_3	−11.7	−16.8	
Southern Cape coast: Cape Town to Mossel Bay plus Humansdorp and Cape St Francis							
					(−8.7)	(−12.5)	(−9.9)
UCT 583	13415	Voelvlei[1,2,10,17]	Pta-8760 560 ± 45	RM_3	−8.4	−11.6	
UCT 67a	13423	Wilderness[9,10,12]	Pta-6821 570 ± 45	LM^2	−7.6	−11.3	
UCT 114	13405	Cape St Francis[1,2]	GrA-23654 650 ± 40	RM^2	−8.2	−9.5	
UCT 83	13403	Cape St Francis[1,2]	GrA-23227 680 ± 40	LM^3	−8.3	−12.4	
UCT 582	13414	Voelvlei[1,2,10,17]	Pta-7178 740 ± 40	RM_3	−7.5	−11.7	
UCT 70	13404	Bredasdorp[1,2,10]	GrA-23074 920 ± 40	LM^3	−7.0	−10.5	
UCT 75	13399	Bredasdorp[1,2,10]	GrA-23069 1340 ± 40	LM_3	−5.2	−10.6	
UCT 109	13402	Humansdorp[1,2]	GrA-23656 1590 ± 50	RM^3	−8.5	−13.4	
SAM-AP 4825	13655	Humansdorp[5,9,10]	Pta-6607 2060 ± 50	RM_2	−11.1	−14.3	−10.9
UCT 78	13400	Cape St Francis[1,2]	GrA-23241 2145 ± 45	$LM_{3/2}$	−10.2	−13.6	
SAM-AP 4210	13652	Humansdorp[5,9,10]	Pta-6654 3760 ± 60	RM^3	−10.2	−13.8	−8.8
UCT 323	13411	Blombos[10]	Pta-8794 6430 ± 80	LM_3	−10.4	−16.4	
SAM-AP 4828	13656	Humansdorp[5,9,10]	Pta-6605 9830 ± 90	LM^3	−10.1	−14.0	−9.9
Southern Cape coast: Wilderness to Tsitsikamma National Park							
					(−9.7)	(−13.3)	(−9.8)
UCT 262	13410	Oakhurst[1,2,6, 9, 10, 12,13]	GrA-23221 510 ± 40	LM_3	−5.8	−9.4	
UCT 254	13409	Plettenberg Bay[5,9,10,12]	Pta-6820 1270 ± 50	RM_3	−11.8	−15.7	−11.9

TABLE 2. (Continued)

Accession no.	UCT no.	Locality	Radiocarbon date (BP)	Tooth sampled	$\delta^{13}C_{enamel}$	$\delta^{13}C_{collagen}$	$\delta^{13}C_{bone\ apatite}$
SAM-AP 1878a	13640	Robberg[5,9,10,12]	Pta-6592 2170 ± 20	LM_2	−9.8	−13.0	−10.9
SAM-AP 1146	13639	Robberg[5,9,10,12]	Pta-6646 2240 ± 20	RM^3	−11.4	−14.7	−11.5
UCT 107	13401	Knysna[5,9,10,12]	Pta-6815 2290 ± 50	RM_3	−9.6	−13.3	−9.2
SAM-AP 34	13642	Touws River Mouth[5,9,10]	Pta-6599 2310 ± 25	RM_3	−12.3	−15.4	−12.1
SAM-AP 1893	13636	Robberg[5,9,10,12]	Pta-6613 2360 ± 20	LM^1	−7.3	−11.7	−9.1
UCT 345	13412	Nelson Bay Cave[9,10,12]	~2750	LM^3	−10.8	−12.5	
	13370	Noetzie 2[10]	UGAMS-2798 3190 ± 40	LM_3	−10.3	−14.5	
SAM-AP 1145	13637	Robberg[1,2,5,9,10,12]	Pta-2284 3210 ± 70	RM_2	−11.7	−12.5	−9.7
UCT 347	13413	Nelson Bay Cave[5,9,10,12]	OxAV2055–35 3236 ± 33	LM^3	−9.5	−14.3	
SAM-AP 1871	13635	Robberg[1,2,5,9,10,12]	Pta-2273 3310 ± 60	LM_2	−8.8	−12.4	−8.3
SAM-AP 1879	13638	Robberg[1,2,5,9,10,12]	Pta-2283 3440 ± 60	LM_3	−7.8	−11.1	−7.7
UCT 161	13407	Plettenberg Bay[1,2,10,12]	OxAV2064–54 3541 ± 26	RM_2	−7.7	−12.1	
	13369	Noetzie 1[10]	UGAMS-2797 3800 ± 40	RM_3	−7.9	−12.9	
SAM-AP 3021	13641	Robberg[5,9,10,12]	Pta-6595 4030 ± 60	LM_3	−10.7	−12.0	−8.9
UCT 191/203	13424	Oakhurst[5,6,9,10,12,13]	Pta-4431 4100 ± 60	LM^2	−9.3	−17.9	
UCT 209	13408	Oakhurst[5,6,9,10,12,13]	Pta-4348 4880 ± 70	M3?	−10.7	−13.4	
UCT 214	13425	Oakhurst[5,6,9,10,12,13]	Pta-4467 4900 ± 60	LM_3	−10.4	−14.4	

References: (1) Stynder (2006); (2) Stynder et al. (2007); (3) Sealy (1989); (4) Hausman (1980); (5) Sealy (1997); (6) Sealy et al. (1992); (7) Lee-Thorp et al. (1989); (8) Sealy (1986); (9) Sealy and Pfeiffer (2000); (10) Sealy (2010); (11) Jerardino et al. (1992); (12) Sealy (2006); (13) Patrick (1989); (14) Dewar (2008); (15) Sealy and van der Merwe (1988); (16) Sealy and van der Merwe (1986); (17) Morris et al. (2004–2005).

Accession No. is the number of the skeleton in the UCT Medical School or Iziko/South African Museum collections. UCT no. is the number of the enamel sample in the register in the stable light isotope laboratory. $\delta^{13}C_{enamel}$ for UCT 164 is an average of two determinations (−8.6 and −7.5 for RM_3 and LM_3 respectively). For specimens from Stompneusbaai and Atlantis Rd Burial 1 reported in Table 1, both values for third molars have been averaged. The averages for each region are presented in brackets.

Further identifying information:

[a] Vredenburg Peninsula.
[b] Fish factory, St Helena.
[c] 5 Gen. Jansens Rd.
[d] 17 Moolman Str.
[e] 32°43′24.2″S; 17°58′31.5′E.
[f] 33°17′9478S; 18°43′7496E.
[g] 33°37.74′S; 18°26.42′E.

Marmor, Cavendish Marble, NBS 18 and NBS 19. Results are reported relative to VPDB, expressed in delta notation (δ) in parts per thousand (‰). The standard deviations for repeated measurements of a homogenous material were better than 0.06 for $\delta^{13}C$.

Six recently recovered skeletons lacked $\delta^{13}C_{collagen}$ values (individuals without collection accession numbers in Table 1, and identified by superscripts b–g). Collagen was prepared from samples of rib bone according to methods described by Sealy (1997). Rib fragments were surface-cleaned using fine sand-paper, compressed air and where necessary, by stripping out rootlets using tweezers. They were left for several days in a solution of ~2.5% HCl until decalcified (soft and translucent). At this stage, any remaining rootlets were removed with tweezers. The samples were washed with distilled water and left in 0.1M NaOH overnight to remove humic contaminants. They were then left to soak for several days in distilled water, changed daily, before being freeze dried.

About 0.45 to 0.65 mg of collagen was weighed into a tin cup and folded tightly to exclude air. Samples were loaded into an automated Thermo Finnigan Flash EA 1112 series elemental analyzer and combusted at 1020°C to produce CO_2 and N_2 gas. These were purified before being introduced to a ThermoElectron Delta Plus XP isotope ratio mass spectrometer via a Conflo III gas control unit, using helium as a carrier gas. Each sample was analyzed in duplicate. Carbon isotope values are reported relative to VPDB, expressed in delta notation (δ) in parts per thousand (‰). Standard deviations of repeated measurements of standard materials were <0.1‰.

RESULTS AND DISCUSSION

Table 1 presents $\delta^{13}C_{enamel}$ for each tooth along half a dental arcade for each of three individuals. The range of values within each mouth varies from 1.1 to 1.6‰, comparable to the intraindividual "background variation" in $\delta^{13}C_{collagen}$, estimated by DeNiro and Schoeninger (1983) at approximately ±1‰. There are no consistent differences between $\delta^{13}C_{enamel}$ of early- compared with late-forming teeth; in other words, we cannot detect a weaning effect. Trophic level effects in $\delta^{13}C$ are small: Fuller et al. (2003) documented a shift of only 1.2‰ ± 0.4‰ between dentine of early and late forming teeth. This degree of variation may simply be subsumed within the range of values we see within each mouth. We do not know whether diet was uniform throughout life, but this study provides an estimate of the degree of intertooth (intraindividual) isotopic variation, which is substantially less than the intrapopulation variation reported below. In these three individuals, any tooth could be used for dietary reconstruction.

$\delta^{13}C_{enamel}$, $\delta^{13}C_{collagen}$, and $\delta^{13}C_{bone\ apatite}$ for all skeletons in this study are presented in Table 2. The specimens are divided into three geographical regions: i) the west coast from the Namibian border southward to Cape Town, where vegetation is mostly C_3, ii) the southern Cape coast from Cape Town to Mossel Bay, as well as

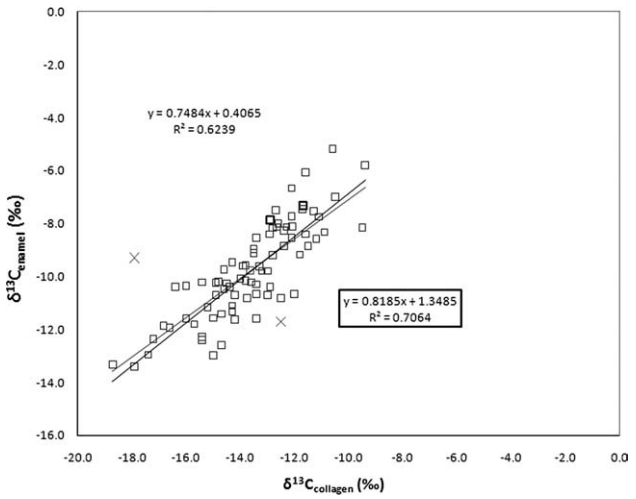

Fig. 2. $\delta^{13}C_{enamel}$ plotted against $\delta^{13}C_{collagen}$. The unboxed regression equation is for the entire dataset, that in the box excludes the two outliers UCT 191/203 and SAM-AP 1145 (marked by crosses).

Humansdorp/Cape St Francis, just west of Port Elizabeth. These areas have terrestrial vegetation that includes significant quantities of C_4 grass, and iii) the southern Cape coast between Wilderness and the Tsitsikamma National Park, where the terrestrial vegetation is predominantly C_3.

$\delta^{13}C_{enamel}$ values for the entire sample set range from -13.4 to -5.2‰, $\delta^{13}C_{collagen}$ from -18.7 to -9.4‰. $\delta^{13}C_{bone\ apatite}$ has been measured for only 33 skeletons, and ranges from -13.5 to -7.7‰. A plot of $\delta^{13}C_{enamel}$ versus $\delta^{13}C_{collagen}$ (Fig. 2) shows a reasonably close correlation between the two ($R^2 = 0.62$). R^2 increases to 0.71 if two outliers (Oakhurst 191/203 and SAM-AP 1145) are excluded.[1]

If the sample is broken down into three geographical regions, the correlations do not differ significantly: in the C_3 dominated region of the western Cape coast the regression equation is $y = 0.8106x + 1.2498$, $R^2 = 0.69$, $n = 48$. In the area with a significant amount of terrestrial C_4 vegetation, from Cape Town to Mossel Bay, the regression equation is $y = 0.703x + 0.1604$, $R^2 = 0.64$, $n = 13$. In the C_3 dominated area of the south coast, from Wilderness to Tsitsikamma, the regression equation is $y = 0.9092x + 2.3554$, $R^2 = 0.70$, $n = 17$. The regression lines were tested for homogeneity across the groups and no significant differences were observed. This issue does, however, warrant further research considering the small size of this dataset. With a larger sample, a different relationship may be observed in the area with more C_4 grasses.

The average $\Delta^{13}C_{collagen\text{-}enamel}$ for the dataset presented here is 3.8 ± 1.0‰ (range 1.3–6.0‰, $n = 79$), with no significant differences emerging if the results are subdivided according to region or radiocarbon date. A comparison of this data set with $\Delta^{13}C_{collagen\text{-}bone\ apatite}$ reported by Lee-Thorp et al. (1989) for the west coast of South Africa, and Sealy (1997) for the south coast using univariate analysis of variance (ANOVA) indicates statistically significant differences between the three groups ($P < 0.001$). The means of the three groups were then compared with one another using t-tests to assess the significance of the observed differences. $\Delta^{13}C_{collagen\text{-}enamel}$ is significantly different from $\Delta^{13}C_{collagen\text{-}bone\ apatite}$ of 2.6‰ ± 1.0‰ ($n = 35$) reported for skeletons from the western Cape coast by Lee-Thorp et al. (1989), ($t = 5.7$; $P < 0.001$), but not significantly different from $\Delta^{13}C_{collagen\text{-}bone\ apatite}$ of 3.7‰ ± 1.0‰ ($n = 58$) on the southern Cape coast (Sealy, 1997), ($t = 1.0$, $P = 0.3$). Harrison and Katzenberg (2003) reported $\Delta^{13}C_{collagen\text{-}bone\ apatite}$ of 2.7‰ ± 0.7‰ ($n = 31$) for coastal fisher–gatherers from San Nicolas Island, California, very similar to the findings of Lee-Thorp et al. (1989). Overall, it seems that $\Delta^{13}C_{collagen\text{-}enamel}$ cannot simply be equated with $\Delta^{13}C_{collagen\text{-}bone\ apatite}$.

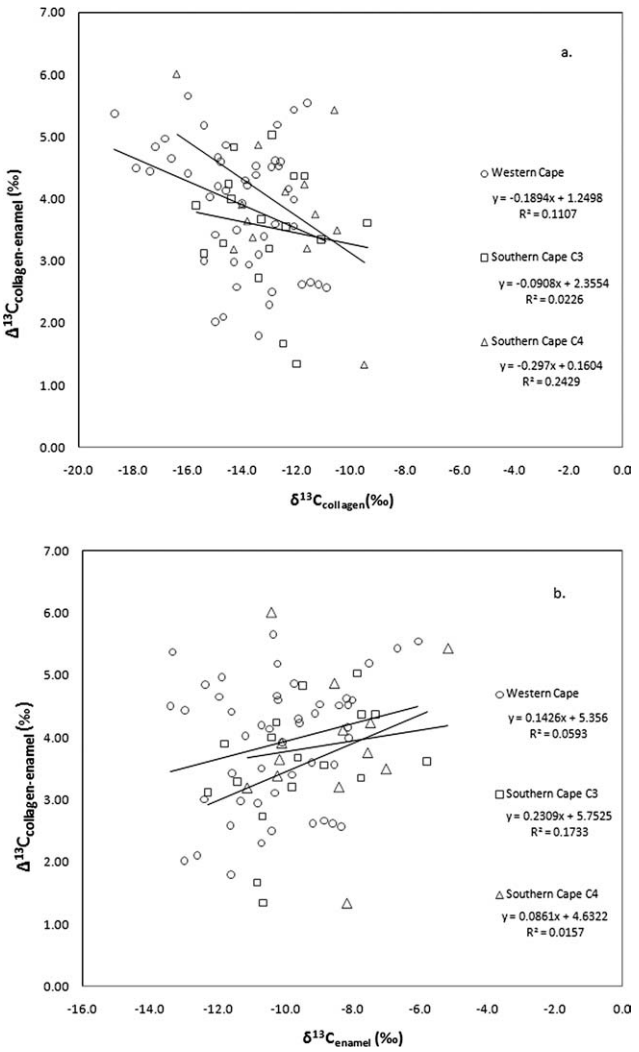

Fig. 3. $\Delta^{13}C_{collagen\text{-}enamel}$ plotted against (a) $\delta^{13}C_{collagen}$ and (b) $\delta^{13}C_{enamel}$, according to geographic region.

Figure 3a shows $\Delta^{13}C_{collagen\text{-}enamel}$ plotted against $\delta^{13}C_{collagen}$ for the skeletons in this study. There is no strong relationship for the data set as a whole, nor within any of the three subregions (all $R^2 < 0.25$). A plot of $\Delta^{13}C_{collagen\text{-}enamel}$ against $\delta^{13}C_{enamel}$ (Fig. 3b) shows even poorer correlations (all $R^2 < 0.2$). This contrasts with the findings of Lee-Thorp et al. (1989), where

[1]Note that these outliers change all relationships disproportionately and have been omitted from calculations of averages and standard deviations, except where noted otherwise.

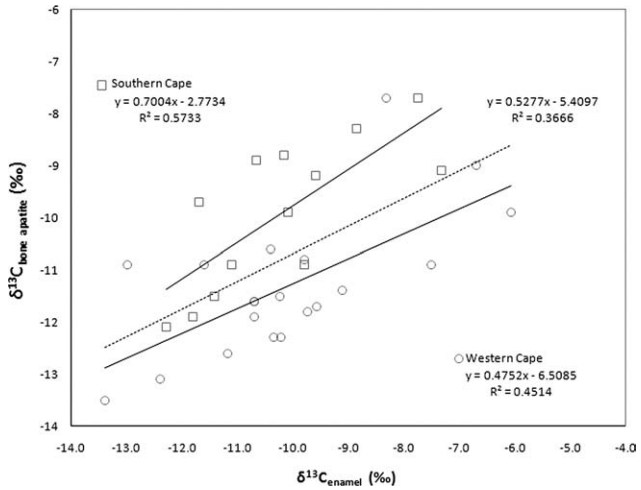

Fig. 4. $\delta^{13}C_{bone\ apatite}$ plotted against $\delta^{13}C_{enamel}$, distinguished by region. The dashed line is the regression line for the entire dataset.

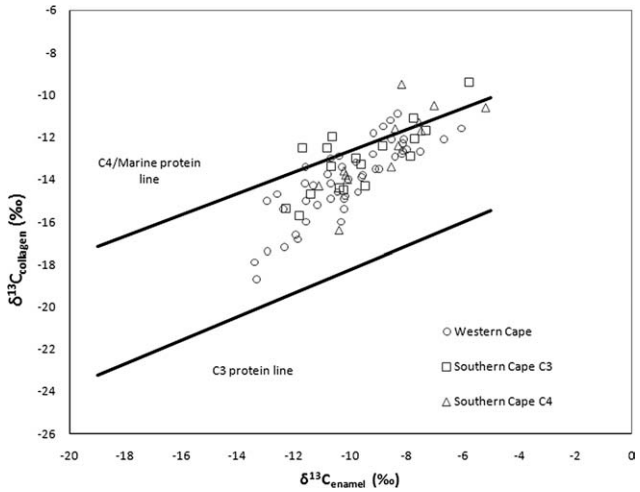

Fig. 5. Data for the western Cape, southern Cape C_3 and southern Cape C_4 regions plotted against the Froehle et al. (2010) model regression lines (based on $\delta^{13}C_{bone\ apatite}$) describing dietary protein.

regression of $\Delta^{13}C_{collagen\text{-}bone\ apatite}$ on $\delta^{13}C_{collagen}$ had $R^2 = 0.39$.

For 33 of the individuals whose $\delta^{13}C_{enamel}$ was analyzed in this study, both $\delta^{13}C_{bone\ apatite}$ and $\delta^{13}C_{collagen}$ values had previously been published in Lee-Thorp et al. (1989) and Sealy (1997). Mean $\Delta^{13}C_{enamel\text{–}bone\ apatite}$ is 0.6‰ ± 1.5‰, significantly smaller than the value of 2.3‰ reported by Warinner and Tuross (2009), for seven experimental pigs ($t(34) = 5.1$, $P \leq 0.001$). In an archeological population that consumed varied mixed diets, there does not appear to be a fixed or "systematic" offset as suggested by Warinner and Tuross (2009). Figure 4 shows a weak correlation between $\delta^{13}C_{bone\ apatite}$ and $\delta^{13}C_{enamel}$ ($R^2 = 0.37$), which improves slightly when the dataset is separated into the western and southern coastal regions ($R^2 = 0.45$, $n = 20$ and $R^2 = 0.57$, $n = 13$, respectively). Thus, the correlation between $\delta^{13}C_{enamel}$ and $\delta^{13}C_{bone\ apatite}$ is weaker than that between $\delta^{13}C_{enamel}$ and $\delta^{13}C_{collagen}$. Because both enamel and bone apatite are synthesized from carbonates and bicarbonates in the blood, this weak correlation is unlikely to be due to metabolic "routing" of different dietary macronutrients. The most likely explanation is that, despite pretreatment, there was some residual diagenetic alteration in some or all of the bone apatite samples. Bone preservation was not systematically investigated at the time that the analyses of bone apatite were carried out, although most samples were of compact femoral bone in good condition, with excellent collagen preservation. Any diagenetic carbonates that may be present are likely to be ^{13}C enriched, since they derive from the shells of marine mollusks in the burial matrices. The effect would therefore be to increase $\Delta^{13}C_{collagen\text{-}bone\ apatite}$. The residual alteration must be small, since we can detect patterning in the relationship between $\delta^{13}C_{bone\ apatite}$ and $\delta^{13}C_{collagen}$ (Lee-Thorp et al. 1989), but large enough to obscure the relationship between $\delta^{13}C_{enamel}$ and $\delta^{13}C_{bone\ apatite}$. Koch et al. (1997) showed that $\delta^{13}C_{bone\ apatite}$ can be influenced by sample pretreatment, even in relatively recent samples.

In Figure 5 the $\delta^{13}C_{collagen}$ and $\delta^{13}C_{enamel}$ values from this study are plotted against the lines of the model developed by Froehle et al. (2010), which was based on $\delta^{13}C_{collagen}$ and $\delta^{13}C_{bone\ apatite}$. Most points fall closest to the C_4/marine protein line with almost none lying near the C_3 protein line. The points do not separate out clearly based on region. While the marine protein input is expected, archeological faunal remains show that C_3 protein from terrestrial animals was also consumed. Individuals with the most negative $\delta^{13}C_{collagen}$ values tend to lie closer to the C_3 protein line. If $\delta^{13}C_{enamel}$ is enriched relative to $\delta^{13}C_{bone\ apatite}$, as data from this study and others indicate, then plotting enamel values on to these lines is likely to overestimate C_4/marine protein consumption.

CONCLUSION

This study comprises the largest comparison to date of $\delta^{13}C_{enamel}$ with $\delta^{13}C_{collagen}$ and $\delta^{13}C_{bone\ apatite}$ in archeological humans. The close correlation between $\delta^{13}C_{enamel}$ and $\delta^{13}C_{collagen}$ is to be expected, given that both enamel and collagen are reliable sample materials for stable isotope analysis of archeological remains. Using two tissues in conjunction can provide additional information about diet but the close correlation suggests that, at least in the environments from which these samples came, one tissue can provide a reasonable approximation of the isotopic character of the whole diet. The unexpectedly poor correlation between bone and enamel apatite may be due to pretreatment effects or diagenetic alteration of the bone apatite. Comparison of intertooth $\delta^{13}C_{enamel}$ within a single individual showed little variation, despite the different ages of crown formation. Elucidating the precise nature of the relationship between $\delta^{13}C_{enamel}$ and $\delta^{13}C_{collagen}$ is important because analysis of older (Pleistocene) human and animal remains will necessarily be of tooth enamel. Most of the existing isotope analyses of coastal hunter–gatherers, in this region and elsewhere in the world, are of collagen. Understanding the relationship between the two is therefore important to enable comparison with this large data set, and interpretation in terms of the range of variation that has been documented among human populations world-wide.

ACKNOWLEDGMENTS

The authors would like to thank Petro Keene and Lalou Meltzer for access to the collection of precolonial human remains at Iziko Museums of Cape Town, Alan Morris for access to the collection at the Department of Human Biology at UCT Medical School, and the Archeology Contracts Office (Department of Archeology, UCT) for access to recently recovered skeletons. Vincent Hare, John Lanham, and Ian Newton provided technical support for which the authors are grateful. They thank Kutlwano Ramaboa, Wendy Black, and Becky Ackermann for advice and help with statistical analyses, and Sally Adam for drafting the figures.

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