Stable isotope-based diet reconstructions of Turkana Basin hominins

Thure E. Cerling[a,b,1], Fredrick Kyalo Manthi[c], Emma N. Mbua[c], Louise N. Leakey[d,e], Meave G. Leakey[d,e], Richard E. Leakey[d,e], Francis H. Brown[b], Frederick E. Grine[e], John A. Hart[f], Prince Kaleme[g], Hélène Roche[h], Kevin T. Uno[b], and Bernard A. Wood[i]

[a]Department of Biology and [b]Department of Geology and Geophysics, University of Utah, Salt Lake City, UT 84112; [c]National Museums of Kenya, Nairobi 00100, Kenya; [d]Turkana Basin Institute, Nairobi 00502,Kenya; [e]Department of Anthropology, Stony Brook University, Stony Brook, NY 11794; [f]Directeur Scientifique et Technique, Lukuru Foundation, Projet Tshuapa-Lomami-Lualaba (TL2), Kinshasa, Democratic Republic of Congo; [g]Maiko National Park, Frankfurt Zoological Society, Lubutu, Maniema, Democratic Republic of Congo; [h]Unité Mixte de Recherche 7055 Préhistoire et Technologie, Centre National de la Recherche Scientifique—Université Paris Ouest Nanterre La Défense, Maison de l'Archéologie et de l'Ethnologie (Boite 3), 92023 Nanterre Cedex, France; and [i]Center for the Advanced Study of Hominid Paleobiology, Department of Anthropology, George Washington University, Washington, DC 20052

Edited by James O'Connell, University of Utah, Salt Lake City, UT, and approved April 15, 2013 (received for review December 23, 2012)

Hominin fossil evidence in the Turkana Basin in Kenya from *ca.* 4.1 to 1.4 Ma samples two archaic early hominin genera and records some of the early evolutionary history of *Paranthropus* and *Homo*. Stable carbon isotopes in fossil tooth enamel are used to estimate the fraction of diet derived from C_3 or C_4 resources in these hominin taxa. The earliest hominin species in the Turkana Basin, *Australopithecus anamensis*, derived nearly all of its diet from C_3 resources. Subsequently, by *ca.* 3.3 Ma, the later *Kenyanthropus platyops* had a very wide dietary range—from virtually a purely C_3 resource-based diet to one dominated by C_4 resources. By *ca.* 2 Ma, hominins in the Turkana Basin had split into two distinct groups: specimens attributable to the genus *Homo* provide evidence for a diet with a *ca.* 65/35 ratio of C_3- to C_4-based resources, whereas *P. boisei* had a higher fraction of C_4-based diet (*ca.* 25/75 ratio). *Homo* sp. increased the fraction of C_4-based resources in the diet through *ca.* 1.5 Ma, whereas *P. boisei* maintained its high dependency on C_4-derived resources.

Theropithecus | hominid

Many approaches have been used to reconstruct the diet of early hominins. Some of the methods focus on the functional morphology of the masticatory system, others focus on tooth wear (both macroscopic and microscopic), and yet others focus on the physicochemical signatures that an animal's diet leaves within its hard tissues (1, 2). Chemical methods include the use of strontium/calcium and barium/calcium ratios (3, 4), but this study focuses on the analysis of stable isotopes of carbon (5–9).

Modern tropical ecosystems differ from those ecosystems that predate the late Miocene. Tropical grasses were rare until the late Miocene, when they greatly expanded in abundance; therefore, by the latest Miocene and Pliocene, many mammals had changed their diets, and some had become dependent on this relatively new dietary resource (10, 11). The study of this dietary evolution is based on the difference in carbon isotope ratios of plants that use either the C_3 or C_4 photosynthetic pathway (12). Plants using the C_3 pathway have $\delta^{13}C$ values that range between *ca.* −24‰ and −32‰ (13); the more positive values are associated with xeric environments, intermediate values are associated with mesic environments, and the most negative values are associated with closed canopy environments (14, 15). Plants using the C_4 pathway have $\delta^{13}C$ values that range from about −10‰ to −14‰, with more positive values associated with mesic environments and more negative values associated with more xeric environments (16). In the tropics, C_3 plants are primarily trees, shrubs, and nongrassy herbs and forbs; C_4 plants are primarily grasses and sedges, with some rare dicots. A third photosynthetic pathway, Crassulacean acid metabolism, has $\delta^{13}C$ values similar to C_4 plants in the tropics; Crassulacean acid metabolism plants are mostly succulents in the African tropics and make up a minor but potentially important dietary resource in some circumstances. Carbon isotope values of animal tissues (e.g., bioapatite) are enriched in ^{13}C compared with the diet; for large herbivorous mammals, bioapatite is enriched *ca.* 12–14‰ relative to dietary materials (5, 17, 18).

Thus, the $\delta^{13}C$ of fossil tooth enamel can distinguish between diets that are predominantly based on C_3 resources (leaves and fruits from trees and shrubs along with nongrassy forbs and herbs and their fruits) and diets that are predominantly based on C_4 resources (primarily grasses or sedges). Meat and most other organic tissues are only slightly enriched in ^{13}C compared with the plant-derived diet (19). Stable carbon isotopes in tooth enamel are unable to distinguish between plant- and meat-based (or insect-based) diet, but they can be used to trace the diet back to the ultimate resource: C_3 or C_4 plants.

The Turkana Basin has an excellent, well-dated record (20–23) of hominin fossils from *ca.* 4 Ma to the present. Thus, the diets of the hominin taxa represented at sites within the Turkana Basin (Fig. 1) can be used to study dietary preferences within the hominin clade across this time interval. All samples come from collections held at the National Museums of Kenya in Nairobi. We analyzed 110 teeth from 94 different individual hominins for their stable carbon isotopes. For practical reasons, we could not always sample specimens with unambiguous taxonomic assignments, and in some cases, we could sample only associated material. Therefore, we discuss the results in the context of generic rather than specific taxonomic attributions. The genera that we discuss include *Australopithecus* (*ca.* 4 Ma), *Kenyanthropus* (*ca.* 3–3.6 Ma), *Paranthropus* (*ca.* 2.5–1.4 Ma), and *Homo* (*ca.* 2.3–0.01 Ma). We use the taxonomy favored by Wood (24) and Wood and Leakey (25), although we make no distinction among earlier *Homo* species (e.g., *H. habilis* and *H. rudolfensis*) because of the limitations of the size and quality of the sample. We then compare the results of our analysis of hominins from sites in the Turkana Basin with data from hominins recovered at other locations in eastern and southern Africa.

Results

In this section, we present the results of the stable isotope analyses. We group the Turkana Basin hominin specimens by their geological age (Fig. 2 and Table S1) and discuss the taxa represented in each of the major age groupings.

Author contributions: T.E.C., F.K.M., L.N.L., M.G.L., R.E.L., F.H.B., J.A.H., P.K., H.R., K.T.U., and B.A.W. designed research; T.E.C., F.K.M., E.N.M., L.N.L., M.G.L., R.E.L., F.H.B., F.E.G., J.A.H., P.K., H.R., K.T.U., and B.A.W. performed research; T.E.C., F.K.M., L.N.L., M.G.L., R.E.L., F.H.B., F.E.G., H.R., K.T.U., and B.A.W. analyzed data; and T.E.C., M.G.L., F.H.B., and B.A.W. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

See Commentary on page 10470.

[1]To whom correspondence should be addressed. E-mail: thure.cerling@utah.edu.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1222568110/-/DCSupplemental.

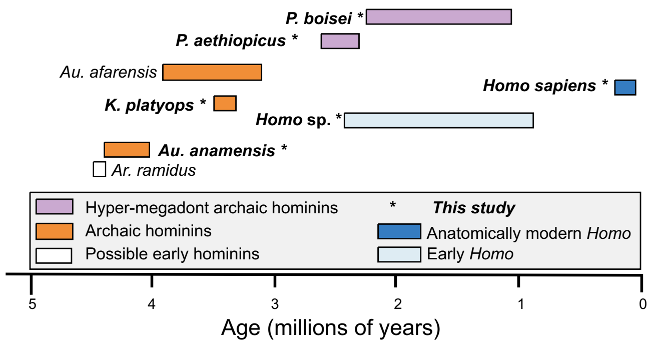

Fig. 1. Age distribution of hominins from East Africa. Isotopic values of lineages in bold are reported in the text.

Intraindividual Variation. For 10 individuals, stable isotope ratios were measured on two to five postcanine teeth. Comparison of the results (Table S1) shows that these individuals have a narrow range of $\delta^{13}C$ and $\delta^{18}O$ values among the teeth sampled, with average ranges of 1.0‰ and 0.7‰, respectively. Such a narrow range indicates that these individuals had a fairly homogeneous diet in terms of C_3- vs. C_4-derived resources across the time period represented by the development of the teeth sampled for each of the 10 individuals (i.e., based on timescales appropriate to isotope attenuation during enamel maturation). Comparison with other large mammals suggests that molar enamel in early hominins has an isotope maturation interval on the order of 1 or 2 y. Tooth enamel maturation involves an initial stage of bioapatite formation followed by a long period, the isotope maturation interval, wherein the enamel continues to increase in density and incorporate stable isotopes into the bioapatite structure (26). Therefore, the $\delta^{13}C$ values of individual teeth, as discussed below, seem to provide a reliable but time-integrated signal reflecting the diet of each of the individuals analyzed.

Temporal Samples. ***4.2–4.0 Ma.*** Fossil evidence of *Au. anamensis* is found in *ca.* 4.0- to 4.2-Ma-old strata in the Turkana Basin; 17 teeth from 12 different individuals were analyzed (Table S1). *Au. anamensis* has a relatively narrow range of $\delta^{13}C$ values, indicating a diet that is C_3-based. The average $\delta^{13}C$ value of −10.7 ± 0.8‰ corresponds to a $\delta^{13}C$ diet value of about −25‰ based on an estimated isotopic enrichment (diet bioapatite) for primates of 14‰ (*Methods*). Such a $\delta^{13}C$ value is compatible with either a 100% C_3 diet in a mesic to xeric environment or a diet that has both C_3- and C_4-derived foods but with the latter making up only *ca.* 10% of the diet. For comparison, modern browsers (*Giraffa camelopardalis*) (9) from the semiarid region of Tsavo, Kenya, have $\delta^{13}C_{1750}$ values of −11.2 ± 1.1‰ ($\delta^{13}C_{1750}$ refers to isotope values corrected for the anthropogenic addition of ^{13}C-depleted CO_2 to Earth's atmosphere) (*Methods*), whereas gorillas (*Gorilla beringei*) from densely forested environments in eastern Democratic Republic of Congo have $\delta^{13}C_{1750}$ values of −13.5 ± 1.2‰ ($n = 1$) (Table S1). Tooth enamel from modern baboons (*Papio*) from Kenya and Ethiopia has $\delta^{13}C_{1750}$ values that average −9.1 ± 3.1‰ ($n = 19$) and range from *ca.* −13‰ to *ca.* −2‰; baboons from forested regions in Democratic Republic of Congo have $\delta^{13}C_{1750}$ values that average −12.2 ± 2.3‰ ($n = 5$) (*Methods* and Table S1).

Thus, the $\delta^{13}C$ results for *Au. anamensis* suggest either a C_3-dominated diet or a diet with a small C_4 component. Published $\delta^{13}C$ values for *Ardipithecus ramidus* are similar: −10.2 ± 1.0‰ ($n = 5$) (27). Intertaxon comparison using ANOVA shows that the diets of *Au. anamensis*, *Ar. ramidus*, and modern *G. camelopardalis* (Tsavo) are indistinguishable in $\delta^{13}C$ space, but the diets of all three taxa are significantly different ($P < 0.0001$) from *G. beringei* from forested habitats (Fig. S1 and Table S1). As is seen below, the diet of *Au. anamensis* differs from the diet of all later hominins from the Turkana Basin.

3.4–3.0 Ma. *K. platyops* is found in the Turkana Basin between *ca.* 3.0 and 3.4 Ma (28). The only hominin recovered from deposits of similar age in the Awash region of Ethiopia is *Au. afarensis* (29); 21 teeth from 18 different individuals assigned to *K. platyops* were analyzed. The observed range in $\delta^{13}C$ of this sample (average = −6.2 ± 2.7, $n = 20$; maximum = −2.7, minimum = −11.1) is broader than any other hominin included in this study. The only other hominins with such a large range of values are *Au. afarensis* (average = −7.5 ± 2.6, $n = 20$; maximum = −2.9, minimum = −13.0) (30) and *Au. africanus* (average = −6.5 ± 2.3, $n = 23$; maximum = −1.8, minimum = −11.3) (data in refs. 6, 8, and 31). Compared with modern taxa with a similar sample size, the range and SD for *K. platyops* is broad and large, respectively (14, 32). The $\delta^{13}C$ values for the 18 *K. platyops* individuals are normally distributed,

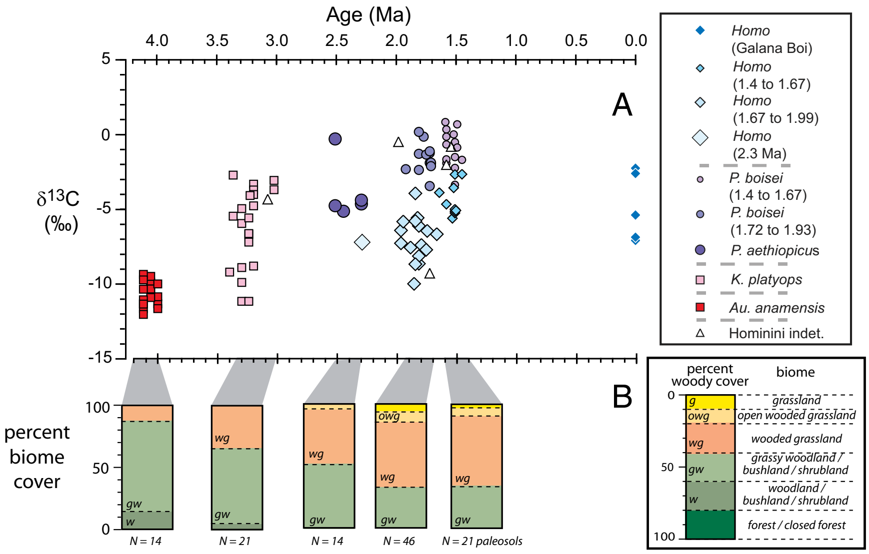

Fig. 2. (*A*) $\delta^{13}C$ values of tooth enamel with respect to age for major hominin groups in the Turkana Basin, Kenya. (*B*) Relative proportions of biomes based on $\delta^{13}C$ from paleosols (52), where the boundaries between biome types are as described in the text.

and the Akaike Information Criterion (33, 34) does not support a bimodal distribution for this population.

One hominin individual (KNM-ER 5431 F) in this time range is assigned to Homininae indet. It is of about the same age as the *K. platyops* samples discussed above, and its $\delta^{13}C$ value (−4.3‰) is within the range of *K. platyops*.

Modern *Papio* in East Africa has a similarly wide range of $\delta^{13}C_{1750}$ values; *Papio* from the Laikipia region of Kenya have values similar to the higher observed values.

2.5–2.3 Ma. Six teeth from five individual hominins in this time range were analyzed in this study. Four individuals attributed to *P. aethiopicus* had $\delta^{13}C$ values ranging between −0.3‰ and −5.1‰. Three of these individuals (KNM-WT 16005, KNM-WT 38351, and KNM-WT 38353) have a very narrow range of $\delta^{13}C$ values—from −4.4‰ to −5.1‰. The fourth individual attributed to *P. aethiopicus*, KNM-WT 17000, is an outlier compared with the other three *Paranthropus* of this age range. Taken together, *P. aethiopicus* has a diet with a consistently high C_4 component (*ca*. 50% or greater) in this time interval.

One specimen has been assigned to *Homo* sp. indet. (KNM-WT 42718) (35), and it gave a $\delta^{13}C$ value of −7.2‰, which is outside the range of the *P. aethiopicus* specimens of the same age, although the sample size of *P. aethiopicus* from this time interval ($n = 4$ individuals) is small.

1.99–1.67 Ma. The sample from this temporal interval includes two morphologically distinctive hominin taxa, *P. boisei* and *Homo* sp. indet.; 13 teeth from 13 different individual *P. boisei* specimens have an average $\delta^{13}C$ value of −1.6 ± 1.0‰ ranging from 0.2‰ to −3.4‰. These values represent a diet dominated by C_4 resources (i.e., a C_3/C_4-based resources ratio of *ca*. 25/75). The 16 *Homo* sp. indet. specimens have $\delta^{13}C$ values significantly different ($P < 0.001$, ANOVA, Tukey posthoc) from the *P. boisei* individuals in the same age range (−7.0 ± 1.5‰, $n = 16$; i.e., a C_3/C_4-based resources ratio of *ca*. 65/35).

Two specimens in this time interval have proven difficult to classify. One of these specimens, KNM-ER 1482 (a taxonomically enigmatic mandible) (24, 36, 37), has a $\delta^{13}C$ value of −0.4‰. The other, KNM-ER 2607 (a taxonomically enigmatic lower molar fragment) (24, 36, 38), has a $\delta^{13}C$ value of −9.2‰.

1.65–1.45 Ma. Both *P. boisei* and *Homo* sp. indet. are represented among the specimens from this time interval. The *P. boisei* individuals ($n = 14$) have an average $\delta^{13}C$ value (−0.9 ± 1.2‰) that does not differ statistically from the *P. boisei* individuals in the 1.99–1.67 Ma time range. The −0.9 ± 1.2‰ value corresponds to a C_3/C_4-based resources ratio of *ca*. 20/80.

Ten *Homo* sp. indet. specimens in this age range have an average $\delta^{13}C$ value of −4.3 ± 1.1‰ (i.e., a C_3/C_4-based resource ratio of *ca*. 45/55). The *Homo* sp. indet. individuals in this age range differ significantly from the coeval *P. boisei* sample (ANOVA, Tukey posthoc test, $P < 0.001$), and they also differ from *Homo* sp. indet. individuals from the earlier (1.99–1.67 Ma) time range in that there is a *ca*. 20% increase in the C_4 diet component (ANOVA, Tukey posthoc test, $P < 0.001$).

Two specimens that cannot be easily assigned to either *Homo* sp. indet. or *Paranthropus*, KNM-ER 2593 and KNM-ER 42705, were both found in the Area 6/6A region near Ileret, where many *P. boisei* specimens have been recovered. They have $\delta^{13}C$ values similar to the values of *Paranthropus* from Area 6/6A and may well be attributable to this genus.

0.01 Ma. Five teeth from four individual hominins from the Galana Boi Formation (Holocene) have an average $\delta^{13}C$ value of −4.8 ± 2.3‰ (i.e., a C_3/C_4-based resources ratio of *ca*. 50/50). These values are not significantly different from the earlier *Homo* sp. indet. samples.

Oxygen isotopes. The range of variation of the stable oxygen isotopes ($\delta^{18}O$) among the groups discussed above is between 0.7‰ and −1.8‰ (the individual range is from *ca*. +4‰ to −4‰); SDs within each group are *ca.* ±1.5‰. These values and ranges of variation are equivalent to water-dependent species such as suids and elephantids (39) as well as carnivores and omnivores. $\delta^{18}O$ as a function of time comparing the different hominins is shown in Fig. S2.

Discussion

Diets of Early Hominins: C_3- and C_4-Based Resources. We use the terms C_3- and C_4-based resources throughout our discussion, because our isotopic method cannot distinguish between a plant-based diet, a meat-based diet, and an omnivorous diet. Thus, based on isotopes alone, we consider that the diets of the early hominins that we have investigated could be primarily herbaceous (C_3 and C_4 plants), or they could be a secondary C_3- or C_4-based diet, an apparent C_3- or C_4-based diet, or an omnivorous diet. A secondary C_3- or C_4-based diet could be a meat- or insect-based diet (in which the $\delta^{13}C$ values are derived from the basal herbaceous diet of the prey). An apparent C_4-based diet is one based on aquatic resources in which algae have elevated $\delta^{13}C$ values because of bicarbonate uptake during photosynthesis (40); for this example, algae or fish then have $\delta^{13}C$ values with an apparent C_4 component (41, 42). Lastly, an omnivorous diet is a combination of the above resources: primary herbaceous diet along with secondary C_3- or C_4-derived components (i.e., meat or insects) or apparent components (i.e., aquatic).

The stable carbon isotope signature of a meat-based diet depends on the nature of the prey: small bovid herbivores less than *ca*. 10 kg (e.g., dik-dik and other neotragines) tend to be browsers and have C_3-based diets (14, 32), whereas large herbivores can have diets that are C_3-based (browsers such as most tragelephines, black rhinos, and giraffes), C_4-based (grazers such as warthogs, zebra, alcelaphines, reducines, and bovines), or mixed (e.g., impala, and some gazelles). Thus, the size of prey may be important in considering possible secondary diet C_3 or C_4 resources. Other small mammals (e.g., hyrax, lagomorphs, or rodents) could have been an important dietary resource and would contribute to isotope mixing lines between C_3- and C_4-based end member values.

Evolution of Hominin Diets Between 4 and 1.4 Ma in Eastern Africa. The earliest hominin taxon sampled in this study, the *ca.* 4 Ma *Au. anamensis*, has a diet comprised primarily of C_3-based resources (an average *ca*. 90/10 ratio of C_3/C_4 diet resources with a range from 100/0 to 80/20 for C_3/C_4-based resources). This finding is not entirely unexpected, because the diets of the obvious outgroups for hominins, *Pan* and *Gorilla*, are both predominantly C_3-based (43–45). It is impossible to refute the hypothesis that some C_4 resources contributed to the diet of these *Au. anamensis* individuals, and we do not attempt to do so. The observed $\delta^{13}C$ range of *Au. anamensis* is narrow, and it is similar to the slightly older *Ar. ramidus* found in the Awash region of Ethiopia (27). A diet with this carbon stable isotope signature is likely dominated by plant foods. The only alternative is a diet based on meat/insect resources based on animals that themselves consume almost entirely C_3 resources. However, note that initial surveys of fossil mammals from the Turkana region show that, by 7 Ma and on, most herbivores in the Turkana Basin had C_4-based diets (10, 11, 46, 47). Thus, *Au. anamensis* would have to have been a very specialized hunter if meat were a significant portion of the diet, because the prey would have to have been exclusively C_3 consumers.

By 3.5 Ma, the diet of the hominins that we sampled had expanded to include significant C_4 resources. The diet of *K. platyops* shows a broad range, with some individuals having strongly C_3-based diets and others having C_4-dominated diets. The range of values for 18 *K. platyops* individuals corresponds to $\delta^{13}C$ diet average values of *ca*. −25‰ to −17‰; the average *K. platyops* diet corresponds to a C_3/C_4-based resources ratio of *ca*. 60/40, but the range of the C_3/C_4-based resources ratio, 95/5 to 35/65, is wide. This wide range of dietary C_3 vs. C_4 resources suggests that *K. platyops* expanded into a dietary niche hitherto unexploited by hominins in the Turkana Basin. $\delta^{13}C$ values of *Au. afarensis* (30), another hominin taxon in East Africa of similar age, and *Au. africanus* in southern Africa of less certain age (6, 8, 31) similarly have a wide range of $\delta^{13}C$ values. The work by Sponheimer et al. (48) discusses these similarities and differences in more detail.

One individual in the small sample of *P. aethiopicus*, the KNM-WT 17000 cranium (49), deserves special comment. Its left M2 has a $\delta^{13}C$ value of –0.3‰, indicating a C_4-dominated diet (*ca*. 15/85 for the C_3/C_4 diet ratio), which is a diet similar to the *P. boisei* sample in the later time range. The dietary breadth within *Paranthropus* by *ca*. 2.3 Ma needs to be investigated with additional samples and analyses.

Between 2.0 and 1.4 Ma, numerous *P. boisei* and *Homo* sp. indet. specimens show that the diets of the two genera were distinct, with *Paranthropus* having a diet comprised of a 20/80 C_3/C_4 diet ratio, whereas *Homo* sp. indet. shows C_3/C_4-derived ratio values that range from 25/75 to 45/55. Previous comparisons with southern African fossils suggest that *P. boisei* from East Africa had a diet that was much narrower in terms of C_3/C_4 resources than *P. robustus* from southern Africa (9), with a wide range of $\delta^{13}C$ values that indicates a much broader dietary niche.

The taxonomically enigmatic mandible KNM-ER 1482 (50) has a $\delta^{13}C$ value of –0.4‰, which is intriguing. It has shifted even farther in the direction of being dependent on C_4 resources than most *P. boisei*. Its mandibular and dental morphology offers little or no evidence to assign it to *P. boisei*. Indeed, some have suggested that it may belong to the same taxon as the KNM-ER 1470 cranium (50) and the KNM-ER 62000 maxilla (51). If this proves to be the case, then within the same region, there may be at least two hominin taxa, almost certainly in different lineages, that have shifted to a diet dominated by C_4 resources.

Paleoecology of the Koobi Fora and Nachukui Formations. Precessional climatic cycles of *ca*. 20,000 y duration are widely recognized in marine and lacustrine sequences in tropical latitudes. Between insolation maxima and minima (separated by *ca*. 10,000 y), there are significant changes in rainfall and ecology. Fluvial strata present challenges for quantitative paleoenvironmental interpretation at timescales less than 20,000 y because of their abrupt and discontinuous mode of deposition. A discrete sedimentary package associated with a particular insolation cycle still does not reveal whether a fossil was deposited at maximum, minimum, or intermediate insolation. Furthermore, matching a fossil with the paleoenvironment in which it lived requires knowing the part of a cycle that a paleoenvironmental indicator (e.g., paleosol carbonate, mineralogy, indicator fossil, or biomarker) records. A vertebrate fossil from the base of a 20,000-y fining upward sequence may have lived during a climatic milieu different from the climate under which carbonate formed in a paleosol at the top of the same sequence. In most situations, it is not feasible to relate a fossil from fluvial sediments to a particular part of a precessional cycle, and it is certainly not possible using legacy collections or surface finds. Nonetheless, long-term ecological changes can be discerned through the 3-Ma record considered here.

Stable isotopes also provide important constraints on the paleoecology of the Turkana Basin. The earliest hominins reported here, from *ca*. 3 to 4 Ma, lived in an environment that was predominantly *ca*. 40–60% woody cover, which was determined from paleosol $\delta^{13}C$ values (52, 53). For this period, the soil carbon contribution from C_3 woody plants, C_3 forbs and herbs, and C_4 grasses would be *ca*. 60–40%, 15–20%, and 25–40%, respectively (Fig. 2*B* and Fig. S3). Such a habitat would be a grassy woodland, grassy shrubland, or grassy bushland (54). Sedimentological evidence (55) shows that the proto-Omo river was present throughout this period; this river likely had a riparian forest (>80 woody cover) that may have been hundreds of meters wide; Δ_{47} measurements on paleosols indicate that the region had soil temperatures between 30 °C and 40 °C (56), indicating a regional temperature regime similar to the temperature regime of today. Thus, this region had a riparian corridor with cooler temperature and little to no C_4 resource availability, but close by, the woodland/shrubland/bushland was a more open habitat with significant C_4 resources and much higher daily temperatures than in the riparian corridor. These paleoecological conditions suggest that, based on dietary considerations, *Au. anamensis* may have been restricted to a narrow riparian corridor, whereas *K. platyops* must have ventured into open habitats to obtain C_4 dietary resources. Thus far, fossils of *K. platyops* are associated with alluvial fans of a large lateral stream on the western basin margin interfingering with deposits of the ancestral Omo River.

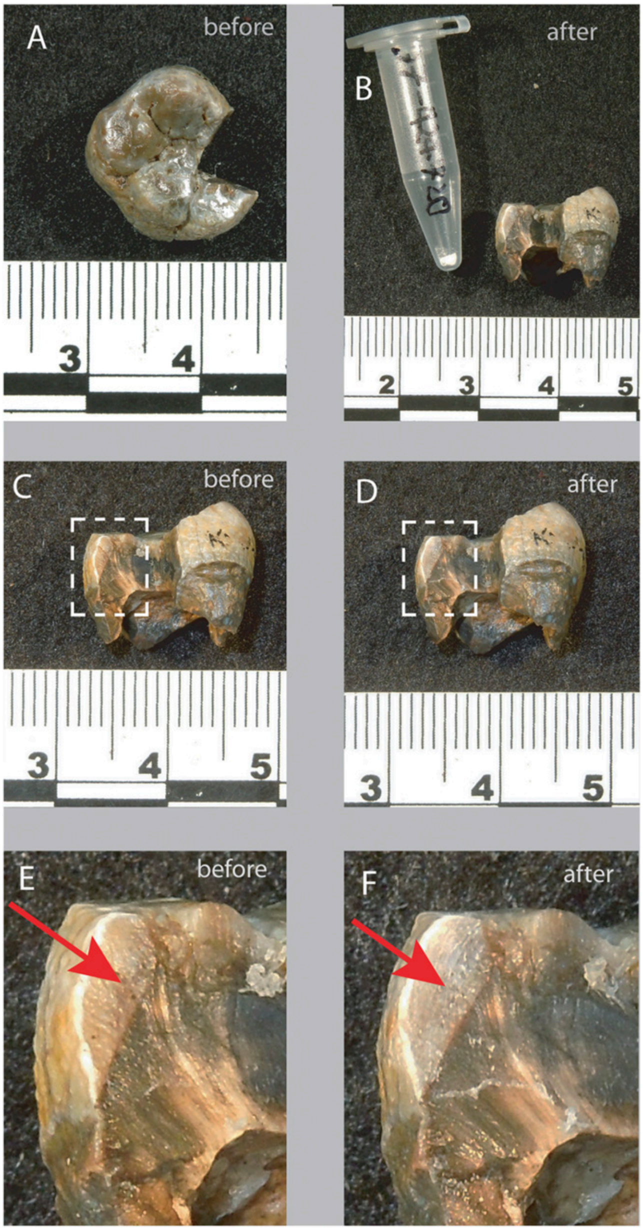

Fig. 3. Images of KNM-ER 45502 before and after sampling. (*A*) Top view of tooth before sampling. (*B*) View of the tooth with sample powder (1.8 mg) after sampling. (*C*) Sample before sampling; the box shows the area chosen for sampling (*E*). (*D*) Sample after sampling (same view as *C*); the boxed area is the close-up view shown in *F*. (*E*) Close-up view of the area sampled for stable isotope analysis before sampling. The red arrow shows the broken enamel surface to be sampled. (*F*) The same area as *E* but after sampling. The red arrow points to the surface sampled.

Woody cover diminished in the region over time; between *ca*. 2.0 and 1.4 Ma, woody cover was 20–40% based on $\delta^{13}C$ in paleosols (52). Such an ecosystem is equivalent to wooded grassland with soil contributions of C_3 woody plants, C_3 forbs and herbs, and C_4 grasses of *ca*. 40–20%, 20–30%, and 40–50%, respectively (Fig. 2*B*); areas of true grassland [<10% woody cover in the United Nations Educational, Scientific, and Cultural Organization terminology (54)] were uncommon, at least on the timescale of paleosol formation (>1,000 y). This change represents a great increase in the availability of C_4 plants for *Homo* and *Paranthropus* compared with the earlier *Australopithecus* and *Kenyanthropus* and a significant opening of the landscape. The 2.0- to 1.4-Ma interval had intermittent lakes fed by the proto-Omo River; however, it may have been diverted to the Nile drainage for some periods during this interval (55). Geochemical and mineralogic evidence shows that some of these lakes were alkaline (56); Δ_{47} evidence from paleosols indicates high mean annual temperatures, similar to the temperatures of today (57).

Thus, from 4.1 to 1.4 Ma, the region had abundant C_4 resources available in the 30-km broad grassy woodland/shrubland to wooded grasslands that bordered narrow (hundreds of meters wide) riparian forests or woodlands associated with the proto-Omo River.

Comparison of Hominin Diets to *Theropithecus*. *Theropithecus* was another large-bodied primate in the Turkana Basin at this time. Stable isotope measurements of tooth enamel show that *Theropithecus* was a heavy C_4 consumer by 4 Ma, with *ca*. 65% C_4 resources contributing to the diet (58). Throughout the period from 4 to 1 Ma, *Theropithecus* had a diet that was as much or more C_4-based than any hominin. *Paranthropus*, from 1.4 to 2.0 Ma, had a diet that was *ca*. 75% C_4-based, whereas the coeval *Theropithecus* had a diet that was *ca*. 75–85% C_4-based (58).

Modern Primates as Analogs for Hominin Diets. Modern gorilla and chimpanzees have diets that are entirely or almost entirely C_3-based (data in Table S2 and refs. 43–45). Only the earliest hominin in this study could be interpreted as having had a C_3-based diet with minimal (if any) C_4 components; the $\delta^{13}C$ of *Au. anamensis* is more positive than gorillas or chimpanzees from closed canopy forests, but it is compatible with a pure C_3 diet from riparian forests or open habitats.

Modern baboons (*P. anubis* and *P. hamadryas*) have a wide range of $\delta^{13}C_{1750}$ values, showing diet strategies that range from essentially pure C_3-based (e.g., from Neshisar NP) (Table S2) to dominated by C_3-based resources but with measureable C_4-based resources (Gona, Olorgesailie, Tsavo, and Turkana) (Table S2) to subequal with respect to C_3- and C_4-based resources (e.g., Laikipia region) (Table S2). Baboons have been suggested as an important study analog for early hominins (59, 60); thus future studies of baboon diets, coupled with stable isotope analyses, will be a fruitful avenue of research.

Conclusions

Within the past 4 Ma, the earliest dietary isotope evidence from hominins in the Turkana Basin comes from a single species, *Au. anamensis*, with a diet dominated by C_3 resources but possibly with a small component of C_4-derived resources. By *ca*. 3.5 Ma, at least one hominin taxon, *Kenyanthropus*, in the Turkana Basin had a diet with a broad range of C_3/C_4-based resources. By the 1.99- to 1.67-Ma time period, at least two morphologically highly distinctive hominin taxa, *P. boisei* and *Homo* sp. indet., had shifted in the direction of consuming higher but different proportions of C_4 resources. We cannot determine from the stable isotopes by themselves what the C_4 resources were that caused this shift in diet.

Methods

Hominin teeth from the National Museums of Kenya were sampled using a high-speed rotary drill to obtain powder (*ca*. 2–5 mg) from each sample. Only hominin teeth with broken surfaces were sampled; sampling was from the exposed broken enamel (Fig. 3). Enamel powder from modern gorilla (*G. beringei*) was obtained from archived samples in Kahuzi-Biega National Park and the Centre de Research National en Sciences Naturelles (CRNS)-Lwiro, Democratic Republic of Congo; most gorilla samples were young adults that had been killed by poachers. Other modern primates were from Democratic Republic of Congo and Kenya. All samples were treated with 0.1 M buffered acetic acid for 30 min to remove secondary carbonates; we had about 50% recovery during this treatment.

Fossil samples (*ca*. 500 μg) were reacted with 105% phosphoric acid at 90 °C in silver capsules on an isotope ratio mass spectrometer after cryogenic separation of CO_2 at the University of Utah Stable Isotope Ratio Facility for Environmental Research (SIRFER). Modern samples from Democratic Republic of Congo were analyzed in the Archaeology Department at the University of Cape Town using a Kiel device coupled to an isotope ratio mass spectrometer. Results are reported using the standard per million (‰) notation, where

$$\delta^{13}C = \left(R_{sample}/R_{standard} - 1\right) \times 1,000$$

and R_{sample} and $R_{standard}$ are the $^{13}C/^{12}C$ ratios in the sample and standard, respectively (Vienna–Pee Dee Belemnite is the standard for carbon isotope measurements). Corrections for temperature-dependent isotope fractionation in oxygen were made using modern and fossil internal reference materials that had been reacted at 25 °C (61). For comparative purposes, modern mammals have had their $\delta^{13}C$ values adjusted to compensate for recent changes in atmospheric $\delta^{13}C$ values (62, 63); these values are referred to in the text as $\delta^{13}C_{1750}$. We use the year of death to calculate the $\delta^{13}C_{1750}$ value; this date will result in a maximum correction, because tooth enamel likely formed *ca*. 10 y before death for these individuals.

Age estimates for each hominin use the correlations and chronology of the >350 volcanic ashes in the basin (20–23, 64); taxonomic assignments are in refs. 24 and 25.

SI Methods has additional information on isotope enrichment factors in mammals, stratigraphic information and age estimates for individual hominins (Table S3), statistical treatment, taxonomic assignments, and biome classifications used in this work.

ACKNOWLEDGMENTS. We thank the governments of Kenya and Democratic Republic of Congo for permission to do this research. We thank the field crew of the Koobi Fora Research Project (1969–2012), whose members discovered most of the specimens analyzed in this study. We also thank John Lanham for assistance at the University of Cape Town and Sandrine Prat and anonymous reviewers for PNAS for helpful comments. This project was initiated by the National Museums of Kenya. This material is based on work supported by National Science Foundation Grant BCS-0621542, National Geographic Society Grant 7767-04, and the Fulbright Foundation.

1. Grine FE, Sponheimer M, Ungar PS, Lee-Thorp JA, Teaford MF (2012) Dental microwear and stable isotopes inform the paleoecology of extinct hominins. *Am J Phys Anthropol* 148(2):285–317.
2. Wood BA, Schoer K (2012) Reconstructing the diet of an extinct hominin taxon: The role of extant primate models. *Int J Primatol* 33:716–742.
3. Copeland SR, et al. (2011) Strontium isotope evidence for landscape use by early hominins. *Nature* 474(7349):76–78.
4. Balter V, Braga J, Télouk P, Thackeray JF (2012) Evidence for dietary change but not landscape use in South African early hominins. *Nature* 489(7417):558–560.
5. Lee-Thorp JA, van der Merwe NJ (1987) Carbon isotope analysis of fossil bone apatite. *S Afr J Sci* 83:712–715.
6. Lee-Thorp JA, van der Merwe NJ, Brain CK (1994) Diet of *Australopithecus robustus* at Swartkrans from stable carbon isotopic analysis. *J Hum Evol* 27:361–372.
7. Sponheimer M, Lee-Thorp JA (1999) Isotopic evidence for the diet of an early hominid, Australopithecus africanus. *Science* 283(5400):368–370.
8. Sponheimer M, et al. (2005) Hominins, sedges, and termites: New carbon isotope data from the Sterkfontein valley and Kruger National Park. *J Hum Evol* 48(3):301–312.
9. Cerling TE, et al. (2011) Diet of Paranthropus boisei in the early Pleistocene of East Africa. *Proc Natl Acad Sci USA* 108(23):9337–9341.
10. Cerling TE, et al. (1997) Global change through the Miocene/Pliocene boundary. *Nature* 389:153–158.
11. Uno KT, et al. (2011) Late Miocene to Pliocene carbon isotope record of differential diet change among East African herbivores. *Proc Natl Acad Sci USA* 108(16):6509–6514.
12. O'Leary M (1981) Carbon isotopic fractionation in plants. *Phytochemistry* 20:553–567.
13. Deines P (1980) The isotopic composition of reduced organic carbon. *Handbook of Environmental Geochemistry*, eds Fritz P, Fontes JB (Elsevier, Amsterdam), Vol 1, pp 329–406.

14. Cerling TE, Harris JM, Passey BH (2003) Dietary preferences of East African Bovidae based on stable isotope analysis. *J Mammal* 84:456–471.
15. Cerling TE, Hart JA, Hart TB (2004) Stable isotope ecology in the Ituri Forest. *Oecologia* 138(1):5–12.
16. Hattersley PW (1982) ^{13}C values of C4 types in grasses. *Aust J Plant Physiol* 9:139–154.
17. Cerling TE, Harris JM (1999) Carbon isotope fractionation between diet and bioapatite in ungulate mammals and implications for ecological and paleoecological studies. *Oecologia* 120:347–363.
18. Passey BH, et al. (2005) Carbon isotopic fractionation between diet, breath, and bioapatite in different mammals. *J Archaeol Sci* 32:1459–1470.
19. DeNiro MJ, Epstein S (1978) Influence of diet on distribution of carbon isotopes in animals. *Geochim Cosmochim Acta* 42:495–506.
20. Brown FH, Sarna-Wojcicki AM, Meyer CE, Haileab B (1992) Correlation of Pliocene and Quaternary tephra layers between the Turkana Basin of East Africa and the Gulf of Aden. *Quat Int* 13/14:55–67.
21. Brown FH, Haileab B, McDougall I (2006) Sequence of tuffs between the KBS Tuff and the Chari Tuff in the Turkana Basin, Kenya and Ethiopia. *J Geol Soc London* 163:185–204.
22. McDougall I, Brown FH (2006) Precise ^{40}Ar/^{39}Ar geochronology for the upper Koobi Fora Formation, Turkana Basin, northern Kenya. *J Geol Soc London* 163:205–220.
23. McDougall I, Brown FH (2008) Geochronology of the pre-KBS Tuff sequence, Omo Group, Turkana Basin. *J Geol Soc London* 165:549–562.
24. Wood BA (1991) *Koobi Fora Research Project Volume 4: Hominid Cranial Remains* (Oxford Univ Press, Oxford), Vol 4.
25. Wood BA, Leakey MG (2011) The Omo-Turkana Basin fossil hominins and their contribution to our understanding of human evolution in Africa. *Evol Anthropol* 20(6): 264–292.
26. Passey BH, Cerling TE (2002) Tooth enamel mineralization in ungulates: Implications for recovering a primary isotopic time-series. *Geochim Cosmochim Acta* 18:3225–3234.
27. White TD, et al. (2009) Macrovertebrate paleontology and the Pliocene habitat of *Ardipithecus ramidus*. *Science* 326(5949):87–93.
28. Leakey MG, et al. (2001) New hominin genus from eastern Africa shows diverse middle Pliocene lineages. *Nature* 410(6827):433–440.
29. Kimbel WH, Delezene LK (2009) "Lucy" redux: A review of research on *Australopithecus afarensis*. *Am J Phys Anthropol* 140(Suppl 49):2–48.
30. Wynn JG, et al. (2013) Diet of Australopithecus afarensis from the Pliocene Hadar Formation, Ethiopia. *Proc Natl Acad Sci USA* 110:10495–10500.
31. van der Merwe NJ, Thackeray JF, Lee-Thorp JA, Luyt J (2003) The carbon isotope ecology and diet of *Australopithecus africanus* at Sterkfontein, South Africa. *J Hum Evol* 44(5):581–597.
32. Sponheimer M, et al. (2003) Diets of southern African bovidae: The stable isotope evidence. *J Mammal* 84:471–479.
33. Hurvich CM, Tsai CL (1989) Regression and time series model selection in small samples. *Biometrika* 76:297–307.
34. Burnham KP, Anderson DR (2004) Multimodel interference: Understanding AIC and BIC in model selection. *Social Methods Res* 33:261–304.
35. Prat S, et al. (2005) First occurrence of early Homo in the Nachukui Formation (West Turkana, Kenya) at 2.3-2.4 Myr. *J Hum Evol* 49(2):230–240.
36. Leakey RE, Wood BA (1974) A hominid mandible from East Rudolf, Kenya. *Am J Phys Anthropol* 41(2):245–249.
37. Beynon AD, Wood BA (1986) Variations in enamel thickness and structure in East African hominids. *Am J Phys Anthropol* 70(2):177–193.
38. Leakey RE (1976) New hominid fossils from the Koobi Fora formation in northern Kenya. *Nature* 261(5561):574–576.
39. Levin NE, Cerling TE, Passey BH, Harris JM, Ehleringer JR (2006) Stable isotopes as a proxy for paleoaridity. *Proc Natl Acad Sci USA* 103:11201–11205.
40. Popp BN, et al. (1998) Effect of phytoplankton cell geometry on carbon isotopic fractionation. *Geochim Cosmochim Acta* 62:69–77.
41. Chisholm BS, Nelson ED, Schwarcz HP (1983) Marine and terrestrial protein in prehistoric diets on the British Columbia coast. *Curr Anthropol* 24:396–398.
42. Schoeninger MJ, DeNiro MJ, Tauber H (1983) Stable nitrogen isotope ratios of bone collagen reflect marine and terrestrial components of prehistoric human diet. *Science* 220(4604):1381–1383.
43. Schoeninger MJ, Moore J, Sept JM (1999) Subsistence strategies of two "savanna" chimpanzee populations: The stable isotope evidence. *Am J Primatol* 49(4):297–314.
44. Sponheimer M, et al. (2006) Do "savanna" chimpanzees consume C4 resources? *J Hum Evol* 51(2):128–133.
45. Smith CC, Morgan ME, Pilbeam D (2010) Isotopic ecology and dietary profiles of Liberian chimpanzees. *J Hum Evol* 58(1):43–55.
46. Harris JM, Cerling TE (2002) Dietary adaptations of extant and Neogene African suids. *J Zool (1987)* 256:45–54.
47. Cerling TE, Harris JM, Leakey MG (2003) Isotope paleoecology of the Nawata and Apak Formations at Lothagam, Turkana Basin, Kenya. *Lothagam: The Dawn of Humanity in Africa*, eds Leakey MG, Harris JM (Columbia Univ Press, New York), pp 605–624.
48. Sponheimer M, et al. (2013) Isotopic evidence of early hominin diets: Past, present, and future. *Proc Natl Acad Sci USA* 110:10513–10518.
49. Walker A, Leakey RE, Harris JM, Brown FH (1986) 2.5 Mr *Australopithecus boisei* from west of Lake Turkana, Kenya. *Nature* 322:517–522.
50. Leakey RE (1973) Evidence for an advanced plio-pleistocene hominid from East Rudolf, Kenya. *Nature* 242(5398):447–450.
51. Leakey MG, et al. (2012) New fossils from Koobi Fora in northern Kenya confirm taxonomic diversity in early Homo. *Nature* 488(7410):201–204.
52. Cerling TE, et al. (2011) Woody cover and hominin environments in the past 6 million years. *Nature* 476(7358):51–56.
53. Levin NE, Brown FH, Behrensmeyer AK, Bobe R, Cerling TE (2011) Paleosol carbonates from the Omo Group: Isotopic records of local and regional environmental change in East Africa. *Palaeogeogr Palaeoecol Palaeoclim* 307:75–89.
54. White F (1983) *The Vegetation of Africa* (United Nations Scientific and Cultural Organization, Paris).
55. Feibel CS, Harris JM, Brown FH (1991) Paleoenvironmental context for the Late Neogene of the Turkana Basin. *Koobi Fora Research Project*, ed Harris JM (Clarendon, Oxford), Vol 3, pp 321–370.
56. Cerling TE (1979) Paleochemistry of Plio-Pleistocene Lake Turkana, Kenya. *Palaeogeogr Palaeoclimatol Palaeoecol* 27:247–285.
57. Passey BH, Levin NE, Cerling TE, Brown FH, Eiler JM (2010) High-temperature environments of human evolution in East Africa based on bond ordering in paleosol carbonates. *Proc Natl Acad Sci USA* 107(25):11245–11249.
58. Cerling TE, Chritz KL, Jablonski NG, Leakey MG, Manthi FK (2013) Diet of *Theropithecus* from 4 to 1 Ma in Kenya. *Proc Natl Acad Sci USA* 110:10507–10512.
59. Codron D, Lee-Thorp JA, Sponheimer M, de Ruiter D, Codron J (2005) Inter- and intrahabitat dietary variability of chacma baboons (*Papio ursinus*) in South African savannas based on fecal δ^{13}C, δ^{15}N, and% N. *Am J Phys Anthropol* 129:204–214.
60. Codron D, Lee-Thorp JA, Sponheimer M, deRuiter D, Codron J (2008) What insights can baboon feeding ecology provide for early hominin niche differentiation? *Int J Primatol* 29:757–772.
61. Passey BH, Cerling TE, Levin NE (2007) Temperature dependence of oxygen isotope acid fractionation for modern and fossil tooth enamels. *Rapid Commun Mass Spectrom* 21(17):2853–2859.
62. Francey RJ, et al. (1999) A 1000-year high precision record of d13C in atmospheric CO2. *Tellus B Chem Phys Meteorol* 51:170–193.
63. Keeling RF, Piper SC, Bollenbacher AF, Walker SJ (2010) Monthly atmospheric 13C/12C isotopic ratios for 11 SIO stations. *Trends: A Compendium of Data on Global Change* (Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, US Department of Energy, Oak Ridge, TN).
64. McDougall I, et al. (2012) New single crystal ^{40}Ar/^{39}Ar ages improve time scale for deposition of the Omo Group, Omo–Turkana Basin, East Africa. *J Geol Soc London* 169:213–226.