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The effectiveness of using carbonate isotope measurements of body tissues to infer diet in human evolution: Evidence from wild western chimpanzees (*Pan troglodytes verus*)☆

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ABSTRACT

Changes in diet throughout hominin evolution have been linked with important evolutionary changes. Stable carbon isotope analysis of inorganic apatite carbonate is the main isotopic method used to reconstruct fossil hominin diets; to test its effectiveness as a paleodietary indicator we present bone and enamel carbonate carbon isotope data from a well-studied population of modern wild western chimpanzees (*Pan troglodytes verus*) of known sex and age from Taï, Cote d'Ivoire. We found a significant effect of age class on bone carbonate values, with adult chimpanzees being more ^{13}C- and ^{18}O-depleted compared to juveniles. Further, to investigate habitat effects, we compared our data to existing apatite data on eastern chimpanzees (*P. troglodytes schweinfurthii*) and found that the Taï chimpanzees are significantly more depleted in enamel $\delta^{13}C_{ap}$ and $\delta^{18}O_{ap}$ compared to their eastern counterparts. Our data are the first to present a range of tissue-specific isotope data from the same group of wild western chimpanzees and, as such, add new data to the growing number of modern non-human primate comparative isotope datasets providing valuable information for the interpretation of diet throughout hominin evolution. By comparing our data to published isotope data on fossil hominins we found that our modern chimpanzee bone and enamel data support hypotheses that the trend towards increased consumption of C_4 foods after 4 Ma (millions of years ago) is unique to hominins.

1. Introduction

Changes in diet throughout hominin evolution have been linked with important evolutionary changes including encephalisation (Milton, 1987; Leonard and Robertson, 1991, 1994) and bipedalism (Darwin, 1871; Dart, 1925; Aiello and Wheeler, 1995). Elucidating the differing dietary strategies employed by ancestral hominins is therefore important in understanding their evolutionary trajectory from plant-eating apes to omnivorous foragers (van der Merwe et al., 2003; Lee-Thorp et al., 2010). A range of methods are employed to investigate hominin dietary ecology including the analysis of bone tools, faunal remains and dentition (Grine and Kay, 1988; Lucas et al., 2009, 2013). Specifically, dental microwear patterns on fossil hominin teeth have been used to provide insights into the dietary habits of fossil hominins (White et al., 2009b; Ungar et al., 2010; Grine et al., 2012) and comparative analyses of hand bones have provided an insight into tool use and manipulation (Marzke, 2013; Kivell et al., 2013). Investigations employing stable isotope analysis investigating diet in early hominins have increased rapidly in the last decade (Clementz, 2012; Cerling et al., 2013; Sponheimer et al., 2013) as they provide additional dietary information (e.g. habitat types, marine versus terrestrial subsistence, complementary to traditional palaeoanthropological methods, and phytolith analysis (Henry, 2012)).

Isotopic studies of extant great apes have increased recently and demonstrate that even amongst great ape species, and subspecies,

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variation is observed in terms of levels of frugivory and folivory (Nelson, 2013), meat eating (Oelze et al., 2011; Fahy et al., 2013) and habitat use (Schoeninger et al., 1999; Cerling et al., 2004; Sponheimer et al., 2006; Fahy et al., 2013; Nelson, 2013) including canopy height preferences (Krigbaum et al., 2013). Similar dietary flexibility has also been found in isotope data from fossil hominins. It has been argued that one of the fundamental distinguishing dietary traits between modern humans and our closet living relatives, chimpanzees (*Pan troglodytes*), is the inclusion of significant quantities of C_4/CAM plants, and animals that feed on them, by hominins (Cerling et al., 2013; Sponheimer et al., 2013; Wynn et al., 2013). Carbon isotope measurements have been successfully applied to a wide range of fossil hominins to determine plant food sources (C_3 versus C_4) (DeNiro and Epstein, 1978; Cerling et al., 2013). White et al. (2009b) suggest that *Ardipithecus ramidus* consumed minimal C_4 vegetation, consistent with inhabiting predominantly forest and woodland areas. The majority of hominin isotope research suggests that our early human ancestors only started adding tropical grasses, sedges and other C_4 flora to their ape-like C_3 diets approximately 3.5 million years ago (Ma) (Cerling et al., 2013; Sponheimer et al., 2013). However, this trend was not identical across Africa; therefore, while consumption of ^{13}C-enriched food increased, the extent of consumption varied by region and species (Sponheimer et al., 2013).

An interesting additional aspect of investigations into fossil hominins is the relationship between adult and juvenile specimens. The fossil record is not made up of only adult specimens but also includes significant juvenile specimens including the type specimen of *Homo habilis*, OH7 (Leakey, 1964), *Paranthropus robustus*, SK 6 and SK 47 (Tobias et al., 1977), and juvenile individuals included in the AL 333 "First Family" (Johanson, 2004). Recent research by L'Engle Williams (2015) reported differences in dental microwear patterns in juveniles compared to adults from Swartkrans. Additionally the author reports Sr/Ca ratios of the juvenile specimen SK 54 as being substantially higher than adults from Swartkrans Member 1 (L'Engle Williams, 2015). Explanations for these differences vary but are likely attributable to differences in life history and diet; research has also suggested that infants and juveniles consumed a more abrasive diet compared to adults (Aiello et al., 1991) due in part to lack of dexterity and proficiency in tool use (L'Engle Williams, 2015). To supplement adult/juvenile comparative research, and as there are also indications that infants consuming their mother's milk can be enriched in both carbon and oxygen isotope values (Wright and Schwarcz, 1999), here we investigate if there are age related differences by testing the effect of age class (e.g. adult versus juvenile) on $\delta^{13}C_{ap}$ and $\delta^{18}O_{ap}$ values in bone and enamel carbonate carbon isotope data from a well-studied population of modern wild western chimpanzees (*P. troglodytes verus*) of known sex and age from Taï, Cote d'Ivoire.

1.1. Stable isotope analysis (SIA)

Stable isotope values of hominin body tissues are related to the isotope values of foods consumed over the lifetime of that individual. Carbon stable isotope ($\delta^{13}C$) analysis of bone collagen and carbonate, and tooth enamel and dentine, is the most widely used isotope method for reconstructing past diet, and enamel carbonate is the main substrate used for isotope measurements of fossil hominins (DeNiro and Epstein, 1978; Lee-Thorp, 1989; Lee-Thorp et al., 2010). Tooth enamel reflects earlier phases of dietary life, whereas bone generally reflects a more mixed dietary picture due to continuous bone remodelling through life (Richards et al., 2002). C_3-plants, including many temperature grasses, trees, shrubs and herbs, are more ^{13}C-depleted compared to C_4-plants, such as tropical grasses and sedges and CAM plants (e.g. succulents) (Vogel, 1978; Lee-Thorp, 1989; Codron et al., 2005). Therefore, enamel carbonate $\delta^{13}C$ measurements can be used to determine the relative proportions of a C_3- and C_4-based foodweb. $\delta^{13}O$ provides information on water intake and excretion (Dansgaard, 1964; Iacumin et al., 1996; van der Merwe et al., 2003; van der Merwe et al., 2008) and can also be used to infer information on water availability, making oxygen isotope data useful palaeoclimatic indicators (Darling et al., 2006; Grine et al., 2012). Furthermore, an important factor in primate dietary research, $\delta^{13}O$ ratios are affected by the degree of water and light stress plants undergo (Nelson, 2013) meaning ^{18}O-depleted plants are generally found on the shaded forest floor rather than high in the canopy.

1.1.1. Tissue-type Stable carbon isotope analysis of organic tissues such as bone collagen, hair keratin and tooth dentine largely provide information reflective of the protein portion of an individual's diet (Sullivan and Krueger, 1981; Krueger and Sullivan, 1984; Ambrose and Norr, 1993; Harrison and Katzenberg, 2003), whereas stable isotope values from bone and enamel apatite carbonate data largely reflect overall diet (i.e. proteins, carbohydrates and lipids) (Ambrose and Norr, 1993; Harrison and Katzenberg, 2003). In general, apatite is more ^{13}C-enriched compared to collagen (DeNiro and Epstein, 1978; Sullivan and Krueger, 1981; Krueger and Sullivan, 1984; Lee-Thorp and van der Merwe, 1987; Lee-Thorp, 1989). Inorganic carbonates survive much longer than organic collagen thereby enabling dietary information to be obtained from very old specimens (van der Merwe et al., 2003; Clementz, 2012); however, limited survival of organic components in specimens encountered in hominin paleodietary research means that stable isotope analysis of carbonates predominate (Garvie-Lok et al., 2004).

While tissue preservation is a large concern in hominin isotopic research, it is less so in modern primate isotopic work. Isotopic studies of wild primates have used a wide range of tissues to infer diet and model the foraging ecologies of extinct and extant species (Schoeninger et al., 1999; Oelze et al., 2011; Fahy et al., 2013). However, data comparison from studies across primate species, and primate body tissues, are often limited due to variation in primate habitats leading to different baseline dietary values and diversity in primate physiology leading to isotopic alteration between tissues (Crowley et al., 2010). The question of comparability across tissue types continues in human evolution research with some researchers finding a high degree of correlation between isotope data obtained from hair, bone collagen and bone apatite (O'Regan et al., 2008) and others suggesting calculation rates need to be checked and updated regularly (O'Connell and Hedges, 1999; O'Connell et al., 2012).

1.2. This study

Currently isotope data exist for nearly all the early African hominin species with significant sample sizes (Sponheimer et al., 2013); however given the inherent problems associated with obtaining unadulterated collagen from fossil specimens and the desire to minimize destruction, insight into the diet of fossil hominins using comparative samples is important. So far only one study compared the $\delta^{13}C$ signatures of chimpanzee dental enamel with those of fossil African hominins (Nelson, 2013). Here we report stable carbon and oxygen isotope data from a group of modern western chimpanzees, which allows us to compare the carbonate isotope data of bone and enamel apatite (the substrate primarily used for fossil hominins) to collagen isotope data (Fahy et al., 2013). Our aim was two-fold: first, to investigate differences in carbonate isotope data between adults and juveniles (ranging in age from 5 to 13 years) from the same community, and second, to investigate intra-species differences by comparing data from the Taï

Table 1
Pre-treated and non-pre-treated inorganic sample data.

S-EVA[a]	Sample	Name	Age	Non pre-treated sample data				Pre-treated sample data			
				Bone apatite		Enamel apatite		Bone apatite		Enamel apatite	
				$\delta^{13}C_{V\text{-}PDB}$ (‰)	$\delta^{18}O_{V\text{-}PDB}$ (‰)	$\delta^{13}C_{V\text{-}PDB}$ (‰)	$\delta^{18}O_{V\text{-}PDB}$ (‰)	$\delta^{13}C_{V\text{-}PDB}$ (‰)	$\delta^{18}O_{V\text{-}PDB}$ (‰)	$\delta^{13}C_{V\text{-}PDB}$ (‰)	$\delta^{18}O_{V\text{-}PDB}$ (‰)
Females											
26915A	Femur	Agathe	15	−14.1	−5.1	−17.0	−1.9			−17.3	−4.4
26916A	Femur	Bijou	19	−16.0	−5.7	−17.3	−2.7	−18.4	−6.2	−17.6	−4.0
26917A	Femur	Castor	23	−15.5	−5.4	−16.9	−2.6	−18.3	−6.1	−17.5	−2.8
26918A	Femur	Fanny	25	−15.2	−5.7	−17.0	−2.7	−18.0	−7.7	−17.8	−4.2
26919A	Femur	Loukoum	27	−15.8	−5.5	−17.5	−2.4	−19.1	−8.4	−18.1	−2.3
26920A	Femur	Venus	27	−16.3	−5.3	−17.0	−2.2			−17.0	0.3
26921A	Femur	Kiri	23	−15.8	−5.5			−18.7	−7.5		
26922A	Femur	Ondine	38	−15.5	−3.4	−17.0	−3.2				
26923A	Femur	Tita	25	−18.4	−5.0			−19.0	−6.0		
26924A	Femur	Unknown1	N/A	−17.5	−5.0	−17.6	−1.9	−18.4	−6.0	−17.8	−1.9
26925A	Femur	Unknown2	N/A	−16.0	−5.6			−18.2	−4.8		
26926A	Femur	Unknown3	N/A	−17.3	−5.3	−17.2	−2.3	−18.7	−7.5	−17.0	−3.6
26927A	Femur	Unknown4	N/A	−15.7	−5.4	−17.7	−3.3	−18.6	−7.6	−17.6	−3.8
Males											
26928A	Femur	Léo	19	−16.3	−6.2	−17.7	−1.9	−18.1	−5.1	−17.3	−3.4
26929A	Femur	Kendo	25	−15.5	−4.9	−17.6	−2.0	−18.6	−8.0	−17.8	−2.1
26930A	Femur	Fitz	20	−14.6	−5.4	−17.6	−5.1	−18.2	−7.4		
26931A	Femur	Rafiki	19	−16.7	−4.9			−19.1	−6.6		
26932A	Humerus	Brutus	46	−15.8	−5.0	−17.2	−4.2	−18.5	−8.1	−17.4	−3.4
26933A	Femur	Unknown1	N/A	−17.6	−2.1			−18.1	−2.9		
26934A	Femur	Unknown2	N/A	−14.9	−5.7	−17.4	−2.9	−17.8	−5.9	−17.8	−3.5
26935A	Femur	Unknown3	N/A	−15.4	−5.2	−18.2	−3.4			−18.3	−5.8
26936A	Femur	Unknown4	N/A	−15.2	−5.7			−18.3	−5.9		

[a] MPI-EVA isotope lab number.

chimpanzees to those of other chimpanzee populations, and to published fossil hominin data (reviewed in Sponheimer et al., 2013).

2. Materials and methods

2.1. Study samples

The Taï chimpanzee skeletal collection is housed at the Max Planck Institute for Evolutionary Anthropology (MPI-EVA), Leipzig, Germany. Individual identifications of chimpanzees under behavioural observation have been recorded since the early 1980s (Boesch and Boesch-Achermann, 2000). Identification of cadavers sampled in this study was based on the field researchers' daily knowledge of the demography of the Taï communities and overlap with the disappearance of individuals. Where accuracy of such identification was reduced due to advanced decay, individuals were identified as part of an on-going genetic investigation into familial relationships at Taï by Linda Vigilant and her team at the Max Planck Institute for Evolutionary Anthropology, Dept. of Primatology, Molecular Genetics Laboratory (Vigilant et al., 2001; Boesch et al., 2006).

2.2. Samples

Bone apatite was extracted from 10 adult female, eight adult male, five juvenile female and four juvenile male chimpanzees (Tables 1 and 2). All but one of the adult samples came from the left femur[1]; juvenile rib bone was sampled on the interior surface at the distal end of the right 5th rib. Enamel apatite from 10 adult females and six adult males with postcranial isotope data was also analysed. The majority of the enamel samples came from permanent first molars, with the exception of one pre-molar sample.[2] All the enamel samples came from the occlusal surface of the tooth. Due to the level of wear on the chimpanzee molars it was not possible to take the sample of enamel from exactly the same position on each tooth; therefore we note that the enamel sampling procedure we used may pool together enamel potentially formed over the full mineralization of the tooth. The bone collagen of individuals sampled for carbonate isotope analysis was previously analysed by Fahy et al. (2013).

Table 2
Juvenile Taï chimpanzee isotope data: bulk bone carbonate data from juvenile rib bone samples.

Females ($n = 5$)					
S-EVA[a]	Name	Age	Cat. no.	$\delta^{13}C_{V\text{-}PDB}$	$\delta^{18}O_{V\text{-}PDB}$
28127	Tina	10	11790	−16.9	−1.4
28128	Kana	11	13437	−16.8	−2.2
28129	Goshu	6	11791	−17.7	−2.8
28136	Zerlina	12	11792	−17.8	−2.1
28135	Manon	5	11783	−17.4	−2.0
Males ($n = 4$)					
S-EVA	Name	Age	Cat. no.	$\delta^{13}C_{V\text{-}PDB}$	$\delta^{18}O_{V\text{-}PDB}$
28131	Max	6	15005	−17.4	−2.3
28132	Noah	7	15011	−16.3	−2.8
28133	Hector	5	12175	−18.0	−2.3
28134	Clyde	13	11779	−17.2	−1.9

[a] MPI-EVA isotope lab number.

[1] Bone growth and turnover rates indicate how much collagen synthesised over the course of a year remains in bone at death (Hedges et al., 2007). Long bones such as the femur reflect longer isotopic time periods (e.g. 10 years) (Hedges et al., 2007); therefore it is assumed that other long bones such as humerii and tibiae follow the same pattern although at present no empirical data exist to support this assumption. However, a review of isotopic turnover rates and half-life by Zanden et al. (2015) highlights the need for more empirical syntheses on the effects of bone-specific turnover rates on isotopic values.

[2] Pre-molar sample from adult male Kendo.

2.3. Sampling methodology

Duplicate samples of 20–30 mg of bone apatite and 8–10 mg of enamel apatite were collected using a diamond-tipped burr on a hand drill. When sampling the enamel, care was taken not to extend into the dentine layer, and to avoid heating the material. The 'A' samples did not undergo any pre-treatment. Along with the juvenile bone samples (no duplicate samples), one set of each adult (bone and enamel) sample went through an acid digestion pre-treatment phase at the Max Planck Institute for Evolutionary Anthropology in Leipzig, Germany. Pre-treatment of the samples for carbonate analysis followed the methods outlined in Koch et al. (1997) with modifications as detailed in Garvie-Lok et al. (2004) and Metcalfe et al. (2009). Stable isotope analysis was carried out by continuous flow – isotope ratio mass spectrometry at Iso-Analytical Limited, Crewe, UK using an ANCA-G gas purification module and 20-20 mass spectrometer (Europa Scientific Ltd, Crewe, UK). The routine external precision of analysis for carbonate samples is 0.2‰ for both isotopes.

2.4. Comparison of chimpanzee populations

To investigate intra-species differences, and to further understand the differing effects of habitat on apatite isotope data, we compared enamel carbonate data from adult Taï western chimpanzees to those of published eastern chimpanzee carbonate data from Kanyawara and Ngogo, Kibale National Park, Uganda (Nelson, 2013) and the Ituri Forest and Itombwe Massif, Democratic Republic of Congo (DRC) (Cerling et al., 2004, 2013). Habitat variation is noticeable both within and between sites with the Kinable National Park samples made up of data from chimpanzees from Kayawara, a moist evergreen forest, and Ngogo, largely primary forest (Nelson, 2013). The samples from Ituri Forest and the Itombwe Massif are representative of a mixed, moist evergreen forest and a mosaic of monodominant forest (Cerling et al., 2013). The historic Ganta chimpanzees are thought to have originated from a primary forest (Smith et al., 2010), similar to the samples from Taï.

2.5. Comparisons with fossil hominin enamel carbonate data

We used carbonate isotope data from Sponheimer et al. (2013) to compare a range of hominin specimens through time with $\delta^{13}C_{ap}$ data from their closest living relatives, chimpanzees. As the majority of published carbonate data comes from tooth enamel, we present only the Taï chimpanzee enamel data for comparison.

2.6. Statistical analysis

All analyses were performed in R (R Core Team citation; Baayen, 2011). We corrected resulting *p* values for multiple testing using the false discovery rate method (Benjamini and Hochberg, 1995). We then ran a likelihood ratio test to determine the significance of the interaction in each model. We checked for model stability by excluding individuals sequentially from the dataset, running the full model and comparing the results with those from the original model. There was no evidence for influential individuals, indicating model stability. To test for the effect of age class on both carbon and oxygen we ran two linear models with Gaussian error structure and identity link function. The significance of age class (e.g. adult versus juvenile) was determined by running a classic F test for each isotope. To test for differences in carbon and oxygen isotope ratios among different chimpanzee sites we ran a Kruskal–Wallis H-test for each isotope separately. *P* values were calculated using a permutation method with 10,000 permutations to account for the very small sample size for the DRC site. *P* values were adjusted as stated above. Subsequent pairwise comparisons were carried out using

Table 3
Stable isotope data: for organic ($\delta^{13}C$ and $\delta^{15}N$) and pre-treated inorganic ($\delta^{13}C$ and $\delta^{18}O$) [uncorrected $\delta^{13}C$ reported] tissue samples of adult male and female Taï chimpanzees.[a]

S-EVA	Sample	Name	Age	Bone apatite		Enamel apatite		Bone collagen		Difference		
				$\delta^{13}C$	$\delta^{18}O$	$\delta^{13}C$	$\delta^{18}O$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C_{Bone\_ap\text{-}Enamel\_ap}$	$\delta^{18}O_{Bone\_ap\text{-}Enamel\_ap}$	$\delta^{13}C_{Bone\_ap\text{-}Bone\_col}$
				(‰)	(‰)	(‰)	(‰)	(‰)	(‰)	(‰)	(‰)	(‰)
Females												
26915A	Femur	Agathe	15			−17.3	−4.4	−22.6	7.8			
26916A	Femur	Bijou	19	−18.4	−6.2	−17.6	−4.0	−23.1	7.3	−0.9	−2.2	4.7
26917A	Femur	Castor	23	−18.3	−6.1	−17.5	−2.8	−23.2	9.0	−0.8	−3.4	4.9
26918A	Femur	Fanny	25	−18.0	−7.7	−17.8	−4.2	−22.9	8.0	−0.2	−3.4	4.9
26919A	Femur	Loukoum	27	−19.1	−8.4	−18.1	−2.3	−23.2	8.0	−1.0	−6.1	4.1
26920A	Femur	Venus	27			−17.0	0.3	−23.5	8.3			
26921A	Femur	Kiri	23	−18.7	−7.5			−23.3	7.4			4.6
26922A	Femur	Ondine	38					−22.7	7.9			
26923A	Femur	Tita	25	−19.0	−6.0			−23.7	7.5			4.6
26924A	Femur	Unknown1	N/A	−18.4	−6.0	−17.8	−1.9	−23.3	7.6	−0.6	−4.2	4.8
26925A	Femur	Unknown2	N/A	−18.2	−4.8			−23.5	7.5			5.3
26926A	Femur	Unknown3	N/A	−18.7	−7.5	−17.0	−3.6	−23.4	7.6	−1.7	−3.8	4.7
26927A	Femur	Unknown4	N/A	−18.6	−7.6	−17.6	−3.8	−23.5	7.8	−0.9	−3.7	4.9
Males												
26928A	Femur	Léo	19	−18.1	−5.1	−17.3	−3.4	−23.0	8.0	−0.7	−1.7	4.9
26929A	Femur	Kendo*	25	−18.6	−8.0	−17.8	−2.1	−23.0	8.4	−0.8	−5.9	4.4
26930A	Femur	Fitz	20	−18.2	−7.4			−22.9	8.2			4.7
26931A	Femur	Rafiki	19	−19.1	−6.6			−23.0	8.3			3.9
26932A	Humerus	Brutus	46	−18.5	−8.1	−17.4	−3.4	−22.8	9.1	−1.2	−4.7	4.2
26933A	Femur	Unknown1	N/A	−18.1	−2.9			−23.2	8.7			5.1
26934A	Femur	Unknown2	N/A	−17.8	−5.9	−17.8	−3.5	−22.6	8.6	−0.1	−2.4	4.8
26935A	Femur	Unknown3	N/A			−18.3	−5.8	−23.4	7.7			
26936A	Femur	Unknown4	N/A	−18.3	−5.9			−23.2	7.8			4.9
Mean	**Females**			**−18.5**	**−6.8**	**−17.5**	**−3.0**	**−23.2**	**7.8**	**−0.9**	**−3.8**	**4.7**
Mean	**Males**			**−18.3**	**−6.2**	**−17.7**	**−3.6**	**−23.0**	**8.3**	**−0.7**	**−3.7**	**4.6**

[a] Mean values for males and females are given in bold.
* Pre-molar sample shows no deviation from molar results indicating similarity in enamel composition.

exact Mann–Whitney U-tests performed in R with the package exactRankTests (Hothorn and Hornik, 2012).

3. Results

3.1. Adult Taï chimpanzees

Table 3 presents all uncorrected collagen and carbonate isotope data for the adult chimpanzees analysed. Average (uncorrected) $\delta^{13}C_{ap}$ ($_{ap}$ = isotope data from bone or enamel apatite with specific material detailed in text) bone and enamel values for adult Taï chimpanzees were −18.4 ± 0.4‰ and −17.6 ± 0.4‰ respectively. Once the bone and enamel apatite data are corrected to account for the effects of changes in atmospheric CO_2 associated with the burning of fossil fuels, average corrected (+1.5‰ after Trudinger et al., 1999) adult Taï chimpanzee bone $\delta^{13}C_{ap}$ data (−16.9 ± 0.4‰) and enamel $\delta^{13}C_{ap}$ data (−16.1 ± 0.4‰) appeared more enriched. Corrected $\delta^{13}C_{ap}$ data were used in statistical analyses. We found no correlation between bone $\delta^{13}C_{col}$ and $\delta^{13}C_{ap}$ in adult females (n = 10, p = 0.61) or males (n = 8, p = 0.69) (Supplementary Online Material [SOM] Fig. S1) and no significant correlation between corrected bone $\delta^{13}C_{ap}$ and $\delta^{15}N_{col}$ in adult females (n = 10, p = 0.44) or adult males (n = 8, p = 0.38) (SOM Fig. S2).

3.2. Adult – juvenile comparison

We found a significant effect of age class on bone carbonate where adult chimpanzees were more ^{13}C- and ^{18}O-depleted compared to juveniles (carbon: $F_{1,24}$ = 8.245, p < 0.0001; oxygen: $F_{1,24}$ = 112.96, p < 0.0001) (Fig. 1). However we found no effect of sex on juvenile $\delta^{13}C_{ap}$ or $\delta^{18}O_{ap}$ values (Fig. 1).

3.3. Comparison of chimpanzee populations

We found a significant difference among sites in both $\delta^{13}C_{ap}$ (Kruskal–Wallis H-test = 21.871, df = 2, p < 0.001) and $\delta^{18}O_{ap}$ (Kruskal–Wallis H-test = 20.726, df = 2, p < 0.001) values indicating that adult Taï chimpanzees are significantly more depleted in enamel $\delta^{13}C_{ap}$ and $\delta^{18}O_{ap}$ compared to their eastern counterparts (Fig. 2). There was a significant difference (U = 0, n_1 = 2, n_2 = 14, p < 0.05) between Taï (mean −17.6‰) and the two DRC sites (Ituri −14.5‰; Itombwe −13.5‰) in $\delta^{13}C_{ap}$ values (average: DRC −14.5‰; Taï −17.6 ± 0.4‰). There was also a highly significant difference (U = 0, n_1 = 13, n_2 = 14, p < 0.0001) in $\delta^{13}C_{ap}$ between Kibale (−14.5 ± 1.0‰) and Taï. No significant difference (U = 3, n_1 = 2, n_2 = 13, p > 0.05) was found between Kibale and the two DRC sites in $\delta^{13}C_{ap}$ values. In contrast there was a significant difference (U = 0, n_1 = 2, n_2 = 13, p > 0.01) in $\delta^{18}O_{ap}$ values between Kibale (0.7 ± 0.4‰ and the two DRC sites (Ituri −1.1‰; Itombwe −3.1‰)). There was also a significant difference (U = 1.5, n_1 = 13, n_2 = 14, p > 0.0001) in $\delta^{18}O_{ap}$ values with Taï (−3.2 ± 1.4‰) being significantly more ^{18}O-depleted compared to Kibale. There was no significant difference (U = 6, n_1 = 2, n_2 = 14, p > 0.05) in $\delta^{18}O_{ap}$ values between Taï and the two DRC sites.

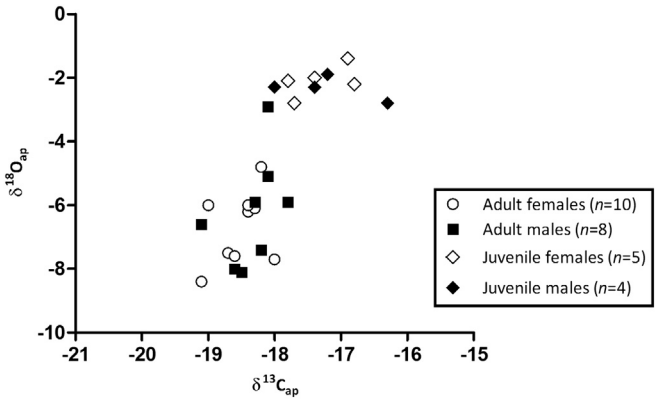

Figure 1. Adult & juvenile Taï chimpanzee bone carbonate $\delta^{18}O_{ap}$ against $\delta^{13}C_{ap}$: adult Taï chimpanzee bone $\delta^{13}C_{ap}$ values ranged from −19.1 to −17.8‰ (mean −18.4 ± 0.4‰). Taï juveniles, ranging in age from 5 to 13 years, were slightly more ^{13}C-enriched with average $\delta^{13}C_{ap}$ values ranging from −18.0 to −16.3‰ (mean −17.3 ± 0.5‰). Adult bone $\delta^{18}O_{ap}$ values were more ^{18}O-depleted and ranged from −8.4 to −2.9‰ (mean −6.5‰) compared to juvenile rib $\delta^{18}O_{ap}$ values which ranged from −2.8 to −1.4‰ (mean −2.2‰).

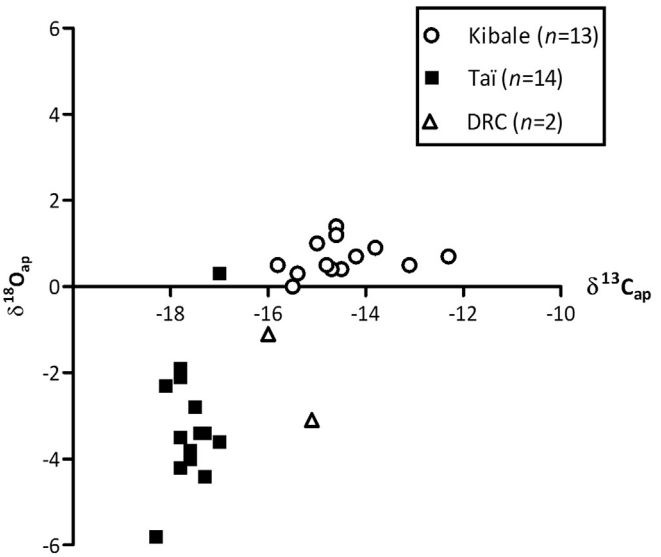

Figure 2. Comparison of adult chimpanzee $\delta^{13}C_{ap}$ and $\delta^{18}O_{ap}$ data: there was a significant difference among sites in both $\delta^{13}C_{ap}$ (Kruskal–Wallis H-test = 21.871, df = 2, p < 0.001) and $\delta^{18}O_{ap}$ (Kruskal–Wallis H-test = 20.726, df = 2, p < 0.001) values indicating that adult Taï chimpanzees are significantly more depleted in enamel $\delta^{13}C_{ap}$ and $\delta^{18}O_{ap}$ compared to their eastern counterparts. Kibale data from Nelson, 2013, Taï data from this study; DRC data from Cerling et al., 2004, 2013.

3.4. Comparisons with fossil hominin enamel carbonate data

Comparison of enamel $\delta^{13}C_{ap}$ from a range of fossil hominins (Sponheimer et al., 2013) with enamel $\delta^{13}C_{ap}$ from the Taï chimpanzees (this study) and chimpanzee enamel $\delta^{13}C_{ap}$ data analysed by Nelson (2013) and Cerling et al. (2004) found that fossil hominins are in general significantly more enriched in $\delta^{13}C_{ap}$ (Fig. 3).

4. Discussion

4.1. Adult Taï chimpanzees

A previous study by us (Fahy et al., 2013) reported average bone $\delta^{13}C_{col}$ ($_{col}$ = isotope data from bone collagen) for adult Taï chimpanzees (−22.9 ± 0.5‰) to reflect their regular consumption of fruit, similar to (adjusted) values reported for the Ganta chimpanzee collection (−22.6‰) from neighbouring Liberia (Smith et al., 2010). Our bone $\delta^{13}C_{ap}$ data and enamel $\delta^{13}C_{ap}$ data also show similar levels of depletion to data reported by Smith et al. (2010) for the Ganta chimpanzees. Additionally, average $\delta^{13}C_{ap}$ and $\delta^{13}C_{col}$ values reported for seven monkey species from Taï National Park (Krigbaum et al., 2013) are comparable to those of the adult Taï chimpanzees (Fahy et al., 2013). Such comparison infers a similar habitat type for chimpanzees and several monkey species

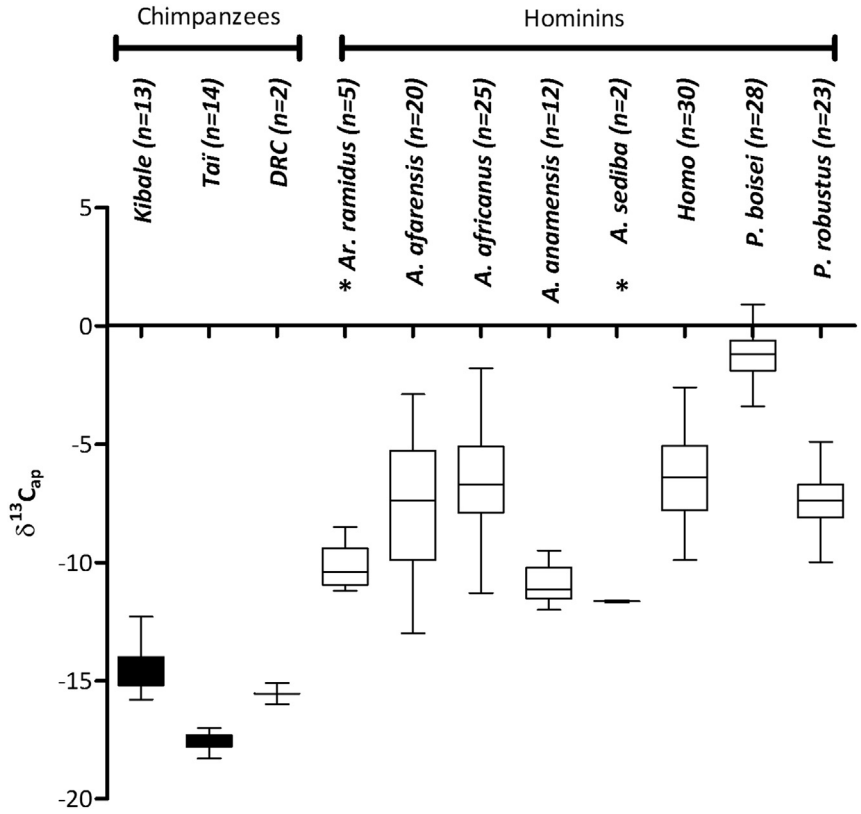

Figure 3. Chimpanzee enamel $\delta^{13}C_{ap}$ data compared with $\delta^{13}C_{ap}$ data for early hominins. Kibale data from Nelson, 2013, Taï data from this study; DRC data from Cerling et al., 2004, 2013; early hominin data as reviewed in Sponheimer et al., 2013. * refers to hominins reported to have stable isotope values reflective of a "chimpanzee-like" diet. Box and Whisker plots represent the mean, 25th and 75th percentiles ±S.D.

from Taï and the Ganta chimpanzees, corresponding with their inhabiting a closed canopy, predominantly C_3-dominant rainforest.

Krigbaum et al. (2013) found that $\delta^{18}O_{ap}$ values for a range of monkey species in the Taï National Park correlated with species canopy height preferences with vertical partitioning visible between taxa feeding at ground, lower and upper canopy levels. However, both bone and enamel carbonate data for the adult Taï chimpanzees are significantly more ^{18}O-depleted compared to values reported by Krigbaum et al. (2013) suggesting that the source of water in the adult Taï chimpanzees' diet is significantly more ^{18}O-depleted compared to the Taï monkeys, irrespective of canopy height; this likely reflects the differing dietary niches occupied by monkeys and chimpanzees in the Taï forest. This is because ^{18}O-enrichment is evident along a vertical gradient in canopied forests (Sternberg et al., 1989) with chimpanzees consuming more ^{18}O-depeted leaves and fruits from the forest floor or lower in the canopy compared to monkeys. However, enamel $\delta^{18}O_{ap}$ values of the Taï are again similar to those reported by Smith et al. (2010) for the neighbouring Ganta (Liberia) chimpanzees. The ^{18}O-depleted values reported here and amongst the Ganta chimpanzees are consistent with observed geographical variation in ^{18}O of precipitation for Taï National Park and the Ganta region (Bowen et al., 2005; Smith et al., 2010).

4.2. Adult-juvenile comparison

A previous study by our group employed stable carbon and nitrogen isotope analysis of tooth root dentine (Fahy et al., 2014) and found that weaning at Taï commences after 2 years of age and is generally complete for female infants by approximately 4.5 years of age, with weaning in male infants taking slightly longer to complete. In contrast we found no effect of sex on juvenile $\delta^{13}C_{ap}$ or $\delta^{18}O_{ap}$ values (Fig. 1). Wright and Schwarcz (1999) found a general ^{13}C-enrichment in consecutively developing teeth with increasing age; similarly Smith et al. (2010) reported a consistent enrichment in ^{13}C from infants to juveniles amongst the Ganta chimpanzees. In contrast our results indicate no association, as sampled juveniles showed no $\delta^{13}C_{ap}$ correlation with age. Tooth enamel reflects earlier phases of dietary life, whereas bone generally reflects a more mixed dietary picture due to continuous bone remodeling through life (Richards et al., 2002). Therefore, it is possible that our use of rib bone carbonate (compared to previous studies using enamel) resulted in differences in the appropriation of isotopic signals due to differing formation and turnover rates and have masked any identifiable age-related signal in our $\delta^{13}C_{ap}$ values. As discussed above, juvenile Taï chimpanzees are also significantly more ^{18}O-enriched compared to adult Taï chimpanzees. $\delta^{18}O$ is recorded in bone mineral at a predictable offset from body water due to the maintenance of constant body temperature in mammals (Longinelli, 1984; Sponheimer and Lee-Thorp, 1999); as breast milk is formed from a female mammal's body water pool it is more ^{18}O-enriched than water the female consumes (Roberts et al., 1988; Wright and Schwarcz, 1999). Many of these studies found a trend towards lighter $\delta^{18}O$ ratios with increasing age. Our isotope data correspond with these observations with adult Taï chimpanzees being significantly more ^{18}O-depleted compared to juveniles.

4.3. Comparison of chimpanzee populations

$\delta^{13}C$ data from forest dwelling *Pan* species (Cerling et al., 2004, 2013; Oelze et al., 2011; Fahy et al., 2013) reflect pure C_3 diets suggestive of plant consumption predominantly from the forest

understory (Wynn et al., 2013). Our data indicate that the Taï chimpanzees consume foods that are extremely ^{13}C- and ^{18}O-depleted, even in comparison to chimpanzees from Kibale (Nelson, 2013) and from two sites in DRC (Cerling et al., 2006, 2013) (Fig. 2) and are in line with data reported by Smith et al. (2010) for the Ganta chimpanzees. Kibale National Park is classified as a moist evergreen forest transitional between lowland and montane forest (Struhsaker, 1997); however it has been suggested that habitat structure and composition vary considerably within the park and are different for chimpanzee communities in Ngogo compared to Kanyawara (Chapman et al., 1997). Therefore it appears that the chimpanzee habitat in Taï National Park is more uniform in comparison, and representative of a primary rainforest environment (Boesch and Boesch-Achermann, 2000). Therefore while habitat types at Taï and Ngogo are similar, both being characterized as moist, evergreen forests with closed canopies (Hohmann et al., 2010), the mixture of data from both Ngogo and Kanyawara chimpanzees by Nelson (2013) may account for differences in $\delta^{13}C_{ap}$ values observed given the more varied habitats encountered by chimpanzees at Kanyawara. Additionally, reported precipitation data for the Taï National Park and the region likely inhabited by the Ganta chimpanzees exhibit $\delta^{18}O$ values that are more negative than those reported for Kibale National Park (Smith et al., 2010), likely contributing to the more depleted $\delta^{18}O_{ap}$ values seen in the Taï chimpanzees.

Our $\delta^{18}O_{ap}$ data confirm that the Taï chimpanzees inhabit a more densely canopied forest compared to eastern chimpanzees. Again in line with the differences observed in carbon between these populations, the $\delta^{18}O_{ap}$ differences are likely artefacts of the various habitat types encountered at Kibale compared to the relatively consistent, and ^{18}O-depleted, dense canopy forest encountered at Taï. Isotopic variation in meteoric water may also play a role in the differences observed as studies have found significant variations across Africa (Craig, 1961; Levin et al., 2009; West et al., 2014).

Overall our data suggest that carbonate isotope differences observed between chimpanzee populations are more reflective of habitat variability rather than contrasting dietary habits. However we feel that data on eastern chimpanzees from Nelson (2013) and Cerling et al. (2004, 2013) indicates some C_4 resource consumption that is not visible in the Taï chimpanzee carbonate data and is again likely an artefact of habitat rather than dietary choice as C_4 resources available for consumption at Taï are negligible (floral stable isotope data from Fahy et al., 2013).

4.4. Comparisons with fossil hominin enamel carbonate data

The emerging picture of dietary evolution from fossil hominin enamel carbonate data is that there is a progression of decreasing C_3-increasing C_4 plant consumption starting with *A. ramidus* (White et al., 2009a,b) that exhibits C_3-enriched $\delta^{13}C$ values described as being similar to those of the great apes (Schoeninger et al., 1999; Sponheimer et al., 2006) indicating that consumption of ^{13}C-depleted plants predominated but with small amounts of ^{13}C-enriched plants included. A similar hypothesis has been suggested for the later *Australopithecus sediba* (Henry et al., 2012). Henry et al. (2012) state that enamel $\delta^{13}C_{ap}$ from two *A. sediba* specimens analysed indicates a nearly pure C_3 diet comparable with savanna chimpanzees (Schoeninger et al., 1999; Sponheimer et al., 2006). Carbon isotope ratios of successive australopith species appear to reflect increased consumption of C_4 plants, with seemingly large inter-and intra-species variation (Lee-Thorp et al., 2010; Cerling et al., 2013; Sponheimer et al., 2013). Most notable is the difference between *Australopithecus anamensis*, which appears to have rarely eaten C_4 foods, compared to its descendant *Australopithecus afarensis* that seemingly consumed significant quantities of C_4 foods, but with a large amount of individual variation (Cerling et al., 2013; Klein, 2013). Interestingly, the sequence of increased C_4-dominance in the diet of early hominins corresponds well with research suggesting that their environments became more open with more grassland along a similar time trajectory (Cerling et al., 2011). Correspondingly, the consumption of C_4 foods continued to increase with subsequent sympatric *Paranthropus boisei* and early *Homo* who both appear to have had a high level of C_4 consumption (van der Merwe et al., 2003). Overall, our modern chimpanzee bone and enamel (Fig. 3) isotope data support hypotheses that the trend towards increased consumption of C_4 foods after 4 Ma is unique to hominins (Sponheimer et al., 2013). Given the correspondence between savanna chimpanzees and *A. ramidus* and *A. sediba*, and the large amount of variation visible in $\delta^{13}C_{ap}$ data from *A. afarensis* and *Australopithecus africanus*, our data suggest that it would be worthwhile investigating variation observed between early hominins and chimpanzees in more detail to determine whether this variation may be mainly due to the effect of habitat type use (e.g. forest dwelling chimpanzees have few C_4 resources available) rather than specific dietary choice.

5. Conclusion

Limited survival of organic components in fossil hominins means that carbon isotopic analysis of enamel carbonate will likely continue to be the main isotope method used to reconstruct palaeodiets. Our data are the first to present a range of tissue-specific isotope data from the same group of wild western chimpanzees and provide a lower boundary for chimpanzees inhabiting dense forest habitats. Our results are consistent with interpretations of site differences in the degree of influence of canopy effect and meteoric water on carbon and oxygen isotope data; given the range of environments known to have been inhabited by our fossil ancestors, and behavioural differences influencing their exploitation, understanding the effect of location and habitat-type on stable isotope ratios, rather than differences in diet alone, should play a large role in data interpretation.

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Supplementary Online Material

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