CARBON AND NITROGEN ISOTOPIC SIGNATURES OF HAIR, NAIL, AND BREATH FROM TROPICAL AFRICAN HUMAN POPULATIONS

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ABSTRACT

Rationale: Stable isotopic analyses are increasingly used to study the diets of past and present human populations. Yet, the carbon and nitrogen isotopic data of modern human diets collected so far are biased towards Europe and North America. Here, we address this gap by reporting on the dietary isotopic signatures of six tropical African communities: El Molo, Turkana (Kerio), Luhya (Webuye), Luhya (Port Victoria), and Luo (Port Victoria) from Kenya, and Baka from Cameroon; representing four subsistence strategies: fishing, pastoralism, agriculturalism, and hunter-gatherer.

Methods: We used EA-CF-IRMS to measure the carbon and nitrogen isotopic ratios of hair (n = 134) and nail (n = 80), and the carbon isotopic ratios of breath (n = 184) from these communities, as well as the carbon and nitrogen isotopic ratios of some food samples from the Kenyan communities.

Results: We expand on the known range of $\delta^{13}C$ values in human hair through the hunter-gatherer Baka, with a diet based on C_3 plants, and through the agriculturalist Luhya (Webuye), with a diet based on C_4 plants. In addition, we found that the consumption of fish from East African lakes is difficult to detect isotopically due to the combined effects of high nitrogen isotopic ratios of plants and the low nitrogen isotopic ratios of fish. Finally, we found that some of the communities studied are markedly changing their diets through increasing sedentism and urbanization.

Conclusion: Our findings contribute substantially to the understanding of the environmental, demographic and economic dynamics that affect the dietary landscape of different tropical populations of Africa. These results highlight the importance of studying a broader sample of human populations and their diet, with a focus on their precise context – both from an isotopic and more general anthropological perspectives.

Keywords food transitions; stable isotopic analysis; East Africa; Central Africa; subsistence strategies

INTRODUCTION

Africa is central to both prehistoric and contemporary human evolution and biology. As the place where humans evolved, it is the cornerstone to research on who we are and how we came to be. Today, Africa continues to be a place of great change due to ongoing political, economic, social, and environmental shifts in the continent [1,2]. However, we still lack focused research on the biology of human activities in both time frames. For instance, there is a surprising lack of fossil and chronologically-controlled archaeological evidence of the events that took place in Africa between the appearance of *Homo sapiens*, 200 000 years ago (200 Ka), and the present day [3]. At the same time, a significant portion of research into current human behaviours relies on sampling of WEIRD populations (Western, educated, industrialized, rich, and democratic) [4].

As a component of the ecological niche that people occupy, diet is a useful lens when studying *both* present and past human populations. Dietary assessment allows for a comprehensive grasp of human social structures, mobility patterns, activities, and overall health [5–8]. The methods most often used for the assessment of *present* diets still rely on dietary reporting (e.g. food frequency questionnaires and 24-hour recalls), although recent developments have led to the emergence of biomarkers as dietary trackers (e.g. analyses of double labelled water, stable isotope ratios, and gut microbiota) [9]. Methods for assessing *past* diets are numerous – they include the study of craniomandibular biomechanics, of dental macro- and microwear, of coprolites, of microfossils, of stable isotope ratios, of parasite relationships, of gut microbiota, and of genomic adaptations [reviewed in 7]. Clearly, dietary assessment in either of these time frames presents its own set of challenges: present populations are rarely accurate when reporting diet and biochemical approaches can be invasive or laborious, whereas the diet of past populations can only be inferred from proxies, all of which have their limitations. In this context, one of the few methods that has been used to great effect to assess both present and past diets is stable isotope analysis. For instance, in present populations, stable isotope analyses have been successfully used to study associations between diet and diseases [e.g. 10,11] and to track food transitions [for a review, see 12]. In past populations, stable isotope analyses were first applied to detect the consumption of maize among prehistoric North American groups [13], and have since provided ample evidence for specific dietary adaptations, such as the high meat consumption among Neanderthals [14], or the consumption of C_4 foods by some species of *Australopithecus* [15].

Here, we investigate the degree to which we can use carbon and nitrogen isotopic data to inform us about the diet of different groups living in different environments in Central and East Africa today. We compare the isotopic data from different samples, to see if:

1. we can observe carbon and nitrogen isotopic differences between groups that are known to have different diets;
2. we can identify dietary factors that result in carbon and nitrogen isotopic changes in hair, nail and breath.

Through this work, we aim to further validate the use of stable isotope analysis as a tool of dietary assessment, to expand our knowledge on the isotopic landscape of present diets, and to contribute to the interpretation of past events in tropical Africa.

SCIENTIFIC BACKGROUND

Stable isotope analysis is one of the few scientific methods that directly quantifies aspects of the diet that an organism consumes throughout its life. This approach is based on the principle that 'we are what we eat', i.e. that the chemical components of ingested food find their way into an organism's tissues in a measurable fashion [16–18]. Although other factors, such as metabolism and environment, also influence stable isotopic signatures, the correlation between certain stable isotope signatures and diet has been well established [19–21]. The most common elements used in diet assessment are carbon and nitrogen. In simple terms, carbon isotope analyses distinguish between the types of plants (C_3 or C_4) that are at the base of the food chain, while nitrogen isotope analyses track an organism's position in the food chain [22–25]. Within archaeology, stable isotope studies are more often performed on bone collagen and bioapatite. However, the technique is applicable to other tissues containing carbon and nitrogen, including hair, nails, skin, and muscle [26,27].

Stable isotope ratios have been validated for use as dietary biomarkers based on three types of work. First, controlled feeding studies, mostly conducted in animals [28], but in some cases also in humans [29,30]; second, observational studies in free-living populations [10,31–34], and finally, large-scale global surveys [35,36]. Nevertheless, additional validation studies are necessary if stable isotope biomarkers are to reach their full potential. On the one hand, new controlled feeding studies in humans may clarify aspects of the isotopic metabolism, such as diet-tissue fractionation, and macronutrient routing. On the other hand, observational studies in diverse populations could evaluate the effect of different diets on isotopic ratios, and

contribute to our understanding of global variation in the chemical uptake of different elements in different human groups [12].

Only a handful of studies have investigated the carbon and nitrogen isotopic ratios of modern groups in Africa [35]. Among these, we find different types of work: (1) forensic studies using isotopic data to assign geographical origin to unknown individuals, with very few samples of African origin [37,38], (2) archaeological studies investigating the diets of historic and prehistoric populations in East and South Africa [39–42], and (3) one observational study that analysed three living populations in the eastern margin of Lake Turkana [43], one of which (El Molo) overlaps with the present study. This paper contributes to our knowledge of the dietary isotopic signature of present-day African populations by targeting groups who have had traditionally different diets, all of whom are undergoing marked changes in subsistence strategy, and focusing on inter-, rather than intra-population dietary changes.

EXPERIMENTAL

ETHNIC GROUPS, SUBJECTS, AND SAMPLES

The subjects sampled for this study come from six communities and five ethnic groups with different traditional diets and living in different environmental settings across tropical Africa (Table 1, and Section S1.1, Supporting Information). These groups were chosen because each maintains relatively traditional subsistence strategies that, combined, represent a high dietary diversity – agriculturalists, pastoralists, hunter gatherers, and fishers. The communities studied include: (1) the El Molo fishers from the south-eastern margin of Lake Turkana, Kenya; (2) the Turkana pastoralists from the Kerio Valley, Turkana County, Kenya; (3) the Luhya agriculturalists from Webuye, Kenya; (4) the Luhya fishers from Lake Victoria, Kenya; (5) the Luo fishers from the northern margin of Lake Victoria, Kenya; and (6) the Baka hunter gatherers from the south-eastern rainforest of Cameroon.

Fieldwork took place between April and September 2015. Samples were collected in as non-invasive manner as possible. Hair and nail were collected to characterise the protein part of the diet, while breath was collected to characterise the overall diet [17,18,44–47].

The participants recruited were in general good health, and did not suffer from any of the diseases known to lead to isotopic fractionation (e.g. liver cirrhosis) [19]. Sampling aimed at a balanced female/male ratio of adult participants, but this was not always possible (Table S1, Supporting Information). Breath was sampled around 11 am each morning, or at least three hours after an individual's last meal in order to minimise any diurnal variation or post-prandial effects [45,48]. Plant and fish foods were also sampled in the field to provide baseline isotopic data against which to compare participants' data.

Fieldwork and Laboratory Methodology

The protocols used were based on standard methods of stable isotope ratio measurements for each type of sample for hair [46, 49], breath [50] and fish collagen [51]. Furthermore, two of the fish samples (*Dagaa* and *Ofulu)* were whole fish, and not just bone. Therefore, these samples were analysed twice: whole, and just bone (post-dissection).

Sample Collection

For hair collection, 20-30 hairs of at least 2 cm length were cut at the nape of the head [49]. Nail samples were collected by clipping the distal edge of a fingernail [46]. Breath was sampled by having the subject blow through a straw into a 12-mL evacuated Exetainer vial (Labco, High Wycombe, UK) [50]. For food samples, each item was placed in a 50-mL plastic container, with silica gel added to fish samples for better preservation.

Sample Preparation

Each hair sample was cleaned by two successive immersions in a 2:1 mixture of methanol and chloroform ($MeOH:CHCl_3$) for 30 minutes (min) each, followed by two 30 min rinses in distilled water. After being freeze-dried, 0.8 ± 0.1 mg of sample was weighed into tin capsules for analysis. Each nail sample was cleaned by sandblasting, followed by one 30 min rinse in distilled water, one 30 min immersion in 2:1 mixture of $MeOH:CHCl_3$, and another 30 min rinse in distilled water, with all three steps taking place in an ultra-sonic bath. The

samples were then freeze dried and 0.8 ± 0.1 mg aliquots were weighed into tin capsules. Breath samples required no preparation prior to analysis.

Extraction of collagen from fish bone was carried out in four steps: defatting; demineralisation; gelatinisation; and freeze-drying [51]. For defatting, fish bones were soaked in 200 mL of 2:1 MeOH:$CHCl_3$ for 30 min in an ultra-sonic bath, and then left standing overnight in the fume hood. If any fat was left, the samples were soaked in new solution, and the procedure was repeated. Once fat was removed, the samples were cleaned by two 30 min rinse in distilled water in an ultra-sonic bath. They were then placed in 8 mL of 0.5 M HCl at 4 °C for several days for demineralisation. During this period, the samples were shaken twice a day and acid was replaced every two to four days. Once demineralised, samples were rinsed three times in distilled water and gelatinised in acidic water (pH 3.0) at 75°C for 48 hours. The solution was then filtered into tubes using an Ezee filter (45 to 90 μm, Elkay products, Basingstoke, UK) and freeze-dried. Finally, 0.8 ± 0.1 mg of the resulting collagen was weighed into tin capsules for analysis.

The plant samples were dried, ground until homogeneous using a mortar and pestle, and then weighed into tin capsules for analysis.

ISOTOPIC ANALYSIS

The isotopic analyses of hair, nail, fish collagen, and plant samples were carried out at the Godwin Laboratory, Department of Earth Sciences, University of Cambridge (Cambridge, UK), using a Costech (Valencia, CA, USA) elemental analyser (EA) coupled in continuous-flow mode to a ThermoFinnigan (Bremen, Germany) Delta V isotope ratio mass spectrometer. Hair and nail samples were analysed in triplicate, with carbon and nitrogen isotopic ratios measured on each run. The carbon and nitrogen results are reported as δ values relative to international standards, VPDB and AIR respectively. Due to the high and variable C/N ratio of plant samples, their carbon isotopic ratios were measured on a first run using a sample mass of 1 ± 0.1 mg, then again using a mass calculated from the C/N ratio that was obtained in the first analysis to measure δ^{15}N values. Repeated measurements using international and in-house standards showed that the analytical error was less than 0.2‰ for both elements. The international isotopic standard used was caffeine (δ^{13}C: −27.5‰; δ^{15}N: +1.0‰, IAEA-600, IAEA, Vienna, Austria), whereas the in-house standards were nylon (size standard, Nylon 6, Sigma-Aldrich, Gillingham, UK), alanine (δ^{13}C: −26.9‰; δ^{15}N: −1.4‰,

L-alanine, Honeywell Fluka, Bucharest, Romania), protein 2 (δ^{13}C: −27.0‰; δ^{15}N: +6.0‰, Protein standard OAS, Elemental Microanalysis, Okehampton, UK) and again caffeine (δ^{13}C: −35.9‰; δ^{15}N: −2.6‰, Elemental Microanalysis).

Isotopic analyses of breath were carried out at Iso-Analytical Limited (Crewe, UK) using gas chromatography (GC) coupled in continuous flow with a Europa Scientific (Crewe. UK) Hydra 20-20 mass spectrometer. These analyses were measured relative to a reference gas (δ^{13}C: −34.56‰, IA-R060, traceable to NBS-19, which is distributed by the IAEA diluted to 3.3% CO_2, and were based on replicate analysis of in-house standards. The analytical errors were less than 0.2‰ for carbon.

Statistical Analysis

Statistical analyses and figure drawing were performed on the R platform (version 3.3.1) [52]. The normality of the data was checked graphically using histograms or quantile-quantile plots, and formally using Shapiro-Wilk tests, while the equality of variances was tested using Levene's test. Inferential tests were adapted to the specific statistical question but, as a rule, when the assumptions of the test were not met, robust versions of the tests were used. Whenever possible, we report the effect sizes of the tests [53(p57)].

Ethics

Fieldwork data collection in Kenya was carried out under the IN-AFRICA Project permit granted to Prof. Marta Mirazón Lahr by the Government of the Republic of Kenya (NACOSTI/P/15/2669/4758), and an exploration and excavation licence from the National Museums of Kenya that specifically includes the present work. During fieldwork, all Kenyan participants signed a consent form or, if illiterate, had the consent form read out to them in their language, and a thumb print was taken instead. Fieldwork in Cameroon was approved by the Centre National de la Recherche Scientifique (CNRS), Agence National de la Recherche (ANR) and Institut de Recherche et Développement (IRD) and was carried out as part of the international agreement between the IRD and the Ministry of Scientific Research and Technology of Cameroon. Consent from participants in Cameroon was obtained orally.

RESULTS

ASSESSMENT OF QUALITY OF RESULTS

A total of 134 hair samples and 82 nail samples produced reliable results on the basis of a C/N ratio in the range of 3.0 to 3.8 [46,49] and low replicate measurement errors (<0.3‰ for $\delta^{13}C$ values and <0.4‰ for $\delta^{15}N$ values). Similarly, a total of 184 breath samples was considered reliable on the basis of CO_2 concentrations greater than 3%. Assessment of the quality of fish (collagen) samples was based on a C/N ratio in the range of 2.9 to 3.6 [54] and the same replicate measurement errors as those of hair and nail (Section S2.1, Supporting Information).

COMPARISON WITHIN AND BETWEEN POPULATIONS

Variation in the isotopic ratios of all body pools within populations was explored in terms of the potential effects of sex and age. No statistically significant associations with age and sex were observed, nor with pregnancy or breastfeeding (Section S2.2 and Tables S4-6, Supporting Information).

Variation in the isotopic ratios of carbon and nitrogen between the sampled populations was explored separately in each of the sample body pools. The statistical results obtained for between-group variation in $\delta^{13}C$ and $\delta^{15}N$ for each body pool are presented below. Figure 1 and Table 2 illustrate and summarise the human isotopic results for all body pools.

HAIR DATA

Robust analysis of variance (rANOVA) found a significant effect of population on both hair $\delta^{13}C$, $F_t = 481.5$, $p < 0.001$, $\xi = 0.95$, and hair $\delta^{15}N$, $F_t = 42.8$, $p < 0.001$, $\xi = 0.79$ (Section S2.2, Supporting Information). Based on the subsequent *post hoc* tests (Table 3(A)), the hair isotopic results show that the Baka hunter gatherers have the lowest carbon and the highest nitrogen isotopic ratios, and are thus, the most distinct group. Among the Kenyan populations, the Webuye Luhya agriculturalists have the highest carbon isotopic ratio, while the remaining four groups overlap considerably in both carbon and nitrogen isotopic ratio. Among these latter four groups, the Turkana (Kerio) pastoralists have the lowest carbon

isotopic ratio and the widest nitrogen isotopic range. The three remaining groups (the Luo, Lake Victoria Luhya and the El Molo) are all fishers and expected to share a dietary isotopic signature, although they do not present the expected ^{15}N enrichment associated with a high trophic position [17]. Nevertheless, the hair isotopic results of the El Molo are closer to those of the Turkana (Kerio) than to the two fishing groups from Port Victoria.

NAIL DATA

A standard ANOVA found a significant effect of population on nail $\delta^{13}C$ values, $F(4, 77) = 38.5$, $p < 0.001$, $\omega^2 = 0.65$, as did the robust ANOVA on nail $\delta^{15}N$ values, $F_t = 7.48$, $p < 0.01$, $\xi = 0.50$ (Section S2.2, Supporting Information). Based on subsequent *post hoc* tests (Table 3(B)), the nail carbon isotopic results show a similar pattern to those of hair data, where the Turkana (Kerio) and the El Molo are more depleted in ^{13}C, followed by the Luo and Luhya from Port Victoria (although these two groups are only significantly different from the Turkana, and not from the El Molo), and finally by the Luhya (Webuye), more enriched in ^{13}C. The *post hoc* analyses of nail found fewer significant comparisons than those of hair, possibly because of the lower sample size available for nail statistical tests. Contrary to the carbon isotopic results, the nitrogen isotopic pattern of nail differs from that of hair. The Luhya (Webuye) are more depleted in ^{15}N than all other groups, who have similar values. Therefore, while the hair nitrogen isotope ratios fail to discriminate the Luhya (Webuye) from both the El Molo and the Turkana (Kerio), the $\delta^{15}N$ values on nail do. This discrepancy in hair and nail $\delta^{15}N$ values could indicate a metabolic difference between the two tissues [46], as discussed in the section Comparison between Body Pools.

BREATH DATA

Before examining inter-population differences in breath $\delta^{13}C$ values, an analysis of covariance (ANCOVA) with time of collection as covariate was performed to test for potential residual effects of diurnal variation. The results show that time of collection was not significantly related to breath $\delta^{13}C$ values, $F(1, 176) = 0.46$, $p = 0.5$, partial $\omega^2 = 0.00$, suggesting that the collection standard (sampling around 11 am) was kept to an adequate level. Hence, a standard ANOVA was applied instead, which found a significant effect of population on breath $\delta^{13}C$ values, $F(5, 178) = 71.5$, $p < 0.001$, $\omega^2 = 0.66$.

Based on the *post hoc* tests that followed (Table 3(C)), the breath carbon isotopic results find three groups: one significantly depleted in ^{13}C represented by the Baka; a second group, also depleted in ^{13}C but to a lesser extent than the Baka, which includes the three fishing populations (El Molo, the Luo, and the Luhya from Port Victoria) and the Turkana (Kerio); and a group comparatively enriched in ^{13}C, the Luhya farmers from Webuye.

The breath $\delta^{13}C$ values varies more within groups than either the nail or hair $\delta^{13}C$ values (Table 2), so that fewer statistically significant differences among groups were observed, and the differences found overlapped with observations already made for $\delta^{13}C$ values in hair and nail. The body isotope pools act as buffers against the effects of short-term fluctuations in diet on the $\delta^{13}C$ values of hair and nail. These buffering mechanisms do not include breath, which therefore, shows a faster isotopic response to dietary changes that results in greater variation in breath $\delta^{13}C$ values among individuals [56,57].

FOOD DATA

Although this work includes a relatively small portion of the range of plants and animals consumed as food by the groups being studied, a few compelling patterns are observed in their isotopic data (Figure 2, Table 4).

Carbon isotopic ratios clearly distinguish between the three food categories: C_3 foods, C_4 foods, and fish. For plants, these isotopic ratios fall within the expected ranges [25], while the ratios of the fish samples that we tested span the entire isotopic range between the C_3 and C_4 foods analysed, probably tracing the plant diet at the base of the food chain [18,58].

The plant $\delta^{15}N$ values varied more than is suggested for either leguminous or non-leguminous plants in the literature [17,59]. This finding is understandable since many factors affect the $\delta^{15}N$ values of plants [60]. In particular, high nitrification rates in soil, within a wet agriculture context [60,61], use of animal-derived fertilisers[21,35,62] and low precipitation and high temperatures[20,21,35] could explain the high $\delta^{15}N$ values observed. In addition, although the overall dispersion of nitrogen isotopic ratios observed in fish is smaller than that of plants, we observe two clusters among fish analysed, one around +5‰ and one around +10‰, which may correspond to a trophic level difference (Section S2.2, Supporting Information). No clear trophic shift is detectable between the fish foods and the plants analysed. Different and not mutually exclusive effects may contribute to this: it could be that the analysed plants are enriched in ^{15}N, as discussed before, that the food chains in these lakes are short, and that the base of the food chain in the two lakes (Turkana and Victoria) is depleted in ^{15}N [63].

Finally, we also observe that food samples from Webuye have relatively low nitrogen isotopic ratios. Given the very small sample size, this finding is tentative; nevertheless, it may explain the lower nitrogen isotopic ratios of the Luhya (Webuye).

Comparison between Body Pools

The present work also considered how the different body pools relate to each other isotopically (Section S2.3, Supporting Information). Here, we must consider the different time scale of growth and sampling for each body pool: 1 cm of hair represents one month's growth[64], 1 cm of nail represents three months' growth[65], and breath will signal a diet change after only three hours, simply associated with digestion time[56], whereas hair sampling is much closer to the time of formation than nail sampling. Therefore, it is possible that a changing diet associated with seasonality could explain the differences between the sampled body pools. However, none of the participants reported substantial changes in diet in the months prior to sampling, suggesting other factors may play a role. In fact, seasonality in this

area is expressed in terms of variation in rainfall, which, for these populations, mostly affects the amount, rather than the type, of food consumed.

We found that hair is slightly enriched in ^{13}C relative to nail, but this enrichment is negligible, as previously suggested [46]. This slight enrichment may be explained by differences in the amino acid composition of the two tissues – hair is richer in cysteine and serine than nail [46] – combined with the enrichment in ^{13}C observed in cysteine and serine [66].

We found that hair is depleted in ^{15}N relative to nail, which is also consistent with previous studies [35,46]. However, we believe that this relationship cannot be modelled with a simple fixed offset [46]. It is possible that the intracellular turnover of amino acids differs between the hair follicle and the nail matrix [46]. This explanation is untested but it is supported by the stronger effect of metabolism on the nitrogen isotope composition (through the de- and transamination of amino acids) [29,49,67], and by the lack of constant offset between the $\delta^{15}N$ values of different tissues and their corresponding pools of free amino acids [68]. Finally, we found that it was not possible to model accurately the isotopic relationship between breath $\delta^{13}C$ values and hair or nail $\delta^{13}C$ values, because the breath $\delta^{13}C$ value is extremely variable due to the rapid response of this body pool to dietary intake, and because hair and nail record diet differently from breath – hair and nail record the protein part of the diet preferentially, while breath records the overall diet. Thus, this relationship will depend on the type of protein source that the populations consume (e.g. C_3/C_4-based or marine) [69,70]. Indeed, the best fitting model was obtained when considering each diet separately. When dealing with mixed diets and complex systems, a simple model fails to explain the relationship between the isotopic ratios of different tissues.

DISCUSSION

The results of this study show that stable isotope analyses distinguish between traditional diets in tropical Africa, although not always in the way expected. Our results can be grouped into those that reveal isotopic patterns that are particular to the tropical African environment, those that uncover recent changes to traditional diets, and (to a lesser extent) those that hint at the complex innerworkings of the human isotope metabolism. These results will be discussed separately, and in order to frame them in the wider context of isotopic research in modern diets, the discussion compares our findings with other isotopic studies on human hair and nail.

Our study found that the inclusion of freshwater fish in the diets of tropical African groups is difficult to detect through the analysis of nitrogen isotopic results, since we detected no signals of a clear trophic shift between fisher (El Molo, Luhya and Luo from Port Victoria) and non-fisher populations (Turkana from Kerio, Luhya from Webuye, and Baka). This observation is likely to result from the combined effects of high nitrogen isotopic ratios in local plants and the low nitrogen isotopic ratios in the lacustrine fish sampled. As discussed earlier, a wet agriculture context [60,61], use of animal-derived fertilisers[21,35,62], and low precipitation and high temperatures [20,21,35] could explain the high δ^{15}N values observed in plants, whereas the low δ^{15}N values of fish could result from short food chains in these lakes or from a ^{15}N-depleted baseline [63,71]. The last two explanations are not mutually exclusive, as there is evidence that food chains in Lake Victoria are short [72], but also that nitrogen-fixing cyanobacteria contribute to ^{15}N depletion at the baseline in both lakes [24,43,71,73–75]. It is worth noting that the nitrogen isotopic ratios of fish obtained in this study are close to those found by other isotopic studies on the aquatic fauna of these two lakes (Lake Turkana and Lake Victoria) [43,63,72]. As such, these findings suggest that lake fish consumption is hard to detect in an East African context, which could be relevant to the interpretation of archaeological isotopic events. However, one must consider that shortening of food webs and proliferation of cyanobacteria in Lake Turkana and Lake Victoria are at least partially the result of recent human activity through the introduction of invasive species (e.g. Nile Perch in Lake Victoria) and pollution [63,72,74].

The hunter-gatherer Baka have a very distinct isotopic signature characterised by low carbon and high nitrogen isotopic ratios. On the one hand, the forest environment where they live, dominated by C_3 plants, easily clarifies the first result, although the "canopy effect" might also contribute to the lowering of carbon isotopic ratios [76]. On the other hand, the high nitrogen isotopic ratios cannot be easily explained by high meat or fish consumption, or by any of the factors that may explain the high nitrogen isotopic ratios of plants in Kenya (wet agriculture context, use of animal-derived fertilisers, combined low precipitation and high temperatures), since none of these factors are present in the forest environment where this foraging population lives. Instead, the ^{15}N enrichment might result from the complex pathways of nitrogen isotopic fixation in tropical forests. This explanation is supported by

isotopic analyses of primate hair in West African forests, which also report relatively high δ^{15}N values[77–79].

Recent Changes to Traditional Diets

The agriculturalists Luhya in Webuye have the highest carbon isotopic ratios, which probably derives from a high consumption of C_4 plant foods. In fact, the carbon isotopic ratio of hair and nail among the Luhya (Webuye) is very close to those obtained for C_4 plant foods in that region, suggesting that the protein component of this group's diet is entirely based on C_4 plants. Therefore, the lower nitrogen isotopic ratio of this group might arise from ^{15}N-depleted staples (Figure 2), and not from a lower consumption of animal and fish foods. It is also relevant that the Luhya from Webuye are known to have traditionally supplemented their diet with termites [80]. However, almost all participants in this study declared they did not eat termites or consumed them in negligible quantities. Furthermore, previous studies [81,82] did not find that insects had particularly high nitrogen isotopic ratios.

The Turkana were chosen as a representative of traditional pastoralists of Kenya. However, the isotopic analyses indicate that the particular community of Turkana from the Kerio Valley has replaced animal foods with C_4 plant foods, probably due to an ongoing sedentarisation process [83,84]. Notwithstanding this change, this was the population with the wider variability in nitrogen isotopic ratios, which indicates that individuals with very different diets might be present within the group. The effects of recent changes in diet are also visible to some extent in the other Kenyan groups, most notably in the Luhya (Webuye). Although some reliance on C_4 plants is expected given the global distribution of such staples, the very high carbon isotopic ratios detected in this study indicate an over-reliance on these foods. This is not consistent with traditional diets – instead, it concurs with famine relief programmes in the case of the Turkana, which often supply maize as nutritional support to populations, and through the increasing participation in market economies in the case of the Luhya (Webuye) [85–89].

Isotopic Differences between Human Body Pools

Isotopic differences between sampled body pools may represent a changing diet recorded by the different time scales of growth and sampling for each body pool, with nail representing the longest time span and breath the shortest. However, we found no strong indication this is

the case in this study. Instead, this study confirmed that hair is slightly ^{13}C-enriched relative to nail, possibly due to disparate amino acid compositions, but that this difference is not significant[35,46]. We also found that a simple offset did not accurately model the relationship of the nitrogen isotopic ratios of hair and nail, which can be explained by differences in the intracellular turnover of amino acids. Similarly, it was not possible to model accurately the isotopic relationship between breath δ^{13}C values and hair or nail δ^{13}C values, possibly due to the high variability in breath δ^{13}C values, which itself is a result of the rapid response of this body pool to dietary intake, but also to the complex relationship between these body pools in the context of mixed diets.

COMPARATIVE FRAMEWORK

When comparing these results with 16 previous isotopic studies on contemporary human hair and nail, totalling over 2000 individual hair and nail samples (Section S3, Supporting Information), we find that both the highest and the lowest carbon isotopic ratios recorded were obtained by the present study – the Luhya (Webuye) and the Baka, respectively. Regarding nitrogen isotopic information, our results are consistent with previous studies that have shown that samples of African origin have higher nitrogen isotopic ratios than European ones, although they originate from populations with a relatively lower consumption of meat and fish. This outcome has been explained by the ^{15}N enrichment observed in hot and arid environments [35]. However, this relationship between rainfall/humidity and δ^{15}N values has been shown to derive from denitrification processes in the soil that directly affect plants [20,90], rather than through physiological responses at the consumer level, as previously suggested [91–94]. Our results are consistent with the plant denitrification hypothesis – variation in the δ^{15}N values of the human populations roughly tracks the values of plant staples, i.e. most plant staples are ^{15}N-enriched as are most of the populations, while the staples from Webuye have lower δ^{15}N values, just like the corresponding human group.

Two earlier isotopic studies included one of the populations sampled in our work, so that we can directly compare the results. The first is that of Kiura[43], who compared multiple aspects of the diet of three populations of northern Kenya who live along the eastern shores of Lake Turkana – two pastoralist groups (Dassanech and Gabra), and the El Molo fishers, who were also included in the present study. Kiura determined the carbon and nitrogen isotopic ratios of eight human hair samples, as well as of the staples consumed directly and indirectly. She reported higher δ^{13}C values (−14.3 ± 0.7 ‰) and lower δ^{15}N values (+8.0 ± 0.6 ‰) in the El

Molo than the present work (δ^{13}C: −16.8 ± 1 ‰; δ^{15}N: +9.0 ± 0.8 ‰) [43(pp312-313)]. Several factors may contribute to the differences between the two studies, including sample size and constitution, and short-term differences in diet. The latter may reflect the fact that there was a severe drought in the area during 1999-2001 followed by erratic rains in 2002, which elicited emergency food aid rich in C_4 foods [95,96], and that the population has since reduced its consumption of C_4 foods and increased its consumption of animal foods. In addition, Kiura found that the agropastoralists Dassanech had higher nitrogen isotopic ratios than the Gabra pastoralists, who in turn had higher nitrogen isotopic ratios than those of the Turkana people in the present study. Kiura attributes the higher Dassanech nitrogen isotopic ratios to the combined consumption of animal and plant products with high δ^{15}N vakues (e.g. sorghum) [43(p381)]. This interpretation is consistent with the high nitrogen isotopic ratios obtained here for the plant samples, but also with the hypothesis that the low nitrogen isotopic ratios of the Turkana communities of the Kerio Valley included in this study reflect a sedentarisation process and decreased consumption of animal products.

The second study is that of Ambrose and DeNiro[39], who studied the isotopic ratios from the bone collagen of 97 archaeological humans from South and East Africa. Relevant to the present work, they found that 7 bone samples from historic Turkana and Dassanech (combined) had lower δ^{13}C values (−14.6 ± 2.9 ‰) and higher δ^{15}N values (+13.8 ± 1.3 ‰) than those reported here for either hair and nail samples among 27 contemporary Turkana [39]. This further endorses the view that there has been a significant recent change in the diet of the Turkana communities living along the Kerio Valley; the differences may also partly reflect the fact that the data for the two populations (Turkana and Dassanech) in this study was combined, as suggested by the comparatively much larger standard deviations of the reported mean values.

The changes in diet detected in these two studies highlight the variation observed not only horizontally within a population, but also vertically through time, which emphasizes the ability of individuals to adjust to new conditions and environments. This is consistent with the differences we observed between the declared diets of the individuals who participated in our study and those traditionally attributed to their communities on a historical basis. For example, not only do different Luhya communities focus on different staple diets in Webuye and Port Victoria, but many individuals from the Webuye community can no longer be described as traditional farmers since they take full part in the regional market economy. Similarly, while traditional nomadic pastoralism was the main subsistence strategy amongst

most Turkana communities historically, most groups today, including those from the Kerio Valley who participated in our study, have become largely sedentary, increasingly depending on a maize-based diet[83,84,97].

Overall, this work provides novel data on the isotopic signatures of tropical African populations. It extends the known range of $\delta^{13}C$ values in human hair by providing evidence on the strongly C_3 plant-based diet of the hunter gatherer Baka, and on the strongly C_4 plant-based diet of the agriculturalist Luhya (Webuye). It also shows that, within an East African context, the consumption of lacustrine fish might be difficult to detect isotopically due to the high nitrogen isotopic ratios of plants and the low nitrogen isotopic ratios of fish. Furthermore, among the ethnic groups for whom dietary information is available at different points during the last 50 years – the El Molo, and the Turkana – we find evidence of relatively recent changes in diet. Taken together, these findings reinforce the view that the dietary diversity of different populations today remains poorly understood, and that stable isotopic analyses are a useful tool for tracking the dynamics of food transitions, such as those associated with urbanisation or with food aid interventions. Finally, this work corroborates the approach that in order to achieve a more complete picture of the isotopic signature of a targeted organism, we should include different types of body pools and all components of the food web in the analyses.

CONCLUSION

We found that the hunter gatherer Baka have low carbon and high nitrogen isotopic ratios, consistent with the tropical forest environment they inhabit. In contrast, the Luhya from Webuye and the Turkana have high carbon and relatively low nitrogen isotopic ratios, indicating they have diverged from a traditional agriculturalist and pastoralist diet, respectively. We also found that the consumption of fish from Lake Victoria and Lake Turkana does not lead to a trophic shift between fisher (El Molo, Luhya and Luo from Port Victoria) and non-fisher populations (Turkana from Kerio, Luhya from Webuye, and Baka). Finally, we confirmed that hair is very slightly ^{13}C-enriched relative to nail, but that the relationship between the $\delta^{15}N$ values of hair and nail is more complex, as is the one between breath $\delta^{13}C$ values and hair or nail $\delta^{13}C$ values. Thus, this study addresses a gap in our knowledge of the isotopic signatures of tropical African populations with well-known

traditional diets, which in some cases are rapidly changing through increasing sedentism and inclusion in market economies.

This study also highlights the need of more information at both population and individual levels. At the population level, it is necessary to collect more data on the isotopic variation of traditional diets across the world. As Western diets or even famine relief foods expand their reach, much of the diversity of human diet is being lost and, with it, the opportunity to understand the significance of such subsistence strategies in a past and present context. At the individual level, it is necessary to better understand the processes that determine isotopic ratios through controlled studies on the isotopic metabolism of the individual. We believe that such knowledge will enable a more effective use of isotopic analyses to track dietary changes, allowing us to better design dietary interventions and to preserve dietary diversity.

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Table 1. Summary of main characteristics of the ethnic groups studied: ecology, livelihood, and main staples of the traditional diet.

	El Molo	Turkana	Luhya	Luo	Baka
Field site	Layeni 02°49’36’’N, 36°41’50’’E Komote 02°51’19’’N, 36°41’33’’E	Nakurio 02°52’23’’N, 36°08’30’’E Lotukomo 02°46’57’’N, 36°12’17’’E	Webuye 0°35’57’’N, 34°46’47’’E Port Victoria 0°5’47’’N, 33°58’42’’E	Port Victoria 0°5’47’’N, 33°58’42’’E	Moangé-Le-Bosquet 3°4’34’’N, 13°31’47’’E
Population size	~ 600	~ 900 000	>5 000 000	>4 000 000	>30 000
Ecology	semi-arid	semi-arid	sub-humid	sub-humid	humid
Temperature	28°C	29°C	20°C	22°C	23°C
Rainfall	<200 mm	~200 mm	>1600 mm	700-1300 mm	>1600 mm
Traditional livelihood	fishers	pastoralists	agriculturalists	fishers and agriculturalists	hunter gatherers
Main staples described in the literature	lake fish, goat, chicken and other lake birds; maize, rice, beans, tea, sugar[88]	milk and blood, meat on special occasions; sorghum, maize, wild plants (e.g. doum palm fruit)[98]	chicken, sheep, goats, beef, fish if close to water bodies; maize, beans, finger millet, sweet potatoes, bananas [85,86,99]	fish, beef, chicken, sheep, and goats; maize, sorghum, and beans, but also cassava and sweet potatoes[87,99]	small fish, wild game, and insects; wild yam, mushrooms, crops like cassava, plantain, taro, and okra[100,101]

Table 2. Summary measures of carbon and nitrogen isotopic results, by body pool and by population.

	Hair			Nail			Breath	
Population	*N*	$\delta^{13}C$/‰	$\delta^{15}N$/‰	*N*	$\delta^{13}C$/‰	$\delta^{15}N$/‰	*N*	$\delta^{13}C$/‰
El Molo	27	-16.8 (1.0)	+9.0 (0.9)	9	-16.6 (0.5)	+10.2 (0.9)	29	-19.8 (1.9)
Turkana	21	-17.5 (1.0)	+8.9 (1.3)	20	-17.5 (1.1)	**+10.4 (1.1)**	32	-18.9 (2.5)
Luhya (Webuye)	17	-13.6 (1.0)	+8.5 (0.5)	15	-13.1 (1.2)	+9.5 (0.5)	32	-15.5 (2.2)
Luhya (Port Victoria)	18	-16.1 (1.8)	+9.7 (0.8)	17	-15.7 (0.9)	+10.6 (0.9)	30	-19.1 (2.5)
Luo (Port Victoria)	18	-15.6 (1.2)	+9.7 (0.6)	21	-15.8 (1.2)	+10.3 (0.4)	29	-19.3 (1.8)
Baka	33	**-23.1 (0.6)**	11.4 (0.9)	—	—	—	32	-25.5 (1.6)

Note. Values are mean (standard deviation). Robust measures of central tendency and dispersion are found in Table S2 (Supporting Information). Values in **bold** indicate a sampling distribution that deviates significantly from normality (Shapiro Wilk test, $p < 0.05$, Table S3, Supporting Information).

Table 3. *Post hoc* results for all body pools: (A) Hair: robust *post hoc* results for both δ^{13}C and δ^{15}N values (with 20% trimmed means, 5000 bootstrap samples, and an FDR correction); (B) Nail: standard *post hoc* tests for δ^{13}C values and robust for δ^{15}N values; (C) Breath: standard *post hoc* tests for δ^{13}C values. The cells above the diagonal represent the pairwise comparisons between groups for hair δ^{15}N, whereas the cells below the diagonal represent the pairwise comparisons between groups for hair δ^{13}C values. Values in each cell are the standard (r) or robust effect size (ξ) for each pairwise comparison. Shaded cells are significant comparisons ($p < 0.05$).

(A)		El Molo	Turkana	Luhya (Webuye)	Luhya (Port Vict.)	Luo (Port Vict.)	Baka
Hair δ^{13}C	El Molo	—	0.2	*0.4*	**0.5**	**0.6**	**0.9**
	Turkana	**0.5**	—	0.1	**0.5**	**0.6**	**0.9**
	Luhya (Webuye)	**1.0**	**1.0**	—	**0.9**	**0.9**	**1.0**
	Luhya (Port Vict.)	*0.4*	**0.6**	**0.8**	—	0.1	**0.9**
	Luo (Port Vict.)	**0.7**	**0.9**	**0.9**	0.3	—	**0.9**
	Baka	**0.9**	**0.9**	**0.9**	**1.0**	**1.0**	—
(B)		—	0.2	**0.7**	*0.4*	0.1	N/A
Nail δ^{13}C	El Molo	*0.4*	—	**0.7**	0.1	*0.4*	N/A
	Luhya (Webuye)	**0.9**	**0.9**	—	**0.9**	**0.9**	N/A
	Luhya (Port Vict.)	**0.5**	**0.7**	**0.8**	—	0.4	N/A
	Luo (Port Vict.)	*0.3*	**0.6**	**0.7**	0.1	—	N/A
(C)	El Molo	—					
Breath δ^{13}C	Turkana	0.2	—				
	Luhya (Webuye)	**0.7**	**0.6**				
	Luhya (Port Vict.)	0.2	0.0	**0.6**	—		
	Luo (Port Vict.)	0.2	0.1	**0.7**	0.1	—	

Baka	**0.8**	**0.8**	**0.9**	**0.8**	**0.9**	—

Note. Interpretation of *r*: small = 0.10, *medium* = 0.30, and **large** = 0.50 [53(p58)];
Interpretation of ξ: small = 0.15, *medium* = 0.35, and **large** = 0.50 [102(p169)].

Table 4. Carbon and nitrogen isotope ratios for individual food samples.

Location (Ethnic Group)	Sample	δ^{13}C/‰	*n*	δ^{15}N/‰	*n*
Layeni (El Molo)	Sorghum (*Sorghum bicolor*)	−12.1	2	+3.6	2
	Maize (*Zea mays*)	−11.3 (0.1)	3	+4.8 (0.1)	3
	Tilapia (*Oreochromis niloticus*)	−13.1 (0.0)	3	+3.8 (0.0)	3
	Golefish (*Hydrocynus forskahlii*)	−16.4 (0.0)	3	+9.3 (0.1)	3
	Flatfish (*Barbus turkanae*)	−24.0 (0.1)	3	+4.9 (0.1)	3
	Catfish (*Bagrus sp.*)	−21.9 (0.1)	3	+9.7 (0.1)	3
	Mudfish (*Clarias sp.*)	−12.9 (0.0)	3	+3.9 (0.1)	3
Nakurio (Turkana)	Doum palm fruit (*Hyphaene ventricosa*)	−25.9 (0.2)	3	+4.0	2
	Sorghum (*Sorghum bicolor*)	−12.1 (0.8)	3	+7.4	2
Webuye (Luhya)	Beans (*Phaseolus vulgaris*)	−30.5 (0.1)	3	+5.2	2
	Cow peas (*Vigna unguiculata*)	−27.6 (0.3)	3	+2.4	2
	Maize (*Zea mays*)	−12.0 (0.4)	3	+1.1	2
Port Victoria (Luhya and Luo)	Soya beans (*Glycine max*)	−28.8 (0.3)	3	+13.2	2
	Beans (*Phaseolus vulgaris*)	−26.6	2	+7.2 (1.5)	3
	Cassava (*Manihot esculenta*)	−24.1 (0.1)	3	—	—
	Rice (*Oryza sativa*)	−28.5 (0.1)	3	+15.7	2
	Cow peas (*Vigna unguiculata*)	−27.5 (0.3)	3	+10.2	2
	Green peas (*Pisum sativum*)	−26.5 (0.2)	3	+2.9 (1.0)	3
	Sorghum (*Sorghum bicolor*)	−12.2	2	+9.1 (1.2)	3
	Finger millet (*Eleusine coracana*)	−13.0 (0.1)	3	+7.7	2
	Yellow maize (*Zea mays*)	−11.9 (0.3)	3	+5.9	2
	Dagaa (*Rastrineobola argentea*)	−18.4	2	+9.4	2
	Dagaa (whole)	−19.4 (0.1)	3	+11.0 (0.1)	3
	Ofulu (*Haplochromis sp.*)	−17.7 (0.1)	3	+9.1 (0.1)	3
	Ofulu (whole)	−19.6 (0.0)	3	+9.8 (0.1)	3
	Nile perch (*Lates nilotius*)	−15.9 (0.1)	3	+8.8 (0.0)	3
	Helicopter fish (*Protopterus sp.*)	−18.2 (0.1)	3	+7.0 (0.0)	3
	Esebu (*Clarias sp.*)	−20.9 (0.0)	3	+5.6 (0.1)	3
	Tilapia (*Oreochromis niloticus*)	−16.1 (0.1)	3	+4.9 (0.1)	3

Notes. Values are mean (standard deviation) *n* = number of replicates.

[a] Isotopic analysis to determine the δ^{15}N values of cassava failed consistently, possibly as a result of its low nitrogen content. Consequently, this datum point is not plotted in Figure 2.

Figure 1
top

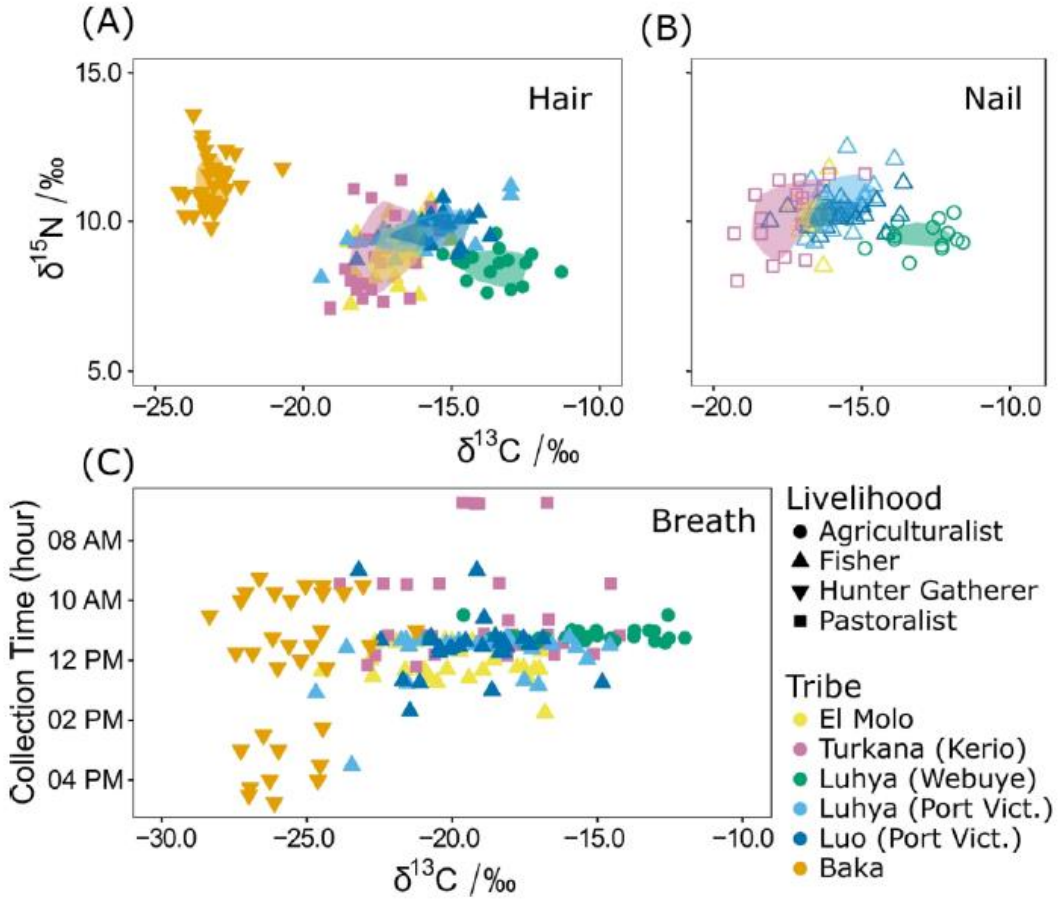

Figure 1. Stable isotopic results of the body pools analysed, by livelihood and population: (A) carbon and nitrogen isotopic ratios of hair; (B) carbon and nitrogen isotopic ratios of nail (shaded areas in the hair and nail plots represent the 'bag' that encloses 50% of the points around the depth median in a bagplot or bivariate boxplot[55]); (C) carbon isotopic ratios of breath according to time of collection (in hours).

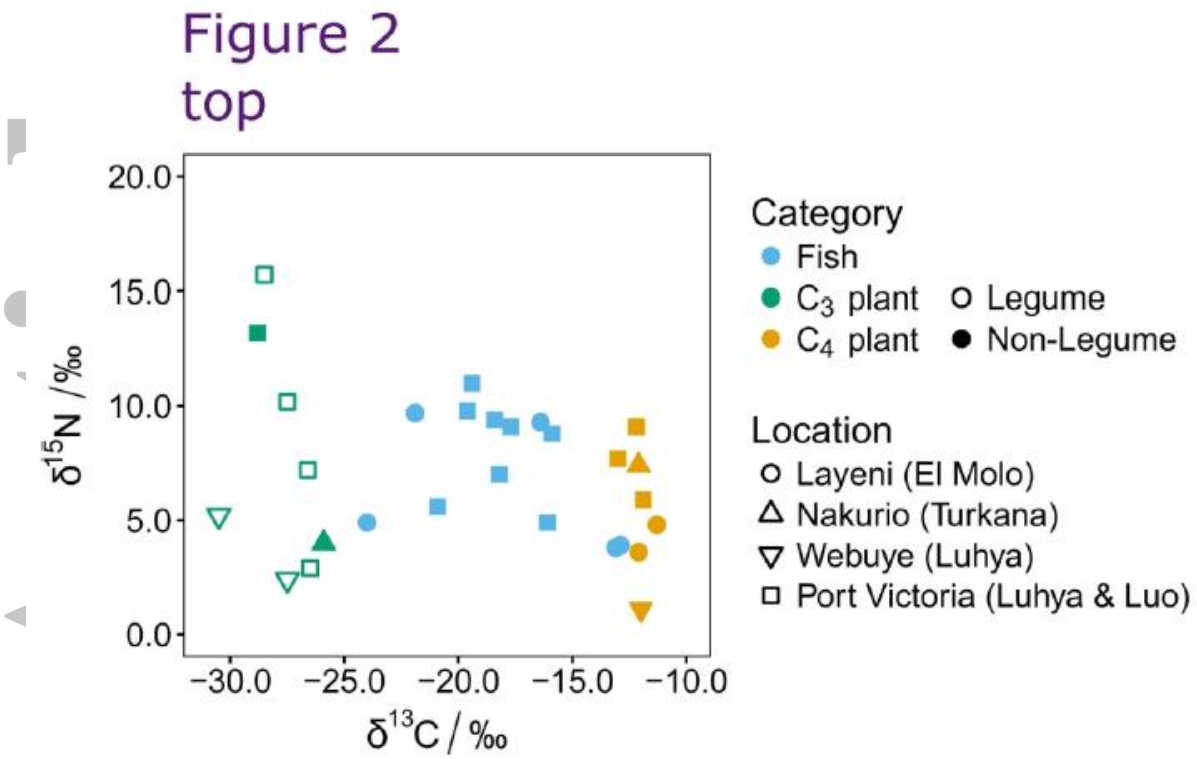

Figure 2. Carbon and nitrogen isotope ratios for individual food samples.