ELECTRONIC SUPPLEMENTARY MATERIALS FOR:

"BODY MASS PREDICTS ISOTOPE ENRICHMENT IN HERBIVOROUS MAMMALS"

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MATERIALS AND METHODS

Stable isotope analyses

Samples of teeth and feces of *Bradypus variegatus* and *Choloepus hoffmanni* were collected from adult individuals raised at the Huachipa Zoo (Lima, Peru), except for one dental sample of *Choloepus hoffmanni* from the Tampa's Lowry Park Zoo (Florida, USA). Individuals were either born at or adopted by the zoos at a young age, and spent several years being fed a controlled diet (spanning 5 to 12 years under a controlled diet feeding regime at the zoo). Samples of living specimens from the Huachipa Zoo were collected by the zoo staff during the annual health inspection. Dental bioapatite was analyzed for $\delta^{13}C$. *Choloepus* and *Bradypus* have ever-growing teeth which in captivity are not sufficiently worn down over time as they are in natural conditions. During health inspections at zoos, excessively grown caniniforms (*Choloepus*) and incisiviforms (*Bradypus*) are trimmed to prevent animals from hurting themselves (in a similar fashion to veterinarian treatments for hamsters). Instead of being discarded, these disposable samples of dental tissues were collected and used for isotopic analyses. Samples were later powdered in the lab using a mortar and pestle.

Powdered samples of dental bioapatite were analyzed for $\delta^{13}C$ and $\delta^{18}O$. Modern samples were first treated for three hours with H_2O_2 to remove organic contaminants, rinsed three times with distilled water, bathed for an hour in 0.1 N acetic acid to remove any secondary carbonates, rinsed three more times with distilled water followed by a fourth rinse with methanol, and finally dried. Samples were run on a Finnigan MAT 252 isotope ratio mass spectometer (IRMS) with a Kiel device. Foodstuff and feces were collected on a daily basis for 11 and 15 days respectively, and analyzed for $\delta^{13}C$ and $\delta^{15}N$. All organic samples were freeze dried and homogenized in a Spex 67000 liquid nitrogen mill. Weight %C and %N were determined using a Carlo-Erba 1500 elemental analyzer after IRMS analysis. All the samples were processed and analyzed at the Department of Geological Sciences, University of Florida (Gainesville).

Fossil dung was freeze dried, homogenized, and processed as for the modern organic samples. Teeth of fossil sloths were sampled using a Dremel® drill and carbide dental burrs. Approximately 0.1 g of powdered outermost dentine was collected and processed as described above.

Values are reported using the conventional permil (‰) notation wherein $\delta^{13}C = (R_{sample}/R_{standard} - 1) \times 1000)$, and $R = {}^{13}C/{}^{12}C$. Values of $\delta^{13}C$ are reported relative to the VPDB standard. Isotope enrichment (ε^*) was calculated as in Cerling and Harris [3], i.e., as a function of α^* (apparent fractionation), as follows: $\varepsilon^* = (\alpha^* - 1) \times 1000$, where $\alpha^* = (1000 + \delta^{13}C_{tissue})/(1000 + \delta^{13}C_{diet})$. The superscript (*) indicates that isotopic equilibrium is not assumed.

Analytical precision of standards:
Inorganic samples (teeth bioapatite, NBS-19 standard)
$\delta^{13}C = 0.02$; $\delta^{18}O = 0.05$
Organic samples (food and feces; USGS-40 standard):
$\delta^{13}C = 0.09$; $\delta^{15}N = 0.22$

Foodstuff

A concentration-weighted linear mixing model [49] was used to calculate the isotopic signatures of any mixed diet. In this model, the proportional contribution of each component to the mixture (and therefore to the consumer) equals the fraction of that component in the mixture multiplied by its elemental concentration (in this case, wt %C and wt %N) and divided by the sum of the products of fraction and concentration of each dietary component, as follows:

$$f_{X,C} = \frac{f_{X,B}[C]_X}{f_{X,B}[C]_X + f_{Y,B}[C]_Y + f_{Z,B}[C]_Z}$$

$$f_{Y,C} = \frac{f_{Y,B}[C]_Y}{f_{X,B}[C]_X + f_{Y,B}[C]_Y + f_{Z,B}[C]_Z}$$

$$f_{Z,C} = \frac{f_{Z,B}[C]_Z}{f_{X,B}[C]_X + f_{Y,B}[C]_Y + f_{Z,B}[C]_Z}$$

Where $f\,(\mathrm{x}, \mathrm{C})$; $f\,(\mathrm{y}, \mathrm{C})$, $f\,(\mathrm{z}, \mathrm{C})$, are the fractions of assimilated C, of components "x", "y", "z" in the mixed diet.

Regression models and traitgram

Data for linear regression models of mammals other than sloths, were collected from the literature (table 2, table S1). Values of $\varepsilon^*_{\text{diet-bioapatite}}$ were regressed against body mass (BM), basal rate of metabolism (BMR), average rectal temperature, and range of rectal temperature variation. All data were log transformed (ln). Statistics and regression modeling were performed with RStudio (v 1.0.136) using packages MASS, foreign, quantreg, MuMIn, and lme4. Traitgram was performed with the R package phytools [50]. We assessed relationships between variables by performing OLS, quantile, and robust regression models; Cook's distance was used as an estimate for the influence of outliers. Linear mixed effect model analyses based on AICc tests were used to assess whether a single variable, reduced model, or various combinatorial model subsets were better supported by the data than a complete global model that includes all the potential predictors. Tree topology for Figure 1 is from O'Leary et al. [51]. Divergence ages (in Ma, or Megannum), only for illustrative purposes (providing approximations for visual representation of the pattern of lineage splitting, but not used in calculations), are mean estimates from the literature (see below).

Regression formulae

This paper proposes three regression formulae (body-mass calibrated) to calculate the C diet-bioapatite enrichment ($\varepsilon^*_{\text{diet-bioapatite}}$) of herbivorous mammals (both extant and extinct). The first formula (A) is based on the regression analysis of correlation of all mammals included:

$$\boldsymbol{\varepsilon * = 2.4 + 0.034\ (BM)} \qquad \textbf{(A)}$$

Where BM is the body mass in kg and should be log transformed (ln). The obtained diet-bioapatite ε* will need to be inverted (e^x) to obtain the ‰ value to be applied for interpretation of the isotopic signature of the herbivorous mammal under study.

This formula (A) should be used when the type of digestive fermentation of the mammal under study is unknown, does not fall within foregut or hindgut types of fermentation (e.g. giant panda), or to get a general or most conservative body-size calibrated value for $\varepsilon^*_{\text{diet-bioapatite}}$.

The two other formulae we propose separate mammals by foregut (B) versus hindgut fermenters (C):

$$\varepsilon * = \mathbf{2.34 + 0.05\ (BM)} \qquad \textbf{(B)}$$

$$\varepsilon * = \mathbf{2.42 + 0.032\ (BM)} \qquad \textbf{(C)}$$

These formulae should be applied when the type of digestive fermentation (foregut vs hindgut) is known or most likely given phylogenetic history of the mammal under study. As for the first formula, BM is in kg and log transformed (ln), and ε* needs to be inverted (e^x) to obtain the ‰ value to be applied for interpretation of the isotopic signature of the herbivore mammal under study. Table 3 documents that multiple models agree closely in terms of regression slopes and intercepts. Rather than select one arbitrarily, we advocate instead for applying a model averaging approach. As discussed by Sears et al. [52], model averaging is appropriate when there is no single, clearly identifiable "correct" model, and given the close agreement across all of the models and that each performs equally well in estimating the variables, use of the mean of the individual estimates is appropriate here.

Sources of divergence ages
Bradypus / *Choloepus*+*Mylodon*: 27 ± 3 Ma [53]; or 29.3 Ma sensu [54].
Mylodon / *Choloepus*: 27.5 Ma [54].
Muridae (*Mus*) / Cricetidae (*Microtus*): 65.8 Ma [55].
Rabbit / Mouse: 65.5 Ma; based on *Mimotona wana* from [51]
Giraffidae / remaining Pecora (i.e. including *Bos*): 19 Ma [56].
Suidae / Ruminantia: 35.2 Ma; based on *Elomeryx crispus* from [51].
Ruminantia / Camelidae: 49-55 Ma; (based on *Pseudamphimeryx*, first ruminant, [57] from [58]).
Camelus / *Lama*: 25 Ma [59].
Equus / *Diceros*: 52-58 Ma; split Ceratomorpha and Hippomorpha [60].
Equus caballus / *Equus burchelli*: 3 Ma [61].
Arytiodactyla / Perissodactyla: 55.4-50.3 Ma, based on *Hyracotherium angustidens* [51].
Carnivora / Eungulata: 84.9 Ma [62].
Afrotheria: 101 Ma [62].
Euarchontoglires / Laurasiatheria: 98.9 Ma [62].
Xenarthra / Epitheria: 101 Ma [62].
Marsupialia / Eutheria: 147.7 Ma [62].

Sources of life history trait values

BM: body mass; BMR: basal metabolic rate, and basal metabolic rate corrected for body mass using the Kleiber value; Temp: body temperature, as average rectal temperature and breadth of range of rectal temperature. † = extinct taxon. Unpublished data from [6] provided by the lead author of that study, B. Passey.

Choloepus hoffmanni
BM: data from this study; BMR [35]; Temp: data from this study and [36].
Notes: Range of temperature is from [36] but average temperature is from specimens analyzed in this study (Huachipa Zoo).

Bradypus variegatus
BM: data from this study; BMR [35], Temp: data from this study and [36].
Notes: same as for *Choloepus*.

†Mylodon darwinii
BM [63].
Note: Although McNab (1985, [35]) provided estimates for BMR and BMR/Kleiber value for *Mylodon listai*, we consider these estimations as unreliable because they are based on highly questionable foundations (body hair length as a proxy for thermal insulation correlated to BMR).

Bos taurus
BM [unpublished data from 6]; BMR [64]; Temp [65].

Sus scrofa
BM [unpublished data from 6]; BMR [64]; Temp [66].

Oryctolagus cuniculus
BM [unpublished data from 6]; BMR [67]; Temp [68].
Notes: BMR based on a close relative, *Lepus alleni*.

Microtus ochrogaster
BM [unpublished data from 6]; BMR [69]; Temp [69,70].
Notes: BMR based on a congener, *Microtus guentheri*.

Mus musculus
BM [5]; BMR [64]; Temp [71,72]

Lama guanicoe
BM [73]; BMR [73,74]; Temp [75].
Notes: BMR based on a congener, *Lama glama*.

Giraffa camelopardalis
BM [76]; BMR [77]; Temp [78].
Notes: BMR calculated from resting metabolic rate (RMR). BMR was assumed to account for ~85% of the RMR.

Diceros bicornis
BM [76,79]; Temp [80].

Camelus bactrianus
BM [67,76,79]; BMR [67]; Temp [81].
Notes: BMR based on a congener, *Camelus dromedarius*.

Equus caballus
BM [82]; BMR [64]; Temp [83].

Equus burchelli
BM [84]; BMR [64]; Temp [84].
Notes: BMR based on a congener, *E. caballus*.

Ailuropoda melanoleuca
BM [85]; BMR [85]; Temp [86].
Notes: BMR calculated from resting metabolic rate.

Loxodonta africana
BM [82]; BMR [64]; Temp [87].
Notes: BMR based on a close relative, *Elephas maximus*

Phascolarctos cinereus
BM [67,82]; BMR [67]; Temp [88].

TAXA	E*(mean)	BM (Kg)	FERM	BMR	BMR/Kleiber value	Temp Mean	Temp Variation	Temp Range
Choloepus hoffmanni	12.6	8.28	fore	0.188	0.44	34.4	33.4-36.2	2.8
Bradypus variegatus	10.3	4.23	fore	0.181	0.42	31	28.4-37.6	9.2
Mylodon darwinii†	15.6	1600	fore	NA	NA	NA	NA	NA
Bos taurus	14.6	322.7	fore	0.17	1.14	38.7	38.3-39.1	0.8
Sus scrofa	13.3	128.6	hind	0.11	0.53	39.3	38.8-39.8	1
Oryctolagus cuniculus	12.8	3.5	hind	0.45	1	39.3	38.1-40.8	2.7
Microtus ochrogaster	11.5	0.05	hind	1.18	0.92	38	37.3-38.7	1.4
Mus musculus	9.1	0.021	hind	3.4	2.12	37.65	36-39.3	3.3
Lama guanicoe	12.9	120	fore	0.23	1.24	38	37.5-38.5	1
Giraffa camelopardalis	14.1	1500	fore	0.13	1.08	38.5	38-39	1
Diceros bicornis	14.4	1089	hind	NA	NA	37.74	36.8-38.6	1.8
Camelus bactrianus	13.7	454	fore	0.1	0.74	37	34-40	6
Equus caballus	13.7	260	hind	0.25	1.65	37.7	37.2-38.2	1
Ailuropoda melanoleuca	10.1	92	NA	0.082	0.42	37.3	37-37.6	0.6
Phascolartos cinereus	10.3	4.8	hind	0.22	0.54	36	34.2-37.7	3.5
Loxodonta africana	14.3	3600	hind	0.15	1.78	36.5	36-37	1
Equus burchelli	13.2	280	hind	NA	NA	39.3	38.4-41.8	3.4

Table S1. Summary of the $\varepsilon^*_{\text{diet-bioapatite}}$ values (highlighted box) for taxa in this study and other life history trait values. Taxonomic selection was driven by: (1) accuracy of dietary $\delta^{13}C$ data and (2) sampling of phylogenetic diversity. Abbreviations: BM: body mass (kg), FERM: type of digestive system (foregut vs hindgut fermentation), BMR: basal rate of metabolism ($cm^3O_2/g*h$), Temp: body temperature (°C). Sources for all values above and in Table S2. † is a recently extinct Pleistocene sloth.

TAXA	ε*	Controlled	Source of ε*
Choloepus hoffmanni	12.62 ± 0.68	Yes	this study
Bradypus variegatus	10.31 ± 1.03	Yes	this study
Mylodon darwinii†	15.63± 0.51	Yes, (diet from dung)	this study
Bos taurus	14.6± 0.3	Yes	[6]
Sus scrofa	12.9± 0.5	Yes	[6]
Oryctolagus cuniculus	12.8± 0.7	Yes	[6]
Microtus ochrogaster	11.5± 0.3	Yes	[6]
Mus musculus	9.1± 1.6	Yes	[5]
Lama guanicoe	12.9	No	[3]
Giraffa camelopardalis	14.1	No	[3]
Diceros bicornis	14.4	No	[3]
Camelus bactrianus	13.7	Partial	[3]
Equus caballus	13.7	No	[3]
Loxodonta africana	14.3	No	[3]
Ailuropoda melanoleuca	10.1	Partial	[89]
Equus burchelli	13.2	No	[90]
Phascolartos cinereus	10.3	Partial	[91]

Table S2. Summary of the $\varepsilon^*_{\text{diet-bioapatite}}$ values included in this study. Selection of taxa was determined by: (1) dietary $\delta^{13}C$ controlled, (2) partially controlled (i.e., monospecific diets), or (3) not controlled but still with a narrow range of reported $\delta^{13}C_{\text{diet}}$ variation in the wild. † is an extinct taxon.

	AICc
Brownian Motion	-11.17615193
Lambda	-9.064478475
Delta	-10.73648238
Kappa	-8.28545515
Early Burst	-8.186953844
Ornstein Uhlenbeck	-10.79611806
White Noise	-11.93408278

Table S3. Summary of AIC analyses comparing different models of trait evolution. Lowest AICc score (gray highlight) corresponds to a White Noise model, which disregards phylogeny. The second lowest AICc score corresponds to Brownian motion (BM), which further emphasizes the lack of phylogenetic signal in the known values of $\varepsilon^*_{\text{diet-bioapatite}}$ across mammals. Data were fitted to the framework phylogenetic tree with the R package GEIGER [92]. Refer to [92] for explanation of each model of trait evolution.

ε* vs:	Body Mass		BMR		BMR/Kleiber value		Temperature (average)		Breadth of temp variation	
	R^2	*p*-value	R^2	*p*-value	R^2	*p*-value	R^2	*p*-value	R^2	*p*-value
All taxa	0.62	0.000	0.24	0.078	0.06	0.366	0.16	0.122	0.12	0.19
Foregut	0.78	0.008	0.15	0.454	0.56	0.089	0.89	0.005	0.51	0.11
Hindgut	0.74	0.003	0.56	0.054	0.00	0.975	0.07	0.482	0.36	0.085

Table S4. Summary statistics for all regression analyses. Highlighted boxes indicate significant values. Only body mass correlated significantly with ε* in all cases (i.e., in analyses with all taxa included as well as for mammals separated by type of digestive system [foregut and hindgut only]). Similar results (no significant correlations of ε* with BMR corrected for body mass, for all mammals or either digestive system subset) when applying the alternative body mass scaling function of [93]; Kleiber value remains the most widely accepted scaling function for BMR/BM covariance, and we present it here because [93] excludes all ruminants, which are a substantial number of the taxa in our analyses and prior studies of carbon isotope enrichment in dental bioapatite.

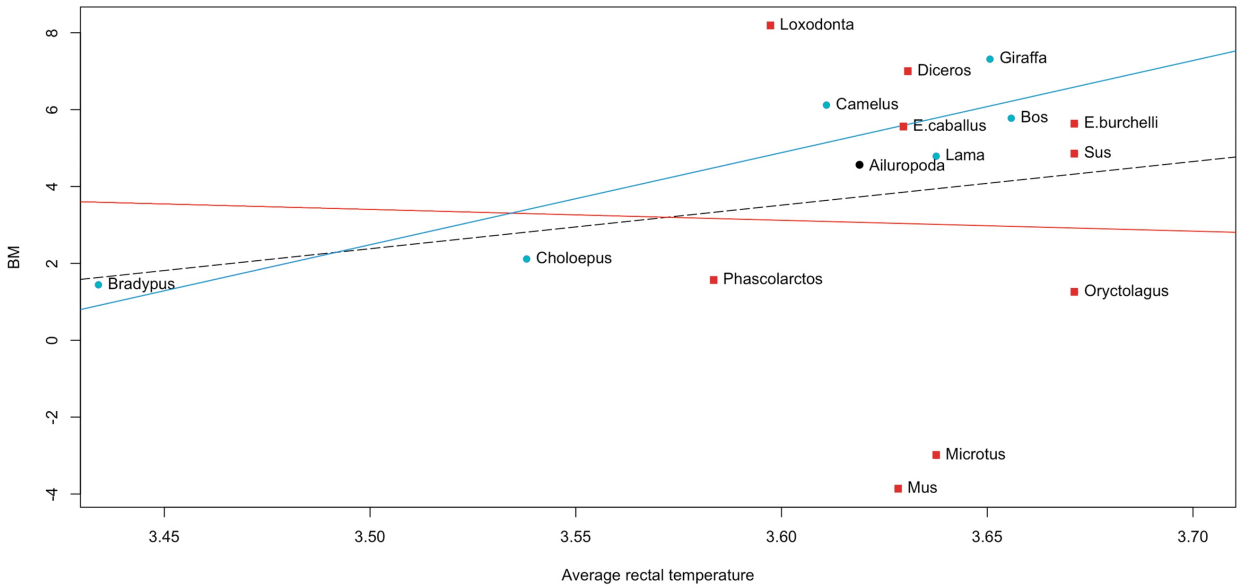

Figure S1. Correlation between body mass (BM) and average rectal temperature. Values are log transformed. All mammals (black dashed line): R^2=0.04, p-value=0.47; hindgut fermenters (red line and symbols): R^2=0.00, p-value=0.95, foregut fermenters (greenish line and symbols): R^2= 0.80, **p-value= 0.02**.

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