Stable carbon and nitrogen isotope enrichment in primate tissues

Brooke E. Crowley · Melinda L. Carter · Sarah M. Karpanty · Adrienne L. Zihlman · Paul L. Koch · Nathaniel J. Dominy

Abstract Isotopic studies of wild primates have used a wide range of tissues to infer diet and model the foraging ecologies of extinct species. The use of mismatched tissues for such comparisons can be problematic because differences in amino acid compositions can lead to small isotopic differences between tissues. Additionally, physiological and dietary differences among primate species could lead to variable offsets between apatite carbonate and collagen. To improve our understanding of the isotopic chemistry of primates, we explored the apparent enrichment ($e^{*}$) between bone collagen and muscle, collagen and fur or hair keratin, muscle and keratin, and collagen and bone carbonate across the primate order. We found that the mean $e^{*}$ values of proteinaceous tissues were small ($\leq 1\%$), and uncorrelated with body size or phylogenetic relatedness. Additionally, $e^{*}$ values did not vary by habitat, sex, age, or manner of death. The mean $e^{*}$ value between bone carbonate and collagen (5.6 ± 1.2%) was consistent with values reported for omnivorous mammals consuming monoisotopic diets. These primate-specific apparent enrichment values will be a valuable tool for cross-species comparisons. Additionally, they will facilitate dietary comparisons between living and fossil primates.

Keywords Stable isotope · Keratin · Muscle · Collagen · Apatite · Carbonate

Introduction

Stable isotope ratios in animal tissues vary with diet, habitat, and environmental conditions, and are often used to assess the foraging ecology and habitat preferences of living and extinct species (West et al. 2006). These studies have varied methodologically, using a range of tissues. For instance, the diets of wild primates have been assessed using isotope values from hair (e.g., Schoeninger et al. 1997, 2006), tooth enamel (e.g., Codron et al. 2005; Fourie et al. 2008; Smith et al. 2010), bone (e.g., Ambrose and DeNiro 1986; Thackeray et al. 1996; Smith et al. 2010),
and feces (e.g., Codron et al. 2006). These data, in turn, have been used to inform paleo-ecological models of extinct species, including early human ancestors (e.g., Thackeray et al. 1996; Codron et al. 2005; Sponheimer et al. 2006, 2010). Due to tissue preservation issues, these studies have frequently had to use different tissues in their modern and ancient comparisons.

Although the availability and state of preservation of specimens are practical constraints, it can be problematic to compare the isotopic composition of different tissues for two reasons. First, carbon and nitrogen isotope values of proteinaceous tissues can differ within an animal because each tissue has a unique amino acid (AA) composition, and the AAs themselves vary isotopically (e.g., Hare et al. 1991; Styting et al. 2010). Second, when studying fossils, researchers generally use the carbonate fraction of biological apatite. The isotopic difference [hereafter termed apparent enrichment, $\varepsilon^*$ (defined below)] between carbon in organic tissues, such as collagen in bone or dentin, and carbon in the carbonate in bone or tooth apatite varies with both digestive physiology and dietary macromolecular composition (reviewed in Hedges 2003).

A wide range of dietary and gut physiological adaptations among primates could lead to differences in $\varepsilon^*$ values for both carbon and nitrogen that could in turn confound ecological or paleoecological interpretations. Many experiments have been conducted on rodents and pigs, but most were focused on carbon isotope differences between carbonate and collagen. Scarcely any work has examined the differences between proteinaceous tissues, let alone unconventional taxa. For instance, only one published study has focused on nonhuman primates (O’Regan et al. 2008) (Table 1). Accordingly, we compared the carbon and nitrogen isotope values in keratin, muscle protein, bone collagen, and bone carbonate (carbon only) for a diverse group of primate species (Table 2). We expected that differences in AA composition would drive $\varepsilon^*$ variation among proteinaceous tissues for both carbon and nitrogen, but that these differences would be small and consistent across individuals and species. We also expected that $\varepsilon^*$ values for carbonate versus collagen carbon would vary among species as a function of both diet and digestive physiology, and factors that correlate with these variables (body size, habitat, etc.).

**Background on variation in $\varepsilon^*$ values**

**Variation among proteinaceous tissues**

The isotopic values of proteinaceous tissues within an individual could vary because (1) the concentration of different AAs varies among tissues, and (2) the isotopic composition of individual amino acids (AAs) shows considerable variation (19.9‰ average range for C, 24.4‰ average range for N; Fig. 1). This variability relates to isotopic differences among ingested AAs, differences in mammalian biosynthetic pathways for non-essential AAs, and the extent to which a mammal either synthesizes or incorporates a particular AA from its diet. This is a complex subject, but a few patterns have emerged. For carbon, glycine and metabolically-related AAs (serine, cysteine) are often $^{13}$C-enriched relative to other non-essential AAs, whereas essential AAs track variation in ingested AAs (Hare et al. 1991; Fogel and Tuross 2003; Jim et al. 2006). For nitrogen, there are a suite of AAs that are $^{15}$N-enriched with each trophic step (e.g., glutamate, asparagine, alanine, isoleucine, valine, proline) and others that do not enrich (e.g., phenylalanine, lysine, glycine) (McClelland and Montoya 2002; Popp et al. 2007). Muscle (myosin) in humans, and most likely other primates, is dominated by $^{15}$N-enriched glutamate and alanine (Bergström et al. 1974). Collagen is mainly composed of $^{13}$C-enriched glycine (33%) and $^{15}$N-enriched aspartate (5%), glutamate (7%), and proline and hydroxyproline (33%). Primate keratin is dominated by $^{13}$C-enriched cysteine (~12–17%) and serine (~10%), and $^{15}$N-enriched glutamate (~17%) (Hrdy and Baden 1973; O’Connell et al. 2001).

**Variation in carbonate-apatite $\varepsilon^*$ values**

The measured mean carbon isotope difference between carbon in carbonate and collagen ($\varepsilon^{13}$C$_{\text{carbonate}-\text{collagen}}$) is ~7‰ or greater in wild large-bodied herbivores and ~3‰ in faunivorous animals (Table 1). There are two potential explanations for this difference that are not mutually exclusive. First, it could result from differences in dietary macromolecular composition (i.e., protein, lipid, and carbohydrate), which affect both diet-to-protein and diet-to-carbonate $\varepsilon^*$ values due to differing $\delta^{13}$C values among macromolecules, and differential routing of macromolecules to particular tissues. Second, it could result from differences in how animals digest plant and animal matter, which only affect diet-to-carbonate $\varepsilon^*$ values (Hedges 2003).

Apatite carbonate, which likely forms in isotopic equilibrium with blood bicarbonate, reflects carbon in bulk diet (i.e., a proportional mixture of carbon from all assimilated macronutrients) (Ambrose and Norr 1993; Passey et al. 2005). The isotopic composition of consumer proteins reflects that of dietary proteins (Ambrose and Norr 1993; Tieszen and Fagre 1993; Ambrose et al. 1997; Howland et al. 2003; Jim et al. 2004, 2006). Essential AAs must be routed directly from the diet, but depending on dietary protein concentration, non-essential AAs can also be routed into consumer tissues or synthesized using carbon from dietary carbohydrates, lipids and proteins. Theoretically,
Table 1  Tissue-tissue carbon and nitrogen fractionation values from previous research on mammals

| Taxon          | Diet   | n   | $\Delta^{13}C$ Range | $\Delta^{15}N$ Range | References |
|----------------|--------|-----|-----------------------|-----------------------|------------|
| Collagen–keratin |        |     |                       |                       |            |
| Mouse          | Captive Mixed | 72  | 2.9 ± 2.2             | 1.1, 7.1$^b$         | 1          |
| Mouse          | Captive Uniform | 24 | 2.5 ± 0.3             | 2.3, 2.7$^b$         | 1          |
| Wolf           | Captive Uniform | 18 | 0.4 ± 0.8             | −0.9, 2.3            | 0.3 ± 0.7  | −1.0, 1.7 | 2          |
| Human          | Modern Mixed | 8   | 1.5 ± 0.5             | 0.8, 2.2             | 0.9 ± 0.2  | 0.7, 1.1  | 3          |
| Macaque        | Wild Mixed | 13  | 0.1 ± 1.1             | −1.5, 1.0$^b$        | 0.4 ± 0.3  | −0.3, 1.1 | 5          |
| Collagen–muscle |        |     |                       |                       |            |
| Mouse          | Captive Mixed | 72  | 2.4 ± 0.4             | 1.7, 2.9$^b$         | 1          |
| Mouse$^d$      | Captive Mixed | 2  | 3.7 ± 0.1             | 3.6, 3.8             | 1          |
| Mouse          | Captive Uniform | 24 | 2.2 ± 0.8             | 1.6, 2.7$^b$         | 1          |
| Mouse$^d$      | Captive Uniform | 6  | 2.4 ± 0.6             | 1.8, 3.5             | 1          |
| Sheep          | Domestic Mixed | 2  | 4.1 ± 0.1             | 4.0, 4.1             | 6          |
| Pig            | Domestic Uniform | 20 | 1.1 ± 1.6             | −0.1, 2.2$^b$        | 0.9 ± 0.6  | 0.5, 1.3  | 7          |
| Wolf           | Captive Uniform | 18 | 1.5 ± 0.7             | 0.4, 3.1             | −0.5 ± 0.8 | −1.8, 1.8 | 2          |
| Gemsbok        | Wild Mixed | 1   | 0.7                   |                       | 6          |
| Hartabeest     | Wild Mixed | 1   | 1.9                   |                       | 6          |
| Impala         | Wild Mixed | 1   | 1.7                   |                       | 6          |
| Kudu           | Wild Uniform | 2  | 2.4 ± 0.6             | 1.9, 2.8             | 6          |
| Springbok      | Wild Mixed | 3   | 2.4 ± 0.6             | 1.8, 3.0             | 6          |
| Warthog        | Wild Mixed | 1   | 1.7                   |                       | 6          |
| Muscle–keratin |        |     |                       |                       |            |
| Gerbil         | Captive Mixed | 37 | −2.3 ± 0.7            | −3.6, −1.3$^b$       | 8          |
| Mouse          | Captive Mixed | 72  | 0.5 ± 2.1             | −1.3, 4.5$^b$        | 1          |
| Mouse          | Captive Uniform | 24 | 0.4 ± 0.5             | 0.0, 0.7$^b$         | 1          |
| Mouse          | Captive All   | 18  | −2.9$^e$              |                       | 9          |
| Mouse          | Captive All   | 18  | 0.3$^e$               |                       | 10         |
| Pig            | Domestic Uniform | 5  | 1.8                   | −0.1                 | 11         |
| Fox            | Captive Mixed | 20  | −1.5$^f$              | 0.2 ± 0.1            | 0.1, 0.2$^b$ | 12         |
| Wolf           | Captive Uniform | 19 | −1.2 ± 0.4            | −2.2, 0.5            | 0.8 ± 0.5  | −0.2, 1.5 | 2          |
| Carbonate-collagen |    |     |                       |                       |            |
| Mouse          | Captive Mixed | 72  | 4.7 ± 3.0             | 1.3, 8.7$^b$         | 1          |
| Rat            | Captive Mixed | 20  | 4.2 ± 4.4             | −0.8, 11.1$^b$       | 13         |
| Rat            | Captive Mixed | 18–60 | 7.2 ± 4.6            | 1.3, 11.3$^b$        | 14         |
| Mouse          | Captive Uniform | 24 | 5.9 ± 1.1             | 5.1, 6.7$^b$         | 1          |
| Rat            | Captive Uniform | 8  | 5.0 ± 0.6             | 4.5, 6.0             | 13         |
| Rat            | Captive Uniform | 3–10 | 5.7                  |                       | 14         |
| Pig            | Domestic Mixed | 5   | 7.5 ± 1.0             | 6.4, 9.1             | 15         |
| Pig            | Domestic Uniform | 1  | 6.0                   |                       | 15         |
| Herbivore      | Wild All      |     | 6.8                   |                       | 16         |
| Giraffe        | Wild Uniform | 4   | 6.9 ± 0.3             | 6.7, 7.4             | 18, 19     |
| Hartabeest     | Wild Mixed | 1   | 8.4                   |                       | 18         |
| Topi           | Wild Uniform | 1   | 10.3                  |                       | 19         |
| Deer           | Wild Mixed | 1   | 6.8                   |                       | 18         |
| Reindeer       | Wild Uniform | 8   | 8.5 ± 0.8             | 7.0, 9.5             | 20         |
| Llama          | Wild Uniform | 6   | 7.1 ± 0.3             | 6.6, 7.3             | 17         |
| Hippo          | Wild Mixed | 4   | 6.6 ± 0.7             | 5.8, 7.5             | 18, 19     |
| Zebra          | Wild Mixed | 2   | 9.0 ± 1.1             | 8.2, 9.7             | 18, 19     |
| Omnivore       | Wild All      |     | 5.2                   |                       | 16         |
Table 1 continued

| Taxon         | Diet | n  | $\Delta^{13}C$ | Range   | $\Delta^{15}N$ | Range | References |
|---------------|------|----|---------------|---------|---------------|-------|------------|
| Macaque       | Wild | Mixed | 11 | 5.7 ± 0.5 | 5.0, 6.1 | 5 |  |
| Carnivore     | Wild | All | 4 | 4.3 | | 16 |  |
| Fur Seal      | Wild | Uniform | 2 | 2.2 ± 0.8 | 1.6, 2.7 | 16 |  |
| Harbor Seal   | Wild | Uniform | 4 | 2.4 ± 1.1 | 1.6, 4.1 | 20 |  |
| Harp Seal     | Wild | Uniform | 4 | 3.6 ± 1.1 | 2.2, 4.5 | 20 |  |

References: (1) Tieszen and Fagre (1993); (2) Fox-Dobbs et al. (2007); (3) O’Connell et al. 2001; (4) O’Regan et al. (2008); (5) Vogel (1978); (6) Hare et al. (1991); (7) Tieszen et al. (1983); (8) DeNiro and Epstein (1978); (9) DeNiro and Epstein (1981); (10) Nardoto et al. (2006); (11) Roth and Hobson (2000); (12) Jim et al. (2004); (13) Ambrose and Norr (1993); (14) Howland et al. (2003); (15) Lee-Thorp et al. (1989); (16) Schoeninger and DeNiro (1982); (17) Sullivan and Krueger (1981); (18) Kellner and Schoeninger (2007); (19) Nelson et al. (1986)

Whenever possible, animal diets were divided into “uniform” (consumed all C3 or all C4) or “mixed” (consumed a combination of C3, C4 or marine). Otherwise, we use the category “All”. Diets for wild animals, which were inferred by the primary authors from each study, were considered mixed if the primary diet source (e.g., C3 or C4) was ≤90%

Standard deviations and ranges were calculated for captive groups fed similar diets, or wild groups living in different regions

Dietary information is not available for these animals. The authors argue that collagen $\delta^{13}C$ values suggest that some individuals may have consumed some C4 resources. However, apatite $\delta^{13}C$ values do not support C4 consumption. Because no comparative plant data are available from the respective habitats, it is not possible to validate or refute C4 consumption

Nursing mothers (n = 2) and suckling babies (n = 6)

Mean $\Delta$ values estimated using Datathief 12.0

Standard deviation and range not presented

animals on high protein diets (e.g., faunivores) should route more carbon from dietary protein to tissue protein, whereas animals on low protein diets (e.g., many herbivores) should synthesize more non-essential amino acids de novo, incorporating carbon from lipid and carbohydrate as well as protein into their tissue protein (Fogel and Tuross 2003; Hedges 2003; Martínez del Río and Wolf 2005). Additionally, because assimilation of $^{13}C$-depleted lipids could lower apatite $\delta^{13}C$ values without affecting body protein $\delta^{13}C$ values (due to routing), faunivores with fat-rich diets (such as seals) should have even smaller $\epsilon^{13}C_{\text{carbonate-collagen}}$ values (Krueger and Sullivan 1984; Lee-Thorp et al. 1989; Hedges 2003). Provided that these animals consume monoisotopic diets (e.g., only C3-derived foods), this should result in larger and smaller $\epsilon^{13}C_{\text{carbonate-collagen}}$ values in herbivores and carnivores, respectively. Whereas, all primates consume a dominantly vegetarian diet (Milton 1987), some genera such as Cebus, Daubentonia, Galago, and Microcebus can consume considerable amounts of animal matter (Milton and May 1976). Based on these dietary differences, we might anticipate that these taxa should have lower $\epsilon^{13}C_{\text{carbonate-collagen}}$ values than more herbivorous species. Importantly, controlled diet studies demonstrate that animals fed a mixture of C3, C4 and marine-derived macronutrients exhibit substantial variation in $\epsilon^{13}C_{\text{carbonate-collagen}}$ values (Table 1). Mixed diets are unlikely in the majority of wild primate species. However, this could be important for captive primates if they consume manufactured pellets containing a mix of C3 and C4 foods.

The isotopic composition of carbonate in bone apatite is also predicted to vary with the extent to which complex carbohydrates are fermented in the gut (Hedges 2003). During fermentation, bacteria break down structural carbohydrates, releasing appreciable amounts of hydrogen, CO2, and volatile fatty acids (VFA) (Jensen 1996). Some of the released CO2 can be reduced to form CH4. This process discriminates heavily against $^{13}C$, leaving the remaining CO2 $^{13}C$ enriched (Metges et al. 1990; Schulze et al. 1997). If even a small amount of this $^{13}C$-enriched CO2 enters the blood bicarbonate pool, it could increase the $\delta^{13}C$ value of apatite carbonate which forms from this pool, thus increasing $\epsilon^{13}C_{\text{carbonate-diet}}$ and $\epsilon^{13}C_{\text{carbonate-collagen}}$ values (Passey et al. 2005). The $\delta^{13}C$ value of collagen is not affected by methane production (e.g., Metges et al. 1990).

Ruminants have been shown to produce copious amounts of methane and large $\Delta_{\text{carbonate-collagen}}$ values (e.g., Crutzen et al. 1986; Metges et al. 1990; Table 1). Although some large, non-ruminant herbivores such as camels and horses also exhibit high levels of methane production and elevated $\Delta_{\text{carbonate-collagen}}$ values (Crutzen et al. 1986; Langer 1987; Table 1), methane production in most single-stomached species is trivial, despite the presence of methanogenic bacteria (Crutzen et al. 1986; Jensen 1996). Acidic conditions in the stomachs and small intestines of single-stomached animals may prevent methane production, but neutral
conditions in the posterior portions of the colon may be more amenable (Jensen 1996). Nevertheless, because gases formed near the end of the gastrointestinal tract do not likely have time to diffuse into the bloodstream, $^{13}$C-depleted methane produced in the posterior portions of the colon have a negligible effect on apatite $\delta^{13}$C values. Little is known about methane production in nonhuman primates. For the most part, it is doubtful that nonhuman primates would differ substantially from other simple-stomached animals. However, colobine monkeys could provide a possible exception. This subfamily of Old World Primates, has been likened to ruminants because they have large sacculated stomachs to facilitate microbial fermentation of leaves (Kay and Davies 1994). Primates with adaptations for caeco-colic fermentation, such as Alouatta palliata (Lambert 1998), may also have increased levels of

| Family and species | Body mass (kg) | Type | Provenance |
|--------------------|---------------|------|------------|
|                     | Male          | Female |         |
| Lorisioidea         |               |       |           |
| Galago senegalensis mohili | 0.2 | 0.2 | Captive | 1 |
| Lemuroidea          |               |       |           |
| Avahi laniger       | 1.0           | 1.3   | Wild      | 2 |
| Cheirogaleus major  | 0.4           | 0.4   | Wild      | 2 |
| D. madagascariensis | 2.6           | 2.5   | Captive   | 1 |
| Eulemur fulvus albifrons | 2.0 | 2.2 | Captive | 1 |
| E. fulvus rufus     | 2.2           | 2.3   | Wild      | 4 |
| E. macaco flavifrons| 2.4           | 2.5   | Captive   | 1 |
| E. mongoz           | 1.6           | 1.6   | Captive   | 1 |
| L. catta            | 3.6           | 3.5   | Captive   | 1 |
| Indri indri         | 5.6           | 6.3   | Wild      | 4 |
| Microcebus griseorufas | 0.05 | 0.06 | Wild | 3 |
| M. marinus          | 0.1           | 0.1   | Captive   | 1 |
| M. rufus            | 0.1           | 0.1   | Wild      | 2 |
| Propithecus coquereli verreauxi | 3.7 | 4.3 | Captive | 1 |
| P. diadema          | 5.9           | 6.3   | Wild      | 9 |
| P. verreaux         | 3.3           | 3.0   | Wild      | 3 |
| V. variagata        | 3.5           | 3.5   | Captive   | 1 |
| Cebioidea           |               |       |           |
| Alouatta palliata   | 6.5           | 4.2   | Wild      | 6–8 |
| A. geoffroyi        | 7.8           | 7.3   | Wild      | 6,7 |
| C. capucinus        | 3.7           | 2.5   | Wild      | 6,7 |
| Cercopithecoida     |               |       |           |
| Cercopithecus ascanius | 3.7 | 2.9 | Wild | 9 |
| Chlorocebus aethiops | 5.0 | 3.5 | Captive | 10 |
| Lophocebus albigena | 8.3           | 6.0   | Wild      | 9 |
| M. malattia         | 11            | 8.8   | Wild      | 11 |
| Papio anubis        | 25.1          | 13.3  | Wild      | 9 |
| P. badius           | 8.4           | 8.2   | Wild      | 9 |
| S. entellus         | 19.2          | 14.8  | Captive   | 10 |
| Hominoida           |               |       |           |
| Gorilla gorilla     | 170.4         | 71.5  | Captive   | 10 |
| Homo sapiens        | 72.1          | 62.1  | Captive   | 12 |
| Hylobates moloch    | 6.6           | 6.3   | Captive   | 10 |
| Pan paniscus        | 42.7          | 33.7  | Captive   | 10 |
| P. troglodytes      | 59.7          | 45.8  | Wild      | 9 |
| P. troglodytes      | 59.7          | 45.8  | Captive   | 10 |
| Pongo pygmaeus      | 78.5          | 35.8  | Captive   | 10 |

---

a Mass estimates are based on Smith and Jungers 1997 (H. sapiens = Danish values), excepting M. griseorufas (Génin 2008).

b Sources: (1) Duke Lemur Center; (2) S.M. Karpanty, Ranomafana National Park, Madagascar, samples collected from raptor nests; (3) Beza Mahafaly Special Reserve; Madagascar, (4) L.R. Godfrey; (5) P.C. Wright; (6) Santa Rosa National Park, Costa Rica, (7) El Zota Research Station, Costa Rica, (8) K.E. Glander; (9) M.E. Carter; (10) Department of Anthropology, UC Santa Cruz; (11) O’Regan et al. 2008; (12) O’Connell et al. 2001.
methane production. This possibility is strengthened by the observation that horses, which are also caeco-colic fermenters, have Δ\text{carbonate-collagen} values (Sullivan and Krueger 1981; Kellner and Schoeninger 2007, Table 1).

Isotopic terminology

Isotope ratios are typically presented using δ notation, where

$$\delta^a_X = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1,000$$  \hspace{1cm} (1)

and $R$ is the heavy-to-light isotope ratio in element $X$. It is expressed in parts per thousand (i.e., per mil, ‰). Carbon isotope values are reported relative to the V-PDB standard (a marine carbonate); nitrogen isotope values are relative to AIR. The offset, or fractionation, between two substances ($a$ and $b$) is often expressed using Δ notation (Martínez del Rio et al. 2009), where

$$\Delta^HX_{a-b} = \delta^HX_a - \delta^HX_b$$  \hspace{1cm} (2)

δ values are trivial to calculate and accurate so long as the differences in δ values among tissues are small. However, Δ values become less accurate as the differences in δ values among tissues increase. We choose to use alternative expressions, the fractionation factor ($\alpha$) and isotope enrichment values ($\epsilon$), which provide exact solutions and are not limited by the isotopic scale on which they are calculated (e.g., PDB vs. SMOW). Δ and ε values are nearly identical when isotopic differences among tissues are <1–2‰, but the two increasingly differ with increasing isotopic differences among tissues. When tissues are ≥10‰, Δ and ε values can differ by as much as 0.5‰ (Cerling and Harris 1999). To calculate ε, we first calculate $\alpha$.

$$\alpha_{a-b} = \left( \frac{\delta^HX_a + 1,000}{\delta^HX_b + 1,000} \right)$$  \hspace{1cm} (3)

$$\epsilon_{a-b} = \left( \alpha_{a-b} - 1 \right) \times 1,000$$  \hspace{1cm} (4)

In animals, the observed $\alpha$ value between two tissues, or between diet and a tissue, is the net result of a large range of biochemical and transport phenomena, not the simple equilibrium and kinetic reactions for which isotopic fractionation factors are typically measured. We recognize the complexity of these physiological systems by denoting these as apparent fractionation factors ($\alpha^*$) and apparent enrichment values ($\epsilon^*$). When referring to values for a particular element, we will use $\epsilon^{13\alpha}$ for carbon and $\epsilon^{15\alpha}$ for nitrogen. Note that the sign of enrichment is dependent on which substance is in the numerator in Eq. 3. Hence $\epsilon^a$ (and $\alpha^*$ and Δ) values must always be reported with subscripts or explicitly defined.

Materials and methods

Sample acquisition

Tissues from captive and wild primates were acquired from cadaveric and osteologic collections in museums, universities and research field stations (Table 2). With a few exceptions, the animals were in good health at the time of death. The main manner of death for captive animals was electrocution, drowning, or short-term illness. However, a few individuals endured chronic illness, and some died at an advanced age. The manner of death for wild animals was largely unknown, but we were able to attribute the deaths of several individuals to predation or automobile
impact (Electronic supplementary material, ESM, Table S1). The acquisition and analysis of tissues was approved by the Chancellor’s Animal Research Committee, University of California, Santa Cruz (approval nos. DOMIN 07.01 and ZIHL 97.12), and the Institutional Animal Care and Use Committee, Stony Brook University (approval no. 20001142). We combined our data with data from three preexisting datasets (Kibale primates: Carter, 2001; modern humans: O’Connell et al. 2001; Macaca mulatta: O’Regan et al. 2008).

Sample preparation and analysis

For each specimen, soft tissues were separated and lyophilized. Bone was defleshed; 20 mg were ground for the analysis of carbonate in bone apatite and 50 mg were crushed coarsely for extraction of collagen. For protein analysis, bone samples were treated with 5 ml of 0.5 N HCl for 72 h to remove the mineral fraction. Samples were rinsed 5× with water and dried. Lipids were removed from all proteinaceous tissues by repeated rinsing and sonication in 5 ml aliquots of petroleum ether for 15 min intervals until all visible lipids were removed. Samples were then rinsed 5× with ultrapure water and lyophilized.

With the exception of keratin—which was cleaned, cut to 1 mm lengths, and homogenized—all soft tissue samples were powdered using a mortar and pestle. Approximately 700 μg of ground soft tissue, homogenized keratin, or bone collagen were then sealed into tin boats and analyzed for δ13C and δ15N values on a ThermoElectron (Finnigan) Delta + XP continuous flow system coupled to an elemental analyzer (EA) at the University of California, Santa Cruz (UCSC) Stable Isotope Laboratory. Analytical precision (±1SD) based on 33 replicates of IAEA Acetanilide was −29.6 ± 0.1‰ for carbon and 1.1 ± 0.1‰ for nitrogen. We ran replicate samples for a subset of our specimens to determine sample precision. The average difference between the absolute value of 14 duplicate tissue samples was 0.2 ± 0.2‰ for carbon and 0.2 ± 0.2‰ for nitrogen. The average difference between the absolute value of five triplicate samples was 0.3 ± 0.1‰ and 0.3 ± 0.3‰ for carbon and nitrogen, respectively.

Bone carbonate samples were prepared using a modified technique from Koch et al. (1997). To oxidize organic materials, 1 ml of 30% laboratory-grade hydrogen peroxide (H2O2) was added to 20 mg of powdered sample and left for 48 h, then rinsed 5× with ultrapure water. To remove non-lattice bound carbonate, samples were reacted for 24 h with 0.5 ml of 1 M acetic acid (buffered to pH 5.0 with calcium acetate). Samples were again rinsed 5× with ultrapure water and lyophilized. For carbonate samples, 1.5 mg of powdered bone were put into steel cups and dried at 65°C for 1 h under vacuum. The samples were then analyzed on a Micromass Optima gas source mass spectrometer integrated with an Isocarb automated carbon device. Samples were dissolved in 100% H3PO4 at 90°C, with concurrent cryogenic distillation of CO2 and H2O and automated CO2 admittance to the mass spectrometer for analysis. Reaction time was set at 740 s and blanks were run between samples. Accuracy and precision (±1SD) based on the international NBS 19 standard analyzed with samples was δ13C = 2.1 ± 0.1‰ (n = 18), very close to the known value of 2.0‰. The average difference between the absolute value of 10 duplicate samples was 0.3 ± 0.2‰.

Data analysis

We were not able to assess dietary composition or digestive physiology carefully for primates included in this study. Although it is tempting to divide primates into broad groups such as folivore, frugivore, or trophic omnivore, these dietary categories would likely be inaccurate for four reasons. First, the majority of primates are generalist primary consumers rather than strict folivores or frugivores. For example, the “frugivorous” lemur Varecia variegata can eat substantial amounts of leaves and fungus (A. Baden, personal communication). Conversely, the diet of Piliocolobus badius, a “folivorous” monkey, frequently contains fruit and flowers (Chapman et al. 2002a). Second, all primates have omnivorous tendencies (Fleagle 1999). In particular, many “frugivorous” primate species supplement their predominantly herbivorous diets either intentionally or inadvertently with insects or vertebrates. For example, among the “frugivorous” species, Hyllobates lar and Lemur catta spend a substantial amount of time feeding on insects in addition to vegetation (Rowe 1996; Yamashita 2002), and Pan troglodytes consumes termites and red colobus monkeys (Boesch and Boesch-Achermann 2000). Third, primate diets can differ substantially between years and between localities (e.g., Chapman et al.2002a, b; González-Zamora et al. 2009). For example diets ranging from 49 to 87% leaves, and 13–49% fruits have been reported for Mexican populations of A. palliata (Cristóbal-Azarate and Arroyo-Rodríguez 2007). Finally, we know little about the diets of most of our captive individuals, including the degree to which they were provisioned with chow.

Instead, we used one-way analysis of variance (ANOVA) and Tukey post-hoc tests of honestly significant differences (HSD) to detect differences in e*-values among habitats (e.g., captive, dry, or moist habitat) that may correlate with diet quality. Diet and digestive physiology may covary with two other variables that we were able to assess: body size and phylogenetic relatedness. In general, diet quality decreases with increasing body size (e.g., Kleiber 1961). More folivorous primates have longer and
more complex guts than frugivorous or insectivorous primates (Chivers and Hladik 1980), and primates that are more closely related should have more similar digestive physiology. We used Pearson correlation coefficients to determine if e* values correlate with body mass, and we tested for the potential confounding effects of phylogenetic relatedness by using the primate phylogeny of Bininda-Emonds et al. (2007) and the PDAP module of Mesquite version 2.5 (Maddison and Maddison 2008) to calculate phylogenetic independent contrasts.

Additionally, we used one-way ANOVA and Tukey HSD to detect differences in e* values among manners of death (grouped into abrupt, short-term illness, long-term illness, and unknown). We used independent sample t tests to detect differences in e* values between sexes. Detailed age information for strepsirrhines from the Duke Lemur Center allowed us to calculate percent lifespan lived. We grouped these individuals into five equal age classes, and used one-way ANOVA and Tukey HSD to detect differences in e* values among age classes. Although there are no theoretical expectations for e* differences among sexes, age classes, or manners of death, we sought to verify that metabolic or dietary differences between these different groups do not affect e* values. Such comparisons are often missing from tissue fractionation and enrichment studies. Analyses were performed using JMP version 5.0.1a for Macintosh with the significance of all tests set at α ≤ 0.05.

Results

Mean and standard deviations for each species are presented in Table 3, and raw δ13C, δ15N, and e* values are available in ESM Table S1. Patterns of apparent enrichment varied little within the Strepsirrhini (Fig. 2) and Haplorrhini (Fig. 3). Across primates, the e13*collagen–keratin, e13*collagen–muscle, e13*muscle–keratin and e13*carbonate–collagen values did not differ (p > 0.05). Whereas, the e15*collagen–keratin and e15*muscle–keratin values also did not differ (p > 0.05), e15*collagen–muscle values did (t = -2.42, df = 18, p = 0.027); however, this result was driven by two Eulemur and Microcebus individuals. The removal of these two individuals resulted in no overall difference among species for e15*collagen–muscle (p > 0.05).

We found small but significant variation at habitat types for both carbon and nitrogen e*collagen–keratin values (carbon: F2,82 = 3.36, p = 0.040; nitrogen: F2,81 = 6.73, p = 0.020). Captive animals had significantly larger e*collagen–keratin values than those from moist habitats (Table 4). Our results for e15*collagen–muscle values showed a similar pattern (F2,44 = 7.03, p = 0.0023), but e13*collagen–muscle values did not differ significantly among habitat types (p > 0.05). Mean e13*muscle–keratin and e13*carbonate–collagen values also did not differ significantly among habitats.

With the exception of e13*collagen–muscle, e* values between proteinaceous tissues did not correlate with body size (p > 0.05; Table 5). If we excluded two captive Microcebus individuals, the relationship between e13*collagen–muscle and body size was insignificant (r² = 0.06, p = 0.10). The relationship between e13*carbonate–collagen and body mass was significant (r² = 0.031, p = 0.038; Table 5). However, because the slope is near 0 and the r² value is low, we suspect that this result is an artifact of sample size. The range in e13*carbonate–collagen values for the smallest and largest species (Microcebus spp. and Gorilla gorilla) are similar (4.5–6.9 and 5.5–7.1%, respectively), and the lowest and highest e13*carbonate–collagen Values, 3.6 and 8.6%, come from two similar-sized species, P. badius and A. palliata (Tables 2 and 3).

Finally, e* values did not differ among males and females (p > 0.05; ESM Table S2), manner of death (p > 0.05; ESM Table S3), or age class (p > 0.05; ESM Table S4). Given the overall consistency of our results, we combined data from all individuals and calculated mean primate e* values between all proteinaceous tissues, and between carbonate and collagen (Table 6).

Discussion

Variation in e* values among proteinaceous tissues

We expected some variation based on differences in amino acid compositions, but that such differences would be small and consistent across taxa. In line with our expectations, we found small (±1%) e* values between collagen and muscle, collagen and keratin, and muscle and keratin for both carbon and nitrogen (Table 6). These mean values are smaller than the majority of the Δ values reported for captive or wild animals (Table 1). It appears that because each tissue is composed of multiple AAs, the effects of isotopic differences among specific AAs are minimized. For example, relatively 13C-enriched glycine in collagen, serine, and cysteine in keratin, and glutamate in muscle may be driving similar δ13C values in all three tissues (Fig. 1). O’Connell et al. (2001) suggest that the relatively elevated levels of serine and threonine in keratin (6–7% vs. ~2% in collagen) tend to lower keratin δ15N values relative to collagen. The 15N-enriched glutamate in muscle may increase its δ15N values relative to keratin.

Variation in e13* carbonate–collagen

We had anticipated that differences in diet (e.g., δ13C differences in dietary sources, differences in
Table 3  Mean carbon and nitrogen apparent enrichment (ε*) values ± one standard deviation for all species and genera

| Superfamily and species | Habitat classes | €_{collagen-keratin} | €_{collagen-muscle} | €_{muscle-keratin} | €_{carbonate-collagen} |
|-------------------------|-----------------|-----------------------|---------------------|-------------------|------------------------|
|                         | n Carbon | Nitrogen | n Carbon | Nitrogen | n Carbon | Nitrogen | n Carbon | Nitrogen |
| **Lotisoidea**           |          |          |          |          |          |          |          |          |
| *Galago senegalensis mohli* | C       | 5 2.3 ± 1.0 | 0.6 ± 1.3 | 3 2.1 ± 0.2 | 0.5 ± 0.9 | 3 0.5 ± 0.9 | 0.8 ± 0.5 | 5 4.9 ± 0.5 |
| **Lemuroidea**           |          |          |          |          |          |          |          |          |
| *Avahi laniger*          | M        | 1 0.0  | −0.4     | 5 0.8 ± 1.0 | −0.7 ± 0.7 | 1 −1.9 | 0.7  | 9 5.1 ± 0.9 |
| *Cheirogaleus major*     | M        | 4 1.5 ± 0.6 | −0.6 ± 1.7 | 4 1.4 ± 1.0 | −1.2 ± 1.2 | 2 0.1 ± 1.4 | 1.3 ± 1.7 | 9 5.5 ± 0.9 |
| *Daubentonia madagascariensis* | C | 2 2.0 ± 0.2 | 0.3 ± 0.5 | 2 2.6 ± 0.6 | −0.6 ± 1.6 | 2 −0.6 ± 0.3 | 0.9 ± 1.2 | 1 3.8 |
| *Eulemur fulus albifrons* | C        | 3 1.0 ± 0.9 | 1.0 ± 0.5 | 3 0.7 ± 1.4 | 0.5 ± 0.7 | 3 0.3 ± 1.5 | 0.5 ± 0.3 | 3 6.1 ± 0.8 |
| **E. fulus rufus**       | M        | 1 1.9 ± 0.6 | 2.3 ± 1.8 | 2 1.6 ± 0.5 | 1.4 ± 0.3 | 2 0.2 ± 1.0 | 1.1 ± 1.3 | 2 5.9 ± 0.3 |
| **E. mongoz**            | C        | 1 0.9  | 2.1      |          |          |          |          |          |
| *Eulemur mean*           |          | 6 1.3 ± 0.8 | 1.6 ± 1.1 | 5 1.1 ± 1.1 | 0.8 ± 0.7 | 5 0.2 ± 1.2 | 0.8 ± 0.8 | 7 6.0 ± 0.5 |
| *Lemur catta*            | C        | 2 1.3 ± 0.3 | 1.1 ± 0.0 | 2 0.6 ± 1.2 | 0.0 ± 0.1 | 2 0.7 ± 1.5 | 0.9 ± 0.3 | 2 6.7 ± 0.0 |
| *Indri indri*            | M        | 1 0.0  | −0.9     |          |          |          |          |          |
| *Microcebus griseornus*  | D        | 2 1.6 ± 0.8 | 0.5 ± 0.3 | 3 3.0 ± 0.4 | 0.3 ± 0.3 | 3 −1.0 ± 0.2 | 0.2 ± 0.3 | 3 5.4 ± 0.8 |
| *M. murinus*             | C        | 3 0.1  | 1.4      | 1 1.4     | 0.0 ± 0.1 | −0.9 ± 0.1 | 0.5 ± 0.7 | 4 6.6 ± 0.9 |
| *M. rufus*               | M        | 4 1.2 ± 1.0 | 0.7 ± 0.5 | 4 2.2 ± 1.0 | 0.2 ± 0.4 | 4 −1.0 ± 0.1 | 0.5 ± 0.7 | 6 5.6 ± 0.9 |
| *Microcebus mean*        |          | 3 0.1 ± 1.2 | 2.0 ± 1.2 | 3 −0.2 ± 0.6 | 0.6 ± 0.6 | 3 0.3 ± 1.7 | 1.4 ± 1.1 | 2 6.1 ± 0.2 |
| *Propithecus coquereli*  | C        | 1 1.4  | 0.6      | 1 0.2     | −0.9 ± 0.1 | 0.5 ± 0.7 | 4 6.6 ± 0.9 |
| *P. diadema*             | M        | 1 1.1  | 0.6      | 1 1.1     | 1.0 ± 0.1 | 2 0.2 ± 0.4 | 0.7 ± 0.4 | 4 6.4 ± 0.9 |
| *P. verreauxi*           | D        | 8 0.8 ± 0.9 | 1.3 ± 0.0 | 2 0.0 ± 0.4 | 0.5 ± 0.1 | 5 0.7 ± 1.3 | 1.4 ± 0.9 | 10 5.0 ± 1.0 |
| *Variecia variegata*     | C        | 8 1.2  | 1.3      | 1 −0.6 | −0.7 ± 0.1 | 0.7 ± 0.1 | 1 −0.8 | 0.7 ± 0.1 | 1 4.8 |
| **Ceboidea**             |          |          |          |          |          |          |          |          |
| *Alouatta palliata*      | M        | 1 1.1  | 0.6      |          |          |          |          |          |
| *A. palliata*            | D        | 7 1.0 ± 0.6 | 0.4 ± 0.4 | 4 0.7 ± 0.5 | −0.6 ± 0.5 | 3 0.0 ± 0.9 | 1.1 ± 0.7 | 8 5.7 ± 0.4 |
| *Alouatta mean*          |          | 8 1.0 ± 0.5 | 0.4 ± 0.4 | 4 0.7 ± 0.5 | −0.6 ± 0.5 | 3 0.0 ± 0.9 | 1.1 ± 0.7 | 22 6.1 ± 1.0 |
| *Ateles geoffroyi*       | M        | 2 1.3 ± 0.7 | 0.3 ± 0.4 |          |          |          |          |          |
| *A. geoffroyi*           | D        | 2 1.3 ± 0.7 | 0.3 ± 0.4 |          |          |          |          |          |
| *Ateles mean*            |          | 2 1.3 ± 0.7 | 0.3 ± 0.4 |          |          |          |          |          |
| *Cebus capucinus*        | M        | 2 −0.5 ± 0.5 | 1.3 ± 0.2 | 2 −0.3 ± 0.5 | −0.1 ± 0.4 | 1 −0.2 | 1.6 ± 0.2 | 1 5.8 |
| *C. capucinus*           | D        | 2 −0.5 ± 0.5 | 1.3 ± 0.2 | 2 −0.3 ± 0.5 | −0.1 ± 0.4 | 1 −0.2 | 1.6 ± 0.2 | 2 5.4 ± 0.6 |
### Table 3 continued

| Superfamily and species                  | Habitat classes<sup>a</sup> | N<sup>b</sup> | Carbon | Nitrogen | N<sup>c</sup> | Carbon | Nitrogen | N<sup>d</sup> | Carbon | Nitrogen | N<sup>e</sup> | Carbon | Nitrogen | N<sup>f</sup> |
|----------------------------------------|-----------------------------|--------------|--------|----------|--------------|--------|----------|--------------|--------|----------|--------------|--------|----------|--------------|
| **Cercopithecoidea**                   |                             |              |        |          |              |        |          |              |        |          |              |        |          |              |
| Cercopithecus ascanius<sup>c</sup>     | M                           | 1            | 2      | 1.9      | 4            | 4.3    | ± 0.4    |              |        |          |              |        |          |              |
| Chlorocebus aethiops                   | C                           | 1            | -0.6   | 1.8      | 1            | -0.4   | 1        | 1            | -0.2   | 0.9      | 1            | 5.9    |          |              |
| Lophocebus albigena<sup>c</sup>        | M                           | 13           | -0.3   | ± 1.2    | 0.4           | ± 0.4  |          |              | 11      | 5.9      | ± 0.7        |        |          |              |
| Macaca mulatta<sup>d</sup>             | M                           | 13           | -0.3   | ± 1.2    | 0.4           | ± 0.4  |          |              | 11      | 5.9      | ± 0.7        |        |          |              |
| Papio anubis<sup>c</sup>               | M                           | 13           | -0.3   | ± 1.2    | 0.4           | ± 0.4  |          |              | 11      | 5.9      | ± 0.7        |        |          |              |
| Piliocolobus badius<sup>c</sup>        | M                           | 13           | -0.3   | ± 1.2    | 0.4           | ± 0.4  |          |              | 11      | 5.9      | ± 0.7        |        |          |              |
| Semnopithecus entellus                 | C                           | 3            | 1.2    | ± 0.4    | 0.7           | ± 0.5  |          |              | 15      | 4.6      | ± 0.8        |        |          |              |
| **Hominioidea**                        |                             |              |        |          |              |        |          |              |        |          |              |        |          |              |
| Gorilla gorilla                        | C                           | 5            | 0.5    | ± 0.2    | 1.4           | ± 0.4  |          |              | 5       | 6.2      | ± 0.8        |        |          |              |
| Homo sapiens<sup>c</sup>               | C                           | 8            | 1.4    | ± 0.5    | 0.9           | ± 0.2  |          |              | 8       | 6.6      | ± 1.5        |        |          |              |
| Hylobates moloch                       | C                           | 2            | 0.6    | ± 0.2    | 1.7           | ± 0.6  |          |              | 2       | 6.3      | ± 0.5        |        |          |              |
| Pan paniscus                           | C                           | 2            | 0.6    | ± 2.4    | 0.1           | ± 0.6  | -1.7     |              | 2       | 6.0      | ± 0.4        |        |          |              |
| P. troglodytes<sup>c</sup>             | M                           | 8            | 6.6    | ± 1.5    | 1             | 6.1    |          |              | 9       | 6.1      | ± 0.7        |        |          |              |
| P. troglodytes mean                    | C                           | 1            | 6.1    | ± 1.5    |              |        |          |              | 1       | 6.1      | ± 0.7        |        |          |              |
| Pan mean                               | 2                           | 6.6           | ± 1.5  | ± 0.7    |              |        |          |              | 11      | 6.1      | ± 0.6        |        |          |              |
| Pongo pygmaeus                         | C                           | 2            | 6.6    | ± 1.5    |              |        |          |              | 2       | 6.0      | ± 0.5        |        |          |              |

<sup>a</sup> Habitat classes are moist (M), dry (D), and captive (C). Moist and dry habitats are differentiated based on rainfall where “Moist” habitats receive >1,000 mm annual precipitation.

<sup>b</sup> Apparent enrichment values are reported in parts per thousand (%).

<sup>c</sup> Data from Carter (2001).

<sup>d</sup> Data from O’Regan et al. (2008).

<sup>e</sup> Data from O’Connell et al. (2001).
herbivory vs. faunivory), and digestive physiology (e.g., degree of fermentation) would lead to differences in $\Delta^{13}C_{\text{carbonate-collagen}}$. Our results, however, suggest that all primates have comparable $\Delta^{13}C_{\text{carbonate-collagen}}$ values regardless of variation in the variables that covary with diet and digestive physiology such as phylogeny, body size, and habitat. Our mean $\Delta^{13}C_{\text{carbonate-collagen}}$ value of 5.6% for primates is similar to the mean fractionation factor ($\Delta_{\text{carbonate-collagen}}$) for wild omnivores (5.5%), captive omnivorous rodents fed mixed and uniform diets (5.5 and 5.4%, respectively), and captive pigs fed uniform diets (6.0%; Table 1). The mean primate $\varepsilon^{13}C_{\text{carbonate-collagen}}$ value is larger than the $\Delta_{\text{carbonate-collagen}}$ value reported for carnivores (3.0%), and smaller than the reported values for both wild ruminant and non-ruminant herbivores (9.0 and 7.8%, respectively; Table 1). Based on the consistency of our results, we conclude that (1) $\delta^{13}C$ values for dietary protein did not differ substantially from whole diet $\delta^{13}C$ values for either captive or wild primates, and (2) that microbial fermentation, to the extent that it occurred in the primates in our study, failed to significantly label the blood pool with $^{13}C$-enriched bicarbonate, irrespective of differences in habitat, gut physiology or body size.

Diet

We had anticipated that more herbivorous primates would have larger $\varepsilon^{13}C_{\text{carbonate-collagen}}$ values than more faunivorous primates. We found some variation but no consistent trends. We found no differences in $\varepsilon^{13}C_{\text{carbonate-collagen}}$ values with body size, despite probable dietary differences between the smallest primates, *Galago* and *Microcebus* spp., which likely consumed more insect matter, and the largest primates, *Gorilla*, *Pan*, and *Pongo*, which likely consumed more vegetation. A single aye-aye (*Daubentonia madagascariensis*), which relies largely on invertebrate prey, had a carnivore-like $\varepsilon^{13}C_{\text{carbonate-collagen}}$ value of 3.7%. However, $\varepsilon^{13}C_{\text{carbonate-collagen}}$ values for the white-faced capuchin (*Cebus capucinus*), which also consumes animal matter, resembled the overall primate mean (5.0 and 5.8% in dry and moist habitat, respectively). Our results likely reflect underlying dietary similarities among all primate species. In spite of apparent differences in the consumption of animal matter, all primates have a predominantly vegetarian diet (Milton 1987). These results agree with the recent findings of Smith et al. (2010), who showed that collagen $\delta^{13}C$ values did not differ between male and female chimpanzees despite observations that males consumed substantially greater amounts of red colobus meat. These authors speculated that either meat consumption did not noticeably affect male collagen $\delta^{13}C$ values, or that consumption of termites elevated female $\delta^{13}C$ values (Smith et al. 2010).

With the exception of a few *M. mulatta* individuals (O’Regan et al. 2008; ESM Table S1), none of the wild primates in our study ate C_4 or marine foods ($\varepsilon_{\text{apatite}}^{13}C=-16.7\%\pm 1.4, \ n=105$; mean $\delta^{13}C$ collagen $=-22.1\%\pm 1.5, \ n=110$). Conversely, the majority

---

Fig. 2 Mean $\varepsilon^{13}C_{\text{collagen-keratin}}$, $\varepsilon^{13}C_{\text{collagen-muscle}}$, $\varepsilon^{13}C_{\text{muscle-keratin}}$, and $\varepsilon^{13}C_{\text{carbonate-collagen}}$ for carbon ($^{13}C$) and nitrogen ($^{15}N$) ± 1 standard deviation for each strepsirrhine genus. Phylogeny based on Orlando et al. (2008). Illustrations by Stephen D. Nash/Conservation International, used with permission.

---

1 We acknowledge that the observed difference between primates and non-ruminant herbivores may stem entirely from a lack of broad comparative data. Non-ruminant data are derived from equids and hippos, two groups of herbivores with substantial methane production rates (Crutzen et al. 1986), and camels, which have $\Delta_{\text{carbonate-collagen}}$ values comparable to ruminants.
of our captive primates appear to have incorporated some C\textsubscript{4} foods into their diets (mean $\delta^{13}$C apatite = $-12.7\% \pm 1.6$, $n = 36$; mean $\delta^{13}$C collagen = $-18.5\% \pm 1.8$, $n = 45$; ESM Table S1). However, despite this addition of C\textsubscript{4} foods, $\delta^{13}$\textsubscript{C} carbonate-collagen values for captive and wild primates do not differ (Table 4). We cannot assess dietary composition in the captive primates quantitatively. Nevertheless, our results suggest that protein and whole diet $\delta^{13}$C values did not differ substantially for captive animals. We note that the laboratory diets for some of the controlled feeding studies listed in Table 1 were designed to maximize possible isotopic differences among tissues. The majority of these diets were not designed to maintain healthy individuals, and most laboratory animals were sacrificed at a young age. In contrast, captive primates are given balanced diets designed to maintain their health and increase their longevity. As a result, diets for captive primates tend to be much more isotopically restricted than experimental laboratory diets consisting of mixed C\textsubscript{3}, C\textsubscript{4}, and marine components. In line with this reasoning, the range in captive primate $\Delta^{13}$\textsubscript{C} carbonate-collagen values (3.8–7.1\%) is similar to the range in $\Delta^{13}$\textsubscript{C} carbonate-collagen values reported for captive animals fed isotopically homogenous diets (4.5–6.7\%), but much smaller than the ranges reported for captive animals fed experimental diets incorporating a mix of C\textsubscript{3}, C\textsubscript{4}, and marine components ($-0.8$ to $11.3\%$, Table 1).

Physiology and fermentation

Despite differences in diet, all primates ferment their food to some degree. More folivorous, gumnivorous, and faunivorous primates break down the structural carbohydrates in vegetation, plant exudates, and arthropod exoskeletons, respectively (Lambert 1998). Nevertheless, carbohydrate fermentation in primates does not appear to produce enough methane and associated $^{13}$C-enriched CO\textsubscript{2} to significantly label blood bicarbonate or bone carbonate. We might have anticipated that taxa with long measured retention times such as Gorilla, Pongo, Lophocebus, Chlorocebus, and Cercopithecus might have larger $\epsilon^{13}$\textsubscript{C} carbonate-collagen values (Kleiber 1961; Langer 1987). Increased retention time may increase methane production during fermentation, and the degree to which $^{13}$C-enriched CO\textsubscript{2} diffuses into the blood (Kleiber 1961; Langer 1987). Our results do not support these expectations. Mean $\epsilon^{13}$\textsubscript{C} carbonate-collagen values for the hominoids (6.2 and 6.0\%, respectively), and the cercopithecines (5.9, 5.9, and 4.3\%, respectively; Table 1) are comparable to or only slightly larger than our mean primate $\epsilon^{13}$\textsubscript{C} carbonate-collagen value (5.6\%). Conversely, the $\epsilon^{13}$\textsubscript{C} carbonate-collagen value for Ateles geoffroyi, which has a fast retention time (6.8\%), is substantially larger than the average primate value.
We had also anticipated that colobine monkeys, represented by *P. badius* and *Semnopithecus entellus*, and the ateline monkey *A. palliata*, would have higher $\varepsilon^{13}$carbonate–collagen values associated with fermentation in their enlarged stomachs and caeca, respectively. Our results do not support these expectations. Despite their potential for increased levels of methane production, both wild and captive colobine monkeys in our dataset had $\varepsilon^{13}$carbonate–collagen values comparable to other primate species (Fig. 2; Tables 3 and ESM S1). Our lowest reported $\varepsilon^{13}$carbonate–collagen value (3.6%) is from a wild *P. badius* individual. This result is in agreement with the lack of methane production observed in two wild *Colobus polykomos* individuals (Ohwaki et al. 1974). It appears that, despite their large “ruminant-like” stomachs, colobines produce little to no methane and associated $^{13}$C-enriched CO$_2$, and their digestion resembles that of small simple-stomached animals rather than ruminants.

We did find a large mean $\varepsilon^{13}$carbonate–collagen value for the mantled howling monkey (*A. palliata*) in a rainforest habitat (7.6%). However, we also found a large $\varepsilon^{13}$carbonate–collagen value (8.4%) for rainforest-dwelling black-handed spider monkeys (*A. geoffroyi*), which does not have a gut designed for extensive fermentation (Chivers and Hladik 1980). Intriguingly, these two species had comparable but lower $\varepsilon^{13}$carbonate–collagen values similar to our primate mean in a seasonally dry forest habitat (5.7 and 5.3%, respectively). Although *A. palliata* and *A. geoffroyi* are typically categorized as folivorous and frugivorous, respectively, both of these species have been observed to have highly variable diets (Cristóbal-Azkarate and Arroyo-

### Table 4 Mean carbon and nitrogen apparent enrichment ($\varepsilon^*$) values ± one standard deviation for primates living in dry, moist, and captive settings

|       | Carbon |          | Nitrogen |          |
|-------|--------|----------|----------|----------|
|       | $\varepsilon^{\%}$ | $\varepsilon^{\%}$ | $\varepsilon^{\%}$ | $\varepsilon^{\%}$ |
|       | collagen-keratin | collagen-muscle | muscle-keratin | carbonate-collagen |
|       | n     |          | n        |          | n          |          | n          |          |
| Dry   | 15    | 0.8 ± 0.8 A | 9        | 0.3 ± 0.6 A | 6          | 0.4 ± 0.9 A | 29        | 5.5 ± 0.8 A |
| Moist | 25    | 0.4 ± 1.2 B | 12       | 0.9 ± 0.9 A | 4          | −0.7 ± 1.2 A | 75        | 5.5 ± 1.2 A |
| Captive | 43     | 1.1 ± 1.0 A | 25       | 1.3 ± 1.2 A | 30         | −0.05 ± 1.3 A | 35        | 5.7 ± 0.8 A |

n.a. Not applicable

* Apparent enrichment values are reported in parts per thousand (%). Mean $\varepsilon^*$ values in the same homogenous subset are given the same letters (α set at 0.05)

### Table 5 Regression results for $\varepsilon^*$ versus the natural logarithm of body mass

|       | Carbon |          | Nitrogen |          |
|-------|--------|----------|----------|----------|
|       | $r^2$ | p        | $r^2$ | p        |
|       |       |          |       |          |
| Collagen–keratin | 83 | 0.046 | 0.051 | 82 | 0.021 | 0.19 |
| Collagen–muscle | 46 | 0.110 | 0.015 | 45 | −0.023 | 0.98 |
| Muscle–keratin | 36 | −0.024 | 0.670 | 36 | −0.019 | 0.55 |
| Carbonate–collagen | 140 | 0.031 | 0.038 | n.a. | n.a. | n.a. |

### Table 6 Suggested $\varepsilon^*$ values for comparing different primate tissue types

| Tissue comparison | Carbon | Nitrogen |
|-------------------|--------|----------|
|                   | Mean ± 1 SD (%) | Mean ± 1 SD (%) |
| Collagen–keratin  | 0.9 ± 1.1 | 0.8 ± 0.9 |
| Collagen–muscle   | 1.0 ± 1.1 | −0.1 ± 1.0 |
| Muscle–keratin    | −0.04 ± 1.2 | 0.9 ± 0.8 |
| Carbonate–collagen | 5.6 ± 1.0 | n.a. |

Fig. 4 The relationship between the natural log of body mass (kg) and $\varepsilon^{13}$carbonate–collagen ($\varepsilon^{13}$carbonate–collagen = 5.24 + 0.163*ln body mass, $r^2 = 0.031, p = 0.038$)

We had also anticipated that colobine monkeys, represented by *P. badius* and *Semnopithecus entellus*, and the ateline monkey *A. palliata*, would have higher $\varepsilon^{13}$carbonate–collagen values associated with fermentation in their enlarged stomachs and caeca, respectively. Our results do not support these expectations. Despite their potential for increased levels of methane production, both wild and captive colobine monkeys in our dataset had $\varepsilon^{13}$carbonate–collagen values comparable to other primate species (Fig. 2; Tables 3 and ESM S1). Our lowest reported $\varepsilon^{13}$carbonate–collagen value (3.6%) is from a wild *P. badius* individual. This result is in agreement with the lack of methane production observed in two wild *Colobus polykomos* individuals (Ohwaki et al. 1974). It appears that, despite their large “ruminant-like” stomachs, colobines produce little to no methane and associated $^{13}$C-enriched CO$_2$, and their digestion resembles that of small simple-stomached animals rather than ruminants.

We did find a large mean $\varepsilon^{13}$carbonate–collagen value for the mantled howling monkey (*A. palliata*) in a rainforest habitat (7.6%). However, we also found a large $\varepsilon^{13}$carbonate–collagen value (8.4%) for rainforest-dwelling black-handed spider monkeys (*A. geoffroyi*), which does not have a gut designed for extensive fermentation (Chivers and Hladik 1980). Intriguingly, these two species had comparable but lower $\varepsilon^{13}$carbonate–collagen values similar to our primate mean in a seasonally dry forest habitat (5.7 and 5.3%, respectively). Although *A. palliata* and *A. geoffroyi* are typically categorized as folivorous and frugivorous, respectively, both of these species have been observed to have highly variable diets (Cristóbal-Azkarate and Arroyo-
Rodríguez 2007; González-Zamora et al. 2009). It is possible that they shared dietary items in the rainforest habitat that were rich in non-starch polysaccharides (NPS), the breakdown of which has been associated with increased methane production in pigs (Jensen 1996). Alternatively, it is possible that the two species shared a food item with elevated δ13C values, (e.g., a CAM plant) which increased their whole diet δ13C values without affecting their dietary protein. This result is interesting and suggests that future work examining species-specific δ13C–collagen values with varying diets could be enlightening. Nevertheless, these are the only two taxa that demonstrate substantial differences in apparent enrichment values among habitats. For example, δ13C–collagen values for C. capucinus from the same two habitats are much more similar (5.8 and 5.0‰ in the moist and dry habitats, respectively). Pan troglodytes exhibits similar δ13C–collagen values among captive and moist habitats (6.1 and 6.6‰, respectively), and all Microcebus taxa have similar δ13C–collagen values in all three habitat types (5.4, 6.0, and 5.7‰ in captive, moist, and dry habitats, respectively). Based on the data available, we therefore advocate using our mean primate δ13C–collagen value (5.6‰) to compare collagen and carbonate δ13C values among primates.

Verification of ε* values

An important outcome of our analyses is the ability to determine mean apparent enrichment values that can be used in existing and future comparisons based on mixed tissues or samples. To validate primate ε* values, we estimated keratin δ13C and δ15N values by applying mean ε* collagen–keratin values to measured collagen δ13C and δ15N values for wild primate populations not included in our apparent enrichment dataset. We then compared these estimated keratin values to measured keratin values from different individuals within the same wild populations (Table 7). Compellingly, the range of estimated keratin isotope values closely matches the measured keratin isotope values.

Conclusions

We have presented data on the apparent isotopic enrichment in carbon and nitrogen isotopes between collagen and keratin, collagen and muscle, and apatite carbonate and collagen in primates. Primates are an extremely diverse group of animals in terms of diet, body size, and gut morphology, yet ε* values are relatively invariant across the order. We recommend applying our calculated mean ε* values when comparing isotope values from different modern primate tissues. Additionally, using these mean apparent enrichment values will be essential for accurately predicting how the isotopic niches of extinct primates compare with those of modern extant primates.

Acknowledgments

We are grateful to institutions that donated cadaveric tissues to the Department of Anthropology, UC Santa Cruz (Oklahoma Zoo, San Francisco Zoo, Ft. Worth Zoo, Milwaukee Zoo, Humboldt Zoo, Chaffee Zoological Gardens, Santa Anna Zoo, Duke Lemur Center, The Gorilla Foundation, The Gibbon Conservation Center). We thank M.J. Schoeninger for providing raw collagen and carbonate δ13C values for wild African herbivores. We are also grateful to the following individuals for samples and assistance: M.R. Blanco, A.D. Cunningham, K.A. Dingess, P. Dolhinow, K.E. Glander, L.R. Godfrey, W. McCandless, S. Matarazzo, I. Mesen, A. Mootnick, G. Pieraccini, M.A. Ramsier, R.B. Segura, C. Underwood, E.R. Vogel, P.C. Wright, and S. Zehr. We thank two anonymous reviewers for useful comments on earlier versions of this manuscript. The

Table 7 Comparing estimated keratin δ13C and δ15N values with measured keratin δ13C and δ15N values from wild and captive primate populations

| Isotope     | n | Mean collagen ± 1 SD (‰) | Estimated keratin (‰) | Measured mean keratin ± 1 SD (‰) | n | Source |
|-------------|---|--------------------------|------------------------|----------------------------------|---|--------|
| Wild        |   |                          |                        |                                  |   |        |
| Cercopithecus ascanius | Carbon 3 | –21.1 ± 0.3 | –22.0 | –22.7 ± 0.2 | 2 | 1 |
| Lophocebus albigena | Carbon 1 | –20.8 | –21.7 | –21.7 ± 0.2 | 2 | 1 |
| Pan troglodytes | Carbon 9 | –21.5 ± 0.7 | –22.4 | –21.8 | 1 | 1 |
| Piliocolobus badius | Carbon 12 | –21.0 ± 0.5 | –21.9 | –22.5 ± 0.4 | 6 | 1 |
| Propithecus verreauxi | Carbon 7 | –21.2 ± 0.7 | –22.1 | –23.1 ± 1.0 | 5 | 2 |
| Nitrogen 7 | 7.3 ± 0.7 | 6.5 | 6.3 ± 1.2 | 5 | 2 |
| Captive     |   |                          |                        |                                  |   |        |
| Pan paniscus | Carbon 1 | –20.2 | –21.1 | –20.7 ± 1.2 | 3 | 2 |
| Nitrogen 1 | 8.3 | 7.5 | 8.0 ± 2.1 | 3 | 2 |
| Semnopithecus entellus | Carbon 3 | –16.5 ± 0.5 | –17.4 | –17.2 ± 0.7 | 2 | 2 |

Estimated keratin isotope values were calculated by applying mean ε* collagen–keratin values (Table 6) to measured collagen isotope values. Data are from (1) Carter (2001); (2) this study.
importation and use of animal tissues was approved by the United States Fish and Wildlife Service (CITES permit nos. 06US130146/9 and 007319). Funding was provided by the David and Lucile Packard Foundation. This is DLC publication #1181.

**Open Access** This article is distributed under the terms of the Creative Commons Attribution Noncommercial License which permits any noncommercial use, distribution, and reproduction in any medium, provided the original author(s) and source are credited.

**References**

Ambrose SH, DeNiro MJ (1986) The isotopic ecology of East African mammals. Oecologia 69:395–406

Ambrose SH, Norr L (1993) Experimental evidence for the relationship of the carbon isotope ratios of whole diet and dietary protein to those of bone collagen and carbonate. In: Lambert JB, Grupe G (eds) Prehistoric human bone—archaeology at the molecular level. Springer, Berlin, pp 1–37

Ambrose SH, Butler BM, Hanson DB, Hunter-Anderson RL, Krueger HW (1997) Stable isotopic analysis of human diet in the Marianas Archipelago, Western Pacific. Am J Phys Anthropol 104:343–361

Bergström J, Furst P, Noree L-O, Vinners E (1974) Intracellular free amino acid concentration in human muscle tissue. J Appl Physiol 36:693–697

Bininda-Emonds ORP, Cardillo M, Jones KE, MacPhee RDE, Beck Ambrose SH, Butler BM, Hanson DB, Hunter-Anderson RL, Krueger HW (1997) Stable isotopic analysis of human diet in the Marianas Archipelago, Western Pacific. Am J Phys Anthropol 104:343–361

Chapman CA, Chapman LJ, Gillespie TR (2002a) Scale issues in the study of primate foraging: red colobus of Kibale National Park. Am J Phys Anthropol 117:349–363. doi:10.1002/ajpa.10053

Chapman CA, Chapman LJ, Gillespie TR (2002a) Scale issues in the study of primate foraging: red colobus of Kibale National Park. Am J Phys Anthropol 117:349–363. doi:10.1002/ajpa.10053

Chapman CA, Chapman LJ, Cords M, Gauthier JM, Gautier-Hion A, Lambert JA, Rode K, Tutin CEG, White LJLT (2002b) Variation in the diets of Cercopithecus species: differences within forests, among forests, and across species. In: Glenn M, Cords M (eds) The Genuons: diversity and adaptation in African monkeys. Kluwer/Plenum, New York, pp 325–350

Chivers DJ, Hladik CM (1980) Morphology of the gastrointestinal tract in primates: comparisons with other mammals in relation to diet. J Morphol 166:337–386

Codron D, Luty J, Lee-Thorp JA, Sponheimer M, de Ruiter D, Codron J (2005) Utilization of savanna-based resources by Plio-Pleistocene baboons. S Afr J Sci 101:245–248

Codron D, Lee-Thorp JA, Sponheimer M, de Ruiter D, Codron J (2006) Inter- and intrahabitat dietary variability of chacma baboons (Papio ursinus) in South African savannas based on fecal δ13C, δ15N, and δ18O. Am J Phys Anthropol 129:204–214. doi:10.1002/ajpa.20253

Corr LT, Sealy JC, Horton MC, Evershed RP (2005) A novel marine dietary indicator utilizing compound-specific bone collagen amino acid δ13C values of ancient humans. J Archaeol Sci 32:321–330. doi:10.1016/j.jas.2004.10.002

Cristóbal-Azkarate J, Arroyo-Rodríguez V (2007) Diet and activity pattern of howler monkeys (Alouatta palliata) in Los Tuxtlas, Mexico: effects of habitat fragmentation and implications for conservation. Am J Primatol 69:1013–1029. doi:10.1002/ajp.20420

Crutzen PJ, Aselmann I, Seiler W (1986) Methane production by domestic animals, wild ruminants, and other herbivore fauna and humans. Tellus Series B 38:271–284

DeNiro MJ, Epstein S (1978) Influence of diet on the distribution of carbon isotopes in animals. Geochim Cosmochim Acta 42:495–506

DeNiro MJ, Epstein S (1981) Influence of diet on the distribution of nitrogen isotopes in animals. Geochim Cosmochim Acta 45:341–351

Fleagle JG (1999) Primate adaptation and evolution, 2nd edn. Academic, San Diego

Fogel ML, Tuross N (2003) Extending the limits of paleodietary studies of humans with compound specific carbon isotope analysis of amino acids. J Archaeol Sci 30:535–545. doi:10.1016/j.jas.2008.06.002

Fourie NH, Lee-Thorp JA, Ackermann RR (2008) Biogeochemical and craniometric investigation of dietary ecology, niche separation, and taxonomy of Plio-Pleistocene cercopithecoids from the Makapansgat Limeworks. Am J Phys Anthropol 135:121–135. doi:10.1002/ajpa.20713

Fox-Dobbs K, Bump JK, Peterson RO, Fox DL, Koch PL (2007) Carnivore-specific stable isotope variables and variation in the foraging ecology of modern and ancient wolf populations: case studies from Isle Royale, Minnesota, and La Brea. Can J Zool 85:458–471. doi:10.1139/Z07-007

Gézin F (2008) Life in unpredictable environments: first investigation of the natural history of Microcebus griseus. Int J Primatol 29:303–321. doi:10.1007/s10764-008-9243-z

González-Zamora A, Arroyo-Rodríguez V, Chaves OM, Sánchez-Lopez S, Stoner KE, Riba-Hernández P (2009) Diet of spider monkeys (Ateles geoffroyi) in Mesoamerica: current knowledge and future directions. Am J Primatol 71:8–20. doi:10.1002/ajp.20625

Groves C (2001) Primate taxonomy. Smithsonian Institution Press, Washington DC

Hare PE, Estep MLF (1983) Carbon and nitrogen isotopic composition of amino acids in modern and fossil collagens. Year B Carnegie Inst Wash 82:410–414

Hare PE, Fogel ML, Stafford TW, Mitchell AD, Hoering TC (1991) The isotopic composition of carbon and nitrogen in individual amino acids isolated from modern and fossil proteins. J Archaeol Sci 18:277–292

Hedges REM (2003) On bone collagen-apatite-carbonate isotopic relationships. Int J Osteoarchaeol 13:66–79. doi:10.1002/oa.660

Howland MR, Corr LT, Young SMM, Jones V, Jim S, van der Merwe NJ, Mitchell AD, Evershed RP (2003) Expression of the dietary isotope signal in the compound-specific δ13C values of pig bone lipids and amino acids. Int J Osteoarchaeol 13:54–65. doi:10.1002/oa.658

Hrdy D, Baden HP (1973) Biochemical variation of hair keratins in man and non-human primates. Am J Phys Anthropol 39:19–24

Jensen BB (1996) Methanogenesis in monogastric animals. Environ Monit Assess 42:99–112

Jim S, Ambrose SH, Evershed RP (2004) Stable carbon isotopic evidence for differences in the dietary origin of bone cholesterol, collagen and apatite: implications for their use in palaeodietary reconstruction. Geochim Cosmochim Acta 68:61–72. doi:10.1016/S0016-7037(03)00216-3

Jim S, Jones V, Ambrose SH, Evershed RP (2006) Quantifying dietary macronutrient sources of carbon for bone collagen biosynthesis using natural abundance stable carbon isotope analysis. Br J Nutr 95:1055–1062. doi:10.1079/BJN20051685
