

# Diet of *Australopithecus afarensis* from the Pliocene Hadar Formation, Ethiopia

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Edited by James O'Connell, University of Utah, Salt Lake City, UT, and approved April 30, 2013 (received for review December 31, 2012)

The enhanced dietary flexibility of early hominins to include consumption of C<sub>4</sub>/crassulacean acid metabolism (CAM) foods (i.e., foods derived from grasses, sedges, and succulents common in tropical savannas and deserts) likely represents a significant ecological and behavioral distinction from both extant great apes and the last common ancestor that we shared with great apes. Here, we use stable carbon isotopic data from 20 samples of *Australopithecus afarensis* from Hadar and Dikika, Ethiopia (>3.4–2.9 Ma) to show that this species consumed a diet with significant C<sub>4</sub>/CAM foods, differing from its putative ancestor *Au. anamensis*. Furthermore, there is no temporal trend in the amount of C<sub>4</sub>/CAM food consumption over the age of the samples analyzed, and the amount of C<sub>4</sub>/CAM food intake was highly variable, even within a single narrow stratigraphic interval. As such, *Au. afarensis* was a key participant in the C<sub>4</sub>/CAM dietary expansion by early australopithecids of the middle Pliocene. The middle Pliocene expansion of the eastern African australopithecid diet to include savanna-based foods represents a shift to use of plant food resources that were already abundant in hominin environments for at least 1 million y and sets the stage for dietary differentiation and niche specialization by subsequent hominin taxa.

stable isotope | bioapatite | carbon-13 | paleodiet | human evolution

One of the traits that distinguishes humans from our closest living relatives, *Pan* and *Gorilla*, is the inclusion of significant quantities of C<sub>4</sub>/crassulacean acid metabolism (CAM) foods in the diet. C<sub>4</sub>/CAM plants include grasses and sedges (C<sub>4</sub>) common in tropical savannas and succulents (CAM) common in deserts. Therefore, the expansion of hominin diets to consume substantial amounts of C<sub>4</sub>/CAM foods signals a major ecological and adaptive divergence from the last common ancestor (LCA) that we shared with African great apes, which mostly occupy closed wooded habitats. C<sub>4</sub>/CAM food consumption is part of a general argument, based on several lines of evidence, that hominin diets diverged from the diets of the LCA during the shift to drier and more open environments in Africa during the Pliocene (1–4). This dietary transition occurred subsequent to the known fossil record of *Ardipithecus ramidus* from Ethiopia at 4.4 Ma (5), which shows little evidence of C<sub>4</sub>/CAM food consumption. The earliest previously published hominins to evince substantial C<sub>4</sub>/CAM food consumption are *Au. africanus* at ≤2.7 Ma (2, 6–8) as well as a small, poorly age-constrained (ca. 3.0–3.5 Ma) sample of *Au. bahrelghazali* from west-central Africa (9). Thus, current evidence places middle Pliocene *Au. afarensis*, a hominin with an extensive and well-defined stratigraphic range (3.7–3.0 Ma) in Ethiopia and elsewhere in eastern Africa at the crux of this hominin dietary change.

In this paper, we use stable isotope analysis to directly investigate the diet of *Au. afarensis* from the Hadar Formation of Ethiopia. Stable isotope analysis of mammalian tooth enamel is a paleodietary tool that allows one to determine the proportions of dietary carbon derived from plants using the C<sub>3</sub> photosynthetic pathway (trees, shrubs, and many herbs) vs. the C<sub>4</sub> and CAM

pathways [C<sub>4</sub> includes tropical grasses and some sedges and CAM includes succulents (6, 10–15)]. Although stable isotope studies alone do not distinguish the specific type of C<sub>4</sub>/CAM food consumed, a C<sub>4</sub>/CAM diet would imply the consumption of foods such as above- or underground portions of C<sub>4</sub> grasses or sedges, CAM succulents, and/or animals that ate these plants. We sampled 20 teeth of *Au. afarensis* (SI Appendix, Table S1) from well-constrained geochronological and refined taphonomic and paleoenvironmental contexts in the Hadar Formation at the geographically and temporally adjacent sites of Hadar and Dikika, Ethiopia (16–22). This sample allows us to investigate not only what *Au. afarensis* ate but also, whether the diet of this species changed through time (23) in response to previously inferred shifts in local environments over a 450,000-y interval (22). We also sampled the associated macromammalian fauna to place the fossil hominin data in a broader context and address questions of diagenesis that can alter primary isotopic signatures.

## Results

The median  $\delta^{13}\text{C}$  value for the sample of *Au. afarensis* is  $-7.4\text{‰}$ , with a range of  $-13.0\text{‰}$  to  $-2.9\text{‰}$  (Fig. 1). There are significant differences in the median  $\delta^{13}\text{C}$  values for *Au. afarensis* and presumptive C<sub>4</sub>-grazing and C<sub>3</sub>-browsing herbivores (Kruskal–Wallis,  $P < 0.0001$ ). Posthoc comparisons show that the median  $\delta^{13}\text{C}$  value for *Au. afarensis* is statistically distinct from  $\delta^{13}\text{C}$  values of the C<sub>3</sub>-browser *Giraffa* (median =  $-10.9\text{‰}$ ,  $n = 15$ , Mann–Whitney U,  $P < 0.0001$ ) and C<sub>4</sub>-grazing alcelaphini (median =  $+1.4\text{‰}$ ,  $n = 10$ , Mann–Whitney U,  $P < 0.0001$ ) (Fig. 1), which are also found in the same Hadar Formation sediments. These end member taxa have  $\delta^{13}\text{C}$  values that validate their presumed diets, implying that diagenesis has not significantly affected the  $\delta^{13}\text{C}$  values of our samples.

The  $\delta^{13}\text{C}$  values for *Au. afarensis* indicate both a significant proportion and range of C<sub>4</sub>/CAM plants in its diet. Using the median values of the two end member taxa as estimates of pure C<sub>3</sub>-browsing and pure C<sub>4</sub>-grazing diets, the nominal percentage of C<sub>4</sub> intake for 20 *Au. afarensis* individuals has a median value of 22% and a range of 0–69%. Posthoc comparisons of the  $\delta^{13}\text{C}$  values from *Au. afarensis* also clearly show more C<sub>4</sub>/CAM consumption than inferred from the  $\delta^{13}\text{C}_{1750}$  values of modern *Pan* (24–26) (median =  $-10.9\text{‰}$ ,  $n = 58$ , Mann–Whitney U,  $P < 0.0001$ ) and *Gorilla* (27) [median  $\delta^{13}\text{C}_{1750} = -13.5\text{‰}$ ,  $n = 15$ , Mann–Whitney U,  $P < 0.0001$ ; modern values are corrected to the year 1750 for the change in the  $\delta^{13}\text{C}$  of atmospheric CO<sub>2</sub> caused by the burning of

Author contributions: J.G.W., M.S., W.H.K., Z.A., K.R., Z.K.B., and J.N.W. designed research, performed research, analyzed data, and wrote the paper.

The authors declare no conflict of interest.

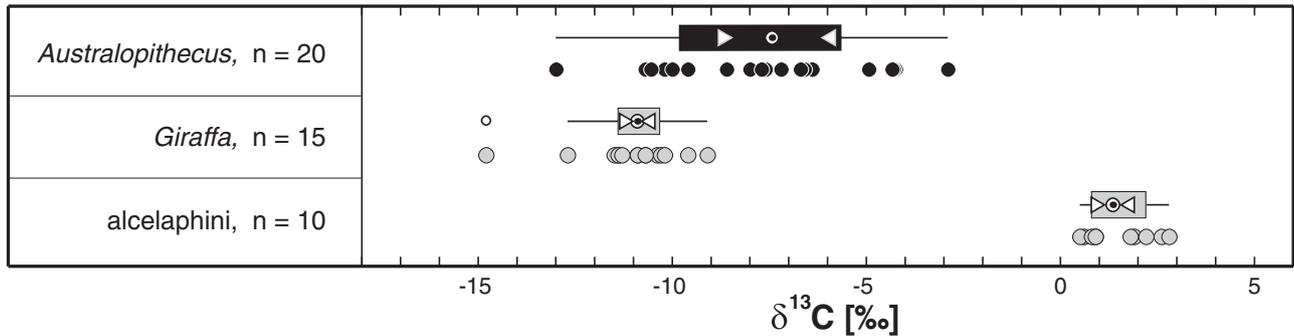
This article is a PNAS Direct Submission.

See Commentary on page 10470.

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This article contains supporting information online at [www.pnas.org/lookup/suppl/doi:10.1073/pnas.1222559110/-DCSupplemental](http://www.pnas.org/lookup/suppl/doi:10.1073/pnas.1222559110/-DCSupplemental).

Taxon, # specimens



**Fig. 1.** Box and whisker plot showing the carbon isotopic composition ( $\delta^{13}\text{C}$  value) of tooth enamel from *Au. afarensis* and two end member taxa ( $\text{C}_3$  browser: *Giraffa*;  $\text{C}_4$  grazer: alcelaphini). The bull's eye at center of each box represents the sample median. Open circles represent outlier values, defined at 1.5 times the interquartile range. Boxes represent 25th and 75th percentiles. Whiskers represent the range exclusive of outliers. Triangles represent the 95% confidence intervals for the medians (thus, if the areas between the triangles for two samples do not overlap, one can conclude with 95% confidence that the true medians differ). Closed circles below the box plots represent individual specimen values. Black, *Australopithecus*; gray, other taxa.

fossil fuels (28)]. There is no statistically significant temporal trend in  $\delta^{13}\text{C}$  values through the Hadar Formation (Fig. 2) ( $r^2 = 0.03$ ,  $P = 0.40$ ). Despite evidence for significant  $\text{C}_4/\text{CAM}$  consumption, the entire range of  $\delta^{13}\text{C}$  values for *Au. afarensis* falls below the modal  $\delta^{13}\text{C}$  value for the Hadar Formation nonhominin mammalian sample analyzed ( $n = 538$ , median =  $-2.2\text{‰}$ , mode =  $-2.5\text{‰}$ , Mann–Whitney U,  $P < 0.0001$ ) (Fig. 3).

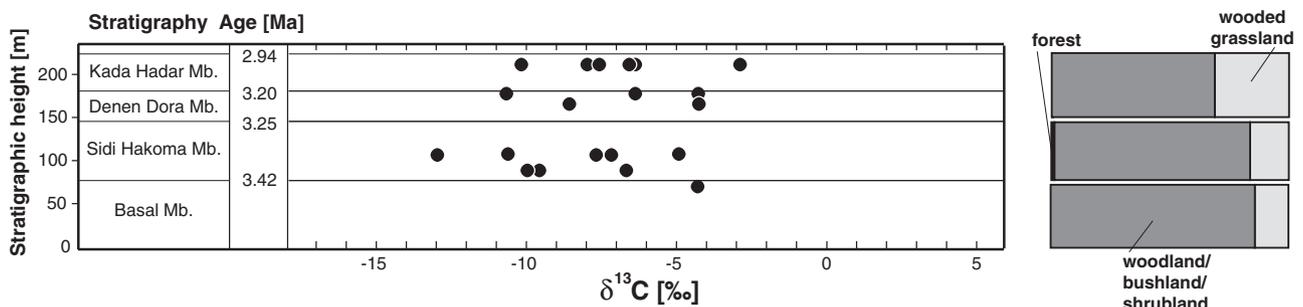
There were significant differences in  $\delta^{13}\text{C}$  values among early hominin taxa, for which a sample of at least five individuals is available (Kruskal–Wallis,  $P < 0.0001$ ). Posthoc comparisons show that the  $\delta^{13}\text{C}$  values for *Au. afarensis* do not differ significantly from the  $\delta^{13}\text{C}$  values of slightly younger *Au. africanus* from South Africa [median =  $-6.8\text{‰}$ ,  $n = 24$ , Mann–Whitney U,  $P = 0.32$  (2, 6–8)]. The ranges of variation in  $\delta^{13}\text{C}$  values for these two taxa broadly overlap (*Au. afarensis*:  $-13.0\text{‰}$  to  $-2.9\text{‰}$ ,  $n = 20$ ; *Au. africanus*:  $-11.3\text{‰}$  to  $-1.8\text{‰}$ ,  $n = 24$ ).  $\delta^{13}\text{C}$  values for *Au. afarensis* show significantly more evidence of  $\text{C}_4/\text{CAM}$  food consumption than in earlier *Ar. ramidus* [4.4 Ma, median =  $-10.4\text{‰}$ ,  $n = 5$ , Mann–Whitney U,  $P = 0.021$  (5)] and *Au. anamensis* (4.0 Ma, median =  $-11.0\text{‰}$ ,  $n = 11$ , Mann–Whitney U,  $P < 0.001$ ). Compared with later australopiths, *Au. afarensis* has similar  $\delta^{13}\text{C}$  values to the  $\delta^{13}\text{C}$  values of *Paranthropus robustus* of southern Africa [median =  $-7.2\text{‰}$ ,  $n = 22$ , Mann–Whitney U,  $P = 0.95$  (7, 13, 29, 30)], but its  $\delta^{13}\text{C}$  values differ significantly from the  $\delta^{13}\text{C}$  values of *P. boisei* of eastern Africa [median =  $-1.3\text{‰}$ ,  $n = 24$ , Mann–Whitney U,  $P < 0.0001$  (31, 32)]. Comparison of  $\delta^{13}\text{C}$  values of *Au. afarensis* with  $\delta^{13}\text{C}$  values from very small samples of *Au. bahrelghazali* from central Africa [median =  $-2.5\text{‰}$ ,  $n = 3$  (9)] and *Au. sediba*

from southern Africa [median =  $-12.15\text{‰}$ ,  $n = 2$  (33)] suggests significant differences in  $\text{C}_4/\text{CAM}$  food consumption among these three taxa.

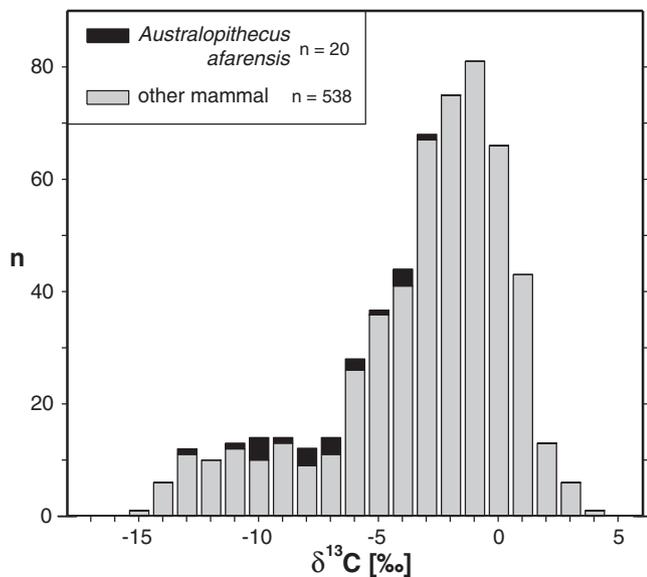
A Kruskal–Wallis test indicates strong divergence in  $\delta^{18}\text{O}$  values, which in part, reflects differences in water dependence (34) between *Au. afarensis* and other mammalian families from the Hadar Formation (Fig. 4) (Kruskal–Wallis,  $P < 0.0001$ ). Posthoc comparisons show that the  $\delta^{18}\text{O}$  values for *Au. afarensis* (median =  $-5.6\text{‰}$ ,  $n = 20$ ) are different from the highly water-independent giraffids (median =  $-0.6\text{‰}$ ,  $n = 30$ , Mann–Whitney U,  $P = 0.0016$ ) as well as the very water-dependent hippopotamids (median =  $-7.5\text{‰}$ ,  $n = 22$ , Mann–Whitney U,  $P = 0.011$ ). *Au. afarensis*  $\delta^{18}\text{O}$  values are, however, statistically indistinguishable from the  $\delta^{18}\text{O}$  values of equids (median =  $-4.8\text{‰}$ ,  $n = 88$ , Mann–Whitney U,  $P = 0.13$ ) and suids (median =  $-4.2\text{‰}$ ,  $n = 104$ , Mann–Whitney U,  $P = 0.10$ ).

**Discussion**

**Middle Pliocene Hominin Dietary Expansion to  $\text{C}_4/\text{CAM}$  Foods.** Forest-dwelling *Gorilla* and *Pan* species have low  $\delta^{13}\text{C}$  values, reflecting pure  $\text{C}_3$  diets of plants predominantly from the forest understory, where vegetation is typically  $^{13}\text{C}$ -depleted by several permil compared with the upper canopy (14). Even the savanna chimpanzee (*P. troglodytes verus*), which frequents relatively open habitats, shows little evidence of consumption of  $\text{C}_4/\text{CAM}$  foods that characterize the savanna portion of their habitat (24). The diets of these extant apes contrast sharply with the diets of our Ethiopian sample of *Au. afarensis*, which shows significant use of  $\text{C}_4/\text{CAM}$  foods. *Au. afarensis* shows a greater range of  $\text{C}_4/\text{CAM}$

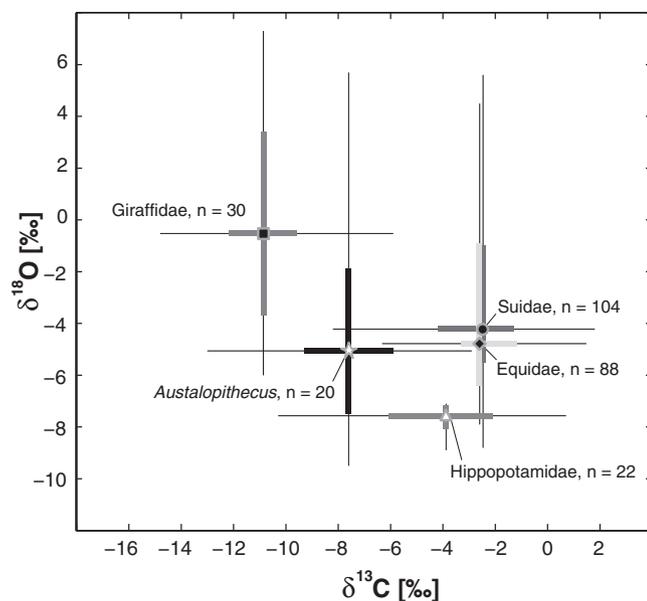


**Fig. 2.** Stratigraphic distribution of the *Australopithecus* isotopic composition shown in Fig. 1. Specimens are divided into submembers of the Hadar Formation and plotted with stratigraphic height. Also shown are the percentages of cover of forest, woodland/bushland/shrubland, and wooded grassland estimated by samples of  $\delta^{13}\text{C}$  of pedogenic carbonates from the Hadar Formation (47).



**Fig. 3.** Histogram showing the carbon isotopic composition ( $\delta^{13}\text{C}$  value) of tooth enamel from *Au. afarensis* and all other mammalian taxa analyzed from the Hadar Formation. Black, *Au. afarensis*; gray, all other mammals analyzed. Data are from this study (hominin samples are in [SI Appendix, Table S1](#), and other samples are in [SI Appendix, Table S2](#), and in ref. 44).

food consumption relative to the earlier east African hominin taxa *Ar. ramidus* and *Au. anamensis*, both of which focused on  $\text{C}_3$  plant foods (5, 27). Although the lowest  $\delta^{13}\text{C}$  value in our *Au. afarensis* sample is the most  $^{13}\text{C}$ -depleted specimen of an early hominin taxon yet recorded [specimen Afar locality (A.L.) 125–11:  $\delta^{13}\text{C} = -13.0\text{‰}$ ], approaching the great ape range (35), the majority of  $\delta^{13}\text{C}$  values for *Au. afarensis* are well above the range of  $\delta^{13}\text{C}_{1750}$  values for extant *Pan* and *Gorilla* (14, 27). Simple mixing calculations of pure  $\text{C}_3$  and  $\text{C}_4$  end member diets



**Fig. 4.** Cross-plot of box and whisker diagrams for carbon and oxygen isotopic composition ( $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values) of tooth enamel from *Au. afarensis* and select mammalian taxa from the Hadar Formation. Boxes represent 25th and 75th percentiles. Whiskers represent the range exclusive of outliers (defined in Fig. 1). Data are from this study (hominin samples are in [SI Appendix, Table S1](#) and in ref. 44).

show that four individuals of *Au. afarensis* consumed only  $\text{C}_3$  foods, at least during the period of tooth crown mineralization, whereas the majority of sampled individuals consumed significant amounts of  $\text{C}_4/\text{CAM}$  foods during this growth period, with four individuals having consumed 50% or more  $\text{C}_4$  vegetation.

The stable isotope data from *Au. afarensis* combined with other data from African early hominins (9, 27, 36) begin to clarify the timing of increased  $\text{C}_4/\text{CAM}$  use in the hominin lineage after the LCA shared with chimpanzees. The data from Hadar and Dikika in this paper, combined with data from *Kenyanthropus* in the Turkana Basin (27) and *Au. bahrelghazali* from Koro Toro, Chad (9), show a pattern of hominin dietary expansion to include  $\text{C}_4/\text{CAM}$  foods during the middle Pliocene by ca. 3.4 Ma (the age uncertainty of the first occurrence of these three taxa is within the age uncertainty of the oldest specimens from the Hadar Formation) (27, 37). Especially conspicuous is the lack of evidence for  $\text{C}_4/\text{CAM}$  plant consumption in *Au. anamensis*, the probable anagenetic ancestor of *Au. afarensis* (27), despite evidence from occlusal microwear that the mechanical properties of foods were essentially the same in these two australopith species (38–41). In contrast, virtually all hominins coeval with and younger than *Au. afarensis* consumed substantial but variable amounts of  $\text{C}_4/\text{CAM}$  foods. Regardless of whether the sampled isolated teeth attributed to *Kenyanthropus* (27) can ultimately be distinguished morphologically from teeth of *Au. afarensis*, their broad range of nominal  $\text{C}_4/\text{CAM}$  intake reinforces our inference of an expansion of the hominin dietary niche by ca. 3.4 Ma. We have no data to address the  $\text{C}_4/\text{CAM}$  consumption patterns of hominins between the youngest  $\text{C}_3$ -focused *Au. anamensis* specimens and the later  $\text{C}_4/\text{CAM}$ -consuming australopiths, but this gap may ultimately be filled by analysis of additional material from Laetoli, Woranso-Mille, and other localities that span this range (42, 43). Among the late Pliocene robust australopiths, *P. robustus* from South Africa retained a range of  $\text{C}_4/\text{CAM}$  intake similar to the range in *Au. afarensis*, *Au. africanus*, and *Kenyanthropus*, whereas *P. boisei* from eastern Africa apparently specialized on a narrow range of  $\text{C}_4/\text{CAM}$  foods (31). A potential exception to this late Pliocene focus on  $\text{C}_4/\text{CAM}$  foods is *Au. sediba* from Malapa, South Africa, at ca. 2 Ma, which hints at an entirely  $\text{C}_3$  diet, but the small sample size ( $n = 2$ ) dictates a cautious interpretation of these data (33).

#### Temporal Patterns in $\text{C}_4/\text{CAM}$ Plant Use Through the Hadar Formation.

Using high-resolution pollen data from Hadar, Bonnefille et al. (17) suggested that *Au. afarensis* was adapted to variable environmental conditions between 3.4 and 2.9 Ma. Our stable isotope data allow us to explore this idea by examining whether this species adjusted its plant food intake in response to environmental changes in the Hadar Formation. Increased  $\text{C}_4/\text{CAM}$  use over time by *Au. afarensis* might be expected given evidence of faunal change and increased aridity [Kada Hadar Member level KH-2 (22, 23)]. Our data, however, reveal no shift in  $\delta^{13}\text{C}$  values over time. The entire range of  $\text{C}_4/\text{CAM}$  consumption, from pure  $\text{C}_3$  to high  $\text{C}_4$ , can be found within the *Au. afarensis* sample from any single stratigraphic member. Because a range of environments (from woodlands to bushland and edaphic grasslands) is represented by the sampled submembers (22, 44, 45), the stable isotopic variation of *Au. afarensis* also occurs across this range of reconstructed paleoenvironments. This observation suggests that fluctuating environmental conditions did not perceptibly influence the  $\text{C}_4/\text{CAM}$  consumption patterns of *Au. afarensis* in the Hadar Formation and that *Au. afarensis* expanded its diet to exploit a range of viable resources, despite habitat variation (Fig. 2).

The lack of a temporal pattern in the isotopic composition of the diet of *Au. afarensis* is consistent with similar findings from occlusal microwear (39), which show little variation in *Au. afarensis* over time. In contrast to the homogeneity of the dental microwear pattern, however, the range of variation in the carbon isotopic

composition of *Au. afarensis* is wide, even within the temporally brief stratigraphic subdivisions of the Hadar Formation (Fig. 2). Assuming that occlusal microwear provides direct insight into the mechanical properties of food consumed (but see ref. 41), these opposing patterns suggest use of foods that varied little in terms of these mechanical properties but differed substantially in photosynthetic pathway (for example, mixtures of C<sub>3</sub> plants and semi-aquatic C<sub>4</sub> plants such as sedges). In any case, taken together, the dietary isotopic and microwear data imply that *Au. afarensis* in the Hadar Formation exploited a similar range of plant foods in the face of local environmental fluctuation over time.

**Paleoecology of *Au. afarensis*.** Although early australopiths at about 3.4 Ma may be the earliest hominins to expand the dietary repertoire to include perceptible amounts of C<sub>4</sub> resources in their diet, we have little insight as to what specific C<sub>4</sub>/CAM foods were consumed. Based on present data, a reasonable conclusion is that *Au. afarensis* was a generalist omnivore consuming isotopically varied resources at a variety of spatial (e.g., microhabitat) and temporal (e.g., inter- or intraannual) scales. C<sub>4</sub>/CAM foods consumed might have included grass seeds and roots seasonally, sedge underground storage organs at other times, termites, succulent CAM plants, and even small game or scavenged carcasses [as suggested by modified bones associated with *Au. afarensis* fossils from Dikika (46)]; all are possibilities consistent with the existing data. Narrowing the range of possibilities will require independent evidence from trace element chemistry, dental calculus, and other sources.

Despite this uncertainty, the stable carbon isotopic results suggest much about the ecology of *Au. afarensis*. Throughout its stratigraphic range, *Au. afarensis* has been found alongside faunal, paleobotanical, and paleosol evidence indicating a spectrum of savanna mosaic environments ranging from relatively closed woodlands to open wetlands and edaphic grasslands (17, 22, 44, 47, 48). It has been a challenge to pinpoint the species' preferred habitats within such a broad paleoenvironmental spectrum. Stable carbon isotope data provide direct evidence that *Au. afarensis* consumed C<sub>4</sub> foods and thus, likely exploited resources in areas with grassy understories and/or water-edge microhabitats, both of which provide ample opportunity for C<sub>4</sub> food consumption. Interestingly, savanna chimpanzees, which are found in such areas today, do not measurably consume the abundant C<sub>4</sub> resources available to them. Thus, the remarkably wide range of C<sub>3</sub> to C<sub>4</sub> diets of by *Au. afarensis* across a temporally fluctuating environmental mosaic supports the inference of a generalist hominoid primate that exploited a broad range of habitats for food resources (1, 22).

Although highly variable, the range of  $\delta^{13}\text{C}$  values for *Au. afarensis* does not incorporate the modal value from the other macromammalian fauna sampled from the Hadar Formation, indicating a diet that was less C<sub>4</sub>-focused than the diet of the abundant representatives of relatively open habitats from the Hadar region's paleoenvironmental spectrum (Fig. 3). Clearly, the dietary ecology of *Au. afarensis* differed from the dietary ecology of specialist taxa, such as *Giraffa* and alcelaphini, but it is also apparent that *Au. afarensis* occupied a relatively underrepresented dietary niche compared with the majority of taxa in the Hadar mammalian community (Figs. 3 and 4).

Likewise, the  $\delta^{18}\text{O}$  values of *Au. afarensis* show that this species was distinct from many contemporaneous taxa in terms of drinking habits and/or other factors affecting oxygen isotopic composition (34). Fig. 4 suggests that *Au. afarensis* was intermediate in its degree of dependence on drinking water between the end members of water-independent giraffids and the water-dependent hippopotamids.

The question remains of what led *Au. afarensis* to consume C<sub>4</sub>/CAM foods, whereas its likely ancestor, *Au. anamensis*, largely avoided C<sub>4</sub>/CAM foods, despite the fact that both species

inhabited similar mosaics of savanna habitats (48). Although the broad range of *Au. afarensis*  $\delta^{13}\text{C}$  values might reflect dietary change in response to shifting habitat distributions, these habitat shifts may have occurred at spatial and/or temporal scales too fine to be resolved with currently available data. If such a relationship does exist, then it may ultimately be possible to characterize the relationship between paleoenvironment and diet with more comprehensive sampling of both taxa from a broader temporal and/or habitat range. The slightly older sample of *Au. afarensis* from Laetoli may provide such an additional test, given the evidence for relatively open paleoenvironments (48). However, such a quest may also prove to be a red herring because a species' dietary preference is not necessarily coupled to the most common habitats within the local environment. Chimpanzees offer an example of a modern analog to this potential disconnect between diet and environment: savanna chimpanzees live in relatively open habitats among abundant C<sub>4</sub>/CAM plants, whereas forest-dwelling chimpanzees do not, but both use almost exclusively C<sub>3</sub> foods. If we were to attempt to reconcile such a paradox using only the slice of data available from the fossil record, even with extensive datasets, presently available methods would likely fail.

#### **Dentognathic Morphology and the Advent of C<sub>4</sub> Diet in Hominins.**

Our research shows a diet for *Au. afarensis* that includes significant consumption of C<sub>4</sub> resources, which has implications for our understanding of the links between hominin morphology and diet. The influential dietary hypothesis of the 1950s by Robinson (49, 50) proposed two morphologically distinct groups of early hominins from southern Africa (the subsequently branded gracile and robust australopiths) with strongly divergent dietary adaptations. Later discoveries of *Au. afarensis* beginning in the 1970s clarified that all Pliocene hominins morphologically expressed a "heavy chewing" signal to one degree or another (51). *Au. afarensis* (3.7–3.0 Ma) and its likely ancestor *Au. anamensis* (4.2–3.9 Ma) are the earliest hominins known to exhibit dentognathic specializations conventionally associated with mastication of mechanically resistant food items, including relatively large, thickly enameled cheek teeth, heavily built jaws, and rugose chewing muscle attachment sites (19, 52). Morphological evidence for Pliocene hominin diets suggests that the australopith lineage was marked by increasing reliance on hard and brittle food items, such as nuts and seeds, or foods with abrasive particles, such as underground storage organs (3, 4, 19, 20, 52). Meanwhile, Grine et al. (39) and Ungar et al. (53) found that *Au. afarensis* shows dental microwear that is dissimilar to dental microwear of known hard-object feeding primates, such as the extant mangabey (*Lophocebus*), and likewise, dissimilar to dental microwear of the fossil hominin *P. robustus*, interpreted to be a hard-object feeder. Furthermore, despite the morphological similarity of *Au. afarensis* and *Au. anamensis*, isotopic data from the latter species lack evidence of C<sub>4</sub>/CAM plant food intake (27). Although the mechanical properties of food items cannot be deciphered from isotopic data, the fact that the occlusal microwear of these two species is essentially the same, yet different from diets of known or inferred primate hard-object feeders (38, 54) implies that the links between early hominin diet (what was eaten) and the various sources of insight on dietary behavior (how food was acquired and consumed) are not as straightforward as it once seemed (55). Recent work has also underscored the importance of distinguishing the abrasion of teeth by nondietary grit from the abrasion produced by food particles (1, 41, 56). Thus, our results caution against dietary behavioral reconstructions that rely on the primacy of any single method or approach.

#### **Conclusions**

Carbon isotopic data from *Au. afarensis* within the Hadar Formation show that this species consumed significant amounts of relatively  $^{13}\text{C}$ -enriched foods (i.e., from C<sub>4</sub>/CAM plants and/or

animal foods derived from such plants). Furthermore, the amount of relatively  $^{13}\text{C}$ -enriched food intake within the sample is highly variable, even within narrow stratigraphic intervals or paleoenvironmental settings. The two known hominin taxa antecedent to *Au. afarensis* with comparable stable isotopic data (*Ardipithecus* and *Au. anamensis*) are, like *Au. afarensis*, sampled from environments characterized by abundant  $\text{C}_4/\text{CAM}$  vegetation (47), but neither apparently engaged in significant  $\text{C}_4/\text{CAM}$  food consumption in their environments. In light of this newly expanded hominin stable isotopic dataset (36), *Au. afarensis* documents a transformational stage in our ecological history, during which hominins in eastern Africa began to expand their dietary resource base to include  $\text{C}_4/\text{CAM}$  foods that had been abundant in their environments for at least 1 million y. Within the presumptive *Au. anamensis*–*Au. afarensis* lineage of eastern African australopithecids (57), the  $\text{C}_4/\text{CAM}$  dietary expansion is clearly demarcated by the contrast between the data from Kanapoi and Allia Bay (4.1–3.9 Ma) (27) and the data from the Hadar Formation (3.4 Ma–2.9 Ma; this work). The detailed portrait of this transition and its causes remains to be filled in with data from australopithecine-bearing sites in the intervening time period.

The stable isotopic data show that middle Pliocene *Au. afarensis* and *Au. africanus* consumed widely varying mixtures of  $\text{C}_3$  and  $\text{C}_4$  foods. This dietary flexibility implies unique landscape use patterns and malleable foraging behavior within a narrow time frame of a single species. Subsequent reduction of dietary isotopic variation in late Pliocene *Paranthropus* and early *Homo* (36) may be associated with terminal Pliocene aridification and concomitant narrowing of terrestrial habitats (58). The middle Pliocene  $\text{C}_4/\text{CAM}$  dietary expansion may also have set the stage for dietary differentiation between these hominins, which differed markedly in their consumption of  $\text{C}_4/\text{CAM}$ -derived foods.

## Methods

Samples were drilled from specimens housed in the National Museum of Ethiopia using a high-speed drill and diamond-encrusted drill bits. Up to ~2 mg powdered enamel were collected entirely from fractured surfaces of enamel, and therefore, future morphological or surface wear studies were

not compromised (detailed photographs of before and after sample collection are in *SI Appendix*). All samples were treated to remove exogenous carbonate. Samples were soaked in 0.1 M buffered acetic acid for 30 min, rinsed with milli-Q water, and dried overnight at 60 °C. Approximately 0.6 mg of pretreated sample were reacted with 103% (vol/vol) phosphoric acid at 25 °C in 4.5-mL Labco exetainers preflushed with ultrapure helium. The isotopic composition of the  $\text{CO}_2$  resulting from this reaction was analyzed on a Thermo Finnigan Delta V isotope ratio mass spectrometer coupled to a Gasbench II preparation device.  $\text{CO}_2$  was separated from other trace gases by gas chromatography, and 10 replicate samples of each  $\text{CO}_2$  sample were analyzed. The isotopic composition of tooth enamel was normalized with respect to two isotopic reference materials (an internal sample of Carrara Marble with  $\delta^{13}\text{C} = 2.01\text{‰}$  and  $\delta^{18}\text{O} = -1.79\text{‰}$  and National Bureau of Standards (NBS)-18 with  $\delta^{13}\text{C} = -5.04\text{‰}$  and  $\delta^{18}\text{O} = -23.05\text{‰}$ ). Replicate measurements of 10 samples of each reference material for this sequence had errors  $<0.15\text{‰}$  for  $\delta^{13}\text{C}$  and  $<0.20\text{‰}$  for  $\delta^{18}\text{O}$ . A selection of other specimens (10 *Giraffa* and 10 alcelaphini) was analyzed in the same sequence as the hominin specimens under the same conditions. Other analyses shown here are part of a larger dataset from the Hadar Formation, and they were analyzed under slightly different conditions that allow for larger sample mass (~2 mg sample in 12-mL exetainers). Sample yields (percent  $\text{CaCO}_3$ ) were calculated using the integrated peak area of voltage measured on mass 44 using the calcite standards Carrara Marble and NBS-18 as a reference of 100%  $\text{CaCO}_3$ . Nominal percent  $\text{C}_4$  dietary intake was calculated using end member values of pure  $\text{C}_4$  and pure  $\text{C}_3$  diets of +1.4‰ and -10.9‰, respectively (the values of end member  $\text{C}_4$ -grazing and  $\text{C}_3$ -browsing taxa alcelaphini and *Giraffa* from this dataset). Comparisons of fossil samples with modern specimens are corrected to the year 1750 for the effect of burning of fossil fuels (28) by adding +1.5‰ to the modern  $\delta^{13}\text{C}$  value or using similarly corrected values in published data. Comparisons of  $\delta^{13}\text{C}$  values of hair and tooth enamel are made using the information in ref. 59. Statistical analyses were performed using Matlab R2008b using Mann–Whitney U and Kruskal–Wallis tests (60). Kruskal–Wallis tests were adjusted for unequal sample size and reported using *P* values of the  $\chi^2$  statistic. A significance level of  $\alpha = 0.05$  was used throughout.

**ACKNOWLEDGMENTS.** We thank the Authority for Research and Conservation of Cultural Heritage, the National Museum of Ethiopia, the Ethiopian Ministry of Culture and Tourism, for permission to undertake this study. Research was funded by National Science Foundation Grant BCS1064030.

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