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The oxygen isotope composition of mammalian enamel carbonate from Morea Estate, South Africa

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Abstract Stable carbon isotope analysis is now an established tool for investigating the diets of fossil taxa, but carbon isotopes provide us with limited information about an animal's ecology. Recent research suggests that mammalian oxygen isotope compositions might also prove profitable sources of ecological information. If we are to exploit this resource, however, we must improve our nascent understanding of oxygen isotope compositions within modern foodwebs. To this end, we have analyzed the oxygen and carbon isotope compositions of nine ecologically diverse, sympatric taxa from Morea Estate, Mpumalanga Province, South Africa. These data show that the Morea Estate faunivores are depleted in ^{18}O compared to herbivores, and among the herbivores, frequent drinkers are relatively depleted in ^{18}O . While more research is needed to address the mechanisms for and universality of these patterns, these results show oxygen isotope analysis to be a promising avenue of paleoecological research.

Keywords Oxygen isotopes · Paleoecology · Paleodiet · Biogeochemistry

Introduction

Stable isotope biogeochemistry of tooth enamel has proved an invaluable tool for investigating the ecology of fossil taxa for the past decade. This research has focused primarily on carbon isotope compositions, from which important dietary information has been obtained (e.g., Lee-Thorp and van der Merwe 1987; Koch et al. 1994; Bocherens et al. 1996; Cerling et al. 1997; Sponheimer

and Lee-Thorp 1999a), but despite these successes, carbon isotopes provide us with an incomplete picture of an animal's ecology. For example, frugivores, folivores, and faunivores can have indistinguishable carbon isotope signatures, despite their dietary and attendant ecological disparities. New research suggests, however, that oxygen isotopes in enamel carbonate may provide us with additional information about the ecology and physiology of fossil taxa (Bocherens et al. 1996; Cerling et al. 1997; Sponheimer and Lee-Thorp 1999b). For example, the oxygen isotope composition of the Pliocene rhinocerotid *Teleoceras* led MacFadden (1998) to suggest that it was completely terrestrial, and not semi-aquatic, despite morphological convergences with the modern *Hippopotamus amphibius* (Prothero 1992). While the promise of oxygen isotopes is tantalizing, interpretations of these data are bedeviled by our incomplete understanding of oxygen isotope compositions within modern foodwebs. For example, Bocherens et al. (1996) found that browsing black rhinoceroses (*Diceros bicornis*) and mixed-feeding elephants (*Loxodonta africana*) are depleted in ^{18}O compared to grazers, whereas Kohn et al. (1996) reported that browsing and mixed-feeding herbivores tend to be enriched in ^{18}O compared to grazing herbivores. While this discord can probably be explained through detailed examination of the ecologies and physiologies of the taxa analyzed in these studies (Kohn 1996; Kohn et al. 1996; Sponheimer and Lee-Thorp 1999b), more modern data are clearly needed to improve our understanding of mammalian oxygen isotope compositions. To this end, we have analyzed a diverse mammalian fauna collected in the early 1970s from Morea Estate, a small, homogenous woodland environment in Mpumalanga Province, South Africa.

Background

The oxygen isotope composition of mammalian tooth enamel is a function of the isotopic composition of oxygen that enters and exits the body [see Bryant and Froelich (1995) and Kohn (1996) for detailed reviews]. The body's

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main oxygen sources are atmospheric O₂, liquid water, and oxygen bound in food, of which only the latter two are likely to cause differences in oxygen isotope values among sympatric taxa. Unlike atmospheric O₂, the oxygen isotope composition of food and liquid water are highly variable, and thus likely to explain any differences found in the oxygen isotope compositions of animals within a local ecosystem. Liquid water enters the body through drinking and as free water in food. In most cases, liquid water in plant roots and stems is isotopically similar to available drinking water, but leaf water is relatively enriched in H₂¹⁸O due to preferential evapotranspiration of the lighter H₂¹⁶O molecule (Gonfiantini et al. 1965; Epstein et al. 1977; Sternberg 1989; Yakir 1992). Thus, animals that derive most of their water from plant leaves (such as *Giraffa* or *Litocranius*) ingest water enriched in ¹⁸O compared to animals that drink regularly (such as *Equus* and *Phacochoerus*). Less is known about the oxygen chemically bound in foods available to mammals. Nonetheless, limited evidence suggests that carbohydrates are enriched in ¹⁸O compared to proteins (Epstein et al. 1977; Sternberg 1989; Yakir 1992; Tredget et al. 1993). Therefore, ceteris paribus, we might expect low-protein herbivorous diets to be more enriched in ¹⁸O than high-protein faunivorous diets.

The main oxygen outputs from the body are respiratory CO₂, liquid water, and water vapor. Liquid water is lost in urine, feces, and sweat and has an isotopic composition similar to body water (Wong et al. 1988), while water vapor is lost through the mouth, nose, and skin and is depleted in ¹⁸O (Wong et al. 1988). This is significant given the different physiological adaptations of animals to heat stress. For example, if the oxygen inputs of two animals are isotopically equal, but one pants and the other sweats to lose heat, the former will have higher δ¹⁸O values because it loses isotopically depleted oxygen while panting.

Materials and methods

Oxygen is present in both phosphate (PO₄³⁻) and carbonate (CO₃²⁻) ions in enamel apatite, and since [¹⁸O_{phosphate} and ¹⁸O_{carbonate} are highly correlated ($r^2=0.98$; Bryant et al. 1996; Iacumin et al. 1996)], both are suitable for our purposes here. Most studies of fossil mammal oxygen isotope compositions have focused on phosphate oxygen because the P-O chemical bond is much stronger than the C-O bond, making it less susceptible to isotopic exchange than carbonate oxygen. Nonetheless, recent studies have shown that reasonable biogenic oxygen signals can be retained in ancient enamel carbonate (Wang and Cerling 1994; Bocherens et al. 1996; Cerling et al. 1997; Sponheimer and Lee-Thorp 1999b). For example, Bocherens et al. (1996) demonstrated that hippos from early and middle Pleistocene sites are depleted in ¹⁸O compared to other herbivores in the local ecosystem, as is also the case today. The ability to obtain biogenic signals from ancient enamel carbonate is particularly important because carbonate oxygen isotope ratios are already conveniently obtained from evolved CO₂ during analysis for carbon isotopes. Therefore, unlike analysis of phosphate oxygen, carbon and oxygen isotope ratios can be measured without additional sampling. For this reason, we have analyzed oxygen (and carbon) isotopes from enamel carbonate.

We chose to analyze specimens from Morea Estate because they were collected from a very small area in a short period of time (~2 years). Thus, differences between taxa are unlikely to be artifacts due to different meteoric water compositions within the study

area or changes in climate over time. Unfortunately, in satisfying these spatial and temporal constraints, we greatly limited the number of collections available for study, and while Morea Estate met our criteria, it is hampered in being a small collection. In the end, we chose experimental control over sample size (22 specimens), while cognizant of the fact that this limits the statistical utility of the study. Students of paleontology are often confronted with the bane of small sample sizes and, as always, the litany of caveats applies here. Nonetheless, such studies may generate hypotheses to be tested in larger, well-controlled studies in the future.

To obtain oxygen isotope ratios, 3 mg of enamel was obtained from permanent molars using a rotary drill equipped with a diamond-tipped drill bit. The resulting powder was then pretreated in 1.5% sodium hypochlorite solution to remove organic contaminants and 0.1 M acetic acid to remove the highly soluble (high carbonate content) mineral component. The enamel was then freeze-dried and placed in individual reaction vessels for reaction with phosphoric acid at 70°C, and CO₂ was obtained by cryogenic distillation (Lee-Thorp et al. 1997; Sponheimer 1999). Replicate analyses using this procedure are within 0.2‰.

Results and discussion

As shown in Table 1, animals that live in the same place at the same time can have δ¹⁸O values that differ by more than 10‰. Thus, as other recent studies have shown (Bocherens et al. 1996; Kohn et al. 1996), the assumption cannot be made that all large animals in an area will have more or less the same oxygen isotope composition (contra Bryant and Froelich 1995). Body size has been suggested as the prime determinant of most of the variation that is found in animals from one area (Bryant and Froelich 1995). However, these data reveal no relationship between estimated body weight and δ¹⁸O. Indeed, the most enriched and most depleted taxa analyzed, *Aepyceros melampus* (impala) and *Orycteropus afer* (aardvark), respectively, are both about the same size (40–80 kg).

These data reveal an interesting relationship between oxygen isotope composition and trophic level. The ranges of the faunivores (mean=25.6‰, SD=1.1‰, $n=4$) and herbivores (mean=30.7‰, SD=2.3‰, $n=18$) do not overlap and their means differ by more than 5‰ ($P=0.0004$; ANOVA and t -test). A number of factors may contribute to the relative depletion in ¹⁸O of faunivores compared to herbivores. First, these faunivores may have been drinking more frequently than the herbivores, although there is no evidence to substantiate this at present. The depletion in the ¹⁸O of faunivores may also result from the fact that the liquid water they ingest from their prey is less enriched in ¹⁸O than free water in most herbivore plant foods. Furthermore, this depletion is also consistent with evidence suggesting that proteins are relatively depleted in ¹⁸O compared to carbohydrates (Tredget et al. 1993; Kohn 1996), and data showing that laboratory rats fed high-protein diets have lower δ¹⁸O values than controls (S.H. Ambrose, personal communication). Future studies should enable us to determine which of these factors is primarily responsible for the depletion in ¹⁸O of faunivores found here.

There is also variation of about 6‰ between the various herbivorous taxa analyzed, which seems to be part

Table 1 $\delta^{13}\text{C}_{\text{PDB}}$ and $\delta^{18}\text{O}_{\text{SNOW}}$ values for the taxa analyzed in this study

Species	Common name	Diet	<i>n</i>	$\delta^{13}\text{C}_{\text{PDB}}$	$\delta^{18}\text{O}_{\text{SNOW}}$
<i>Crocuta crocuta</i>	Spotted hyena	Faunivore	2	-7.7±0.1	26.3±1.4
<i>Orycteropus afer</i>	Aardvark	Faunivore	2	-6.9±2.0	25.0±0.4
<i>Cercopithecus aethiops</i>	Vervet monkey	Herbivore/faunivore	2	-13.3±1.7	27.8±0.6
<i>Giraffa camelopardalis</i>	Giraffe	Herbivore-browser	3	-16.8±0.6	29.4±2.1
<i>Aepyceros melampus</i>	Impala	Herbivore-mixed	3	-3.3±3.3	34.5±1.2
<i>Connochaetes taurinus</i>	Wildebeest	Herbivore-grazer	2	+2.8±0.5	30.7±1.2
<i>Damaliscus lunatus</i>	Tsessebe	Herbivore-grazer	3	+2.3±0.6	32.0±0.9
<i>Kobus ellipsiprymnus</i>	Waterbuck	Herbivore-grazer	2	+1.6±1.1	29.7±1.6
<i>Phacochoerus africanus</i>	Warthog	Herbivore-grazer	3	-1.3±0.1	29.8±0.5

tered in predictable ways. Even though the sample sizes are small, the impala (*A. melampus*) specimens are significantly enriched in ^{18}O compared to other herbivores ($P < 0.001$; ANOVA and *t*-test). The next most enriched herbivores are the tsessebe (*Damaliscus lunatus*) and the blue wildebeest (*Connochaetes taurinus*). These alcelaphines, in turn, are more enriched in ^{18}O than common warthogs (*Phacochoerus africanus*), waterbucks (*Kobus ellipsiprymnus*), and giraffes (*Giraffa camelopardalis*). Of the non-dedicated faunivores, the vervet monkey (*Cercopithecus aethiops*) is the most depleted in ^{18}O . Most cercopithecids have a significant faunivorous component to their diet, which might explain their liminal positions between dedicated herbivores and dedicated faunivores. This will be discussed in more detail below.

As discussed earlier, the primary determinants of the differing $\delta^{18}\text{O}$ values between sympatric taxa are believed to be differences in the oxygen isotope composition of their water sources, food, and thermophysiological adaptations (e.g., Kohn et al. 1996; Sponheimer and Lee-Thorp 1999b). Although the number of specimens analyzed for this study is small, these data seem to support this idea. For example, the enrichment in ^{18}O of the impala was predictable. Impalas inhabit ecotones between grassland and woodland and are frequently found amidst riverine woodland. They are rarely found far from water, and are known to visit water holes regularly in some areas (Kingdon 1982, 1997; Spinage 1986; Estes 1991). Lamprey (1963) claims that impalas do not need to drink when green vegetation is available, though Maloiy and Hopcraft (1971) doubt that they could maintain their water balance without occasional drinking. Regardless, impalas derive less of their total water intake from drinking than do the other antelopes in this study (Taylor 1968; Taylor et al. 1969; Maloiy and Hopcraft 1971), and are likely enriched in ^{18}O because they obtain relatively more of their water from isotopically enriched leaf water. Moreover, since they require less water in the first place, relatively more of their oxygen input comes from very enriched oxygen sources (primarily inspired oxygen and, to a lesser extent, oxygen bound in food).

Although not statistically significant due to the small sample sizes, the relatively depleted $\delta^{18}\text{O}$ values of blue wildebeests (*C. taurinus*) compared to tsessebes (*D. lunatus*) are consistent with what is known of their physiologies and ecologies. Detailed physiological stud-

ies of tsessebes are lacking, but the ecology of the species suggests that they obtain less of their water from drinking than do wildebeests. While tsessebes prefer grassland, they are more tolerant than wildebeests of bushy and woodland environments (Kingdon 1982, 1997; Spinage 1986; Estes 1991). Tsessebes are also able to go without drinking during most of the rainy season, and drink about every 2 days during the dry season when their fodder is dry (Huntley 1972). Wildebeests, on the other hand, require more open environments and tend to drink daily for much of the year (Western 1975). Thus, wildebeests obtain more of their water from drinking than do tsessebes, and hence are relatively depleted in ^{18}O . Physiological studies have shown that wildebeests also obtain a higher portion of their water intake from drinking than does their other close relative *Alcelaphus* (Taylor 1968; Maloiy and Hopcraft 1971). Wildebeests also have relatively wide premaxillas and crop grasses more quickly than tsessebes, which select leaf more carefully with their narrow muzzles (Kingdon 1982, 1997; Spinage 1986; Estes 1991). Consequently, wildebeests may eat more stem than tsessebes do. As discussed earlier, stem water is depleted in ^{18}O compared to leaf water (Sternberg 1989; Yakir 1992), which might also lead to the relative depletion of wildebeests compared to tsessebes found here.

The further depleted $\delta^{18}\text{O}$ values of the warthog (*P. africanus*) and waterbuck (*K. ellipsiprymnus*) are also predictable. The waterbuck, so named by Dutch settlers at the Cape because it was never found far from water, is a grazer that requires cover and water. It is an ecotone species that is often found around riparian woodland (Kingdon 1982, 1997; Spinage 1986; Estes 1991). At both mild and stressful temperatures, the waterbuck requires more water than domestic cattle, and more than three times the water (per kilogram) needed by the impala (Taylor et al. 1969; Maloiy and Hopcraft 1971). Therefore, it must drink daily to satisfy its tremendous need for water. The reason for this dependence appears to be twofold. First, waterbucks can only survive on very high protein diets, and thus must expel a considerable amount of urea. Second, waterbucks are unable to concentrate their urine, much like domestic cattle (Taylor and Lyman 1967; Taylor et al. 1969). Indeed, their urine-concentrating ability is about half that of hartebeests, and about 40% that of impalas (Maloiy and Hopcraft 1971).

The high-protein diet, coupled with the inability to significantly concentrate its urine, necessitates a continuously high urine volume, even under conditions of heat stress and dehydration. In addition, the waterbuck cools itself by sweating and panting about equally, while the previously discussed taxa primarily pant (Taylor et al. 1969). Thus, the waterbuck does not lose as much depleted H_2O through panting, which contributes to its relative depletion in ^{18}O (Wong et al. 1988). All told, the waterbuck needs a tremendous amount of water, most of which it obtains from drinking. All of these factors cause the waterbuck to be depleted in ^{18}O compared to the impala and alcelaphines.

Comparatively little is known about the physiology of the warthog (*P. africanus*). Nonetheless, its ecology suggests that it should also be depleted compared to alcelaphines and the impala. The warthog is a grazer that inhabits lightly wooded areas (Cumming 1975; Kingdon 1982, 1997; Estes 1991). It drinks daily, and will also wallow daily when hot. The preponderance of its water intake comes from drinking. In addition, during the dry season when grasses are desiccated, the warthog digs up roots, bulbs, and tubers with its specialized snout. The water in these underground storage organs has a similar $\delta^{18}O$ value to that of much available drinking water, and the carbohydrates in these organs have less ^{18}O than carbohydrates in leaves (Sternberg 1989; Yakir 1992). This combination of dependence on drinking water and ^{18}O -depleted foods suggests that *Phacochoerus* should have among the lowest $\delta^{18}O$ values of all grazing taxa. Indeed, this is precisely what the Morea Estate data show.

The depletion in ^{18}O of the only primate studied here, the vervet monkey (*C. aethiops*), is also predictable. Vervets are found in virtually all wooded habitats (excepting rainforests) throughout Africa, and they can also invade relatively treeless areas as long as they are transected by riverine forest (Struhsaker 1967; Kingdon 1982, 1997; Estes 1991). Given the vervets' small size and unimpressive speed, they are never found far from trees. Vervets tend to prefer fruit but have catholic diets that include seeds, leaves, flowers, forbs, invertebrates and, less frequently, vertebrates. Some vervet populations drink about every other day (Struhsaker 1967), while others drink very rarely (Hall and Gartlan 1965). Thus, the relationship between the $\delta^{18}O$ values of vervets and other local fauna may vary depending upon their drinking behavior in a given location. However, we might expect vervets to be depleted in ^{18}O compared to most sympatric herbivores given their consistent, if small, consumption of animal foods. Furthermore, fruit water is depleted in ^{18}O compared to leaf water (Dunbar and Wilson 1983; Yakir 1992), so one might expect a frugivore to be depleted in ^{18}O compared to a folivore.

Given what is known of their individual ecologies and physiologies, the $\delta^{18}O$ values of the taxa discussed to this point were more or less anticipated. However, *G. camelopardalis* was not enriched in ^{18}O as expected. While Cerling et al. (1997) found that giraffids are consistently enriched in ^{18}O compared to other herbivores in

the local ecosystem, the giraffids from Morea Estate have lower $\delta^{18}O$ values than all taxa analyzed save the vervet and dedicated faunivores. Giraffes inhabit primarily wooded grassland and open woodlands. They are widely dispersed during the rains, but tend to gather around watercourses during the dry season. Because giraffes browse from trees that can support growth through much of the year, they are able to obtain much of their water through food and by drinking every 3 days or so (Innis 1958). Thus, we might expect giraffes to be enriched, since they derive a large percentage of their water from plant leaves.

There are at least two possible explanations for the depletion in ^{18}O of the Morea Estate giraffes. One possibility is that the Morea Estate giraffes drank more frequently than the Kenyan giraffes analyzed by Cerling et al. (1997). This is not a particularly far-fetched idea, as Innis (1958) noted that giraffes drink regularly in some areas where water is plentiful. Thus, the depletion in ^{18}O of the Morea Estate giraffes may simply reflect the fact that these giraffes (from the well-watered South African lowveld) are drinking more regularly than their brethren from more sere climes to the north. This raises an important point. One must be careful to recognize that taxa alter their behavior in a variety of different ways as the environment changes. Thus, we cannot always expect the relationships between $\delta^{18}O$ values of taxa to be the same from place to place. In other words, giraffes (and other taxa) might quite naturally be relatively depleted in ^{18}O in one place, but enriched in ^{18}O in another. While this complicates the picture considerably, it also offers us the opportunity to describe the more subtle interactions between taxa and their environments.

Another possible explanation for the unanticipated oxygen isotope composition of the Morea Estate giraffes is essentially procedural. Unlike the other taxa examined here, only sub-adult giraffes were available for analysis. Therefore dP_4s and not permanent molars (as for all other taxa) were sampled. While analyses of ungulate tooth rows have produced no evidence that deciduous dentition is systematically depleted in ^{18}O compared to permanent dentition (Bryant 1995; M. Sponheimer, unpublished data), this explanation cannot be excluded at present. This question will be addressed in future studies.

This study reveals some important trends in mammalian oxygen isotope composition. For example, animals that obtain most of their liquid water from ^{18}O -enriched plant sources like leaves tend to be enriched in ^{18}O compared to animals that drink regularly. Furthermore, faunivores appear to be depleted in ^{18}O compared to sympatric herbivores. Additional research might well prove these generalizations to be correct but overly simplistic. Our aim here, however, is not to build a model explicating oxygen isotope compositions in mammalian taxa, but to probe for the limits of variation between sympatric, yet ecologically and physiologically distinct species. Our hope is that this and future studies will help establish rules of thumb that might enable us to resolve ecological and physiological differences between closely

related fossil taxa. For example, if two fossil alcelaphines have grazing $\delta^{13}\text{C}$ values, but one is very enriched in ^{18}O and the other is depleted, we may be able to infer that the former was less dependent on drinking than the latter. This type of information may ultimately aid us in making paleoenvironmental inferences from fossil assemblages but, equally importantly, should enable us to better observe the evolutionary processes that shaped contemporary mammalian lineages.

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