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# High-resolution palaeodietary reconstruction: Amino acid $\delta^{13}C$ analysis of keratin from single hairs of mummified human individuals

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Abstract

Stable isotope analysis of mummified human hair allows the reconstruction of the recent life histories of individuals that died thousands of years ago. The objective of this study is to improve the resolution of hair palaeodietary reconstruction by sequentially analyzing 0.5 cm segments of a single hair using liquid chromatography-isotope ratio mass spectrometry (LC/IRMS).

The subsistence strategies of seven individuals, spanning different cultures/periods (Chinchorro, Formative, Inca) and covering different geographic areas (coasts and hinterland) of the Atacama Desert, northern Chile, were reconstructed by analyzing $\delta^{13}C$ amino acid compositions using an improved methodology that requires only 0.5 cm segments of a single hair. The amino acid $\delta^{13}C$ values were supplemented with bulk carbon, nitrogen and sulfur isotope analysis performed on multiple hairs.

Our results show that the archaic hunter-gatherers strongly relied on aquatic resources, as did the first transitional Formative individuals living on the coasts. Conversely, the Formative inhabitants of the nearby valley exhibit consumption of terrestrial resources in a seasonal pattern. A broader dietary spectrum made of mixed terrestrial and aquatic foods is identified in the Inca individual.

The sequential analysis of 0.5 cm increments from a single hair has permitted the high-resolution reconstruction (approximately fortnightly) of the recent life history of these pre-Columbian individuals, discerning short-term nutritional changes related to agricultural cycle, multiple dietary intakes or mobility. Although bulk methods can detect changes in diet and track seasonal shifts, the variations in the carbon isotope signal can be highly attenuated with respect to the dietary intake because of the use of multiple hairs.

## 1. Introduction

Stable isotope analysis of bone collagen preserved in archaeological remains has long been a major source of bioarchaeological information, providing direct palaeodietary and palaeoenvironmental information (Ambrose and DeNiro, 1986; Drucker et al., 2012). However, while the persistence of bone collagen in the archaeological record for tens of thousands of years in temperate environments is an advantage, a consequence of its turnover during the lifetime of an individual is an indistinct dietary signal. When a collagen sample is measured it represents the ultimate years or decades of dietary intake (Hedges et al., 2007). Information pertaining to more relevant human timescales, such as how much an individual's diet varied over the course of their life or even how it may have changed through the course of a year, is unresolved. So, whilst analysis of bone collagen isotope compositions is an invaluable part of bioarchaeology, alternative stable isotope approaches are constantly sought to add detail to dietary reconstructions of ancient individuals (Beaumont et al., 2013).

Mummified bodies are a powerful archaeological resource (Lynnerup, 2007) that enables detailed bioarchaeological investigations especially when stable isotope studies are included (Knudson and Stojanowski, 2008). Although a variety of tissues (skeletal and non-skeletal) can be examined in a mummified body, hair has been proposed as the ideal biomaterial for isotopic studies because hair is mainly constituted by protein (i.e. keratin) (Harkey, 1993) and it retains the unaltered isotope signature recorded incrementally as the tissue grows (Petzke et al., 2010); furthermore, sampling is minimally invasive. All these characteristics potentially allow the high-resolution reconstruction of the recent life history of ancient individuals, with respect to diet (and especially seasonal changes in diet), mobility and physio-pathological conditions.

Palaeodietary reconstructions conducted to date on South American mummy hair have mainly relied upon bulk stable isotope analysis, e.g. (Horn et al., 2009; Knudson et al., 2007; Webb et al., 2013; White et al., 2009; Williams and Katzenberg, 2012), requiring the use of 1 (Webb et al., 2013; Williams and Katzenberg, 2012) or 2 cm (Knudson et al., 2007; White et al., 2009) segments of multiple hairs. Detecting rapid dietary changes in the hair isotope signature is complex as the hair requires time to isotopically equilibrate to a new diet (O'Connell and Hedges, 1999), in part due to the buffering effect of the bodily endogenous amino acid pool (Jackson, 2007), meaning that the complete isotopic equilibrium between diet and scalp hair keratin after a dietary change may take up to ~ 4-12 months for carbon and nitrogen isotope compositions (McCullagh et al., 2005; O'Connell and Hedges, 1999), although the latter appears to have a faster response (Huelsemann et al., 2009; O'Connell and Hedges, 1999). This is further complicated by the complexity of the hair growth cycle (Stenn and Paus, 2001) and by the inter-individual variability of growth rates (Loussouarn et al., 2005), which means that any two hairs may be temporally out of phase. The consequent misalignment of multiple hairs used in a bundle for isotopic analysis will generate an 'averaged' isotope signal that may be isotopically attenuated with respect to the original dietary signal (Remien et al., 2014).

The present study describes an improved method that refines human palaeodietary reconstructions by using compound-specific carbon stable isotope analysis of hair keratin amino acids from 0.5 cm segments of a single hair. This approach presents two major advantages over the traditional bulk method: (1) the analysis of a single hair avoids the potential problem of non-contemporaneous hairs being analyzed in bulk samples (i.e. growth cycle error) (Williams et al., 2011); (2) the use of minimal sample amount enhances the temporal resolution at an approximate fortnightly scale [considering a growth rate of approx. 1cm/month (Krause and Foitzik, 2006)], which is favorable in detecting the onset of a new

diet or short-term nutritional changes with respect to seasonality, mobility, environmental stress or disease (although diet-tissue equilibration time remains a potential source of error). Moreover, the application of a compound-specific approach can help to discriminate different sources of the isotopic signal via comparison of $\delta^{13}C$ values of *essential* amino acids (EAAs) with *non-essential* AAs (NEAAs). Although these classifications are a matter of some debate (Wu, 2013), broadly speaking in healthy individuals, EAAs are amino acids whose carbon skeletons are not synthesizable by the human body and are routed from ingested proteins to body tissues, while NEAAs are produced through various metabolic pathways (i.e. *de novo* synthesis), in addition to direct routing. NEAAs in hair will therefore potentially contain carbon derived from the whole diet (proteins, lipids, carbohydrates), whereas EAAs will reflect only protein intake. Despite the potential of individual amino acid (AA) $\delta^{13}C$ analysis to provide detailed information about distinct dietary components useful in reconstructing metabolic characteristics and nutritional stress among ancient populations, there are few published studies that contain reference data on compound-specific carbon isotope analysis of archaeological human tissues (Choy et al., 2010; Corr et al., 2009; Corr et al., 2005; Fogel and Tuross, 2003; Honch et al., 2012; McCullagh et al., 2006; Richards et al., 2007), especially for hair keratin, e.g. (Raghavan et al., 2010).

The technique was applied to hair samples from seven mummies from archaeological sites located along the coasts and the nearby valleys of the Atacama Desert, northern Chile (Table 1 and Fig. 1) and supplemented with bulk carbon, nitrogen and sulfur isotope analysis performed on multiple hairs. The individuals were selected in order to cover a wide chronological spectrum, from the Late Archaic Period (~6,000-4,000 B.P.) to the Late Period (~500-420 B.P.), and different geographic areas of the coasts (Arica, Camarones) and coastal valley (Azapa) of northern Chile. The study of intra-individual variation provides detailed insights into the subsistence strategies employed by pre-Columbian individuals from the

perspective of the individual's life history, thus generating for the first time information on individual flexibility or persistence in dietary intake at a fortnightly scale from the analysis of a single hair.

Table 1. Summary of pre-Columbian individuals analyzed.

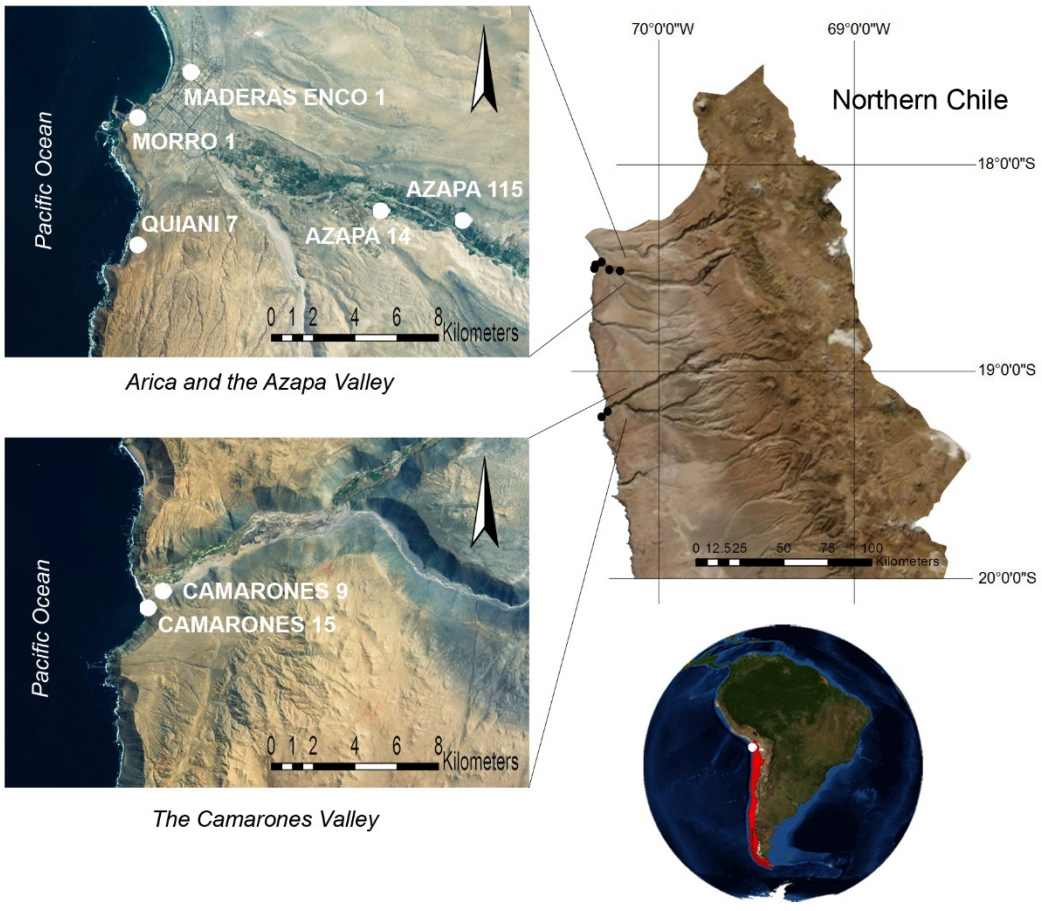


Fig. 1. Map of northern Chile showing the location of the archaeological sites outlined in this research (created using ArcGIS® software by Esri).

## 2. Environmental context

Although the Atacama Desert has been characterized by unique dryness for millennia (Houston, 2006), the early human communities of the coastal areas had access to an exceptionally rich marine ecosystem. The maritime resources were abundant and reliable (except during El Niño-Southern Oscillation events), and complemented by estuarine resources retrieved at the mouths of rivers (Grosjean et al., 2007) (wetlands). Perennial and ephemeral rivers, recharged by melted snow from the Andes, ran through the deep valleys creating other favorable habitats to human occupation (Nester et al., 2007). The existence of terrestrial food resources was (and still is) favored by upwelling groundwater, as well as by the humidity generated by the semi-permanent fog (Cereceda et al., 2008).

Environmental records show climatic and hydrological fluctuations, with a wetter phase coeval with the earliest colonization of the region (~ 13,000 cal yr B.P.) (Gayo et al., 2015; Latorre et al., 2006; Latorre et al., 2013; Sáez et al., 2016). The mid-Holocene (9,000-4,000 cal yr B.P.) was characterized by more arid conditions (even more arid than today), although, at a century-scale, intermittent wet and dry cycles have been documented (Grosjean et al., 2003; Núñez et al., 2013). The most disruptive climatic conditions were represented by the ENSO events, whose frequency increased after ~ 3,500 cal yr B.P. (Sandweiss et al., 2001); these events caused drought in the highlands, and decline in marine resources because of seawater warming (Williams et al., 2008).

## 3. Archaeological background

The early inhabitants of the Pacific Ocean's rocky shore were specialized fishers, as well as skilled in hunting marine game and gathering mollusks (Olguín et al., 2015; Rebolledo et al., 2016; Santoro et al., 2012). This existence of a marine-focused diet is supported by studies on material culture (toolkits and grave goods) (Standen, 2003), chronic pathology (Arriaza, 1995a; Standen et al., 1984), bone injury (Arriaza, 1995a; Costa-Junqueira et al., 2000; Standen et al., 1984), and dental health (Kelley et al., 1991). Terrestrial plants and animals were presumably an additional food intake, as suggested by archaeofaunal remains and rock art motifs (Standen, 2003; Valenzuela et al., 2015). Furthermore, it is thought that Chinchorros were involved in exchange networks with highland populations (Sepúlveda et al., 2013; Standen, 2003).

Following the Archaic Period (~ 10,000-4,000 B.P.), the dietary pattern moved to a mixed agro-maritime intake, consisting mainly of terrestrial foods (Valenzuela et al., 2015), as indicated by material culture (Muñoz Ovalle, 2004a; Rivera, 2008) and dental disease (Watson et al., 2010), although the persistence of a fishing tradition is recorded among the coastal groups (Muñoz Ovalle, 2011; Standen et al., 1997; Watson et al., 2013). During the Formative Period (~ 4,000-1,500 B.P.), the agro-pastoral experimentations led to successful domestication of cultigens and camelids (Mengoni Goñalons, 2008; Rivera, 2008; Stahl, 2008), associated with an increase in population and formation of villages (Rivera, 2008). Since the Formative Period, the role of camelids became crucial (Valenzuela et al., 2015), as a source of food, by-products (textiles, tools), and especially as a beast of burden in the large-scale system of exchange among the coast, valley and highland regions (Cases et al., 2008).

The Late Period (~ 500-420 B.P.) is characterized by the expansion of the Inca Empire to the lowlands, resulting in more wide-scale and deliberate control and distribution of the local

coastal resources (e.g. dried fish, salt, guano) (Muñoz Ovalle, 1989; Rivera, 2008). The advanced road system facilitated the mobility of people and goods (Covey, 2008). During the Late Period the dietary patterns continued to be a mixture of terrestrial and marine resources (Standen et al., 1997; Valenzuela et al., 2015).

## 4. Materials and methods

The scalp hairs analyzed in this study were taken from seven mummified individuals (Maderas Enco 1-C2, Morro 1-T28 C8, Camarones 15A-T14, Quiani 7-T13, Azapa 14-T31, Azapa 115-T9, Camarones 9-T39) that are part of the Archaeology Museum collection of the University of Tarapacá (UTA), Anthropology Department, Arica (Chile).

Methods are only outlined in brief here, further details can be found in Supporting Information 1.

### 4.1. LC/IRMS analysis

A single hair from each individual has been cut into 0.5 cm segments from root to tip and analyzed. The CAM15A-T14, QUI7-T13 and ME-C2 individuals have been sampled twice [(I), (II)]. The orientation and the distance from scalp of ME-C2 (I) and (II) hairs were unknown.

Single amino acid carbon isotope analysis was conducted at the La Trobe Institute for Molecular Sciences (LIMS, La Trobe University, Melbourne, Australia) using a Thermo Scientific LC/IRMS system consisting of an Accela 600 pump connected to a Delta V Plus Isotope Ratio Mass Spectrometer via a Thermo Scientific LC Isolink. Keratin amino acids

were separated and their carbon isotope content was measured using the three phase method described by Smith et al. (2009), but modified for a narrower column (Primesep A column, 2.1x250mm, 100Å, 5µm, SIELC Technologies Prospect Heights).

The quality of hair preservation at the molecular level was assessed by investigating the amino acid peak area values [Vs], which represent the sum of the peak areas for the ion currents at *m/z* 44, 45 and 46. The archaeological hair samples present good keratin preservation, with the exception of three segments (1.5cm, 4cm, 5cm) of CAM15A-T14 (I) hair.

### 4.2. Bulk isotope analysis

Two bundles of hair from each individual were sectioned into approximately 1 cm segments from root to tip for both carbon and nitrogen, and sulfur isotope analyses. When incremental sampling was not applicable (ME-C2, M1-T28-C8), the non-oriented samples were analyzed in duplicate. Analysis of the carbon, nitrogen and sulfur isotope compositions was performed at the University of Bradford (Department of Archaeological Sciences, UK) using a Thermo Flash EA 1112 coupled to a Delta Plus XL via a Conflo III interface (Thermo Scientific, Bremen, Germany).

#### 4.2.1. Stable isotope mixing model

A Bayesian mixing model has been applied to the bulk isotope data in order to better quantify the contribution of food sources to the diets of the individuals analyzed. In particular, we preferred FRUITS (Food Reconstruction Using Isotopic Transferred Signals) (Fernandes et al., 2014) as it incorporates concentration dependence and isotopic routing in the model.

## 5. Results

### 5.1. Bulk stable isotopes

A summary of the hair keratin stable isotope compositions ($\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$) for each individual is provided in Table 2.

Table 2. Carbon, nitrogen, and sulfur isotope compositions of 1 cm increments (starting from the scalp) of multiple hairs for the individuals analyzed.

The C/N atomic ratios for the majority of hair segments fall inside the empirical range of values for modern hair (2.9-3.8) (O'Connell and Hedges, 1999). A few samples present slightly elevated C/N ratios (3.9), though such results are commonly accepted for archaeological hair (Knudson et al., 2015; Webb et al., 2013). Three hair segments (from 5 to 9 cm) of the AZ115-T9 individual contained an insufficient amount of nitrogen and carbon, and were excluded from further discussion. Although the content by weight of sulfur (average 2.8±0.5%) is lower than in modern hair (Lehn et al., 2011; Nehlich, 2015), there is only a weak correlation ($\rho$=.529, n=36, p=.001) between $\delta^{34}S$ (‰) and S% (weight) which we interpret as implying that the sulfur isotope compositions are not biased by sulfur content. Although quality indicators for sulfur isotope analysis have been discussed for bone collagen (Privat et al., 2007), to date no empirical ranges have been proposed as an integrity standard for hair keratin (Nehlich, 2015). As a precaution, sulfur isotope data will be treated with caution, applying a high uncertainty to the $\delta^{34}S$ values, considering also the low reproducibility of sulfur measurements.

The bulk stable isotope compositions are similar to those published in previous studies of hair from populations of the same archaeological sites (Macko et al., 1999), or elsewhere in Chile (Aufderheide et al., 1994; Knudson et al., 2012; Silva Pinto et al., 2014) and Peru (Carmichael et al., 2014; Horn et al., 2009; Knudson et al., 2007; Knudson et al., 2015; Panzer et al., 2014; Webb et al., 2013; Webb et al., 2015; White et al., 2009; Williams and Katzenberg, 2012; Wilson et al., 2007) (Fig. 2). In particular, the individuals ME-C2, M1-T28-C8, CAM15A-T14, and QUI7-T13 cluster with other Chilean coastal individuals characterized by a marine-focused diet complemented by very limited terrestrial plant intake (Aufderheide et al., 1994; Knudson et al., 2012; Macko et al., 1999), while the coastal CAM9-T39 individual displays higher carbon isotope compositions. The AZ14-T31 isotope compositions are similar to the ones measured in a Formative individual from the same coastal valley previously interpreted as alternately relying mainly on $C_4$ or $C_3$ terrestrial resources (Silva Pinto et al., 2014). The AZ115-T9 isotope compositions are close, though being generally lower in $\delta^{15}N$ than those of south Peruvian individuals identified in previous studies as consuming mixed $C_3$-$C_4$ terrestrial foods, as well as marine resources (Knudson et al., 2007; Knudson et al., 2015).

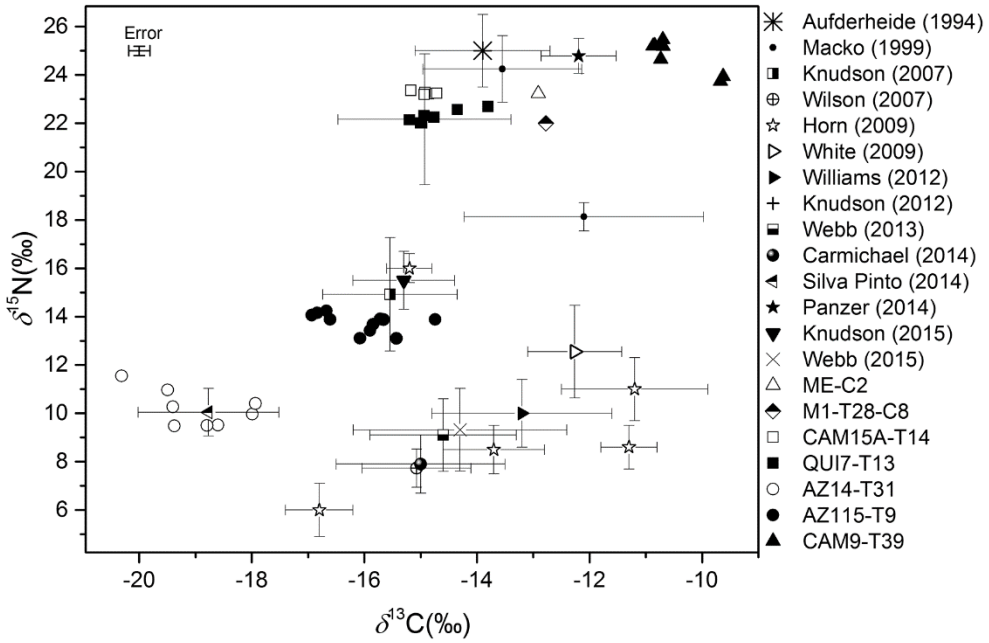


Fig. 2. Plot of $\delta^{15}$N values versus $\delta^{13}$C values for human hair analyzed in this study and in other studies on Chilean and Peruvian pre-Columbian populations.

The bi-plots of $\delta^{15}$N vs. $\delta^{13}$C and $\delta^{34}$S vs. $\delta^{13}$C values compare the isotope compositions of the hair keratins to the edible portion of the local flora and fauna (Figs. 3-4).

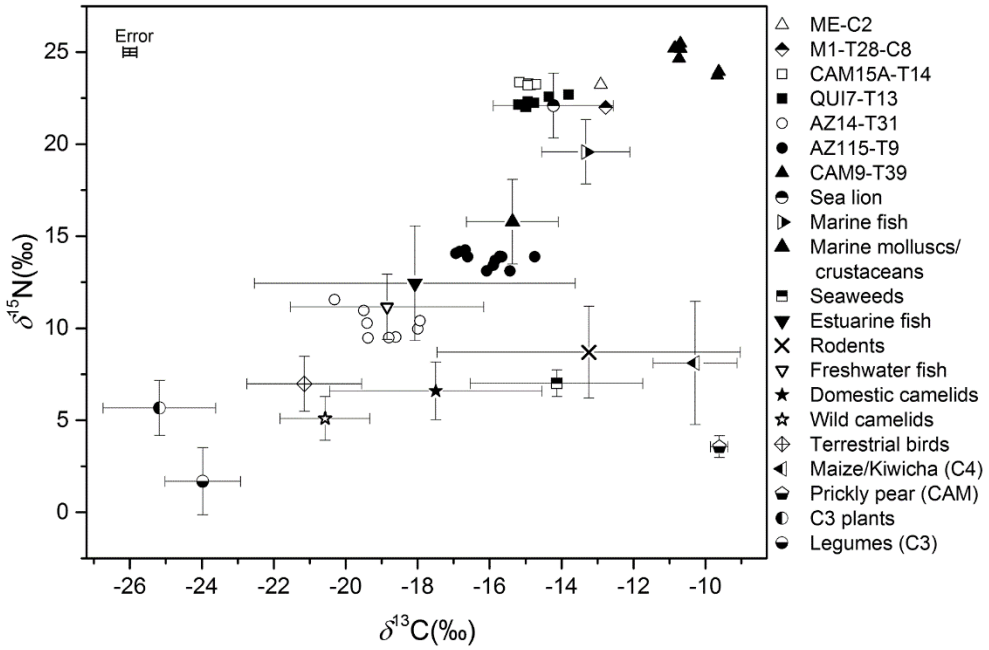


Fig. 3. Plot of $\delta^{15}$N values versus $\delta^{13}$C values for human hair analyzed in this study compared against edible portions of South American flora and fauna.

South American flora and fauna consist of: *Otaria flavescens* (Sea lion); *Trachurus murphyi*, *Genypterus chilensis*, *Cilus gilberti*, *Aplodactylus punctatus*, *Trachurus symmetricus*, *Merluccius gayi*, *Engraulis ringens, Coelorinchus chilensis*, *Clupea bentincki* as 'Marine fish'; *Tegula atra*, *Choromytilus chorus*, *Mesodesma donacium*, *Concholepas concholepas*, *Dosidicus gigas*, *Euphausia mucronata* as 'Marine molluscs and crustaceans'; *Ulva lactuca*, *Grateloupia doryphora*, *Cryptopleura cryptoneuron* as'Seaweeds'; *Mugil curema*, *Micropogonias furnieri*, *Mugil liza*, *Astyanax fasciatus*, *Lycengraulis grossidens* as 'Estuarine fish'; *Myocastor coypus*, *Phyllotis* sp., *Cavia porcellus* as 'Rodents'; *Crenicichla missioneira*, *Oligosarcus robustus*, *Trachelyopterus galeatus*, *Serrasalmus spilopleura* as 'Freshwater fish'; *Vicugna pacos*, *Lama glama* as 'Domestic camelids'; *Lama guanicoe* as 'Wild camelids'; *Pterocnemia pennata*, *Chloephaga melanoptera* as 'Terrestrial birds'; *Zea mays* (Maize) and *Amaranthus caudatus* (Kiwicha); *Opuntia ficus-indica* (Prickly pear); *Chenopodium quinoa*, *Cucurbita maxima*, *Solanum tuberosum*, *Oxalis tuberosa*, *Capsicum annuum*, *Ullucus tuberosus* as '$C_3$ plants'; *Phaseolus lunatus*, *Phaseolus vulgaris*, *Lupinus mutabilis*, *Prosopis chilensis* as 'Legumes'.

Data taken from: DeNiro and Hastorf (1985), van Der Merwe et al. (1993), Aufderheide et al. (1994), Tieszen and Chapman (1992), Finucane et al. (2006), Falabella et al. (2007), Hückstädt et al. (2007), Gil et al. (2011), Hoeinghaus et al. (2011), Szpak et al. (2013), Burress et al. (2013), Szpak et al. (2014), Thornton et al. (2011), Szpak et al. (2015), and DeNiro (1988).

A correction of 1.5‰ was applied to the $\delta^{13}$C values of modern samples to account for the burning of fossil fuels that has decreased the $\delta^{13}$C values of the atmospheric $CO_2$ (Suess effect) (Marino and McElroy, 1991; Schloesser et al., 2009). The isotope compositions of the edible portions were estimated applying the offsets proposed in previous studies (Codron et al., 2005; Hobson and Clark, 1992; Hobson et al., 1996; Kelly, 2000; Mateo et al., 2008; Sealy et al., 1987; Sholto-Douglas et al., 1991; Vogel, 1978; Warinner and Tuross, 2010; Yoneyama and Ohtani, 1983).

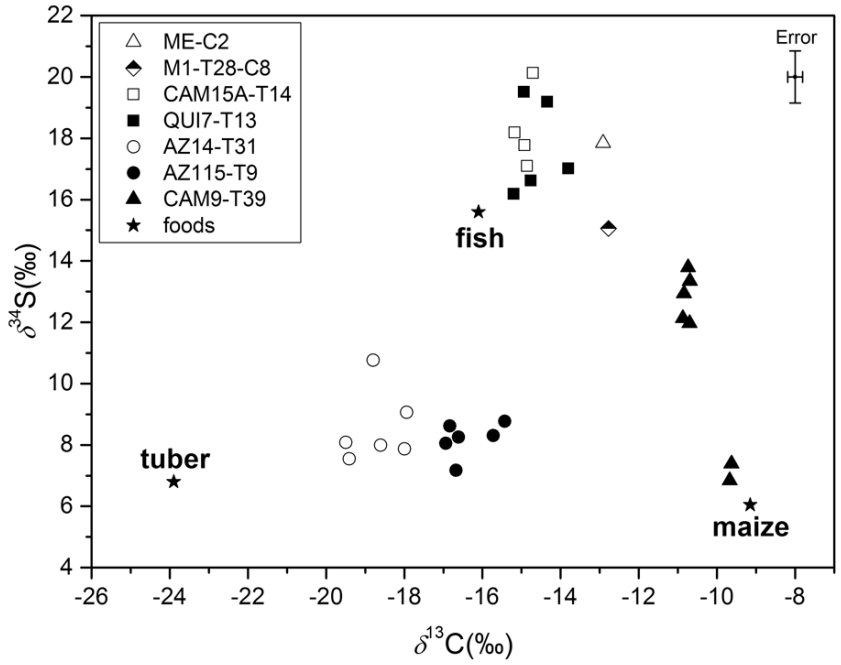


Fig. 4. Plot of $\delta^{34}$S values versus $\delta^{13}$C values for human hair analyzed in this study compared against South American flora and fauna data. South American flora and fauna data taken from Macko et al. (1999).

According to the dietary estimates (Table 3 and Fig. 5) generated by the FRUITS model, the coastal dwellers had a consistent caloric contribution from proteins (42-44%), specifically from aquatic foods (74-79%). These findings are validated by the $\delta^{34}$S hair isotope values which are in accordance with previous studies (Aufderheide et al., 1994; Aufderheide et al., 1993) reporting a range of ~ +15.4 to +18.5‰ for individuals living on the coasts of northern Chile with an almost total maritime diet. The marine fauna provided at least half of the calorie contribution (48-51%) to the diet of these coastal individuals, and it was complemented by terrestrial plants. In detail, $C_3$ cultigens (38-39%) were predominant in the diet of CAM15A-T14 and QUI7-T13 individuals, while $C_4$ crops (42%) were mostly contributing to CAM9-T39 dietary calories. Although the Inca individual presents an overall strong caloric dependence on both $C_4$ and marine foods, in the two hair segments nearest to

the scalp (approx. 'final' two months of life), the calorie contribution from $C_4$ crops becomes predominant (moving from 39% to 49%) (Supporting Information 2), and the hair sulfur isotope signature indicates a terrestrial dietary intake ($\delta^{34}S \sim +7‰$). The model suggests that the coastal valley inhabitants mainly retrieved their calories from carbohydrates and lipids (72% for AZ14-T31, 64% for AZ115-T9), predominantly from $C_3$ plant foods (70% for AZ14-T31, 41% for AZ115-T9). The sources of dietary proteins were $C_3$ plants (65% for AZ14-T31, 36% for AZ115-T9) and terrestrial animals (e.g. camelid meat) (21% for AZ14-T31, 37% for AZ115-T9). The intake of terrestrial foods is confirmed by the sulfur isotope data, which is comparable to published values (Macko et al., 1999) reporting $\delta^{34}S <+11‰$ for Chilean individuals relying on terrestrial resources. In both the Azapa individuals' diets the calorie contribution of $C_3$ and $C_4$ plants fluctuate through time in an alternating manner suggesting the existence of an agricultural cycle (Supporting Information 2). This hypothesis is supported by the cyclical changes in bulk $\delta^{13}C$ and $\delta^{15}N$ values, having a range of <1.6‰ and <2.1‰ for AZ14-T31, and <2.2‰ and <1.1‰ for AZ115-T9, which is comparable to those published in Webb et al. (2013) and Williams and Katzenberg (2012).

Table 3. Dietary estimates, based on the isotope compositions of hair, generated by FRUITS.

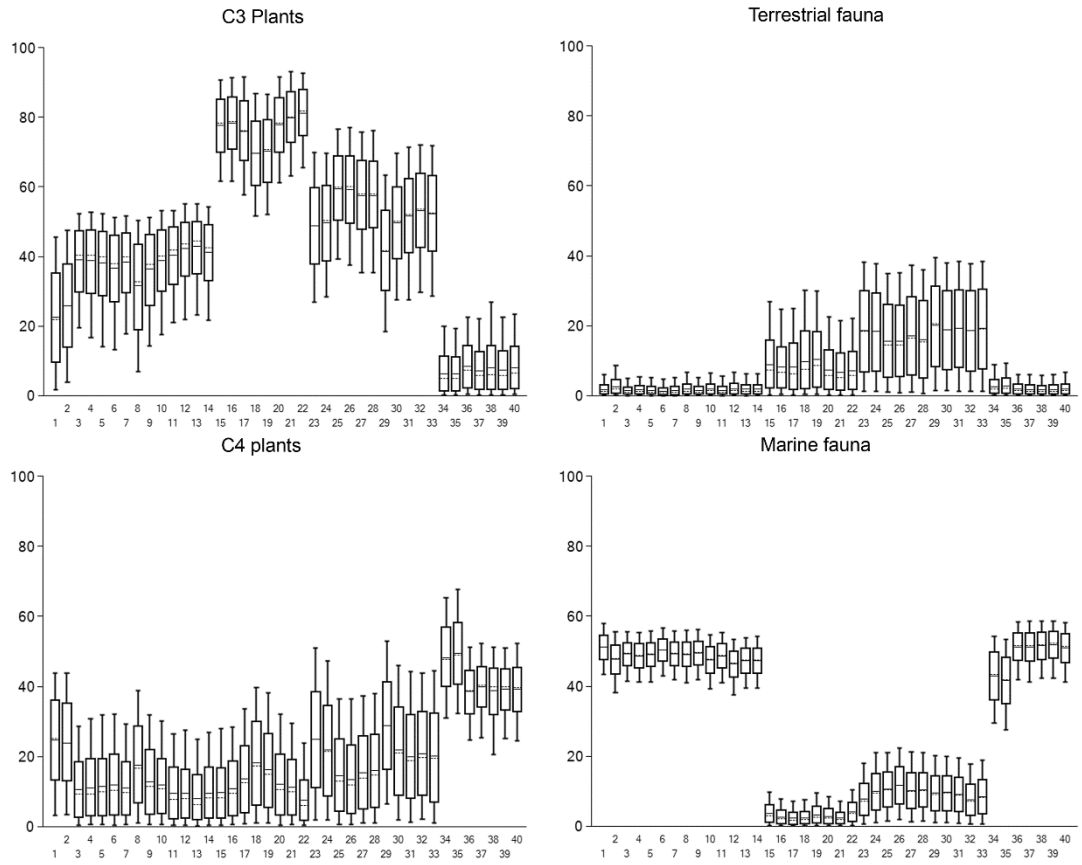


Fig. 5. FRUITS model estimates of individual calorie intake recorded by each hair segment (1 cm increments).

1= ME-C2; 2= M1-T28-C8; 3-7= CAM15A-T14; 8-14= QUI7-T13; 15-22= AZ14-T31; 23-33= AZ115-T9; 34-40= CAM9-T39. 68% credible interval represented by boxes, 95% credible interval represented by whiskers.

## 5.2. Individual amino acid carbon isotopes

A summary of the hair keratin single amino acid $\delta^{13}C$ values for each individual is provided in Table 4. The complete data set of amino acid $\delta^{13}C$ values is presented in Supporting Information 3.

Table 4. Summary of amino acid $\delta^{13}C$ (‰) values.

The existence of autocorrelation among amino acid isotope compositions measured from the same hair renders the application of principal component analysis (PCA) inappropriate. To address this issue, we decided to perform functional PCA using curves as measurement units, which is an approach similar to that of Caussinus and Ferre (1992) when performing PCA on growth curves. Briefly, for each amino acid and hair, we fitted a generalized least squares (GLS) model with amino acid values as outcome and distance from scalp as the explanatory variable (predictor). To model the curvature shown by the data, we included the linear and quadratic effects of distance. As a result of model fitting, we obtained, from each model, the three numbers that characterize the curve representing the intercept estimate, and the linear and quadratic effects of distance. We collected all these estimates in a data matrix with 10 rows (one row for each hair) and 42 columns (i.e. 3 columns for each amino acid), and performed principal component analysis on this data matrix.

The first three principal components explain 81.5% of the variation shown by curves from all the single hairs. The first principal component (PC1), which is mainly influenced by the $\delta^{13}C$ amino acid values of leucine and aspartic acid, groups hairs taken from the same individual, and separates the consumers of aquatic proteins (or coastal individuals) (ME-C2, M1-T28-C8, CAM15A-T14, QUI7-T13, CAM9-T39) from the consumers of terrestrial foods (or coastal valley individuals) (AZ14-T31, AZ115-T9) (Fig. 6). In particular, at the extreme negative scale of PC1 we find the AZ14-T31 individual characterized by the lowest protein intake (as determined by the FRUITS model), while at the extreme positive scale of PC1 we find one of the individuals with the highest protein consumption (ME-C2). The PC2 is also strongly influenced by leucine, but also arginine and lysine. Although all the individuals plot with positive PC2 scores, among the terrestrial resource consumers, the individual with the greatest proportion of $C_3$ foods in their diet (AZ14-T31) has a more positive PC2 score than the individual (AZ115-T9) who, according to the FRUITS model based on bulk isotope data,

consumed a high proportion of $C_4$ resources. For these reasons, PC1 might be useful in distinguishing between terrestrial and aquatic resource consumers, while PC2 might track differences within terrestrial diets (e.g. $C_3$, $C_4$). Lastly, the third principal component, whose dominant loadings are serine, proline and lysine, clearly separates the CAM9-T39 individual (positive PC3) from the other individuals (negative PC3).

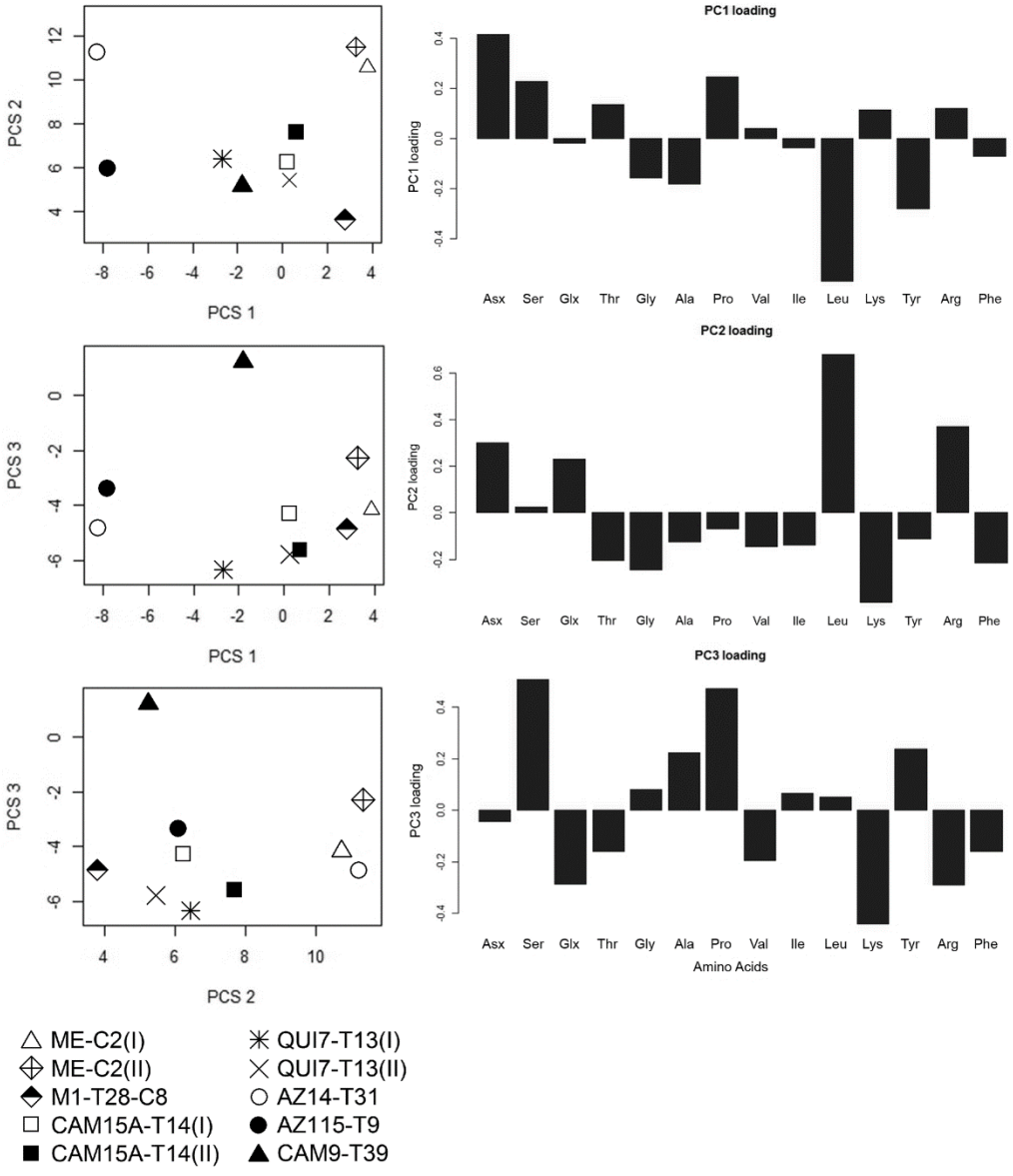


Fig. 6. Functional Principal Component Analysis output for $\delta^{13}C$ amino acid values: scores plots and component loadings.

Overall, the terrestrial resource consumers present more negative $\delta^{13}C$ amino acid values compared to the high-aquatic protein consumers (Table 4). The difference between the highest and the lowest $\delta^{13}C$ values of each of the amino acids for the aquatic resource consumers (ME-C2, M1-T28-C8, CAM15A-T14, QUI7-T13) is small (<6‰) suggesting a consistent diet through time, as well as a predominant dependence on proteins (mostly aquatic) with respect to other macronutrients. In fact, in high protein diets non-essential amino acids (e.g. glycine) are preferentially assimilated from the dietary proteins rather being synthesized *de novo,* as it is energetically more efficient (Corr et al., 2005; Jim et al., 2006). Conversely, the range of $\delta^{13}C$ amino acid values for the terrestrial resource consumers (AZ14-T31, AZ115-T9) is much larger, especially for the non-essential amino acids glutamic acid/glutamine and alanine (range >16‰) in AZ115-T9, indicating a carbohydrate contribution from diverse crops ($C_3$, $C_4$). Glutamate/glutamine (both contributing to measured glutamic acid as a result of hydrolysis in sample preparation) and alanine are predominantly synthesized by the human body (Jim et al., 2006), even when high quantities of proteins are available (Fernandes et al., 2012).

Glutamate is synthesized from α-ketoglutarate, an intermediate of the tricarboxylic acid cycle (TCA) (Brosnan and Brosnan, 2013). The TCA cycle and its intermediates facilitate the interrelationship among amino acids, lipids and carbohydrates and their carbon skeletons (Gropper et al., 2008). Alanine is derived from pyruvate, which is in turn formed from glucose, as an end product of glycolysis (Brosnan and Brosnan, 2013). Therefore, alanine, and especially glutamic acid, are expected to closely reflect the whole diet carbon (Howland et al., 2003; Jim et al., 2006) potentially making it possible to track the dietary carbohydrate. The spread of the $\delta^{13}C$ phenylalanine values (~ 8‰) of AZ115-T9 suggests that this individual retrieved dietary proteins from varied sources of food (e.g. legumes and meat),

because the essential phenylalanine is directly incorporated into body tissues (i.e. minimal fractionation between diet and proteinaceous tissues) and closely reflects the dietary amino acids (Howland et al., 2003; Jim et al., 2006).

The $\delta^{13}$C glycine values of the high aquatic protein consumers (ME-C2, M1-T28-C8, CAM15A-T14, QUI7-T13, CAM9-T39) are more negative than the $\delta^{13}$C serine values along the length of the hair, while the opposite is generally true for AZ14-T31 (a high-carbohydrate consumer) and (to a lesser extent) for AZ115-T9 (a mixed terrestrial resource consumer) (Supporting Information 4). Serine and glycine both derive from glucose, as serine is synthesized from a glycolytic intermediate (3-phosphoglycerate), and then it can be converted into glycine (by a one-step reversible reaction) (Brosnan and Brosnan, 2013). In mixed diets consisting of sufficient amounts of carbohydrate and protein (as it could be for AZ115-T9), the process of *de novo* synthesis of a non-essential amino acid such as glycine is expected to exceed that of direct assimilation (Howland et al., 2003). Whereas under condition of high-marine protein diets, the pathway of *de novo* synthesis of glycine is preferentially substituted by direct routing of dietary glycine (Corr et al., 2005). In addition if it is feasible in humans to convert fatty acids into glucose (Fernandes et al., 2012; Kaleta et al., 2011), this might explain why in marine diets rich in proteins and lipids (but poor in carbohydrates) $\delta^{13}$C glycine values are more negative than serine values [as for Greenlanders (Raghavan et al., 2010)], despite glycine being predominantly derived from marine foodstuffs that have very high $\delta^{13}$C glycine values (Corr et al., 2009).

Based on the observation of the variation of the essential amino acid carbon isotope compositions along the hair fiber, it appears that the relationship among $\delta^{13}$C phenylalanine, valine and leucine values may be useful in tracking the source (terrestrial or aquatic) of the dietary amino acid pool (Supporting Information 4). The $\delta^{13}$C phenylalanine values are more negative than the valine and leucine values for the ME-C2, M1-T28-C8, CAM15A-T14, and

QUI7-T13 individuals, whereas they are generally more positive for the terrestrial consumers (AZ14-T31, AZ115-T9). The CAM9-T39 individual presents an intermediate situation with $\delta^{13}$C phenylalanine values more negative than those of valine, but approximately equal to leucine.

Taking into account the pattern of single AA carbon isotope compositions along the hair fiber and the output of functional PCA (showing the largest magnitude among the PC1 and PC2 component loadings for leucine), it is plausible to suppose that $\delta^{13}$C leucine values may be used not only to differentiate between terrestrial and aquatic consumers, but also $C_3$ and $C_4$ terrestrial consumers. Collagen $\delta^{13}$C leucine and phenylalanine values were reported to be strongly correlated with those of diet in a feeding experiment of pigs raised on omnivorous diets (Howland et al., 2003), showing minimal isotopic fractionation between dietary amino acids and body tissues. When $\delta^{13}$C phenylalanine values are plotted against $\delta^{13}$C leucine values for our dataset, terrestrial and marine consumers are separated, and strong correlations exist within the terrestrial consumers (AZ14-T31, AZ115-T9) ($\rho$=.937, n=104, p=.001, 2-tailed) and within the aquatic consumers (ME-C2, M1-T28-C8, CAM15A-T14, QUI7-T13) ($\rho$=.876, n=103, p=.001, 2-tailed), while the CAM9-T39 hair segments are spread between the terrestrial and marine consumers (Fig. 7).

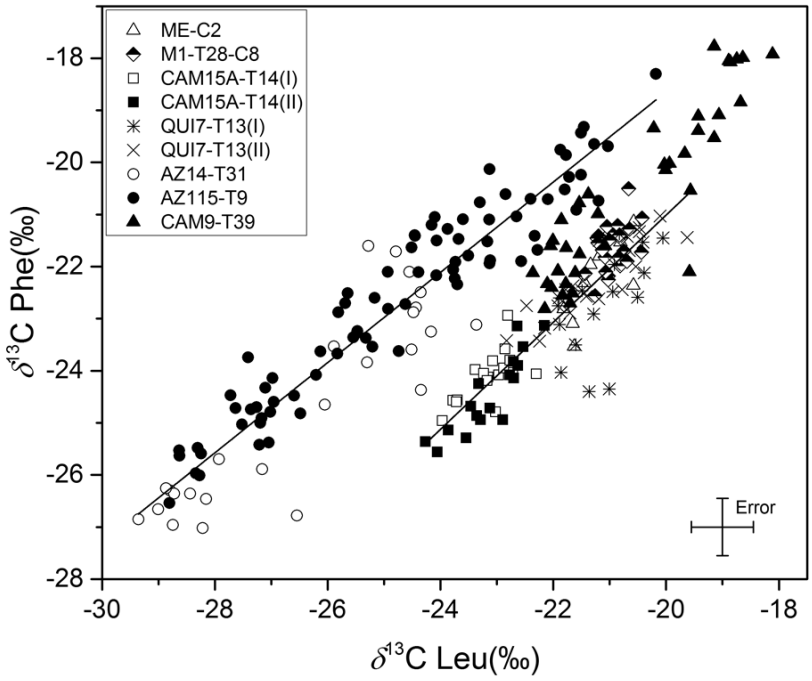


Fig. 7. Plot of $\delta^{13}$C phenylalanine values versus $\delta^{13}$C leucine values for all the segments of mummy hair analyzed in this study.

The study of other potential biplots shows that $\delta^{13}$C Phe vs. $\delta^{13}$C Ile, and $\delta^{13}$C Phe vs. $\delta^{13}$C Val scatterplots generate a similar distribution of the hair samples (respectively, for terrestrial consumers ρ=.843, n=104, p=.001, 2-tailed, and for aquatic consumers ρ=.854, n=103, p=.001, 2-tailed; for terrestrial consumers ρ=.869, n=104, p=.001, 2-tailed, and for aquatic consumers ρ=.847, n=103, p=.001, 2-tailed) (Figs. 8-9). In fact, isoleucine, leucine, valine and phenylalanine are all essential amino acids, directly routed from the diet (Brosnan and Brosnan, 2013). Despite the fact that leucine contributes slightly more carbon (C weight %) to the keratin than valine [8.5% and 6.6% respectively (Robbins and Kelly, 1970; Wolfram, 2003)], valine is preferred over leucine for making dietary interpretation because the chromatographic peak of Val is better resolved than Leu.

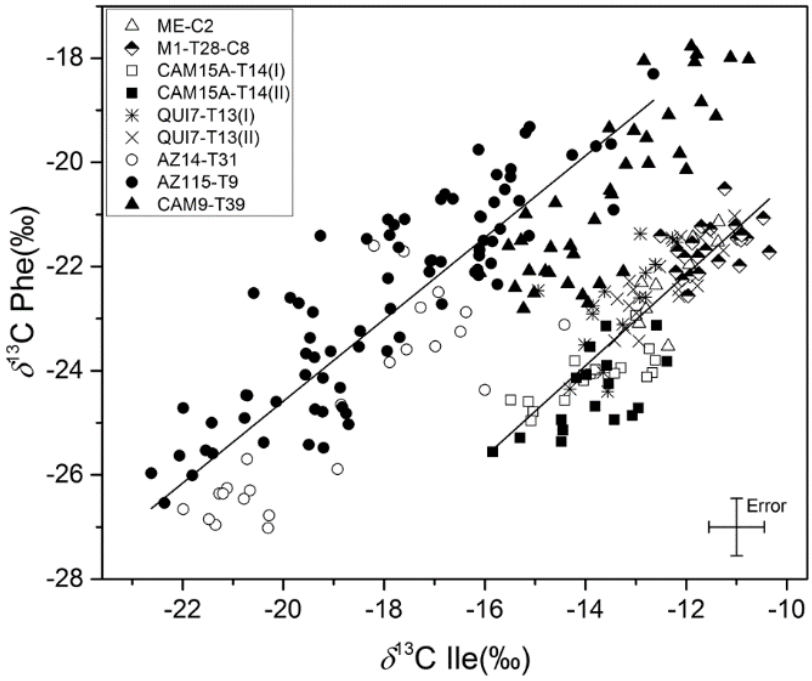


Fig. 8. Plot of $\delta^{13}$C phenylalanine values versus $\delta^{13}$C isoleucine values for all the segments of mummy hair analyzed in this study.

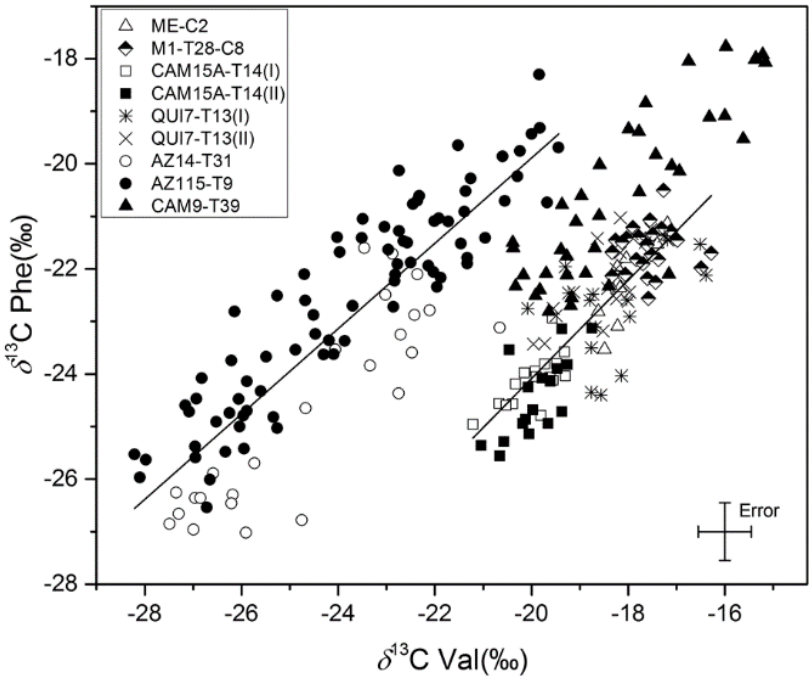


Fig. 9. Plot of $\delta^{13}$C phenylalanine values versus $\delta^{13}$C valine values for all the segments of mummy hair analyzed in this study.

Recently, Honch et al. (2012) proposed the dietary marker $\Delta^{13}C_{Val\text{-}Phe}$ for bone collagen to give the best differentiation between groups with known diet. Despite compositional differences between the two proteinaceous tissues (collagen and keratin) and their different physiology (formation, metabolism), $\Delta^{13}C_{Val\text{-}Phe}$ is a suitable indicator to track carbon sources (terrestrial vs. aquatic) in hair isotope analysis. As shown by Fig. 10, this dietary marker is able to clearly separate terrestrial (AZ14-T31, AZ115-T9) and aquatic consumers (ME-C2, M1-T28-C8, CAM15A-T14, QUI7-T13), with individuals consuming a mixed diet, such as CAM9-T39, plotting in the gap between these two end-members.

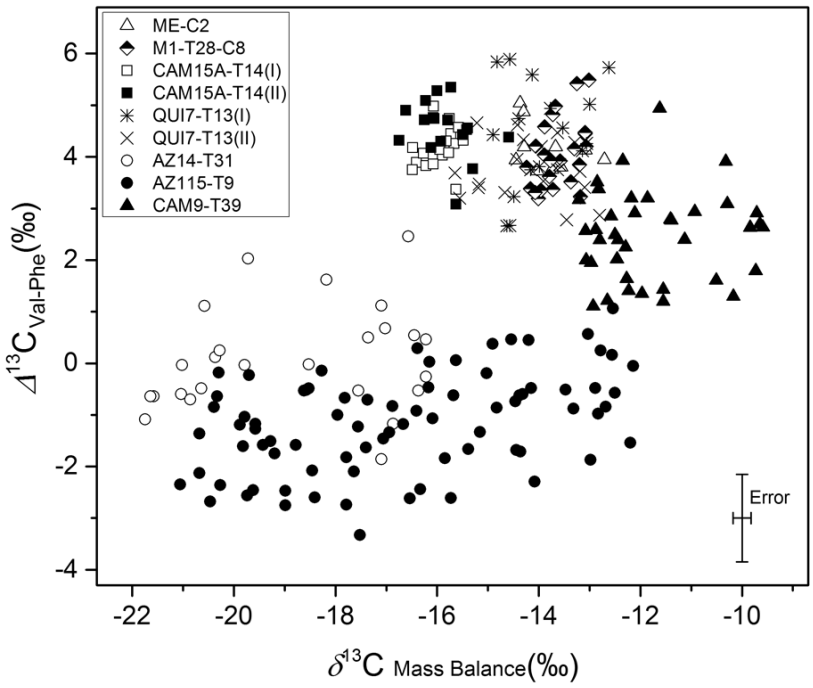


Fig. 10. Plot of $\Delta^{13}C$ valine-phenylalanine values versus $\delta^{13}C$ Mass balance values for all the segments of mummy hair.

The $\delta^{13}C$ leucine values measured for bone collagen isolated from humans consuming maize/non-maize diets (Fogel and Tuross, 2003) and from animals raised on $C_3/C_4$ macronutrients (Copley et al., 2004; Jim et al., 2006) were shown to directly derive from the dietary amino acid (leucine), thus the origin of the protein component, from either $C_3$ or $C_4$ plants, can be traced. Given this premise, the $\delta^{13}C$ Phe vs. $\delta^{13}C$ Leu plot (Fig. 7) appears to be effective in discriminating $C_3$- and $C_4$- terrestrial consumers with $C_4$ consumers having $\delta^{13}C$ leucine values enriched in the $^{13}C$ isotope compared with the $C_3$ terrestrial consumers, as it is true for the $\delta^{13}C$ leucine values of the plant themselves (Copley et al., 2004; Fogel and Tuross, 2003). When the $\delta^{13}C$ Phe vs. $\delta^{13}C$ Leu scatterplot is compared to the $\delta^{13}C$ Phe vs. $\delta^{13}C$ Val plot proposed by Honch et al. (2012) for bone collagen, it appears that a similar sample distribution is produced, suggesting that the $\delta^{13}C$ Phe vs. $\delta^{13}C$ Val plot could be used as a dietary indicator for terrestrial $C_3$- or $C_4$- consumers even for hair keratin samples.

In our $\delta^{13}C$ Phe vs. $\delta^{13}C$ Val plot (Fig. 9) from hair keratin values it is interesting to note that CAM15A-T14 hair segments have slightly lower phenylalanine and valine $\delta^{13}C$ values compared to other aquatic consumers (ME-C2, M1-T28-C8, QUI7-T13), which could be interpreted as a mixed marine and freshwater diet. Data reported by Honch et al. (2012) for human bone collagen show that high freshwater protein consumers have more negative phenylalanine and valine $\delta^{13}C$ values relative to high marine protein consumers. This is because the organic matter of riverine origin has lower $\delta^{13}C$ Phe and Val values compared to the sediments in the marine ecosystem (Keil and Fogel, 2001), and these amino acids are assimilated into the tissues of the consumers without incurring extensive isotopic fractionation, especially phenylalanine.

Glycine has been identified in previous studies (Corr et al., 2009; Corr et al., 2005) as a suitable marker for high marine protein intake. In particular, glycine $\delta^{13}C$ values have been found to be higher in marine animals and in humans feeding on them (Corr et al., 2009; Corr

et al., 2005). This occurs because, despite being a non-essential amino acid, glycine is preferentially assimilated from diet instead of being synthesized in high-protein diets (Corr et al., 2005; Jim et al., 2006). This means that glycine $\delta^{13}C$ values in high aquatic protein consumers are expected to reflect the glycine $\delta^{13}C$ values of their ecosystem-specific foods (marine or freshwater), with $^{13}C$-enriched glycine originating from marine-derived organic matter (e.g. planktonic pool) compared to the $^{13}C$-depleted matter of freshwater origin (Keil and Fogel, 2001). This pattern has been observed in glycine $\delta^{13}C$ values from human bone collagen (mean $\delta^{13}C$ Gly for high marine protein consumers: –7.0±1.3‰; mean $\delta^{13}C$ Gly for high freshwater protein consumers: –10.8±1.3‰) (Honch et al., 2012). The glycine $\delta^{13}C$ values of hairs from the QUI7-T13 individual, and especially the CAM15A-T14 individual, are lower than ME-C2 and M1-T28-C8 (Fig. 11) and might be evidence that QUI7-T13 and CAM15A-T14 have mixed freshwater and marine resources in their diets. However, it remains to be demonstrated whether the differences in $\delta^{13}C$ glycine values among high aquatic protein consumers in this study is related to diverse production origins of carbon sources (freshwater vs. marine ecosystems), or a result of geographic, temporal or environmental factors, as the samples come from different areas (Arica area vs. Camarones area). Use of estuarine areas could potentially present mixed aquatic resources with diverse isotope compositions. Further investigations on a broader dataset are needed in order to confirm this hypothesis.

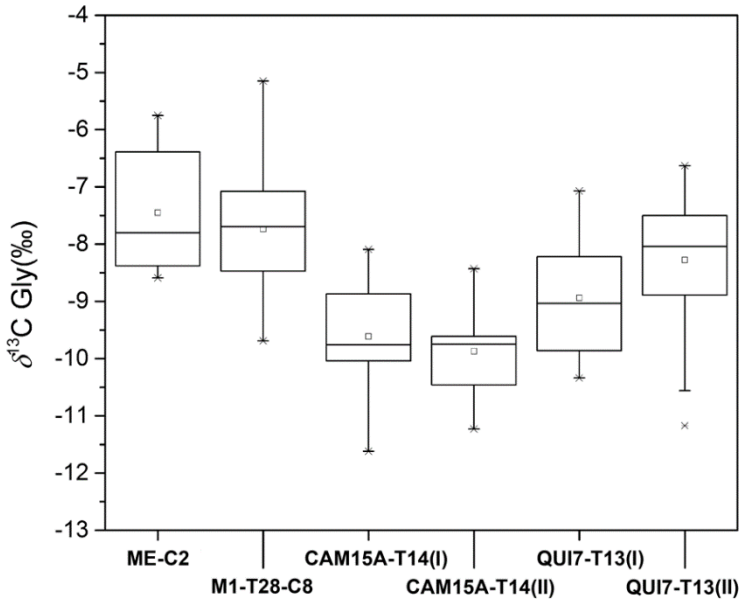


Fig. 11. Boxplots of $\delta^{13}$C glycine values for high aquatic protein consumers.

## 6. Discussion

The following discussion details brief isotopic histories of the individuals according to their archaeological period and culture.

### 6.1. Chinchorro individuals: Maderas Enco 1-C2 and Morro 1-T28 C8

Past studies on Chinchorros have highlighted their specialization in exploiting the abundant and reliable marine resources along the Pacific littoral of the Atacama Desert (Arriaza et al., 2008; Aufderheide et al., 1993; Santoro et al., 2012; Standen et al., 2004). The coastal economy was complemented to a minor extent by hunting terrestrial game (e.g. rodents, guanaco) and gathering wild plants (e.g. *Opuntia ficus-indica*, Cyperaceae seeds) at the mouth of river valleys (Reinhard et al., 2011; Santoro et al., 2012).

Isotope data measured for the hair from the ME-C2 mummy indicate an almost total reliance on marine foods (e.g. fish, mollusks, seabirds, sea mammals) ($\delta^{13}$C Val range=–18.6‰/–17.4‰; $\delta^{13}C_{\text{Mass Balance}}$ range=–14.5‰/–12.7‰; $\delta^{13}$C=–12.9±0.1‰, $\delta^{15}$N=+23.2±0.0‰, $\delta^{34}$S=+17.8±1.6‰; $\Delta^{13}C_{\text{Val-Phe}}$ range=3.7/5.0) (Tables 2, 4; Fig. 10). According to the FRUITS estimates, the ME-C2 diet was made of at least 51% by marine fauna and for the remaining by wild plants, either $C_3$, $C_4$ or CAM. Aquatic proteins greatly contributed (44%) to the caloric intake of this individual (Table 3). The orientation and the distance from the scalp of the hair segments analyzed are unknown; however, they appear to reflect the same dietary resources consumed but during two different time periods.

The M1-T28-C8 individual shows a strong dependence on marine resources for the period of approximately 1 year ($\delta^{13}$C Val range=–18.3‰/–16.3‰; $\delta^{13}C_{\text{Mass Balance}}$ range=–14.2‰/–13.0‰; $\delta^{13}$C=–12.8±0.1‰, $\delta^{15}$N=+22.0±0.0‰, $\delta^{34}$S=+15.1‰; $\Delta^{13}C_{\text{Val-Phe}}$ range=3.2/5.5) (Tables 2, 4; Fig. 10). The caloric contribution from proteins was estimated to be 43% by the FRUITS model, specifically from marine foods (75%) and complemented by terrestrial plants (Table 3), broadly similar to that of ME-C2. These findings are in line with archaeological and anthropological studies published on Morro 1 communities: namely a significant percentage of individuals appear to have been affected by external auditory exostosis [29%, N=52 (Standen et al., 1997)] and bone injuries, both connected to marine food acquisition (Arriaza, 1995b; Standen et al., 1984). Furthermore, the majority of grave goods found associated with those mummies relate to activities such as fishing (e.g. *Sardinops sagax*), gathering mollusks (e.g. *Concholepas concholepas* and *Choromytilus chorus*) and hunting marine game (e.g. *Otaria flavescens*, *Pelecanus thagus*) (Standen, 2003). The isotopic evidence confirms this and implies that the marine dietary strategy was used year-round.

6.2. Early Formative individuals: Camarones 15A-T14 and Quiani 7-T13

The amino acid and bulk results recorded for both the single hairs of the CAM15A-T14 individual ($\delta^{13}C$=–14.9±0.2‰, $\delta^{15}N$=+23.3±0.1‰, $\delta^{34}S$=+18.3±1.3‰; $\delta^{13}C$ Val range=–21.2‰/–18.8‰; $\delta^{13}C_{Mass\ Balance}$ range=–16.8‰/–14.6‰; $\Delta^{13}C_{Val\text{-}Phe}$ range=3.1/5.4) are consistent with a protein-rich diet derived from aquatic resources (Tables 2, 4; Fig. 10). FRUITS estimates indicate that the CAM15A-T14 individual retrieved 44% of the caloric contribution from proteins similar to the antecedent Chinchorros, with the aquatic foods representing 49% of the dietary intake (Table 3). However, the remaining fraction of the diet was mainly composed of $C_3$ plants (38%), supporting the hypothesis of the initial experimentation with cultigens for the Early Formative communities. Lower phenylalanine, valine and glycine $\delta^{13}C$ values (relative to other marine consumers) (Figs. 9, 11) suggest that the exploitation of marine resources was combined with some freshwater resources (e.g. mollusks, fish, birds). It is plausible to infer that coastal individuals living at the mouth of the rivers, such as the Camarones River, were involved in explorations of nearby freshwater ecosystems, and doing so, diversified their food-sourcing. While the two hairs analyzed do not clearly match with each other (and therefore likely represent two separate periods covering more than 18 months), they do show similar overall dietary intakes for their respective periods, implying a consistent strategy that procures resources with a distinct isotopic range, with no evidence of total marine consumption for periods of longer than 2 weeks.

Similarly, it appears that the two single hairs of the QUI7-T13 individual were not contemporaneous as they recorded slightly different dietary choices. Overall the isotope compositions ($\delta^{13}C$ Val range= –20.1‰/–16.4‰; $\delta^{13}C_{Mass\ Balance}$ range= –15.7‰/–12.6‰; $\delta^{13}C$=–14.7±0.5‰, $\delta^{15}N$=+22.3±0.3‰, $\delta^{34}S$=+17.7±1.5‰; $\Delta^{13}C_{Val\text{-}Phe}$ range=2.7/5.9) (Tables 2, 4; Fig. 10) measured in QUI7-T13 hair indicate a strong exploitation of marine resources,

representing 48% of the food intake, with additional consumption of mostly terrestrial $C_3$ plants (39%) (according to FRUITS estimates, Table 3), which might have been cultivated by exploiting the upwelling groundwater or simply gathered. The hairs record periods of approximately 8 to 9 months with a diverse range of isotope values, implying dietary diversity, but mainly within the estuarine ecosystem.

The coastal populations such as Camarones 15 and Quiani 7 played a key role in the transition from the Chinchorro foragers to the agro-pastoral Formative economy. During the critical and gradual process of agricultural experimentation with limited productivity, the traditional marine resources continued to be part of a convenient subsistence strategy (Muñoz Ovalle, 2004a, 2011; Watson et al., 2013; Watson et al., 2010). The persistence of fishing economies on the coasts at the beginning of the Formative Period has been recorded by means of cultural material (Muñoz Ovalle, 2011) and evidence of dental diseases (Watson et al., 2013; Watson et al., 2010) as well as in the isotopic compositions of these individuals, which display a continuous reliance on aquatic resources.

### 6.3. Formative Period individuals: Azapa 14-T31 and Azapa 115-T9

The Formative individuals both exhibit a predominant intake of terrestrial resources of plant origin (88% for AZ14-T31, 73% for AZ115-T9, estimated from FRUITS, Table 3), resulting in a high caloric contribution to their diets from carbohydrates and lipids (72% for AZ14-T31, 64% for AZ115-T9). The consumption of $C_3$ cultigens (70%-81% for AZ14-T31, 42%-59% for AZ115-T9) and $C_4$ crops (8%-18% for AZ14-T31, 13%-29% for AZ115-T9) appears to fluctuate alternately with time suggesting the existence of a harvesting cycle for the periods measured in the bulk isotope analysis (Supporting Information 2). An agricultural pattern is evident further in notable fluctuations in single amino acid isotope compositions

(Supporting Information 4). If we suppose that the cultigens were cultivated in the same production zone (i.e. lower coastal valley), it is unlikely that the cyclical fluctuations in $\delta^{13}C$ and $\delta^{15}N$ values were caused by seasonal variations in environmental factors, as in the region the significant differences between wet or dry conditions happen during El Niño or La Niña years (ENSO events), rather than linked to seasonality on a monthly basis (Ehleringer et al., 1998).

The northern coasts and coastal valleys of the Atacama Desert were characterized, and still are characterized by extreme aridity (0.9 mm/year mean annual precipitation in Arica, modern times). During the austral summer (November to April) some (very limited) rainfall occurs in the region (1.5 mm mean January precipitation in Arica, in modern times) (Houston, 2006). Precipitation is scant on the coasts, but increases towards the Andes, permitting the recharge of the few perennial rivers, which reach their maximum flow regime during the summer (Houston, 2006). The ancient farmers would have followed the natural cycle of seasons, consisting of 'wet summer' and 'dry winter', by sowing the maize in November and beginning the harvest in February, continuing this activity until May/June (Dantas et al., 2014). The harvest of other grain crops as well as tubers and legumes would have likely started in these months (May/June) leading to a generalized isotopic oscillation between production, and potentially intake of, $C_4$ and $C_3$ cultigens (Williams and Katzenberg, 2012).

AZ14-T31 isotope compositions are consistent with alternate periods of intake of predominantly $C_3$ resources [e.g. *Capsicum annum*, *Phaseolus vulgaris*, *Cucurbita pepo*, *Chenopodium quinoa, Solanum tuberosum* (Muñoz Ovalle, 2004b; Szpak et al., 2013)] or mixed $C_3$-$C_4$ resources ($\delta^{13}C$ Leu range =–29.4‰/–23.4‰; $\delta^{13}C_{\text{Mass Balance}}$ range=–21.8‰/–16.2‰; $\Delta^{13}C_{\text{Val-Phe}}$ range=–1.9/2.5). The AZ115-T9 results show a similar pattern but richer

in $C_4$ resources [e.g. *Amaranthus sp*., *Zea mays* (Cadwallader et al., 2012)] ($\delta^{13}C$ Leu range=–28.8‰/–20.2‰; $\delta^{13}C_{\text{Mass Balance}}$ range=–21.1‰/–12.1‰; $\Delta^{13}C_{\text{Val-Phe}}$ range=–3.3/1.1) (Table 4, Fig. 10). These fluctuations in $\delta^{13}C$ amino acid values are generally tracked by both essential and non-essential amino acids along the hair fibers (Supporting Information 4), implying that proteins, carbohydrates and lipids were gleaned through the intake of foods containing carbon from the same source ($C_3$ or $C_4$), either of plant or animal origin.

The variation of bulk carbon isotope values along the seasonal shift of both individuals is attenuated by several permill when compared to the amino acid carbon compositions (Fig. 12). The attenuation of the bulk isotope compositions may be induced by the 'averaged' carbon signal measured in a bundle of multiple hairs caused by presence of different growing phases and/or rates in combined hairs (Remien et al., 2014).

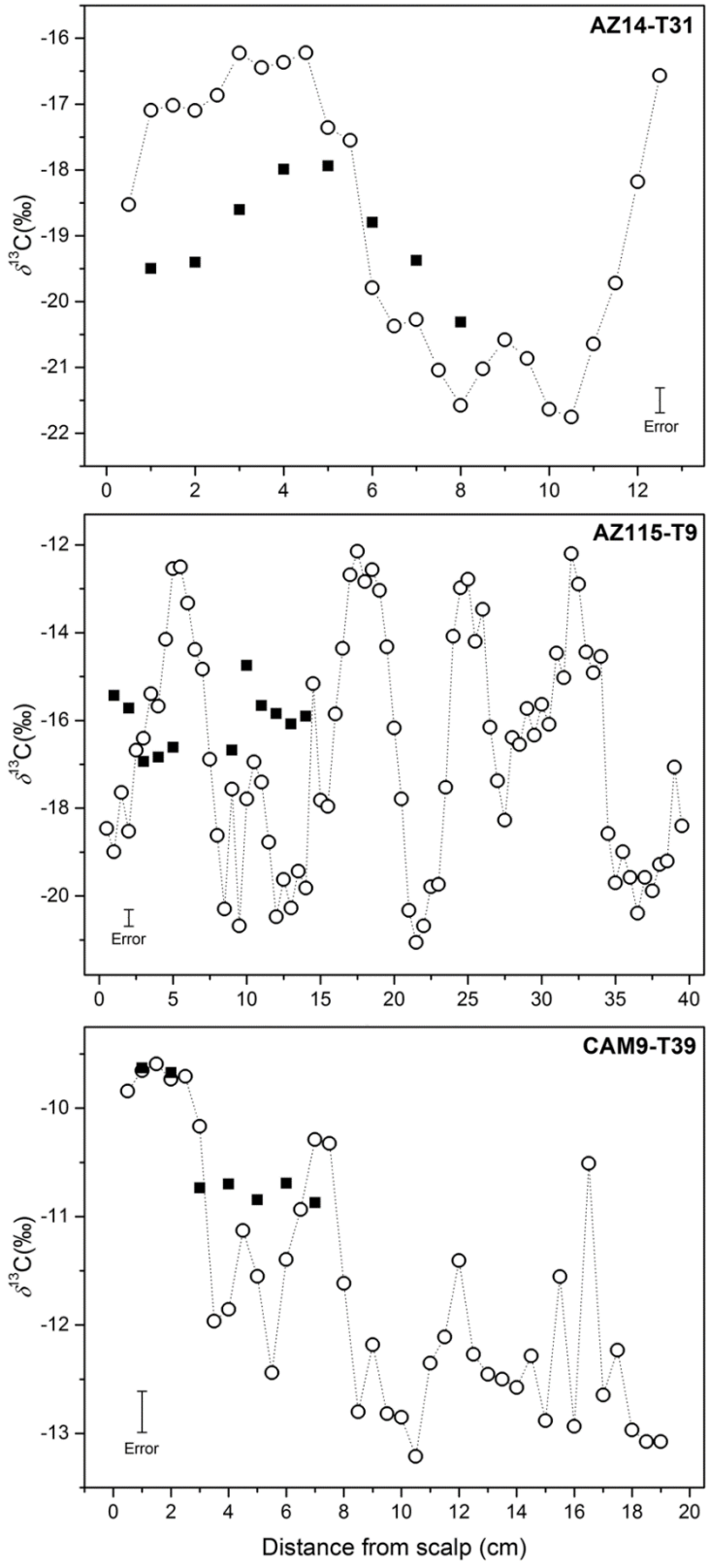


Fig. 12. Plot of $\delta^{13}C$ mass balance values (white points) and $\delta^{13}C$ bulk values (black squares) for Azapa 14-T31, Azapa 115-T9, and Camarones 9-T39 individuals.

The AZ14-T31 hair displays approximately 1 year of data indicating a subsistence pattern that presents two moderately stable periods of dietary intake lasting approximately 4 months each (~ –25‰ and ~ –28.5‰ in $\delta^{13}$C Leu values) (Fig. 12, Supporting Information 4). The shift in $\delta^{13}$C and $\delta^{15}$N values (2.4‰, 2.1‰ respectively) (Table 2) along the hair shaft are consistent with dietary changes related to agricultural cycles combined with a limited intake of terrestrial meat (8%) (Table 3) taken presumably from animals raised on the same crops (i.e. $C_3$ or $C_4$). In the most distal portions of hair (11.0-12.5 cm of amino acid $\delta^{13}$C values) of AZ14-T31 there is an indication of consumption of non-terrestrial resources ($\Delta^{13}C_{Val\text{-}Phe}$ >1.5) (Fig. 10), based on the distribution of isotope compositions in Figs. 7-9, and on previous bone collagen isotope data (Honch et al., 2012). This is a suggestion of sporadic yet significant use of alternative resources only once for a period of perhaps 6 weeks in the (approx.) 1 year period recorded by the hair. If this is a typical example of the Formative populations of the lower Azapa valley it displays a fully agro-pastoral shift, though maintaining some access to coastal resources on an occasional basis. However, the presence of external auditory exostosis among the Azapa 14 population (20%, N=10) (Standen et al., 1997) indicates that some individuals were indeed specialized fishermen and it can be assumed that aquatic resources would have been an ideal dietary supplement during periods of decreased agricultural productivity (Muñoz Ovalle, 2004a; Watson et al., 2013; Watson et al., 2010).

An agricultural pattern, to some extent similar to that of AZ14-T31, is also detected in the AZ115-T9 hair, but it is complicated by short-term dietary changes (Fig. 12, Supporting Information 4). The periods of dietary stability are limited in time, although not varying at a monthly scale. The carbon isotopic composition of hair fluctuates between –28.8‰ and –20.2‰ for $\delta^{13}$C Leu values as the proportions of $C_3$ and $C_4$ plants in the diet vary through time. It is possible that the AZ115-T9 individual had access to multiple ecozones (though

limited to lower altitudes), where the isotope compositions of plants (and consequently of animals) were influenced by the distribution of $C_4$ and $C_3$ plant species, as well by environmental factors (e.g. elevation, precipitation) (Ehleringer et al., 1998; Szpak et al., 2013). The high bulk $\delta^{15}N$ values of AZ115-T9 (when compared to those of AZ14-T31 individual, Table 2) are likely induced by the intake of local terrestrial animal meat. In fact, the highest source of dietary proteins for AZ115-T9 was terrestrial animals (37%) (Table 3), presumably camelids [based on the ubiquity of camelid remains in the archaeological record of the Formative Period (Valenzuela et al., 2015)]. Furthermore, it is not excluded that cultigens could have been fertilized by camelid dung (Horn et al., 2009). This manuring practice would have increased the nitrogen isotope compositions (by about ~ 2-4‰) of cultivated plants (Szpak et al., 2012b). It is plausible to suppose that the AZ115-T9 individual could have been involved in camelid herding with mobility between coasts and lower valleys (<1000 masl), where the camelid meat would have obtained higher nitrogen isotope compositions, compared to high-altitude animals (Szpak et al., 2015; Thornton et al., 2011).

In the AZ115-T9 hair there is no evidence of intake of either marine or freshwater resources for a period of more than 3 years, indicative of a reliance on terrestrial resources ($\Delta^{13}C_{Val\text{-}Phe}$ <1.1) (Fig. 10).

The resulting reconstructions of AZ14-T31 and AZ115-T9 diets are in agreement with the change in subsistence economy towards a larger contribution of agricultural products and terrestrial animal meat by about 4,000-3,000 BP. Throughout the Formative Period, highland influences promoted agricultural experimentation in the inland valleys that evolved into the domestication of local and foreign cultigens by exploitation of the upwelling groundwater (Muñoz Ovalle, 2012; Muñoz Ovalle and Chacama, 2012). The individuals analyzed here show an almost complete reliance on terrestrial resources with use of $C_3$ and $C_4$ resources in a broadly seasonal periodicity even though riverine and marine resources were accessible.

6.4. Inca individual: Camarones 9-T39

The isotope compositions ($\delta^{13}$C Val range=–20.4‰/–15.2‰; $\delta^{13}C_{\text{Mass Balance}}$ range=–13.2‰/–9.6‰; $\delta^{13}$C=–10.5±0.6‰, $\delta^{15}$N=+24.8±0.7‰, $\delta^{34}$S=+11.2±2.5‰; $\Delta^{13}C_{\text{Val-Phe}}$ =1.1/4.9) (Tables 2, 4; Fig. 10) from CAM9-T39 hair indicate a predominant consumption of marine resources, which contributed to the 79% of the dietary proteins of this individual, but also a high intake of $C_4$ plants (FRUITS estimates, Table 3). During the Late Period a diverse range of food resources would have been available with fishing carried out on the coast, and agriculture and animal husbandry in the valleys and puna (Núñez et al., 2010; Rivera, 2008). The Inca were adept at moving food surpluses (and other related goods such as salt and guano) around the landscape via fully-developed exchange networks between local lowland groups and Andean populations that helped to foster socio-economic relationships (Muñoz Ovalle, 1989; Núñez et al., 2010; Rivera, 2008).

The isotope compositions recorded in the two hair segments closest to the scalp (approx. 'final' two months of CAM9-T39's life) indicate a greater consumption of $C_4$ foods. During this period the calorie contribution from $C_4$ plants exceeds that of marine foods (moving from 39% to 49%, FRUITS estimates, Fig. 3 and Supporting Information 2), resulting in a shift toward the new diet of ~2.5‰ in $\delta^{13}$C Val values and ~6‰ in $\delta^{34}$S values (Table 2 and Supporting Information 4). In these hair segments closest to the CAM9-T39's scalp the $\delta^{13}$C lysine values become less negative and more stable compared to the other Lys $\delta^{13}$C values recorded along the hair fiber (Supporting Information 4). A similar shift is visible for other essential amino acids, in particular valine and phenylalanine. These higher amino acid carbon isotope compositions suggest that the CAM9-T39 individual was predominantly consuming maize combined with limited marine foods during this timeframe. Published bone collagen isotope data (Honch et al., 2012) for maize consumers report very high Val, Lys and Phe $\delta^{13}$C values. However, it is difficult to explain how the significant consumption of maize, which is

deficient in lysine (i.e. it contains an amount lower than the metabolic requirement) (Keeney, 1970), could produce the high lysine $\delta^{13}C$ value measured in CAM9-T39's keratin. It is likely that lysine was obtained from marine-derived proteins (Schmidt et al., 2016) via direct routing, as fish meat is rich in Lys (Carpenter, 1960), but also through break down and reuse of protein reserves containing lysine of marine origin (stored when CAM9-T39 was consuming high quantities of marine foodstuffs). Furthermore, the concurrent lysine synthesis from gut microflora may have played an important role. Past studies (Metges, 2000; Torrallardona et al., 2003) of essential amino acids have suggested that the human body may meet the metabolic requirement of lysine thanks to intestinal microflora, which may synthesize lysine from endogenous amino acids. Microbial lysine is produced and absorbed by the host tissue even under conditions of low protein intake (Metges et al., 2006).

The high nitrogen isotope compositions of CAM9-T39 hair may be induced by the use of seabird guano as maize fertilizer (Szpak et al., 2012a; Szpak et al., 2012b) (a common agricultural practice among Inca populations), although this assumes that guano does not significantly affect the $\delta^{34}S$ values of plants. The low sulfur content (1.4%) measured in the guano from the Antarctic penguin (*Pygoscelis adeliae*), compared to that of nitrogen (10.9%) (Zdanowski et al., 2005), supports this hypothesis.

The change in diet in the most recent months could be indirectly caused by the predominant consumption of a non-local staple in the individual's diet or be the result of travel towards the interior, as in the Inca Empire extensive mobility of goods and people around the landscape was common (Covey, 2008). If way stations were used as crop storage (Wilson et al., 2007), the hypothesis of a journey causing the shift in carbon isotope signature of CAM9-T39 hair is viable. During the Inca Empire the mobilization of people was a common practice for ritual, political and economic purposes and often associated with food rituals. For instance, young boys and girls were selected and moved to the mountain peaks of the Andes to be sacrificed,

and during the time leading to these ritual ceremonies victims were fed with maize and fermented maize drink (*chicha*) (Panzer et al., 2014; Wilson et al., 2013; Wilson et al., 2007). Non-Inca elites also travelled to the Andean centers to take part in political gatherings and feasts involving the consumption of maize beer (Alconini and Malpass, 2010; Arriaza et al., 2015; Covey, 2008) and specialized groups of males were mobilized to accomplish compulsory *mit'a* services (Acuto, 2005; Hastorf, 1991). Males undergoing labor tribute, as military and agricultural services, were commonly rewarded with meat, maize and *chicha* (Hastorf, 1991). However, the characteristics of the CAM9-T39 individual, an adult female (35-40yrs) affected by *spina bifida occulta* [a congenital malformation possibly caused by the chronic exposure to high level of arsenic found in the Camarones river valley (Silva-Pinto et al., 2010)], does not fit the profile of the aforementioned travelers.

It is however true that the production of *chicha* was undertaken by selected women and a diet richer in maize and meat may be evidence of this high status (Bray, 2003; Valdez et al., 2010). Further analysis (in progress) to establish the alcohol ingestion in the final months of CAM9-T39 life, recorded by hair keratin, will clarify the causes of the dietary change and the role of this individual within the community.

In the hair segments closest to the scalp of the CAM9-T39 individual (where the $C_4$ intake is predominant) the bulk $\delta^{13}C$ values exhibit a similar shift to that of mass balance $\delta^{13}C$ values (Fig. 12), indicating that the bulk isotope signal has not been attenuated despite the use of multiple hairs. A plausible explanation is that a period of monotonous diet (i.e. terrestrial $C_4$ foods) may increase the likelihood of multiple hairs registering the same signal.

## 7. Conclusion

This study shows that stable carbon isotope analysis of keratin amino acids extracted from 0.5 cm segments of a single human hair highly enhances the resolution of palaeodietary reconstruction, permitting the discrimination of specific diet components at a resolution invisible to traditional methods. Moreover, the LC/IRMS technique requires a single strand of hair, reducing the damage to the archaeological remains.

This work highlights the importance of the relationship between $\delta^{13}$C Phe vs. $\delta^{13}$C Val values for discriminating between terrestrial consumers and high aquatic protein consumers using hair keratin amino acid carbon isotope data, as previously applied by Honch et al. (2012) for bone collagen.

Our results show the importance of improving the palaeodietary reconstruction by applying a Bayesian mixing model (e.g. FRUITS) to the bulk isotope data that accounts for the different elemental concentrations in the foods, as well as for the differential contribution of macronutrients (proteins, carbohydrates, lipids) to the synthesis of the body tissue under investigation.

Although bulk methods can detect changes in diet and track seasonal shifts, the variations in carbon isotope signal can be highly attenuated with respect to the dietary intake because of the use of multiple hairs. This could potentially result in misleading reconstructions of mixed diets, where the magnitude of the isotopic range of dietary intake is underestimated. Controversial dietary interpretations and occasional nutritional intakes are more easily addressed by using compound-specific isotope analysis.

The isotope data confirms that the archaic hunter-gatherers and the first transitional Formative individuals found at coastal sites of modern-day northern Chile showed adaptive

behavior in food acquisition that strongly relied on aquatic resources, primarily maritime (ME-C2, M1-T28-C8, CAM15A-T14, QUI7-T13). These individuals were highly specialized in exploiting the abundant and localized littoral resources all year-round (ME-C2, M1-T28-C8). It is likely that they limited excessive protein intake (Cordain et al., 2000) and reduced foraging risks (Winterhalder, 1981) with the addition of wild plants, gathered at the river mouth (Reinhard et al., 2011). In early Formative individuals CAM15A-T14 and QUI7-T13 supplementary intake of freshwater resources is inferred. This may demonstrate their mobility towards the interior along the river valleys exploring potential niches suitable for the incipient agriculture (Muñoz Ovalle, 2004a), or may even have occurred as a result of disruptive climatic conditions (El-Niño) leading to a decline in marine resources (Williams et al., 2008), demonstrating their flexibility in food acquisition. The analysis of coastal valley Formative (Alto Ramírez) individuals highlights the almost total reliance on crops and terrestrial animal meat (AZ14-T31, AZ115-T9), with only occasional use of aquatic foods as a short-term strategy (AZ14-T31) (Muñoz Ovalle, 2004a). A pattern of seasonality, presumably generated by the occurrence of a harvesting cycle, is introduced in the diets of AZ14-T31 and AZ115-T9 individuals. A broader dietary spectrum made of mixed terrestrial and aquatic resources is identified in the Inca individual (CAM9-T39).

Palaeodietary reconstructions using stable isotopes (especially those based on bulk bone collagen analysis) generally characterize diets of individuals (e.g. as being ‘terrestrial’ or ‘marine’), mainly as a consequence of collagen turnover leading to a lack of temporal resolution. Whilst this is arguably a one-dimensional approach to interpretation, the conservative dietary intakes of many of the individuals measured here (i.e. almost complete reliance on either terrestrial resources or marine resources) does provide support for such interpretations, at least as a first approximation. In most cases analyzed here, a degree of dietary monotony is indeed observed, consistent with simple dietary characterizations.

However, the intra-individual variability observed in our results reveals how misleading such interpretations can be, especially in cases where individuals have mixed diets. For example, the AZ115-T9 individual, if characterized on a single mean carbon isotope measurement would be characterized as a mixed $C_3/C_4$ consumer. Such an interpretation can accommodate a number of different isotopic biographies, for example a temporally monotonous 50/50 $C_3/C_4$ dietary split, or a variety of proportions with a variety of temporal patterns.

The approach applied here demonstrates a method of creating detailed isotopic biographies of individuals. Whilst the subject of 'individuals' in archaeology remains a topic of debate (Knapp and van Dommelen, 2008), we assert that this is of interest not only for its power to illuminate personal histories at a relevant human timescale, but also to be a part of an integrated approach to a diachronic palaeodietary study. Whilst the approach is time consuming (the seven individuals analyzed here represent approximately 50 days of instrument time alone) it is an invaluable way to access information that traditional methods cannot. Through further analysis of more individuals the significance of intra-individual isotope variation will become clearer, as individuals become populations (at least within the limits of the archaeological record) and inferences concerning population coherence and variability can be made, through inter-individual comparisons of intra-individual variability.

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Table 1. Summary of pre-Columbian individuals analyzed.

| Site and Burial | Abbreviation | Chronology | Culture or Period | Site | Location | Mummification |
|---|---|---|---|---|---|---|
| Maderas Enco 1-C2 | ME-C2 | ~4,800 B.P. | Chinchorro | Arica | Coast | Artificial |
| Morro 1-T28 C8 | M1-T28-C8 | ~3,800 B.P. | Chinchorro | Arica | Coast | Natural |
| Camarones 15A-T14 | CAM15A-T14 | ~3,500 B.P. | Early Formative | Camarones | Coast | Natural |
| Quiani 7-T13 | QUI7-T13 | ~3,600 B.P. | Early Formative | Arica | Coast | Natural |
| Azapa 14-T31 | AZ14-T31 | ~2,000 B.P. | Formative | Azapa Valley | 8km Inland | Natural |
| Azapa 115-T9 | AZ115-T9 | ~1,500 B.P. | Formative | Azapa Valley | 12km Inland | Natural |
| Camarones 9-T39 | CAM9-T39 | ~550 B.P. | Inca | Camarones | Coast | Natural |

Table 2. Carbon, nitrogen, and sulfur isotope compositions of 1 cm increments (starting from the scalp) of multiple hairs for the individuals analyzed.

| Individual | Seg (cm)* | $\delta^{15}$N/‰ | %N | $\delta^{13}$C/‰ | %C | C/N | $\delta^{34}$S/‰ | %S |
|---|---|---|---|---|---|---|---|---|
| ME-C2 † | a | +23.25 | 12.1 | –12.95 | 39.6 | 3.8 | +16.74 | 3.2 |
| | b | +23.21 | 12.5 | –12.87 | 41.1 | 3.8 | +18.95 | 3.8 |
| | *mean±1σ* | *+23.23±0.03* | | *–12.91±0.06* | | | *+17.84±1.56* | |
| M1-T28-C8 † | a | +21.99 | 13.3 | –12.69 | 41.2 | 3.6 | +15.06 | 2.6 |
| | b | +22.01 | 13.2 | –12.85 | 41.3 | 3.7 | | |
| | *mean±1σ* | *+22.00±0.02* | | *–12.77±0.11* | | | | |
| CAM15A-T14 | 0-1 | +23.36 | 12.9 | –15.18 | 41.2 | 3.7 | +18.20 | 3.7 |
| | 1-2 | +23.22 | 12.7 | –14.86 | 40.8 | 3.7 | +17.10 | 3.3 |
| | 2-3 | +23.28 | 12.5 | –14.92 | 40.1 | 3.7 | +17.77 | 3.2 |
| | 3-4 | +23.24 | 12.8 | –14.71 | 40.9 | 3.7 | +20.13 | 3.5 |
| | 4-5 | +23.20 | 12.7 | –14.94 | 40.8 | 3.8 | | |
| QUI7-T13 | 0-1 | +22.69 | 12.4 | –13.80 | 41.5 | 3.9 | +17.01 | 2.9 |
| | 1-2 | +22.57 | 12.1 | –14.35 | 40.6 | 3.9 | +19.19 | 3.1 |
| | 2-3 | +22.25 | 12.3 | –14.76 | 40.7 | 3.9 | +16.62 | 2.7 |
| | 3-4 | +22.31 | 12.0 | –14.94 | 40.2 | 3.9 | +19.52 | 2.9 |
| | 4-5 | +22.14 | 12.2 | –15.20 | 40.7 | 3.9 | +16.19 | 2.4 |
| | 5-6 | +22.01 | 12.5 | –15.00 | 41.4 | 3.9 | | |
| | 6-7 | +22.02 | 12.5 | –14.98 | 41.6 | 3.9 | | |
| AZ14-T31 | 0-1 | +10.97 | 13.4 | –19.50 | 42.6 | 3.7 | +8.08 | 3.4 |
| | 1-2 | +10.27 | 13.6 | –19.40 | 42.6 | 3.7 | +7.55 | 3.1 |
| | 2-3 | +9.52 | 13.5 | –18.60 | 42.7 | 3.7 | +7.99 | 3.1 |
| | 3-4 | +9.97 | 13.3 | –17.99 | 42.4 | 3.7 | +7.87 | 3.1 |
| | 4-5 | +10.41 | 13.4 | –17.94 | 42.6 | 3.7 | +9.06 | 3.1 |
| | 5-6 | +9.50 | 13.3 | –18.80 | 42.2 | 3.7 | +10.76 | 3.4 |
| | 6-7 | +9.48 | 13.2 | –19.38 | 41.8 | 3.7 | | |
| | 7-8 | +11.55 | 13.1 | –20.31 | 41.8 | 3.7 | | |
| AZ115-T9 | 0-1 | +13.10 | 13.3 | –15.43 | 42.3 | 3.7 | +8.77 | 2.4 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1-2 | +13.90 | 12.9 | –15.72 | 41.3 | 3.7 | +8.31 | 2.2 |
| | 2-3 | +14.06 | 13.2 | –16.93 | 42.4 | 3.7 | +8.06 | 1.9 |
| | 3-4 | +14.16 | 12.6 | –16.84 | 40.4 | 3.7 | +8.62 | 1.9 |
| | 4-5 | +13.88 | 13.3 | –16.61 | 42.2 | 3.7 | +8.25 | 1.8 |
| | 5-6 | +14.02 | 10.0 | –16.38 | 32.1 | 3.8 | +6.78 | 2.0 |
| | 6-7 | +13.97 | 5.8 | –15.05 | 18.5 | 3.7 | +10.27 | 2.3 |
| | 7-8 | +14.23 | 9.0 | –14.55 | 28.7 | 3.7 | +9.45 | 2.3 |
| | 8-9 | +14.24 | 13.0 | –16.67 | 41.6 | 3.7 | +7.17 | 2.2 |
| | 9-10 | +13.88 | 13.2 | –14.74 | 42.0 | 3.7 | | |
| | 10-11 | +13.88 | 13.3 | –15.66 | 42.2 | 3.7 | | |
| | 11-12 | +13.69 | 13.4 | –15.84 | 42.1 | 3.7 | | |
| | 12-13 | +13.11 | 13.2 | –16.08 | 41.0 | 3.6 | | |
| | 13-14 | +13.43 | 13.2 | –15.90 | 41.7 | 3.7 | | |
| CAM9-T39 | 0-1 | +23.94 | 13.6 | –9.63 | 43.2 | 3.7 | +7.39 | 2.6 |
| | 1-2 | +23.74 | 12.8 | –9.67 | 40.8 | 3.7 | +6.85 | 2.5 |
| | 2-3 | +24.66 | 13.4 | –10.74 | 42.6 | 3.7 | +13.79 | 3.1 |
| | 3-4 | +25.47 | 13.8 | –10.70 | 42.1 | 3.5 | +11.97 | 3.2 |
| | 4-5 | +25.21 | 12.2 | –10.84 | 37.5 | 3.6 | +12.94 | 3.1 |
| | 5-6 | +25.18 | 13.4 | –10.69 | 41.8 | 3.6 | +13.35 | 3.2 |
| | 6-7 | +25.20 | 13.2 | –10.87 | 41.6 | 3.7 | +12.13 | 2.9 |
| | 7-8 | | | | | | +10.90 | 3.1 |
| | 8-9 | | | | | | +11.38 | 2.9 |

Two bundles of hair have been used for each individual, one for $\delta^{13}C$ and $\delta^{15}N$ analysis and one for $\delta^{34}S$ isotope analysis, maintaining the alignment at the roots.

* Distance of the hair segment from the scalp in cm.

† ME-C2 and M1-T28-C8 hair samples have been analyzed in duplicate (a, b) because incremental sampling was not applicable. Their isotopic means and standard deviations (mean±1σ) are listed.

Table 3. Dietary estimates, based on the isotope compositions of hair, generated by FRUITS.

| Individual | ME-C2 | | M1-T28-C8 | | CAM15A-T14 | | QUI7-T13 | | AZ14-T31 | | AZ115-T9 | | CAM9-T39 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Food (%) | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| C3 plants | 22 | 12 | 26 | 12 | 38 | 1 | 39 | 4 | 76 | 4 | 53 | 5 | 7 | 1 |
| C4 plants | 25 | 11 | 24 | 11 | 11 | 1 | 11 | 3 | 12 | 3 | 20 | 5 | 42 | 5 |
| Terrestrial Fauna | 2 | 2 | 3 | 2 | 1 | 1 | 2 | 1 | 8 | 1 | 18 | 2 | 2 | 1 |
| Marine Fauna | 51 | 4 | 48 | 4 | 49 | 1 | 48 | 1 | 3 | 1 | 10 | 1 | 49 | 4 |
| Food fractions (%) | | | | | | | | | | | | | | |
| Protein | 44 | 1 | 43 | 1 | 44 | 1 | 44 | 1 | 28 | 1 | 36 | 1 | 42 | 2 |
| Energy | 56 | 1 | 57 | 1 | 56 | 1 | 56 | 1 | 72 | 1 | 64 | 1 | 58 | 2 |
| Dietary proxies (Food)(%) | | | | | | | | | | | | | | |
| $\delta^{13}C$ (C3 plants) | 15 | 8 | 17 | 8 | 25 | 1 | 26 | 3 | 70 | 4 | 41 | 4 | 5 | 1 |
| $\delta^{13}C$ (C4 plants) | 13 | 6 | 13 | 6 | 6 | 1 | 6 | 2 | 9 | 3 | 12 | 3 | 22 | 4 |
| $\delta^{13}C$ (Terr. Fauna) | 3 | 3 | 4 | 4 | 2 | 1 | 3 | 1 | 16 | 2 | 31 | 3 | 3 | 1 |
| $\delta^{13}C$ (Marine Fauna) | 70 | 5 | 66 | 5 | 67 | 1 | 65 | 1 | 5 | 1 | 15 | 2 | 70 | 4 |
| $\delta^{15}N$ (C3 plants) | 11 | 6 | 13 | 6 | 19 | 1 | 20 | 2 | 65 | 4 | 36 | 4 | 4 | 1 |
| $\delta^{15}N$ (C4 plants) | 7 | 4 | 7 | 4 | 3 | 1 | 3 | 1 | 7 | 2 | 8 | 2 | 13 | 2 |
| $\delta^{15}N$ (Terr. Fauna) | 3 | 3 | 5 | 4 | 3 | 1 | 3 | 1 | 21 | 3 | 37 | 3 | 4 | 1 |
| $\delta^{15}N$ (Marine Fauna) | 79 | 4 | 75 | 5 | 75 | 1 | 74 | 1 | 7 | 1 | 19 | 2 | 79 | 3 |

The means and standard deviations for ME-C2 and M1-T28-C8 individuals are the estimates generated by the FRUITS model. The means and standard deviations for CAM15A-T14, QUI7-T13, AZ14-T31, AZ115-T9, and CAM9-T39 are calculated on the means and standard deviations estimated by the FRUITS model for the various hair segments.

According to Fernandes et al. (2015), Food (%) represents the calorie contribution of the food groups; Fraction (%) represents the calorie contribution of the food fractions; Dietary proxy (Food)(%) represents the calorie contribution of the food groups to the dietary proxies.

Table 4. Summary of amino acid $\delta^{13}C$ (‰) values.

| | Essential Amino Acids | | | | | | Non-essential Amino Acids | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Phe | Val | Leu | Ile | Lys | Thr | Ala | Ser | Gly | Asx | Glx | Pro | Arg | Tyr | MB[a] |
| ME-C2(I) | | | | | | | | | | | | | | | |
| Mean | -22.1 | -17.9 | -21.1 | -12.0 | -14.9 | -8.1 | -13.0 | -5.9 | -7.4 | -11.9 | -12.2 | -13.1 | -15.2 | -20.4 | -13.7 |
| Std. Deviation | 0.9 | 0.4 | 0.4 | 0.5 | 0.5 | 0.8 | 0.6 | 0.5 | 0.7 | 0.6 | 0.6 | 0.5 | 0.6 | 1.4 | 0.5 |
| Range | 2.1 | 1.1 | 1.1 | 1.2 | 1.4 | 1.8 | 1.5 | 1.3 | 1.7 | 1.8 | 1.4 | 1.3 | 1.6 | 3.9 | 1.3 |
| Minimum | -23.5 | -18.5 | -21.6 | -12.6 | -15.7 | -8.9 | -13.6 | -6.7 | -8.0 | -12.7 | -12.7 | -13.8 | -16.1 | -22.4 | -14.4 |
| Maximum | -21.4 | -17.4 | -20.6 | -11.4 | -14.3 | -7.2 | -12.1 | -5.4 | -6.4 | -10.9 | -11.3 | -12.5 | -14.5 | -18.5 | -13.1 |
| ME-C2(II) | | | | | | | | | | | | | | | |
| Mean | -22.3 | -18.1 | -21.4 | -12.4 | -15.3 | -8.6 | -14.7 | -6.4 | -7.5 | -12.0 | -12.9 | -13.2 | -15.5 | -20.6 | -14.0 |
| Std. Deviation | 0.8 | 0.5 | 0.5 | 0.7 | 0.3 | 1.0 | 0.4 | 1.0 | 1.3 | 0.5 | 0.4 | 0.7 | 0.7 | 0.4 | 0.7 |
| Range | 2.0 | 1.4 | 1.2 | 1.6 | 0.7 | 2.4 | 1.0 | 2.2 | 2.8 | 1.3 | 0.9 | 1.8 | 1.5 | 0.9 | 1.7 |
| Minimum | -23.1 | -18.6 | -21.8 | -12.9 | -15.8 | -9.5 | -15.2 | -7.5 | -8.6 | -12.5 | -13.3 | -13.9 | -16.0 | -20.9 | -14.5 |
| Maximum | -21.1 | -17.2 | -20.6 | -11.4 | -15.1 | -7.2 | -14.2 | -5.2 | -5.8 | -11.2 | -12.4 | -12.1 | -14.4 | -20.0 | -12.7 |
| M1-T28-C8 | | | | | | | | | | | | | | | |
| Mean | -21.6 | -17.6 | -20.9 | -11.5 | -14.5 | -10.3 | -15.3 | -4.3 | -7.7 | -12.4 | -12.4 | -12.2 | -14.7 | -19.6 | -13.6 |
| Std. Deviation | 0.4 | 0.5 | 0.3 | 0.6 | 0.7 | 0.6 | 0.9 | 1.0 | 1.1 | 0.5 | 0.8 | 0.4 | 0.3 | 0.7 | 0.4 |
| Range | 2.1 | 2.1 | 1.0 | 2.2 | 2.8 | 2.1 | 3.2 | 3.6 | 4.5 | 2.2 | 3.0 | 1.8 | 1.4 | 3.4 | 1.2 |
| Minimum | -22.6 | -18.3 | -21.4 | -12.5 | -16.0 | -11.3 | -17.2 | -6.0 | -9.7 | -13.7 | -13.7 | -13.3 | -15.6 | -21.1 | -14.2 |
| Maximum | -20.5 | -16.3 | -20.4 | -10.4 | -13.3 | -9.1 | -14.0 | -2.4 | -5.2 | -11.4 | -10.8 | -11.5 | -14.2 | -17.7 | -13.0 |
| CAM15A-T14(I) | | | | | | | | | | | | | | | |
| Mean | -24.1 | -20.0 | -23.1 | -13.9 | -17.1 | -9.6 | -18.2 | -8.5 | -9.6 | -14.8 | -14.8 | -15.1 | -17.0 | -22.6 | -16.0 |
| Std. Deviation | 0.5 | 0.5 | 0.4 | 0.9 | 0.4 | 0.8 | 0.8 | 0.8 | 0.9 | 0.4 | 0.5 | 0.4 | 0.5 | 0.8 | 0.3 |
| Range | 2.0 | 1.9 | 1.7 | 2.9 | 1.8 | 3.0 | 3.0 | 2.9 | 3.5 | 1.4 | 2.5 | 1.6 | 1.3 | 3.7 | 1.0 |
| Minimum | -25.0 | -21.2 | -24.0 | -15.5 | -17.9 | -11.0 | -20.0 | -10.0 | -11.6 | -15.4 | -16.0 | -15.9 | -17.8 | -24.8 | -16.5 |
| Maximum | -22.9 | -19.3 | -22.3 | -12.6 | -16.1 | -8.0 | -17.1 | -7.2 | -8.1 | -14.0 | -13.6 | -14.3 | -16.6 | -21.1 | -15.5 |
| CAM15A-T14(II) | | | | | | | | | | | | | | | |
| Mean | -24.4 | -19.9 | -23.1 | -13.9 | -17.2 | -8.8 | -18.2 | -8.0 | -9.9 | -14.5 | -14.7 | -15.3 | -16.9 | -22.9 | -15.8 |
| Std. Deviation | 0.8 | 0.6 | 0.6 | 0.9 | 0.4 | 0.7 | 1.1 | 1.0 | 0.8 | 0.6 | 0.5 | 0.6 | 0.7 | 0.7 | 0.5 |
| Range | 2.4 | 2.3 | 2.1 | 3.5 | 1.4 | 3.0 | 4.3 | 3.4 | 2.8 | 2.1 | 1.9 | 2.5 | 2.4 | 2.7 | 2.2 |
| Minimum | -25.6 | -21.0 | -24.3 | -15.8 | -17.9 | -10.4 | -20.4 | -9.3 | -11.2 | -15.7 | -15.5 | -16.5 | -18.0 | -24.2 | -16.8 |
| Maximum | -23.1 | -18.8 | -22.2 | -12.4 | -16.4 | -7.4 | -16.1 | -5.9 | -8.4 | -13.6 | -13.6 | -14.1 | -15.6 | -21.5 | -14.6 |
| QUI7-T13(I) | | | | | | | | | | | | | | | |
| Mean | -22.7 | -18.3 | -21.2 | -13.4 | -14.6 | -3.4 | -18.2 | -6.0 | -8.9 | -14.6 | -13.0 | -12.5 | -16.0 | -21.0 | -14.0 |
| Std. Deviation | 1.0 | 1.0 | 0.6 | 0.8 | 0.6 | 1.9 | 1.5 | 1.2 | 1.1 | 1.5 | 1.2 | 1.2 | 1.0 | 0.7 | 0.7 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Range | 3.0 | 3.7 | 1.9 | 2.8 | 2.4 | 5.4 | 5.6 | 4.1 | 3.3 | 4.4 | 4.6 | 3.9 | 3.5 | 2.3 | 2.3 |
| Minimum | -24.4 | -20.1 | -21.9 | -14.9 | -15.4 | -6.4 | -20.7 | -7.8 | -10.3 | -16.7 | -15.4 | -14.6 | -18.0 | -22.1 | -14.9 |
| Maximum | -21.4 | -16.4 | -20.1 | -12.2 | -13.1 | -1.1 | -15.1 | -3.7 | -7.1 | -12.2 | -10.8 | -10.7 | -14.6 | -19.8 | -12.6 |
| QUI7-T13(II) | | | | | | | | | | | | | | | |
| Mean | -22.2 | -18.5 | -21.2 | -12.4 | -15.5 | -7.9 | -16.8 | -5.8 | -8.3 | -13.3 | -13.3 | -12.7 | -15.5 | -19.7 | -14.2 |
| Std. Deviation | 0.8 | 0.8 | 0.9 | 0.8 | 0.8 | 0.8 | 1.5 | 1.3 | 1.2 | 1.3 | 1.4 | 0.9 | 0.8 | 1.1 | 0.9 |
| Range | 2.4 | 2.8 | 3.2 | 2.4 | 2.8 | 2.9 | 4.3 | 4.5 | 4.5 | 4.2 | 4.0 | 3.1 | 2.7 | 3.9 | 2.9 |
| Minimum | -23.4 | -20.0 | -22.8 | -13.4 | -17.0 | -9.4 | -19.2 | -8.2 | -11.2 | -15.6 | -15.1 | -14.4 | -16.8 | -21.6 | -15.7 |
| Maximum | -21.0 | -17.2 | -19.6 | -11.0 | -14.2 | -6.5 | -14.8 | -3.7 | -6.6 | -11.4 | -11.1 | -11.2 | -14.1 | -17.7 | -12.8 |
| AZ14-T31 | | | | | | | | | | | | | | | |
| Mean | -24.7 | -24.6 | -26.5 | -18.8 | -18.7 | -7.3 | -20.9 | -13.4 | -11.5 | -19.6 | -18.1 | -18.3 | -19.4 | -23.7 | -18.8 |
| Std. Deviation | 1.9 | 2.1 | 2.0 | 2.2 | 1.6 | 3.7 | 2.9 | 2.3 | 2.1 | 2.2 | 2.2 | 1.7 | 2.3 | 2.1 | 2.0 |
| Range | 5.4 | 6.8 | 6.0 | 7.6 | 5.3 | 12.2 | 9.5 | 7.2 | 7.7 | 7.2 | 6.7 | 7.4 | 7.0 | 6.3 | 5.5 |
| Minimum | -27.0 | -27.5 | -29.4 | -22.0 | -21.0 | -14.9 | -25.0 | -16.7 | -14.9 | -23.3 | -20.9 | -22.4 | -22.9 | -26.9 | -21.8 |
| Maximum | -21.6 | -20.7 | -23.4 | -14.4 | -15.8 | -2.7 | -15.5 | -9.5 | -7.3 | -16.1 | -14.2 | -15.0 | -15.8 | -20.6 | -16.2 |
| AZ115-T9 | | | | | | | | | | | | | | | |
| Mean | -22.6 | -23.7 | -24.7 | -17.9 | -19.4 | -9.3 | -18.5 | -10.2 | -9.9 | -16.5 | -14.3 | -14.3 | -17.3 | -21.2 | -16.7 |
| Std. Deviation | 1.9 | 2.3 | 2.3 | 2.3 | 1.7 | 2.7 | 4.1 | 3.3 | 2.2 | 3.4 | 4.1 | 2.9 | 2.8 | 2.2 | 2.6 |
| Range | 8.2 | 8.8 | 8.6 | 10.0 | 7.3 | 11.5 | 16.6 | 12.2 | 9.8 | 12.7 | 16.7 | 11.8 | 10.8 | 9.9 | 8.9 |
| Minimum | -26.5 | -28.2 | -28.8 | -22.6 | -22.2 | -15.0 | -27.1 | -15.6 | -15.0 | -21.9 | -22.1 | -19.9 | -22.1 | -25.5 | -21.1 |
| Maximum | -18.3 | -19.4 | -20.2 | -12.7 | -14.9 | -3.6 | -10.5 | -3.4 | -5.2 | -9.2 | -5.4 | -8.1 | -11.4 | -15.7 | -12.1 |
| CAM9-T39 | | | | | | | | | | | | | | | |
| Mean | -20.6 | -18.1 | -20.6 | -13.6 | -15.1 | -4.1 | -12.6 | -3.1 | -7.7 | -10.4 | -7.0 | -10.7 | -13.6 | -19.4 | -11.7 |
| Std. Deviation | 1.6 | 1.6 | 1.3 | 1.4 | 1.4 | 1.5 | 2.0 | 1.6 | 1.3 | 1.3 | 1.3 | 1.9 | 1.7 | 1.9 | 1.1 |
| Range | 5.0 | 5.2 | 4.3 | 5.3 | 4.2 | 6.1 | 7.1 | 6.3 | 5.0 | 6.0 | 5.4 | 6.7 | 6.9 | 6.9 | 3.6 |
| Minimum | -22.8 | -20.4 | -22.4 | -16.1 | -17.2 | -7.1 | -16.2 | -6.4 | -10.0 | -14.2 | -9.2 | -13.4 | -16.2 | -22.2 | -13.2 |
| Maximum | -17.8 | -15.2 | -18.1 | -10.8 | -13.0 | -1.0 | -9.1 | -0.1 | -5.1 | -8.2 | -3.9 | -6.7 | -9.3 | -15.3 | -9.6 |

[a]MB indicates calculated $\delta^{13}C$ Mass Balance values.

# Supporting Information 1

## Methods

### 3.1 LC/IRMS analysis

A single hair from each individual has been analyzed, with the exception of the CAM15A-T14, QUI7-T13 and ME-C2 individuals who have been sampled twice [(I), (II)]. The orientation and the distance from scalp of ME-C2 (I) and (II) hairs were unknown as this friable sample fragmented soon after sampling the mummy.

A single hair sample was selected from an oriented bundle of hair following the criteria of condition of preservation, presence of visible root and maximum length. Preservation was assessed qualitatively by visual examination of the external structure of the hair fiber using a portable microscope (Dino-Lite Handheld Digital Microscope USB, 20x-100x, AnMo Electronics Corporation). Further microscopic analyses [i.e. studies of microscopic characteristics under a high-magnification microscope in longitudinal or cross-sectional mount (Ogle and Fox, 1998; Oien, 2009)] were precluded as the samples were subject to quarantine restrictions and could not be removed from the laboratory.

Hairs were measured and cleaned superficially using non-electrostatic wipes soaked in ethanol (Merck KGaA). Each hair was sequentially cut using sterile blades into 0.5 cm segments from root to tip, with the exception of some tip ends that presented the last segment longer than half cm. Each segment was weighed and inserted into the hydrolysis tube. The samples were soaked in methanol (Merck KGaA) overnight to remove lipids and other organic residues. Hair segments were hydrolyzed under vacuum in amino acid-free 6M HCl (200µL) (Sigma-Aldrich) at 110°C until the hair dissolved. As a consequence of acid hydrolysis, asparagine and glutamine are deaminated to aspartic acid and glutamic acid. Moreover, methionine may be oxidized and cysteine difficult to detect (Fountoulakis and Lahm, 1998). After hydrolysis the samples were transferred into glass

vials and dried in a rotary vacuum concentrator (Martin Christ) overnight and stored in a freezer. Prior to LC/IRMS analysis, the samples were dissolved under sonication in Milli-Q water (Milli-Q® Advantage A10 Water Purification System, Merck Millipore) with the addition of an internal standard (10µL of a 1mmol solution of 2-aminoisobutyric acid, Sigma-Aldrich). Sample amounts were adjusted to contain approximately 0.7-1µg/µL of keratin hydrolysate for each injection to maximize the LC/IRMS output.

Mobile phases were made as follows: Phase A (1L) = 30µL of 1:50 Suprapur® 96% $H_2SO_4$ (Merck KGaA) in Milli-Q water; Phase B (1L) = 1mL Suprapur® 96% $H_2SO_4$ and 2.48g of ≥98% $K_3PO_4$ (Sigma-Aldrich) in Milli-Q water; Phase C (1L) = 3mL Suprapur® 96% $H_2SO_4$ in Milli-Q water. Reagents for the LC Isolink Interface consisted of an acid catalyst made of 82mL extra pure 89% $H_3PO_4$ (Merck KGaA) in 920mL of Milli-Q water, and an oxidant made of 5g of EMSURE® $Na_2S_2O_8$ (Merck KGaA) in 1L Milli-Q water. All the solutions were sonicated and degassed under vacuum for 1h. During analysis the regassing was prevented by helium sparging.

Laboratory analyses were conducted at the La Trobe Institute for Molecular Sciences (LIMS, La Trobe University, Melbourne, Australia). Separation and analysis of mummy hair keratin hydrolysates were carried out using a Thermo Scientific LC/IRMS system consisting of an Accela 600 pump connected to a Delta V Plus Isotope Ratio Mass Spectrometer via a Thermo Scientific LC Isolink. Individual amino acids in the sample mixture were separated and their carbon isotope content was measured using the three phase method described by Smith et al. (2009), but modified for a narrower column (Table I). The chromatographic separation of the amino acids was performed by a Primesep A column (2.1x250mm, 100Å, 5µm, SIELC Technologies Prospect Heights). Each sample analysis was preceded by an initial conditioning run of a mixture of B and C phases to prepare the column and avoid baseline fluctuations during the sample analytical run. The percentage of the two phases used in the conditioning run was assigned to improve the peak separation

depending on the pH of the mobile phases used. In our research the values varied between 85B:15C and 95B:5C (110µL/min) to avoid the co-elution of glutamic acid with serine or threonine.

**Table I.** *Gradient program for conditioning and analytical runs of liquid chromatography-isotope ratio mass spectrometer with Primesep A column (2.1x250mm, 100Å, 5µm).*

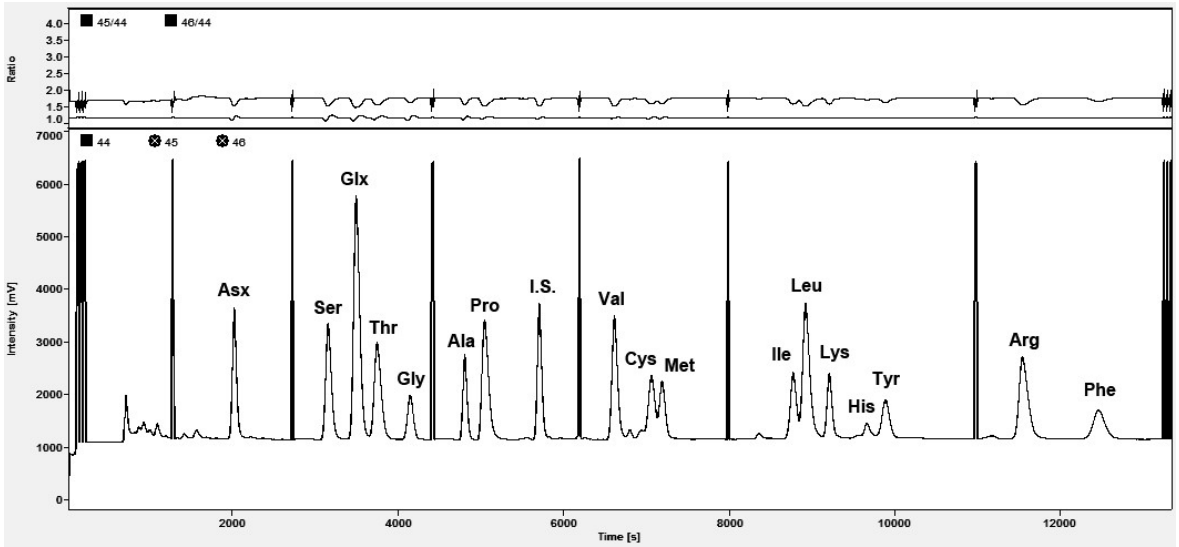
| **Time (min)** | **Phase A (%)** | **Phase B (%)** | **Phase C (%)** | **Flow rate (µL/min)** |
|---|---|---|---|---|
| Conditioning run | | | | |
| 0 | 0 | 90 | 10 | 110 |
| 35 | 0 | 90 | 10 | 110 |
| 36 | 100 | 0 | 0 | 110 |
| 55 | 100 | 0 | 0 | 110 |
| Analytical run | | | | |
| 0 | 100 | 0 | 0 | 60 |
| 45 | 100 | 0 | 0 | 60 |
| 65 | 60 | 40 | 0 | 60 |
| 75 | 40 | 25 | 35 | 80 |
| 140 | 0 | 0 | 100 | 80 |
| 180 | 0 | 0 | 100 | 80 |

A mixture made of standard amino acids (Asp, Hyp, Ser, Glu, Thr, Gly, Ala, Pro, 2-amino-isobutyric acid, Val, Met, Ile, Leu, Lys, His, Tyr, Arg, Phe) was analyzed after changes of mobile phases to check the quality of conditioning and analytical runs, and to monitor the precision on the measurement of standard amino acids, which was in all cases below 0.6‰. A sample volume of 15µL was injected (no waste injection mode) by an Accela autosampler onto the column at the beginning of the analytical run. The Primesep A column was maintained at room temperature. Subsequent to the chromatographic separation, the individual amino acids were oxidized by the two reagents (at 35µL/min flow rate each) through an oxidation reactor set at a constant temperature of 99.9˚C. The LC IsoLink interface separates the analyte $CO_2$ gas from the liquid phase by a Helium carrier gas counter flow (He 5.0, Coregas) that delivers the $CO_2$ to the mass spectrometer. The $\delta^{13}C$ values of each amino acid were measured using a Delta V Plus Isotope Ratio Mass Spectrometer. Measurements were made relative to an in-house laboratory $CO_2$ gas ($\delta^{13}C_{VPDB}$= –2.76‰) which

in turn was calibrated against the international standard USGS-40 L-Glutamic Acid ($\delta^{13}$CVPDB-LSVEC= –26.39±0.04‰) (U.S. Geological Survey, Reston, USA).

Reference gas pulses, lasting 20sec each, were set throughout the analytical run allowing drift-correction to be made. The amino acid peaks were assigned by retention time and were manually integrated and background subtracted using the ISODAT 2.0 software (Thermo Scientific). Non-protein components eluted first followed by the characteristic pattern of 17 amino acids in hair keratin (Fig. I). The co-elution of cysteine and methionine could not be avoided. For this reason, cysteine and methionine $\delta^{13}$C values were considered not applicable. The isotope compositions of histidine have been removed from any statistical analysis because they were missing in a high proportion of samples. As a result, ~ 88.1% in weight of the carbon present in hair keratin has been measured in this study. The three missing values for the variable tyrosine have been imputed using the "Visualization and Imputation of Missing values" package (VIM) (Templ et al., 2011a; Templ et al., 2011b) of R software (R Core Team, 2014).

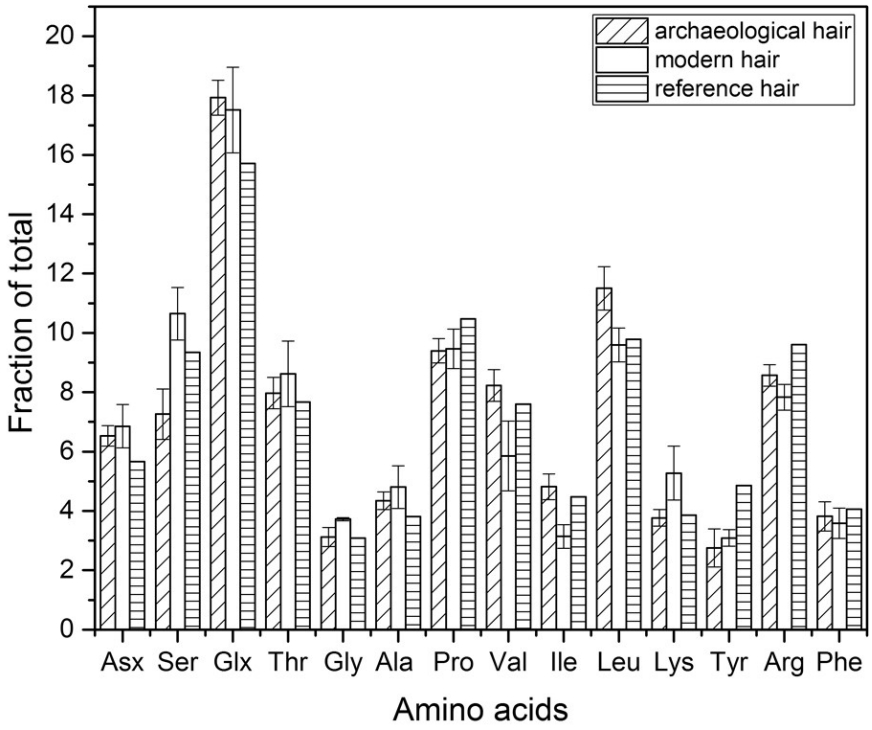
***Fig. I.*** *LC-IRMS chromatogram of keratin hydrolysate of archaeological human hair sample (0.5 cm segment) from CAM15A-T14 (II) individual. Single amino acid and internal standard (I.S.) and reference gas pulses are displayed.*



The quality of hair preservation at the molecular level was assessed by comparing the amino acid composition of archaeological hair to that of modern hair and to the amino acid profiles of human hair published in Robbins and Kelly (1970) and Wolfram (2003) (Fig. II). Modern hairs were taken from two of the authors (AM, CS), and prepared following the same steps described above for LC/IRMS analysis, with the addition of an initial soaking in methanol : chloroform (2:1 v/v) (Merck KGaA) to remove any sebum lipids or detergent residues as in O'Connell and Hedges (1999). Modern and archaeological hair samples were analyzed under similar chromatographic conditions. The amino acid peak area values [Vs], which represent the sum of the peak areas for the ion currents at *m/z* 44, 45 and 46, were converted in fractions of total (%) in order to make comparable chromatographic runs with differential absolute intensities. For the reference human hair, the amino acid carbon weights (%) were calculated from the amino acid residues (micromoles/gram) and then converted to fractions of total (%).

By comparison of amino acid carbon profiles expressed in fractions of total (%), it appears that three segments (1.5cm, 4cm, 5cm) of CAM15A-T14 (I) hair had different amino acid carbon content induced by either unsuccessful laboratory procedure (hydrolysis, chromatography) or degradation processes. These three hair segments have been rejected from further discussions. The remaining archaeological hair samples present amino acid profiles similar to those of modern samples indicating good keratin preservation.

***Fig. II.*** *Fractions of total (%) of amino acid peak areas measured in archaeological and modern hair (mean ± sd, 1σ) and of amino acid carbon weights (%) from human hair of reference.*




The major limitation of our study is that the limited amount of keratin hydrolysate did not allow samples to be run in duplicate. When using hair length as a parameter, we found that this provided only enough for one measurement in most cases, although thicker hairs may provide enough sample for duplicates. The high temporal resolution in this study comes at the cost of repeatability of the measurements.

### 3.2 Bulk isotope analysis

A bundle of hair from each individual was selected following the criterion of maximum length. The multiple hairs were aligned at the root and secured with aluminium foil bands. The orientation of the bundle was marked. Samples were immersed in methanol : chloroform (2:1 v/v), sonicated for

about 20 min, and soaked overnight to remove lipids and contaminants. After two sonications (20 min each), the solution was inspected. If transparent, the bundle of hair was rinsed at least three times in MilliQ water sonicating for about 20 min each time; otherwise the methanol : chloroform mixture was replaced until cleaning was complete. Once the water was discarded, samples were left in fume hood to dry. Samples were frozen, and subsequently freeze-dried for 12h. Then the hair sample was separated into two bundles, one for carbon-nitrogen isotope analysis and one for sulfur isotope analysis. Each hair bundle was sectioned into approximately 1 cm segments from root to tip and weighed into tin capsules for isotope analyses, with vanadium pentoxide added as catalyzer in samples for sulfur isotope analysis. When incremental sampling was not applicable (ME-C2, M1-T28-C8), the non-oriented samples were analyzed in duplicate. Analysis of the carbon, nitrogen and sulfur isotope compositions was performed at the University of Bradford (Department of Archaeological Sciences, UK) using a Thermo Flash EA 1112 coupled to a Delta Plus XL via a Conflo III interface (Thermo Scientific, Bremen, Germany). The $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ values are reported relative to international standards: V-PDB, AIR and V-CDT, respectively. International and laboratory standards used as secondary (reference) materials to monitor analytical precision and accuracy were IAEA-CH-6 (accepted –10.45±0.03‰), IAEA-CH-3 (accepted –24.72±0.04‰), IAEA-600 (accepted –27.77±0.04‰), Fish gel (accepted –15.52‰), Bovine Liver Standard (accepted –21.59±0.25‰) for $\delta^{13}C$ measurements; IAEA-600 (accepted +1.0±0.2‰), IAEA-N-1 (accepted +0.4±0.2‰), IAEA-N-2 (accepted +20.3±0.2‰), Fish gel (accepted +14.45‰), Bovine Liver Standard (accepted +7.65±0.25‰) for $\delta^{15}N$ measurements; NBS-127 (accepted +20.3‰), IAEA-S-1 (accepted –0.30‰), Methionine (accepted +12.6‰) for $\delta^{34}S$ measurements. Accuracy was < ±0.2‰ for both $\delta^{13}C$ and $\delta^{15}N$, and < ±0.8‰ for $\delta^{34}S$. Reproducibility (1σ) was ±0.2‰ for $\delta^{15}N$, < ±0.2‰ for $\delta^{13}C$, and ±0.9‰ for $\delta^{34}S$.

### 3.2.1 Stable isotope mixing model

We chose to apply a Bayesian mixing model to our bulk isotope data in order to better quantify the contribution of food sources to the diets of the individuals analyzed. In particular, we preferred FRUITS (Food Reconstruction Using Isotopic Transferred Signals) (Fernandes et al., 2014) as it incorporates concentration dependence and isotopic routing in the model. When dealing with diets of omnivores, as the ones studied here, food sources may present different elemental concentrations (e.g. lower [N] in plants than in meat), which will affect the contribution of the sources to the consumer's diet resulting in over- or under- estimates of certain sources, if not taken into account (Phillips and Koch, 2002). Furthermore, the effect of isotopic routing needs to be tackled when the diet consists of foods that are very different in terms of protein, carbohydrate and lipid content (Phillips et al., 2014) (e.g. marine/maize mixed diet), because the three macronutrients will differentially contribute to a specific dietary proxy (Fernandes et al., 2014), such as $\delta^{13}C$ hair keratin. How the carbon from the dietary macronutrients (proteins, carbohydrates and lipids) is routed to the body tissue (directly or through various metabolic pathways) should be considered in the model (Phillips et al., 2014).

In the FRUITS model, carbon, nitrogen and sulfur isotope compositions measured for the hair segments of individuals were used as dietary proxies. At the dietary proxies ($\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$) an uncertainty of 0.5‰ for carbon and nitrogen isotope compositions, and 1‰ for sulfur isotope compositions was used in the model to account for analytical uncertainty. Each hair segment was treated as single individual, thus providing 40 consumers in our model. It was also assumed that the $\delta^{13}C$, $\delta^{15}N$ and the $\delta^{34}S$ values are in phase (i.e. representing the same time period), despite being measured from two different hair bundles, as the sampling of hair bundles with a minimum of 25 hairs increases the likelihood of measuring the isotope signature of growing hair (Mekota et al., 2006). The missing values of $\delta^{34}S$ for CAM15A-T14, QUI7-T13, AZ14-T31 and AZ115-T9 individuals, due to shorter hair bundles for sulfur isotope analysis, were replaced by averages. We

justify this estimation because the aforementioned individuals present consistent marine or terrestrial diets, which are the main controlling factors on sulfur isotope compositions in this system.

The food groups were set in order to include all the viable food sources and combining the ones not significantly different in their isotope compositions (Phillips et al., 2014). The mean $\delta^{13}C$, $\delta^{15}N$ values (±SEM, standard error of the mean) for the edible portion of plants or for the collagen of animals comprising the four groupings of foodstuffs used in the model were: –24.7±0.3‰ and +4.1±0.6‰ for "C3 plants" ($C_3$ plants and legumes, n=21), –10.3±0.4‰ and +8.1±1.2‰ for "C4 plants" (n=8), –15.7±0.2 ‰ and +6.9±0.1‰ for "terrestrial fauna" (domestic and wild camelids, rodents and birds, n=236), and –12.5±0.3‰ and +17.2±0.5‰ for "marine fauna" (sea lions, marine fish, crustaceans and mollusks, n=24). The $\delta^{34}S$ values for "C3 plants", "C4 plants" and "marine fauna" food groups are taken from Macko et al. (1999), while the $\delta^{34}S$ value representative for the terrestrial animals was assumed to be close to the $\delta^{34}S$ values of terrestrial plants, having the same geographical origin. An isotope offset of –2‰ and +0.5‰ was applied to $\delta^{13}C$ values of plants for estimating respectively protein and carbohydrate isotope compositions (Fernandes et al., 2015). Collagen $\delta^{13}C$ values of terrestrial animals were adjusted for isotope offsets of –2‰ for protein, and –8‰ for lipids (Fernandes et al., 2015). Collagen $\delta^{13}C$ values of marine animals were adjusted for isotope offsets of –1‰ for protein, and –7‰ for lipids (Fernandes et al., 2015). For all animals, the offset between collagen $\delta^{15}N$ values and protein was set at +2‰ (Fernandes et al., 2015). An uncertainty of 1‰ was been applied to bulk and estimated isotope compositions.

The concentration of protein, lipid and carbohydrate for each food group was estimated by retrieving macronutrient composition of reference foods from the Nutrient Database of the United States Department of Agriculture (USDA Food Composition Databases), with the exception of alpaca and llama meats (Cristofanelli et al., 2004; Polidori et al., 2010). The foodstuffs used for calculations were: maize, amaranth grain for "C4 plants"; potato, quinoa, squash, pepper, Lima

beans, lupin for “C3 plants”; game meat of deer, antelope and beaver, goose, alpaca, llama for “terrestrial fauna”; sea lion, eel, herring, anchovy, sardine, cod, drum, squid, abalone, clam, mackerel for “marine fauna”. The reported values of macronutrients (g/100g) were adjusted for the carbon content considering 52% of C for protein, 75% for lipid and 45% for carbohydrate (Newsome et al., 2004). In the FRUITS model the contribution of lipids and carbohydrates are then entered combined as Energy (Fernandes et al., 2014) (Table II).

***Table II***. *Isotope compositions and concentrations of the food fractions for each food group provided as source input for FRUITS.*

| Food group | Food fraction | $\delta^{13}C$ | $\delta^{15}N$ | $\delta^{34}S$ | Conc. |
|---|---|---|---|---|---|
| C3 plants | Bulk | | | +6.8±1‰ | 100 |
| | Protein | −26.7±1‰ | +4.1±1‰ | | 23±2.5 |
| | Energy[a] | −24.2±1‰ | | | 77±2.5 |
| C4 plants | Bulk | | | +6.1±1‰ | 100 |
| | Protein | −12.3±1‰ | +8.1±1‰ | | 14±2.5 |
| | Energy[a] | −9.8±1‰ | | | 86±2.5 |
| Terr. Fauna | Bulk | | | +6.4±1‰ | 100 |
| | Protein | −17.7±1‰ | +8.9±1‰ | | 78±2.5 |
| | Energy[a] | −23.7±1‰ | | | 22±2.5 |
| Marine Fauna | Bulk | | | +15.6±1‰ | 100 |
| | Protein | −13.5±1‰ | +19.2±1‰ | | 68±2.5 |
| | Energy[a] | −19.5±1‰ | | | 32±2.5 |

[a]Energy combines the contribution of lipids and carbohydrates.

It has been statistically estimated by Fernandes et al. (2012) that the carbon in bone collagen is routed mostly from dietary protein (74±4%), but also from carbohydrates and lipids (26%). The carbon contribution from energetic macronutrients (carbohydrates + lipids) is thought to be mainly (but not only) driven by three non-essential amino acids, namely alanine, serine and glycine (Fernandes et al., 2012), which are synthesized from pyruvate or other three-carbon glycolytic intermediates, derived from glucose (Brosnan and Brosnan, 2013). Despite the numerous nutritional and metabolic studies on human hair [e.g. (Choy et al., 2013; Hedges et al., 2009; Petzke and

Lemke, 2009)], a clear model for carbon routing in hair keratin has not yet been proposed. Hereafter we will assume a contribution of 14% for $\delta^{13}C$ hair keratin from carbohydrate and lipid carbon, considering that the combined amino acid carbon weight (%) for alanine, serine and glycine in the human hair keratin is 14.1% (Robbins and Kelly, 1970; Wolfram, 2003). We are aware of the large differences between the two tissues, bone collagen and hair keratin, with respect to amino acid composition and physiology; for these reasons we applied a conservative uncertainty of 5% to our estimation.

The diet-to-keratin offsets used in this model are 3.9±0.8‰ for $\delta^{13}C$ and 4.9±0.8‰ for $\delta^{15}N$, which are based on past and recent estimations from controlled dietary studies of contemporary human populations (Hedges et al., 2009; Naito et al., 2015; O'Connell et al., 2012; Yoshinaga et al., 1996). Conversely, the fractionation of sulfur isotopes between diet and keratin is thought to be minimal (Nehlich, 2015; Richards et al., 2003). Herein, the diet-to-keratin $\delta^{34}S$ offset is set at 0, but with an associated uncertainty of 1‰ to account for possible variability resulting from nutritionally different diets (Richards et al., 2003).

The dietary estimates generated by the FRUITS model show an excessively high caloric contribution from protein for the coastal individuals (59±3.3%), whose source is mostly (85±3.6%) marine fauna. Although it has already been reported for Chinchorros that they had high-protein diets, made mainly of marine (80%) and terrestrial (12%) meat (Aufderheide, 1996), it is widely recognized that protein intake should be kept under a certain limit in order to prevent protein toxicity (Cordain et al., 2000). Therefore, a protein carbon contribution lower than 45% has been included in the original model as *a priori* constraint (Fernandes et al., 2014; Otten et al., 2006).

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**Supporting Information 2.** Estimates from FRUITS Bayesian mixing model for the bulk isotope compositions of human hair analyzed in this study.

| Individual | **ME-C2** | | **M1-T28-C8** | | **CAM15A-T14** | | | | | | | **QUI7-T13** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cut (cm) | | | | | 0-1 | 1-2 | 2-3 | 3-4 | 4-5 | | | 0-1 | 1-2 | 2-3 | 3-4 | 4-5 | 5-6 | 6-7 | | |
| **Food (%)** | Mean | SD | Mean | SD | | | | | | Mean | SD | | | | | | | | Mean | SD |
| C3 plants | 22 | 12 | 26 | 12 | 39 | 39 | 38 | 37 | 38 | 38 | 1 | 32 | 36 | 39 | 40 | 42 | 43 | 41 | 39 | 4 |
| C4 plants | 25 | 11 | 24 | 11 | 10 | 11 | 11 | 12 | 11 | 11 | 1 | 18 | 13 | 12 | 9 | 9 | 8 | 10 | 11 | 3 |
| Terrestrial Fauna | 2 | 2 | 3 | 2 | 1 | 2 | 2 | 1 | 1 | 1 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 1 |
| Marine Fauna | 51 | 4 | 48 | 4 | 49 | 49 | 49 | 50 | 49 | 49 | 1 | 49 | 49 | 47 | 49 | 46 | 47 | 47 | 48 | 1 |
| **Food fractions (%)** | | | | | | | | | | | | | | | | | | | | |
| Protein | 44 | 1 | 43 | 1 | 44 | 44 | 44 | 44 | 44 | 44 | 1 | 44 | 44 | 44 | 44 | 43 | 44 | 44 | 44 | 1 |
| Energy | 56 | 1 | 57 | 1 | 56 | 56 | 56 | 56 | 56 | 56 | 1 | 56 | 56 | 56 | 56 | 57 | 56 | 56 | 56 | 1 |
| **Dietary proxies (Food)(%)** | | | | | | | | | | | | | | | | | | | | |
| $\delta^{13}$C (C3 plants) | 15 | 8 | 17 | 8 | 26 | 25 | 25 | 24 | 25 | 25 | 1 | 21 | 24 | 26 | 27 | 28 | 29 | 27 | 26 | 3 |
| $\delta^{13}$C (C4 plants) | 13 | 6 | 13 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 1 | 9 | 7 | 6 | 5 | 5 | 4 | 5 | 6 | 2 |
| $\delta^{13}$C (Terr. Fauna) | 3 | 3 | 4 | 4 | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 3 | 2 | 3 | 2 | 3 | 3 | 3 | 3 | 1 |
| $\delta^{13}$C (Marine Fauna) | 70 | 5 | 66 | 5 | 67 | 66 | 67 | 68 | 67 | 67 | 1 | 67 | 67 | 65 | 66 | 64 | 64 | 65 | 65 | 1 |
| $\delta^{15}$N (C3 plants) | 11 | 6 | 13 | 6 | 19 | 19 | 19 | 18 | 19 | 19 | 1 | 16 | 18 | 20 | 20 | 21 | 21 | 21 | 20 | 2 |
| $\delta^{15}$N (C4 plants) | 7 | 4 | 7 | 4 | 3 | 3 | 3 | 4 | 3 | 3 | 1 | 5 | 4 | 4 | 3 | 3 | 3 | 3 | 3 | 1 |
| $\delta^{15}$N (Terr. Fauna) | 3 | 3 | 5 | 4 | 2 | 3 | 3 | 2 | 3 | 3 | 1 | 3 | 3 | 3 | 3 | 4 | 3 | 3 | 3 | 1 |
| $\delta^{15}$N (Marine Fauna) | 79 | 4 | 75 | 5 | 75 | 75 | 75 | 76 | 75 | 75 | 1 | 76 | 76 | 73 | 74 | 72 | 73 | 73 | 74 | 1 |

| Individual | **AZ14-T31** | | | | | | | | | | **AZ115-T9** | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cut (cm) | 0-1 | 1-2 | 2-3 | 3-4 | 4-5 | 5-6 | 6-7 | 7-8 | | | 0-1 | 1-2 | 2-3 | 3-4 | 4-5 | 8-9 | 9-10 | 10-11 | 11-12 | 12-13 | 13-14 | | |
| **Food (%)** | | | | | | | | | Mean | SD | | | | | | | | | | | | Mean | SD |
| C3 plants | 78 | 78 | 76 | 70 | 70 | 78 | 80 | 81 | 76 | 4 | 49 | 50 | 59 | 59 | 58 | 58 | 42 | 50 | 52 | 53 | 52 | 53 | 5 |
| C4 plants | 10 | 11 | 14 | 18 | 16 | 12 | 11 | 8 | 12 | 3 | 25 | 22 | 14 | 13 | 15 | 16 | 29 | 22 | 20 | 21 | 20 | 20 | 5 |
| Terrestrial Fauna | 9 | 8 | 8 | 10 | 10 | 7 | 7 | 7 | 8 | 1 | 19 | 18 | 16 | 16 | 17 | 16 | 20 | 19 | 19 | 19 | 19 | 18 | 2 |
| Marine Fauna | 4 | 3 | 2 | 2 | 3 | 3 | 2 | 4 | 3 | 1 | 8 | 10 | 11 | 12 | 10 | 10 | 9 | 10 | 9 | 8 | 8 | 10 | 1 |
| **Food fractions (%)** | | | | | | | | | | | | | | | | | | | | | | | |
| Protein | 29 | 28 | 27 | 28 | 29 | 27 | 27 | 28 | 28 | 1 | 34 | 36 | 35 | 36 | 36 | 35 | 36 | 36 | 36 | 35 | 36 | 36 | 1 |
| Energy | 71 | 72 | 73 | 72 | 71 | 73 | 73 | 72 | 72 | 1 | 66 | 64 | 65 | 64 | 64 | 65 | 64 | 64 | 64 | 65 | 64 | 64 | 1 |

| **Dietary proxies (Food)(%)** | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $\delta^{13}$C (C3 plants) | 70 | 71 | 70 | 64 | 63 | 72 | 74 | 73 | 70 | 4 | 39 | 39 | 47 | 47 | 45 | 45 | 32 | 39 | 40 | 42 | 41 | 41 | 4 |
| $\delta^{13}$C (C4 plants) | 7 | 8 | 10 | 13 | 12 | 9 | 8 | 5 | 9 | 3 | 16 | 14 | 9 | 8 | 10 | 10 | 18 | 14 | 12 | 13 | 13 | 12 | 3 |
| $\delta^{13}$C (Terr. Fauna) | 17 | 16 | 16 | 19 | 20 | 14 | 13 | 14 | 16 | 2 | 32 | 32 | 27 | 26 | 29 | 28 | 34 | 32 | 33 | 32 | 33 | 31 | 3 |
| $\delta^{13}$C (Marine Fauna) | 6 | 5 | 4 | 4 | 6 | 5 | 4 | 7 | 5 | 1 | 12 | 16 | 17 | 19 | 16 | 17 | 15 | 15 | 15 | 12 | 14 | 15 | 2 |
| $\delta^{15}$N (C3 plants) | 64 | 67 | 66 | 60 | 59 | 68 | 70 | 68 | 65 | 4 | 34 | 33 | 40 | 40 | 39 | 39 | 28 | 33 | 34 | 37 | 35 | 36 | 4 |
| $\delta^{15}$N (C4 plants) | 5 | 6 | 7 | 10 | 8 | 6 | 6 | 4 | 7 | 2 | 11 | 9 | 6 | 6 | 6 | 7 | 12 | 9 | 8 | 9 | 9 | 8 | 2 |
| $\delta^{15}$N (Terr. Fauna) | 22 | 21 | 21 | 24 | 25 | 19 | 18 | 18 | 21 | 3 | 39 | 38 | 33 | 32 | 35 | 34 | 42 | 39 | 40 | 39 | 40 | 37 | 3 |
| $\delta^{15}$N (Marine Fauna) | 8 | 6 | 6 | 6 | 8 | 7 | 6 | 10 | 7 | 1 | 15 | 19 | 21 | 22 | 20 | 21 | 18 | 19 | 18 | 15 | 16 | 19 | 2 |

| Individual | **CAM9-T39** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Cut (cm) | 0-1 | 1-2 | 2-3 | 3-4 | 4-5 | 5-6 | 6-7 | | |
| **Food (%)** | | | | | | | | Mean | SD |
| C3 plants | 6 | 6 | 8 | 7 | 8 | 7 | 8 | 7 | 1 |
| C4 plants | 48 | 49 | 39 | 40 | 39 | 39 | 39 | 42 | 5 |
| Terrestrial Fauna | 3 | 3 | 2 | 2 | 2 | 2 | 2 | 2 | 1 |
| Marine Fauna | 43 | 42 | 51 | 51 | 52 | 52 | 51 | 49 | 4 |
| **Food fractions (%)** | | | | | | | | | |
| Protein | 39 | 38 | 43 | 43 | 43 | 43 | 43 | 42 | 2 |
| Energy | 61 | 62 | 57 | 57 | 57 | 57 | 57 | 58 | 2 |
| **Dietary proxies (Food)(%)** | | | | | | | | | |
| $\delta^{13}$C (C3 plants) | 4 | 4 | 6 | 5 | 5 | 5 | 5 | 5 | 1 |
| $\delta^{13}$C (C4 plants) | 27 | 28 | 20 | 20 | 20 | 20 | 20 | 22 | 4 |
| $\delta^{13}$C (Terr. Fauna) | 4 | 5 | 3 | 3 | 3 | 3 | 3 | 3 | 1 |
| $\delta^{13}$C (Marine Fauna) | 65 | 63 | 72 | 72 | 72 | 73 | 72 | 70 | 4 |
| $\delta^{15}$N (C3 plants) | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 1 |
| $\delta^{15}$N (C4 plants) | 16 | 17 | 11 | 12 | 11 | 11 | 11 | 13 | 2 |
| $\delta^{15}$N (Terr. Fauna) | 5 | 6 | 3 | 3 | 3 | 3 | 3 | 4 | 1 |
| $\delta^{15}$N (Marine Fauna) | 75 | 74 | 81 | 82 | 82 | 82 | 81 | 79 | 3 |

The means and standard deviations for ME-C2 and M1-T28-C8 individuals are the estimates generated by the FRUITS model. The means and standard deviations for CAM15A-T14, QUI7-T13, AZ14-T31, AZ115-T9, and CAM9-T39 are calculated on the means estimated by the FRUITS model for the various hair segments.

**Supporting Information 3.** Individual amino acid $\delta^{13}C$ values for 0.5 cm increments of single hair.

| Individual | Dist. *cm* | $\delta^{13}C$ Asx | $\delta^{13}C$ Ser | $\delta^{13}C$ Glx | $\delta^{13}C$ Thr* | $\delta^{13}C$ Gly | $\delta^{13}C$ Ala | $\delta^{13}C$ Pro | $\delta^{13}C$ Val* | $\delta^{13}C$ Ile* | $\delta^{13}C$ Leu* | $\delta^{13}C$ Lys* | $\delta^{13}C$ Tyr | $\delta^{13}C$ Arg | $\delta^{13}C$ Phe* | $\delta^{13}C$ Mass Balance |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **ME-C2(I)** | 0.5 | -12.73 | -6.71 | -12.73 | -8.04 | -8.04 | -13.42 | -13.79 | -18.49 | -12.36 | -21.63 | -15.67 | -22.38 | -16.14 | -23.53 | -14.37 |
| **ME-C2(I)** | 1 | -12.04 | -5.39 | -12.48 | -7.16 | -6.36 | -13.62 | -13.38 | -18.17 | -12.60 | -20.58 | -14.58 | -20.24 | -15.39 | -22.36 | -13.67 |
| **ME-C2(I)** | 1.5 | -11.90 | -5.84 | -12.74 | -8.87 | -8.01 | -12.09 | -13.09 | -18.04 | -11.78 | -21.19 | -14.95 | -20.04 | -14.97 | -21.80 | -13.74 |
| **ME-C2(I)** | 2 | -11.93 | -5.82 | -11.96 | -8.92 | -7.59 | -13.03 | -12.45 | -17.64 | -11.87 | -21.07 | -15.01 | -20.83 | -14.73 | -21.44 | -13.56 |
| **ME-C2(I)** | 2.5 | -10.93 | -5.84 | -11.31 | -7.55 | -6.90 | -12.80 | -12.71 | -17.40 | -11.37 | -20.81 | -14.26 | -18.53 | -14.52 | -21.53 | -13.08 |
| **ME-C2(II)** | 0.5 | -11.23 | -7.45 | -12.44 | -7.98 | -8.38 | -14.98 | -13.85 | -18.62 | -12.80 | -21.81 | -15.18 | -20.89 | -15.96 | -22.81 | -14.29 |
| **ME-C2(II)** | 1 | -11.84 | -6.59 | -13.30 | -8.85 | -8.48 | -14.22 | -12.85 | -18.22 | -12.94 | -21.66 | -15.78 | -20.20 | -15.93 | -23.09 | -14.30 |
| **ME-C2(II)** | 1.5 | -12.49 | -7.19 | -13.22 | -9.33 | -8.59 | -15.19 | -13.66 | -18.37 | -12.89 | -21.45 | -15.11 | -20.87 | -15.75 | -22.31 | -14.45 |
| **ME-C2(II)** | 2 | -12.52 | -5.67 | -12.66 | -9.53 | -6.39 | -14.65 | -13.54 | -18.23 | -11.94 | -21.34 | -15.05 | -20.87 | -15.18 | -21.96 | -14.03 |
| **ME-C2(II)** | 2.5 | -11.86 | -5.23 | -12.65 | -7.15 | -5.75 | -14.24 | -12.07 | -17.19 | -11.36 | -20.58 | -15.46 |  | -14.43 | -21.14 | -12.71 |
| **M1-T28-C8** | 0.5 | -12.49 | -5.42 | -12.44 | -10.62 | -8.42 | -16.44 | -12.46 | -17.60 | -11.92 | -21.02 | -15.11 | -19.50 | -14.43 | -22.18 | -13.88 |
| **M1-T28-C8** | 1 | -11.95 | -4.39 | -12.30 | -9.45 | -6.61 | -14.97 | -11.75 | -17.12 | -11.52 | -20.65 | -14.34 | -19.30 | -14.55 | -21.28 | -13.30 |
| **M1-T28-C8** | 1.5 | -11.93 | -3.52 | -11.83 | -9.37 | -7.54 | -14.61 | -12.16 | -16.28 | -12.17 | -20.75 | -15.29 | -20.30 | -14.54 | -21.70 | -13.25 |
| **M1-T28-C8** | 2 | -12.36 | -3.15 | -12.43 | -9.93 | -7.87 | -14.66 | -12.67 | -17.43 | -12.08 | -21.03 | -16.02 | -20.63 | -15.56 | -22.25 | -13.72 |
| **M1-T28-C8** | 2.5 | -12.27 | -4.33 | -12.14 | -10.33 | -8.20 | -14.13 | -11.94 | -17.58 | -11.96 | -21.26 | -14.42 | -20.52 | -15.19 | -22.56 | -13.67 |
| **M1-T28-C8** | 3 | -12.90 | -4.04 | -11.99 | -10.14 | -9.32 | -15.12 | -13.25 | -17.83 | -11.77 | -21.08 | -14.60 | -19.29 | -15.04 | -21.82 | -13.82 |
| **M1-T28-C8** | 3.5 | -12.26 | -3.46 | -12.03 | -10.96 | -7.93 | -14.74 | -12.20 | -17.31 | -11.70 | -21.05 | -15.57 | -20.38 | -15.05 | -21.23 | -13.58 |
| **M1-T28-C8** | 4 | -11.48 | -3.97 | -10.75 | -9.93 | -6.26 | -14.48 | -11.48 | -16.49 | -10.94 | -20.81 | -14.48 | -18.86 | -14.84 | -21.98 | -13.01 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **M1-T28-C8** | 4.5 | -11.85 | -3.35 | -11.25 | -10.88 | -7.40 | -14.02 | -11.90 | -17.49 | -10.92 | -20.89 | -14.13 | -18.96 | -14.46 | -21.34 | -13.20 |
| **M1-T28-C8** | 5 | -11.43 | -2.65 | -11.30 | -9.13 | -6.40 | -14.05 | -12.26 | -17.37 | -12.03 | -20.84 | -14.58 | -19.10 | -14.68 | -21.82 | -13.09 |
| **M1-T28-C8** | 5.5 | -11.99 | -2.35 | -11.57 | -10.71 | -6.22 | -14.54 | -11.65 | -16.98 | -10.99 | -20.81 | -14.29 | -19.51 | -14.32 | -21.45 | -13.09 |
| **M1-T28-C8** | 6 | -12.31 | -4.80 | -11.84 | -11.07 | -7.47 | -15.09 | -11.82 | -17.55 | -10.47 | -20.43 | -13.26 | -19.58 | -14.61 | -21.07 | -13.37 |
| **M1-T28-C8** | 6.5 | -12.45 | -2.63 | -11.76 | -10.06 | -7.76 | -14.50 | -12.05 | -17.52 | -10.35 | -20.44 | -13.43 | -19.44 | -14.25 | -21.72 | -13.07 |
| **M1-T28-C8** | 7 | -12.87 | -5.99 | -13.15 | -11.25 | -7.10 | -15.79 | -12.21 | -18.03 | -12.51 | -21.20 | -15.63 | -20.45 | -14.67 | -21.41 | -14.16 |
| **M1-T28-C8** | 7.5 | -12.48 | -4.72 | -13.21 | -11.08 | -7.63 | -16.03 | -11.94 | -18.06 | -12.20 | -21.07 | -14.27 | -19.20 | -14.72 | -22.10 | -13.89 |
| **M1-T28-C8** | 8 | -12.53 | -5.47 | -13.73 | -9.21 | -9.69 | -16.41 | -12.98 | -18.33 | -11.75 | -21.43 | -14.69 | -19.55 | -14.74 | -22.13 | -14.24 |
| **M1-T28-C8** | 8.5 | -12.77 | -4.98 | -12.77 | -10.43 | -9.40 | -16.28 | -12.36 | -18.26 | -10.80 | -21.22 | -15.03 | -21.06 | -14.99 | -21.45 | -14.01 |
| **M1-T28-C8** | 9 | -12.55 | -4.72 | -12.50 | -9.86 | -9.25 | -15.51 | -12.05 | -18.12 | -10.91 | -21.01 | -14.30 | -20.27 | -14.92 | -21.49 | -13.72 |
| **M1-T28-C8** | 9.5 | -12.60 | -4.97 | -12.68 | -10.15 | -8.52 | -15.35 | -11.90 | -17.61 | -11.88 | -21.21 | -14.41 | -19.56 | -14.96 | -21.54 | -13.77 |
| **M1-T28-C8** | 10 | -12.52 | -4.47 | -13.29 | -10.16 | -8.24 | -16.44 | -12.53 | -18.33 | -11.61 | -21.13 | -14.04 | -20.06 | -15.01 | -21.68 | -13.96 |
| **M1-T28-C8** | 10.5 | -13.66 | -5.01 | -13.58 | -10.81 | -8.58 | -17.24 | -12.17 | -17.69 | -11.36 | -20.69 | -14.39 | -18.88 | -14.57 | -21.90 | -14.06 |
| **M1-T28-C8** | 11 | -12.71 | -5.82 | -13.42 | -10.56 | -7.62 | -16.47 | -12.71 | -17.90 | -11.02 | -20.86 | -14.51 | -19.61 | -14.65 | -21.21 | -14.07 |
| **M1-T28-C8** | 11.5 | -12.96 | -5.78 | -12.65 | -10.57 | -7.05 | -14.79 | -12.15 | -17.77 | -10.90 | -20.82 | -14.05 | -19.41 | -14.77 | -21.39 | -13.79 |
| **M1-T28-C8** | 12 | -12.17 | -4.33 | -11.92 | -10.11 | -5.15 | -14.53 | -12.20 | -17.27 | -11.23 | -20.66 | -13.76 | -17.69 | -14.21 | -20.50 | -13.18 |
| **CAM15A-T14(I)** | 0.5 | -14.90 | -9.29 | -15.37 | -9.14 | -11.62 | -19.07 | -15.94 | -20.39 | -14.41 | -23.78 | -16.83 | -22.85 | -17.84 | -24.57 | -16.48 |
| **CAM15A-T14(I)** | 1 | -15.44 | -9.11 | -13.58 | -9.08 | -9.83 | -18.01 | -14.87 | -19.61 | -13.90 | -22.30 | -16.07 | -22.88 | -16.64 | -24.06 | -15.73 |
| **CAM15A-T14(I)** | 2 | -14.93 | -7.21 | -14.91 | -9.26 | -9.76 | -20.03 | -14.84 | -19.32 | -12.73 | -22.86 | -16.97 | -21.84 | -16.88 | -23.58 | -15.67 |
| **CAM15A-T14(I)** | 2.5 | -14.35 | -10.04 | -14.86 | -7.98 | -10.40 | -18.85 | -14.30 | -19.77 | -14.03 | -22.97 | -16.76 | -22.62 | -16.69 | -24.08 | -15.82 |
| **CAM15A-T14(I)** | 3 | -14.88 | -9.41 | -15.06 | -9.62 | -10.20 | -18.89 | -15.14 | -20.67 | -15.49 | -23.70 | -17.00 | -23.31 | -17.80 | -24.56 | -16.41 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **CAM15A-T14(I)** | 3.5 | -14.24 | -9.20 | -15.16 | -9.05 | -9.65 | -18.07 | -14.38 | -19.81 | -15.04 | -23.02 | -16.94 | -24.75 | -17.30 | -24.79 | -16.07 |
| **CAM15A-T14(I)** | 4.5 | -14.62 | -8.11 | -16.03 | -9.72 | -8.87 | -18.61 | -15.29 | -21.21 | -15.08 | -23.97 | -17.56 | -23.47 | -17.64 | -24.96 | -16.49 |
| **CAM15A-T14(I)** | 5.5 | -14.37 | -7.94 | -14.77 | -9.74 | -9.69 | -18.27 | -15.15 | -19.30 | -12.67 | -22.72 | -17.61 | -22.77 | -16.73 | -24.04 | -15.76 |
| **CAM15A-T14(I)** | 6 | -15.08 | -8.96 | -14.39 | -10.21 | -9.57 | -17.63 | -14.83 | -19.92 | -13.30 | -22.88 | -17.22 | -21.35 | -16.69 | -23.95 | -15.90 |
| **CAM15A-T14(I)** | 6.5 | -14.04 | -7.72 | -14.14 | -9.32 | -9.85 | -17.08 | -14.98 | -19.55 | -12.78 | -23.15 | -17.55 | -22.67 | -16.75 | -24.12 | -15.58 |
| **CAM15A-T14(I)** | 7 | -15.18 | -7.19 | -14.51 | -9.12 | -8.80 | -17.83 | -15.14 | -19.48 | -12.61 | -22.77 | -17.01 | -21.96 | -16.64 | -23.80 | -15.49 |
| **CAM15A-T14(I)** | 7.5 | -14.92 | -9.32 | -15.01 | -9.77 | -9.84 | -18.26 | -15.45 | -20.15 | -13.80 | -23.39 | -17.11 | -22.04 | -16.81 | -23.98 | -16.22 |
| **CAM15A-T14(I)** | 8 | -14.93 | -8.17 | -14.95 | -9.66 | -8.79 | -18.28 | -15.69 | -19.91 | -13.42 | -23.23 | -17.88 | -22.42 | -16.69 | -24.05 | -15.95 |
| **CAM15A-T14(I)** | 8.5 | -14.98 | -8.02 | -14.63 | -8.73 | -8.13 | -18.21 | -15.02 | -19.73 | -14.21 | -23.07 | -17.43 | -22.84 | -16.79 | -23.81 | -15.77 |
| **CAM15A-T14(I)** | 9 | -14.54 | -7.75 | -14.78 | -10.55 | -8.09 | -17.16 | -15.22 | -19.57 | -13.00 | -22.81 | -16.98 | -22.23 | -16.57 | -22.94 | -15.63 |
| **CAM15A-T14(I)** | 9.5 | -14.70 | -8.16 | -15.01 | -10.96 | -10.27 | -17.12 | -14.73 | -20.33 | -14.04 | -23.18 | -16.70 | -21.10 | -17.31 | -24.19 | -16.08 |
| **CAM15A-T14(I)** | 10 | -15.38 | -8.11 | -14.52 | -10.81 | -10.04 | -17.38 | -15.64 | -20.53 | -15.13 | -23.72 | -17.10 | -22.90 | -17.63 | -24.60 | -16.27 |
| **CAM15A-T14(II)** | 0.5 | -15.05 | -9.16 | -14.74 | -8.26 | -9.81 | -19.24 | -15.31 | -20.57 | -15.30 | -23.55 | -16.89 | -23.24 | -17.69 | -25.29 | -16.25 |
| **CAM15A-T14(II)** | 1 | -14.29 | -8.59 | -15.16 | -9.42 | -9.65 | -18.68 | -15.25 | -20.05 | -14.45 | -23.86 | -16.92 | -23.65 | -17.47 | -25.14 | -16.23 |
| **CAM15A-T14(II)** | 1.5 | -13.60 | -8.42 | -14.75 | -8.75 | -8.59 | -17.61 | -14.92 | -19.97 | -13.81 | -23.46 | -16.65 | -23.51 | -17.30 | -24.68 | -15.79 |
| **CAM15A-T14(II)** | 2 | -15.22 | -8.07 | -15.07 | -9.53 | -8.43 | -18.64 | -14.98 | -19.66 | -14.48 | -22.89 | -17.09 | -23.46 | -17.48 | -24.94 | -16.00 |
| **CAM15A-T14(II)** | 2.5 | -15.72 | -9.06 | -15.47 | -8.99 | -10.46 | -18.88 | -15.32 | -20.66 | -15.84 | -24.06 | -17.61 | -24.20 | -17.87 | -25.56 | -16.62 |
| **CAM15A-T14(II)** | 3 | -14.45 | -7.45 | -14.29 | -7.85 | -9.75 | -16.93 | -15.17 | -19.61 | -14.18 | -22.70 | -17.14 | -21.98 | -16.28 | -24.14 | -15.42 |
| **CAM15A-T14(II)** | 3.5 | -14.49 | -7.68 | -14.85 | -9.10 | -10.19 | -17.08 | -15.10 | -20.46 | -13.91 | -22.53 | -16.96 | -22.47 | -15.91 | -23.54 | -15.63 |
| **CAM15A-T14(II)** | 4 | -14.40 | -8.48 | -14.07 | -9.10 | -9.72 | -17.48 | -14.94 | -19.47 | -13.58 | -22.63 | -17.15 | -22.17 | -15.97 | -23.90 | -15.50 |
| **CAM15A-T14(II)** | 4.5 | -14.64 | -6.91 | -14.05 | -8.96 | -9.67 | -17.12 | -14.63 | -19.37 | -13.59 | -22.64 | -17.21 | -22.33 | -16.44 | -23.14 | -15.30 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **CAM15A-T14(II)** | 5 | -13.95 | -7.23 | -14.39 | -8.52 | -9.61 | -17.78 | -14.78 | -19.26 | -12.38 | -22.71 | -17.38 | -23.19 | -16.42 | -23.82 | -15.40 |
| **CAM15A-T14(II)** | 5.5 | -14.31 | -8.49 | -14.56 | -8.79 | -10.78 | -17.59 | -16.53 | -19.78 | -13.98 | -22.77 | -17.21 | -22.57 | -16.83 | -24.08 | -15.93 |
| **CAM15A-T14(II)** | 6 | -13.65 | -5.93 | -13.59 | -7.41 | -9.18 | -16.07 | -14.06 | -18.75 | -12.59 | -22.16 | -16.43 | -21.51 | -15.63 | -23.13 | -14.59 |
| **CAM15A-T14(II)** | 6.5 | -15.15 | -7.62 | -14.90 | -9.24 | -10.89 | -19.62 | -15.28 | -20.12 | -13.07 | -23.35 | -17.25 | -23.02 | -17.17 | -24.86 | -16.06 |
| **CAM15A-T14(II)** | 7 | -14.78 | -8.41 | -15.21 | -10.40 | -11.23 | -18.45 | -16.53 | -21.04 | -14.48 | -24.27 | -17.86 | -23.36 | -18.01 | -25.36 | -16.75 |
| **CAM15A-T14(II)** | 7.5 | -13.96 | -9.30 | -14.95 | -8.74 | -10.77 | -19.46 | -15.58 | -20.07 | -13.54 | -23.32 | -17.77 | -22.88 | -16.96 | -24.25 | -16.12 |
| **CAM15A-T14(II)** | 8 | -13.67 | -8.23 | -15.07 | -7.67 | -9.17 | -20.36 | -15.91 | -20.18 | -13.43 | -23.29 | -17.27 | -23.24 | -17.59 | -24.94 | -16.08 |
| **CAM15A-T14(II)** | 8.8 | -14.51 | -6.28 | -14.82 | -9.52 | -9.88 | -17.75 | -15.84 | -19.37 | -12.95 | -23.12 | -17.03 | -22.66 | -16.60 | -24.72 | -15.73 |
| **QUI7-T13(I)** | 0.5 | -14.18 | -5.62 | -13.44 | -4.10 | -8.01 | -19.94 | -11.52 | -18.76 | -14.31 | -21.01 | -15.39 | -21.56 | -15.67 | -24.35 | -14.13 |
| **QUI7-T13(I)** | 1 | -14.89 | -5.28 | -14.10 | -4.27 | -10.34 | -18.50 | -13.29 | -20.08 | -13.85 | -21.92 | -15.37 | -21.01 | -16.21 | -22.75 | -14.57 |
| **QUI7-T13(I)** | 1.5 | -14.52 | -6.15 | -13.17 | -5.01 | -8.84 | -17.46 | -10.83 | -18.79 | -12.93 | -21.90 | -14.62 | -21.91 | -15.36 | -22.60 | -13.99 |
| **QUI7-T13(I)** | 2 | -14.15 | -4.59 | -12.87 | -4.40 | -9.77 | -18.82 | -11.11 | -17.97 | -13.86 | -21.29 | -14.51 | -20.97 | -16.00 | -22.91 | -13.77 |
| **QUI7-T13(I)** | 2.5 | -16.54 | -6.96 | -14.37 | -4.64 | -8.49 | -20.67 | -12.65 | -18.56 | -13.56 | -21.37 | -15.09 | -21.52 | -16.94 | -24.40 | -14.82 |
| **QUI7-T13(I)** | 3 | -16.39 | -7.42 | -13.63 | -4.39 | -10.14 | -17.89 | -10.67 | -18.76 | -14.02 | -21.60 | -15.44 | -19.82 | -16.32 | -23.50 | -14.39 |
| **QUI7-T13(I)** | 3.5 | -15.90 | -7.42 | -13.86 | -5.31 | -9.23 | -18.07 | -12.37 | -19.23 | -14.94 | -21.49 | -14.97 | | -16.49 | -22.46 | -14.49 |
| **QUI7-T13(I)** | 4 | -16.54 | -5.87 | -12.63 | -6.43 | -9.88 | -19.84 | -14.35 | -18.68 | -13.26 | -21.89 | -14.41 | -20.13 | -17.86 | -23.11 | -14.90 |
| **QUI7-T13(I)** | 4.5 | -14.69 | -7.07 | -12.56 | -5.79 | -10.16 | -17.35 | -12.51 | -18.14 | -13.65 | -21.86 | -14.61 | -22.12 | -18.04 | -24.03 | -14.57 |
| **QUI7-T13(I)** | 5 | -12.56 | -5.07 | -11.90 | -1.98 | -7.19 | -15.09 | -12.85 | -16.51 | -12.18 | -20.41 | -14.50 | -20.72 | -15.95 | -21.53 | -13.00 |
| **QUI7-T13(I)** | 5.5 | -12.22 | -5.04 | -11.83 | -1.42 | -8.43 | -16.22 | -13.95 | -17.25 | -12.91 | -20.83 | -14.15 | -20.27 | -15.07 | -21.37 | -13.14 |
| **QUI7-T13(I)** | 6 | -12.48 | -6.28 | -12.30 | -1.41 | -7.70 | -16.64 | -12.43 | -17.19 | -12.28 | -20.06 | -14.63 | -20.31 | -14.57 | -21.45 | -13.06 |
| **QUI7-T13(I)** | 7 | -16.65 | -6.85 | -15.41 | -1.57 | -9.34 | -18.65 | -14.55 | -19.30 | -12.61 | -20.92 | -14.01 | -21.25 | -16.05 | -21.96 | -14.63 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **QUI7-T13(I)** | 7.5 | -14.02 | -7.79 | -13.45 | -1.28 | -8.60 | -19.82 | -13.17 | -18.73 | -13.62 | -20.95 | -14.84 | -21.75 | -15.32 | -22.48 | -14.16 |
| **QUI7-T13(I)** | 8 | -14.80 | -4.93 | -11.94 | -1.06 | -9.84 | -18.35 | -13.22 | -18.03 | -12.81 | -20.51 | -14.25 | -21.78 | -15.21 | -22.59 | -13.53 |
| **QUI7-T13(I)** | 8.5 | -12.84 | -3.67 | -10.78 | -1.74 | -7.07 | -17.98 | -10.90 | -16.39 | -12.81 | -20.39 | -13.07 | -20.25 | -14.96 | -22.12 | -12.62 |
| **QUI7-T13(II)** | 0.5 | -13.81 | -7.23 | -14.84 | -8.44 | -9.23 | -19.06 | -14.06 | -19.49 | -12.99 | -21.70 | -16.75 | -20.73 | -15.89 | -22.89 | -15.19 |
| **QUI7-T13(II)** | 1 | -12.25 | -5.39 | -12.63 | -7.62 | -6.63 | -16.76 | -11.99 | -17.16 | -11.72 | -19.63 | -14.52 | -19.58 | -14.10 | -21.44 | -13.29 |
| **QUI7-T13(II)** | 1.5 | -14.21 | -5.52 | -13.72 | -7.98 | -8.12 | -19.16 | -12.99 | -19.13 | -13.05 | -21.78 | -16.97 | -20.73 | -16.21 | -22.44 | -14.67 |
| **QUI7-T13(II)** | 2 | -13.11 | -4.86 | -13.83 | -8.22 | -8.89 | -17.81 | -12.94 | -18.24 | -12.14 | -21.41 | -15.76 | -20.43 | -15.74 | -22.57 | -14.30 |
| **QUI7-T13(II)** | 2.5 | -12.43 | -5.23 | -12.47 | -6.64 | -7.97 | -16.84 | -12.06 | -18.25 | -12.53 | -20.54 | -15.45 | -20.28 | -15.07 | -22.00 | -13.63 |
| **QUI7-T13(II)** | 3 | -14.29 | -6.07 | -14.32 | -8.46 | -11.17 | -17.93 | -14.35 | -19.96 | -12.94 | -22.24 | -16.08 | -21.56 | -16.12 | -23.43 | -15.17 |
| **QUI7-T13(II)** | 3.5 | -13.34 | -5.71 | -13.77 | -7.98 | -8.35 | -17.45 | -13.68 | -18.42 | -11.78 | -21.65 | -14.71 | -19.76 | -15.58 | -22.37 | -14.43 |
| **QUI7-T13(II)** | 4 | -13.75 | -5.43 | -13.79 | -8.86 | -7.57 | -16.48 | -12.22 | -18.16 | -11.26 | -20.66 | -14.96 | -17.68 | -14.46 | -21.69 | -13.91 |
| **QUI7-T13(II)** | 4.5 | -15.56 | -8.14 | -14.95 | -8.85 | -10.56 | -17.96 | -13.83 | -19.55 | -13.13 | -22.47 | -16.73 | -20.40 | -16.41 | -22.75 | -15.56 |
| **QUI7-T13(II)** | 5 | -14.61 | -5.83 | -15.09 | -6.50 | -7.04 | -17.20 | -12.52 | -18.00 | -13.37 | -21.19 | -15.09 | -19.91 | -15.88 | -22.62 | -14.42 |
| **QUI7-T13(II)** | 5.5 | -15.00 | -8.16 | -14.73 | -8.94 | -8.68 | -18.34 | -12.98 | -18.52 | -13.22 | -22.16 | -15.69 | -19.85 | -16.43 | -23.18 | -15.22 |
| **QUI7-T13(II)** | 6 | -15.44 | -7.69 | -15.10 | -9.36 | -9.47 | -17.88 | -13.71 | -19.73 | -13.42 | -22.83 | -15.64 | -21.13 | -16.83 | -23.42 | -15.65 |
| **QUI7-T13(II)** | 6.5 | -12.86 | -6.15 | -12.41 | -7.43 | -7.03 | -14.89 | -12.02 | -17.65 | -12.26 | -21.18 | -15.00 | -17.89 | -14.78 | -21.50 | -13.59 |
| **QUI7-T13(II)** | 7 | -12.36 | -5.37 | -12.15 | -7.93 | -8.04 | -15.41 | -12.42 | -17.97 | -12.15 | -20.78 | -15.22 | -17.94 | -15.00 | -22.44 | -13.63 |
| **QUI7-T13(II)** | 7.5 | -11.96 | -5.64 | -12.37 | -7.93 | -7.65 | -15.33 | -12.46 | -18.08 | -13.13 | -21.47 | -15.79 | -19.16 | -16.00 | -22.28 | -13.95 |
| **QUI7-T13(II)** | 8 | -11.40 | -3.75 | -11.85 | -7.52 | -7.50 | -15.05 | -11.90 | -17.53 | -11.55 | -20.47 | -15.29 | -18.92 | -15.15 | -21.25 | -13.19 |
| **QUI7-T13(II)** | 8.5 | -11.77 | -3.65 | -11.06 | -6.53 | -7.14 | -14.82 | -11.23 | -18.16 | -11.04 | -20.11 | -14.91 | -20.22 | -14.39 | -21.03 | -12.80 |
| **QUI7-T13(II)** | 9 | -12.10 | -4.23 | -11.36 | -7.13 | -7.58 | -14.99 | -11.65 | -17.92 | -11.60 | -20.47 | -14.16 | -19.21 | -14.70 | -21.33 | -13.10 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **QUI7-T13(II)** | 9.5 | -12.12 | -5.26 | -11.62 | -8.01 | -8.64 | -15.18 | -11.93 | -18.64 | -12.11 | -20.69 | -15.23 | -19.31 | -14.83 | -21.42 | -13.45 |
| **AZ14-T31** | 0.5 | -19.01 | -12.53 | -19.41 | -3.97 | -11.43 | -23.13 | -16.89 | -24.67 | -18.85 | -26.05 | -18.30 | -23.37 | -19.25 | -24.65 | -18.53 |
| **AZ14-T31** | 1 | -17.75 | -10.02 | -18.19 | -3.33 | -12.11 | -20.63 | -14.99 | -22.47 | -17.55 | -24.51 | -17.66 | -22.56 | -17.80 | -23.59 | -17.10 |
| **AZ14-T31** | 1.5 | -17.58 | -10.53 | -17.98 | -3.56 | -7.28 | -20.48 | -16.24 | -22.11 | -17.27 | -24.43 | -17.50 | -21.91 | -18.17 | -22.79 | -17.02 |
| **AZ14-T31** | 2 | -18.10 | -11.90 | -16.24 | -3.80 | -11.02 | -18.35 | -17.08 | -23.46 | -18.20 | -25.28 | -17.31 | -21.38 | -17.26 | -21.60 | -17.10 |
| **AZ14-T31** | 2.5 | -18.14 | -11.33 | -15.74 | -2.97 | -11.81 | -17.67 | -17.26 | -22.88 | -17.61 | -24.79 | -17.91 | -22.96 | -16.68 | -21.71 | -16.87 |
| **AZ14-T31** | 3 | -16.09 | -9.49 | -15.26 | -2.89 | -8.79 | -17.46 | -17.75 | -22.36 | -16.18 | -24.55 | -17.01 | -21.09 | -16.61 | -22.10 | -16.22 |
| **AZ14-T31** | 3.5 | -17.82 | -11.24 | -14.80 | -4.27 | -10.31 | -15.52 | -18.15 | -22.71 | -16.48 | -24.17 | -16.16 | -20.57 | -15.84 | -23.25 | -16.45 |
| **AZ14-T31** | 4 | -17.27 | -11.45 | -14.17 | -3.20 | -12.09 | -16.95 | -17.09 | -23.02 | -16.92 | -24.35 | -17.23 | -20.88 | -16.24 | -22.49 | -16.37 |
| **AZ14-T31** | 4.5 | -16.74 | -11.16 | -14.51 | -5.01 | -8.44 | -15.64 | -16.75 | -22.42 | -16.37 | -24.48 | -16.77 | -20.89 | -16.14 | -22.88 | -16.22 |
| **AZ14-T31** | 5 | -18.05 | -11.68 | -15.01 | -8.32 | -9.70 | -18.10 | -16.89 | -23.34 | -17.89 | -25.30 | -17.98 | -22.64 | -17.65 | -23.84 | -17.36 |
| **AZ14-T31** | 5.5 | -17.69 | -11.76 | -15.59 | -6.77 | -12.08 | -17.90 | -17.51 | -24.06 | -16.98 | -25.89 | -18.67 | -23.13 | -17.73 | -23.53 | -17.55 |
| **AZ14-T31** | 6 | -19.77 | -14.75 | -18.60 | -7.34 | -13.10 | -21.65 | -19.13 | -25.73 | -20.72 | -27.93 | -19.37 | -25.33 | -20.39 | -25.70 | -19.79 |
| **AZ14-T31** | 6.5 | -20.93 | -15.31 | -19.71 | -7.73 | -13.02 | -23.02 | -18.39 | -26.18 | -20.66 | -28.79 | -20.53 | -25.92 | -21.12 | -26.30 | -20.37 |
| **AZ14-T31** | 7 | -20.86 | -14.70 | -19.73 | -8.99 | -14.93 | -22.48 | -18.37 | -26.21 | -20.78 | -28.16 | -20.06 | -25.63 | -20.66 | -26.46 | -20.28 |
| **AZ14-T31** | 7.5 | -21.05 | -16.18 | -20.56 | -9.05 | -12.63 | -25.03 | -19.79 | -26.96 | -21.27 | -28.72 | -20.92 | -26.44 | -21.40 | -26.36 | -21.04 |
| **AZ14-T31** | 8 | -23.20 | -15.58 | -20.72 | -12.79 | -13.10 | -23.04 | -19.82 | -27.30 | -21.99 | -29.01 | -20.44 | -26.90 | -22.67 | -26.66 | -21.58 |
| **AZ14-T31** | 8.5 | -22.50 | -16.66 | -20.33 | -8.75 | -12.39 | -22.92 | -19.13 | -26.99 | -21.35 | -28.75 | -21.01 | -25.80 | -22.12 | -26.96 | -21.02 |
| **AZ14-T31** | 9 | -20.71 | -14.56 | -19.69 | -9.20 | -10.31 | -23.09 | -21.19 | -25.91 | -20.30 | -28.22 | -20.49 | -25.92 | -21.95 | -27.02 | -20.58 |
| **AZ14-T31** | 9.5 | -22.00 | -15.40 | -20.89 | -14.88 | -14.37 | -23.48 | -19.94 | -26.59 | -18.92 | -27.16 | -18.18 | -22.53 | -20.77 | -25.89 | -20.86 |
| **AZ14-T31** | 10 | -23.31 | -16.40 | -19.74 | -12.92 | -13.44 | -24.51 | -20.53 | -27.49 | -21.48 | -29.36 | -20.84 | -26.50 | -22.52 | -26.85 | -21.64 |

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **AZ14-T31** | 10.5 | -22.34 | -16.44 | -19.59 | -13.36 | -14.81 | -24.70 | -22.42 | -27.35 | -21.12 | -28.87 | -20.31 | -26.28 | -22.86 | -26.26 | -21.75 |
| **AZ14-T31** | 11 | -22.30 | -16.04 | -19.06 | -9.76 | -12.04 | -22.80 | -19.59 | -26.85 | -21.20 | -28.44 | -19.93 | -25.10 | -21.29 | -26.36 | -20.64 |
| **AZ14-T31** | 11.5 | -20.80 | -15.09 | -19.65 | -8.78 | -10.73 | -22.03 | -18.40 | -24.75 | -20.28 | -26.55 | -19.53 | -24.08 | -20.27 | -26.78 | -19.72 |
| **AZ14-T31** | 12 | -19.37 | -12.63 | -18.41 | -7.65 | -10.02 | -21.47 | -17.86 | -22.75 | -16.00 | -24.34 | -16.82 | -23.49 | -19.41 | -24.37 | -18.18 |
| **AZ14-T31** | 12.7 | -17.01 | -11.83 | -18.12 | -2.72 | -7.83 | -20.25 | -15.82 | -20.66 | -14.42 | -23.36 | -15.76 | -21.89 | -17.47 | -23.12 | -16.57 |
| **AZ115-T9** | 0.5 | -20.70 | -13.57 | -18.11 | -7.12 | -13.72 | -22.12 | -15.61 | -24.68 | -19.86 | -25.17 | -18.38 | -22.36 | -18.84 | -22.60 | -18.46 |
| **AZ115-T9** | 1 | -21.76 | -12.81 | -18.03 | -8.84 | -11.14 | -26.79 | -15.56 | -25.26 | -20.59 | -25.64 | -19.32 | -22.29 | -20.47 | -22.51 | -18.99 |
| **AZ115-T9** | 1.5 | -20.54 | -12.38 | -17.75 | -6.37 | -12.38 | -21.57 | -13.65 | -23.51 | -19.26 | -24.45 | -17.88 | -20.86 | -18.78 | -21.41 | -17.64 |
| **AZ115-T9** | 2 | -20.79 | -14.67 | -16.41 | -8.53 | -11.50 | -25.36 | -15.58 | -23.86 | -19.47 | -25.32 | -18.58 | -22.08 | -20.09 | -23.37 | -18.53 |
| **AZ115-T9** | 2.5 | -20.67 | -10.02 | -16.77 | -4.16 | -9.23 | -25.70 | -12.52 | -22.65 | -18.34 | -23.68 | -17.97 | -19.96 | -17.47 | -21.47 | -16.67 |
| **AZ115-T9** | 3 | -18.81 | -10.69 | -16.28 | -5.73 | -10.64 | -21.43 | -11.10 | -22.01 | -17.59 | -23.60 | -17.65 | -20.76 | -18.38 | -21.09 | -16.41 |
| **AZ115-T9** | 3.5 | -17.97 | -8.55 | -15.57 | -4.09 | -7.90 | -21.76 | -10.79 | -22.36 | -16.63 | -22.40 | -16.83 | -19.87 | -16.46 | -20.70 | -15.39 |
| **AZ115-T9** | 4 | -18.21 | -8.89 | -14.51 | -6.23 | -7.93 | -22.00 | -11.56 | -21.72 | -17.92 | -23.14 | -17.08 | -20.31 | -16.46 | -21.10 | -15.68 |
| **AZ115-T9** | 4.5 | -16.58 | -6.57 | -13.26 | -5.19 | -6.90 | -21.82 | -9.09 | -20.24 | -16.12 | -21.88 | -16.56 | -18.96 | -14.40 | -19.76 | -14.15 |
| **AZ115-T9** | 5 | -13.48 | -3.98 | -8.87 | -3.55 | -6.44 | -20.07 | -8.37 | -19.68 | -15.31 | -21.20 | -16.29 | -18.55 | -13.24 | -20.74 | -12.54 |
| **AZ115-T9** | 5.5 | -12.52 | -6.16 | -7.97 | -3.70 | -5.85 | -21.08 | -8.06 | -20.00 | -15.19 | -21.51 | -15.71 | -18.58 | -13.03 | -19.43 | -12.50 |
| **AZ115-T9** | 6 | -11.35 | -5.44 | -7.57 | -8.92 | -6.60 | -12.90 | -12.23 | -21.92 | -16.09 | -22.65 | -18.25 | -18.78 | -12.99 | -21.04 | -13.32 |
| **AZ115-T9** | 6.5 | -11.51 | -6.62 | -8.43 | -10.24 | -10.55 | -14.05 | -14.43 | -22.50 | -17.05 | -23.12 | -18.15 | -20.17 | -13.88 | -21.88 | -14.38 |
| **AZ115-T9** | 7 | -13.53 | -6.70 | -9.80 | -9.11 | -8.67 | -14.66 | -13.61 | -22.77 | -16.87 | -23.73 | -18.77 | -20.71 | -15.68 | -21.91 | -14.83 |
| **AZ115-T9** | 7.5 | -16.67 | -11.64 | -14.20 | -10.18 | -10.21 | -13.86 | -13.45 | -24.19 | -17.69 | -25.54 | -19.46 | -22.71 | -17.18 | -23.36 | -16.88 |
| **AZ115-T9** | 8 | -19.07 | -13.33 | -15.18 | -9.40 | -14.73 | -17.48 | -16.00 | -25.95 | -19.49 | -27.21 | -21.36 | -24.02 | -19.70 | -25.42 | -18.62 |

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **AZ115-T9** | 8.5 | -20.43 | -12.98 | -18.16 | -10.34 | -12.32 | -21.17 | -19.00 | -26.72 | -22.36 | -28.80 | -22.06 | -25.51 | -21.92 | -26.54 | -20.30 |
| **AZ115-T9** | 9 | -19.00 | -12.41 | -15.07 | -7.86 | -11.49 | -19.11 | -14.94 | -24.47 | -18.47 | -25.48 | -19.48 | -21.94 | -18.47 | -23.24 | -17.56 |
| **AZ115-T9** | 9.5 | -20.78 | -13.70 | -19.57 | -10.77 | -12.11 | -22.92 | -19.04 | -28.10 | -22.62 | -28.34 | -22.03 | -24.46 | -21.49 | -25.97 | -20.68 |
| **AZ115-T9** | 10 | -18.00 | -12.26 | -15.52 | -8.67 | -11.17 | -18.39 | -15.69 | -25.49 | -19.55 | -25.83 | -19.50 | -22.01 | -17.99 | -23.67 | -17.79 |
| **AZ115-T9** | 10.5 | -15.91 | -11.56 | -14.20 | -7.74 | -8.35 | -17.06 | -15.35 | -24.88 | -18.50 | -25.21 | -19.22 | -21.52 | -17.73 | -23.54 | -16.94 |
| **AZ115-T9** | 11 | -17.43 | -12.20 | -14.61 | -8.26 | -7.66 | -17.85 | -15.15 | -24.51 | -19.41 | -25.81 | -20.00 | -22.35 | -18.79 | -22.88 | -17.40 |
| **AZ115-T9** | 11.5 | -18.01 | -12.10 | -16.90 | -7.28 | -11.28 | -21.14 | -16.86 | -26.96 | -20.39 | -27.04 | -21.01 | -22.00 | -21.08 | -25.38 | -18.78 |
| **AZ115-T9** | 12 | -21.22 | -14.95 | -19.01 | -11.02 | -9.69 | -22.39 | -17.98 | -28.21 | -21.54 | -28.64 | -21.41 | -25.24 | -21.28 | -25.53 | -20.47 |
| **AZ115-T9** | 12.5 | -19.77 | -12.47 | -18.53 | -11.54 | -10.63 | -21.51 | -17.48 | -26.93 | -20.74 | -27.72 | -20.92 | -23.21 | -20.38 | -24.47 | -19.62 |
| **AZ115-T9** | 13 | -21.17 | -15.59 | -18.85 | -10.36 | -11.93 | -22.69 | -18.51 | -27.08 | -21.99 | -27.64 | -21.24 | -24.23 | -20.75 | -24.72 | -20.27 |
| **AZ115-T9** | 13.5 | -19.84 | -13.99 | -19.15 | -8.65 | -10.74 | -22.82 | -17.63 | -26.06 | -20.71 | -26.59 | -21.12 | -23.56 | -19.38 | -24.48 | -19.43 |
| **AZ115-T9** | 14 | -19.89 | -14.43 | -18.75 | -11.40 | -11.29 | -22.24 | -17.71 | -26.52 | -20.77 | -27.17 | -21.01 | -23.22 | -20.25 | -24.91 | -19.82 |
| **AZ115-T9** | 14.5 | -16.77 | -7.13 | -11.47 | -6.19 | -7.56 | -14.19 | -14.23 | -22.96 | -17.71 | -24.51 | -18.61 | -20.08 | -15.38 | -21.63 | -15.16 |
| **AZ115-T9** | 15 | -19.09 | -11.33 | -15.37 | -8.80 | -8.20 | -18.80 | -16.22 | -24.30 | -19.06 | -26.13 | -20.76 | -23.06 | -18.53 | -23.63 | -17.82 |
| **AZ115-T9** | 15.5 | -18.52 | -11.31 | -16.93 | -10.09 | -8.87 | -20.21 | -16.73 | -23.70 | -19.69 | -25.69 | -19.42 | -22.92 | -17.55 | -22.70 | -17.96 |
| **AZ115-T9** | 16 | -16.40 | -8.78 | -13.12 | -7.94 | -8.49 | -17.40 | -13.32 | -23.04 | -17.80 | -24.16 | -19.29 | -21.02 | -16.56 | -21.20 | -15.85 |
| **AZ115-T9** | 16.5 | -15.49 | -8.85 | -10.83 | -5.52 | -8.26 | -14.74 | -12.73 | -22.32 | -16.79 | -22.85 | -17.76 | | -15.07 | -20.61 | -14.36 |
| **AZ115-T9** | 17 | -12.57 | -4.43 | -8.02 | -6.17 | -8.47 | -10.73 | -9.35 | -21.36 | -15.60 | -21.80 | -18.79 | -18.71 | -12.97 | -20.52 | -12.68 |
| **AZ115-T9** | 17.5 | -10.59 | -3.43 | -6.32 | -4.98 | -7.59 | -14.24 | -10.00 | -20.29 | -15.76 | -21.51 | -17.51 | -18.81 | -13.14 | -20.24 | -12.14 |
| **AZ115-T9** | 18 | -9.21 | -6.48 | -6.01 | -6.29 | -9.02 | -15.82 | -12.15 | -21.26 | -15.49 | -21.72 | -17.01 | -18.64 | -13.56 | -20.28 | -12.83 |
| **AZ115-T9** | 18.5 | -10.19 | -5.53 | -5.44 | -5.83 | -9.72 | -12.97 | -11.04 | -20.55 | -16.87 | -22.09 | -17.71 | -19.37 | -13.23 | -20.71 | -12.56 |
| **AZ115-T9** | 19 | -9.84 | -6.04 | -6.07 | -5.70 | -9.04 | -13.74 | -11.52 | -21.33 | -17.08 | -22.57 | -18.41 | -19.98 | -13.82 | -21.90 | -13.03 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **AZ115-T9** | 19.5 | -9.63 | -7.68 | -7.99 | -7.20 | -9.85 | -15.87 | -13.13 | -22.83 | -17.92 | -23.75 | -18.76 | -20.44 | -15.66 | -22.23 | -14.32 |
| **AZ115-T9** | 20 | -15.69 | -8.61 | -12.41 | -8.61 | -10.34 | -15.16 | -14.09 | -24.09 | -17.94 | -24.74 | -19.64 | -22.05 | -17.20 | -23.62 | -16.17 |
| **AZ115-T9** | 20.5 | -17.78 | -10.22 | -14.66 | -9.07 | -9.73 | -17.23 | -16.07 | -26.82 | -19.56 | -26.21 | -20.65 | -22.90 | -19.21 | -24.08 | -17.79 |
| **AZ115-T9** | 21 | -21.86 | -13.70 | -20.39 | -8.05 | -8.26 | -23.51 | -19.04 | -26.65 | -21.81 | -28.27 | -21.38 | -23.57 | -21.76 | -26.01 | -20.33 |
| **AZ115-T9** | 21.5 | -21.39 | -14.82 | -22.11 | -7.35 | -12.62 | -24.29 | -19.86 | -27.98 | -22.06 | -28.62 | -21.35 | -24.41 | -22.07 | -25.63 | -21.06 |
| **AZ115-T9** | 22 | -17.49 | -15.56 | -19.56 | -10.08 | -11.08 | -27.14 | -19.77 | -26.95 | -21.40 | -28.24 | -21.78 | -24.92 | -22.14 | -25.59 | -20.68 |
| **AZ115-T9** | 22.5 | -17.45 | -15.55 | -17.82 | -8.70 | -11.26 | -25.81 | -18.31 | -26.04 | -21.42 | -27.19 | -21.35 | -24.53 | -20.88 | -25.00 | -19.79 |
| **AZ115-T9** | 23 | -19.11 | -14.65 | -19.32 | -9.33 | -10.62 | -22.69 | -17.53 | -27.16 | -20.14 | -26.96 | -20.63 | -23.28 | -21.01 | -24.60 | -19.74 |
| **AZ115-T9** | 23.5 | -17.74 | -10.24 | -15.67 | -9.07 | -9.75 | -19.08 | -14.88 | -26.14 | -17.87 | -24.93 | -19.97 | -23.29 | -18.19 | -22.81 | -17.52 |
| **AZ115-T9** | 24 | -12.72 | -5.49 | -10.50 | -7.47 | -7.37 | -12.79 | -11.80 | -23.97 | -16.10 | -22.28 | -18.43 | -19.79 | -14.32 | -21.68 | -14.08 |
| **AZ115-T9** | 24.5 | -11.77 | -5.39 | -10.09 | -8.33 | -9.36 | -12.13 | -10.00 | -21.52 | -13.49 | -21.27 | -18.02 | -16.09 | -12.27 | -19.65 | -12.98 |
| **AZ115-T9** | 25 | -11.58 | -6.31 | -8.83 | -10.59 | -5.21 | -10.53 | -11.20 | -19.44 | -13.79 | -21.03 | -16.96 | -15.65 | -12.76 | -19.69 | -12.78 |
| **AZ115-T9** | 25.5 | -12.69 | -7.15 | -9.83 | -12.03 | -9.18 | -11.94 | -12.01 | -20.96 | -15.12 | -22.33 | -18.94 | -18.31 | -14.58 | -21.41 | -14.20 |
| **AZ115-T9** | 26 | -12.54 | -6.12 | -10.17 | -10.43 | -5.94 | -12.73 | -11.31 | -19.83 | -15.11 | -21.46 | -17.62 | -18.46 | -13.42 | -19.32 | -13.47 |
| **AZ115-T9** | 26.5 | -15.79 | -10.11 | -13.59 | -12.79 | -9.04 | -14.54 | -13.83 | -22.03 | -16.14 | -23.77 | -19.28 | -20.62 | -16.33 | -22.06 | -16.15 |
| **AZ115-T9** | 27 | -18.15 | -11.45 | -16.34 | -11.94 | -9.34 | -18.19 | -15.27 | -22.82 | -16.19 | -24.39 | -19.38 | -20.83 | -18.02 | -22.11 | -17.37 |
| **AZ115-T9** | 27.5 | -19.68 | -12.76 | -18.29 | -13.51 | -9.99 | -19.95 | -15.50 | -22.86 | -16.85 | -24.62 | -19.86 | -20.91 | -18.38 | -22.72 | -18.27 |
| **AZ115-T9** | 28 | -16.96 | -8.53 | -15.18 | -11.13 | -8.03 | -17.62 | -12.77 | -21.88 | -16.12 | -24.08 | -19.77 | -20.78 | -17.81 | -22.17 | -16.39 |
| **AZ115-T9** | 28.5 | -15.50 | -9.62 | -14.62 | -11.64 | -10.62 | -17.45 | -13.91 | -24.02 | -17.89 | -24.46 | -19.93 | -19.42 | -15.80 | -21.40 | -16.54 |
| **AZ115-T9** | 29 | -15.64 | -8.77 | -14.76 | -9.43 | -9.54 | -18.08 | -13.01 | -22.74 | -15.48 | -23.13 | -18.82 | -19.69 | -15.10 | -20.13 | -15.73 |
| **AZ115-T9** | 29.5 | -16.18 | -9.04 | -14.03 | -12.18 | -11.94 | -19.33 | -12.64 | -23.49 | -16.07 | -24.10 | -19.44 | -19.60 | -16.24 | -21.05 | -16.33 |
| **AZ115-T9** | 30 | -15.77 | -9.01 | -12.87 | -10.73 | -9.07 | -16.75 | -13.66 | -21.46 | -15.85 | -23.17 | -19.37 | -19.75 | -15.75 | -21.52 | -15.63 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **AZ115-T9** | 30.5 | -15.42 | -7.88 | -14.48 | -10.58 | -9.83 | -17.57 | -13.32 | -22.57 | -16.02 | -24.07 | -19.67 | -19.92 | -16.72 | -21.50 | -16.09 |
| **AZ115-T9** | 31 | -14.81 | -8.47 | -12.13 | -9.37 | -7.00 | -15.92 | -11.62 | -20.60 | -14.26 | -21.77 | -18.16 | -19.08 | -14.32 | -19.86 | -14.46 |
| **AZ115-T9** | 31.5 | -14.36 | -7.94 | -10.77 | -9.88 | -9.30 | -15.77 | -12.42 | -22.13 | -15.87 | -23.13 | -19.53 | -21.80 | -15.08 | -21.94 | -15.03 |
| **AZ115-T9** | 32 | -10.99 | -7.24 | -8.24 | -8.14 | -7.65 | -10.87 | -9.98 | -19.84 | -12.65 | -20.18 | -16.16 | -15.78 | -11.35 | -18.30 | -12.20 |
| **AZ115-T9** | 32.5 | -11.15 | -7.62 | -8.02 | -8.44 | -7.85 | -10.99 | -10.46 | -21.39 | -13.44 | -21.59 | -14.94 | -16.78 | -13.67 | -20.91 | -12.89 |
| **AZ115-T9** | 33 | -12.26 | -5.97 | -10.59 | -9.52 | -10.28 | -13.82 | -12.59 | -22.45 | -15.81 | -23.30 | -19.00 | -19.38 | -15.14 | -20.77 | -14.44 |
| **AZ115-T9** | 33.5 | -13.72 | -7.60 | -11.12 | -8.72 | -8.34 | -15.93 | -12.70 | -21.96 | -15.75 | -23.70 | -19.17 | -19.78 | -15.88 | -22.34 | -14.91 |
| **AZ115-T9** | 34 | -12.83 | -6.14 | -11.09 | -8.11 | -8.13 | -15.18 | -12.53 | -21.33 | -16.11 | -23.51 | -18.52 | -20.73 | -15.39 | -21.79 | -14.54 |
| **AZ115-T9** | 34.5 | -16.97 | -12.44 | -16.77 | -11.33 | -12.58 | -20.71 | -16.61 | -25.34 | -18.75 | -26.48 | -20.58 | -21.44 | -19.13 | -24.82 | -18.58 |
| **AZ115-T9** | 35 | -18.67 | -13.35 | -18.57 | -13.93 | -13.28 | -22.06 | -17.38 | -25.26 | -18.70 | -27.52 | -21.57 | -23.79 | -19.86 | -25.03 | -19.70 |
| **AZ115-T9** | 35.5 | -17.31 | -12.23 | -17.03 | -12.32 | -14.61 | -20.05 | -16.55 | -26.21 | -19.38 | -27.41 | -21.97 | -22.94 | -19.16 | -23.74 | -18.99 |
| **AZ115-T9** | 36 | -18.86 | -13.96 | -17.76 | -14.72 | -14.99 | -20.99 | -16.44 | -25.60 | -18.87 | -27.11 | -21.50 | -23.41 | -19.72 | -24.33 | -19.58 |
| **AZ115-T9** | 36.5 | -20.57 | -14.50 | -19.24 | -12.73 | -14.73 | -23.40 | -17.74 | -26.33 | -19.20 | -28.31 | -22.22 | -23.61 | -20.90 | -25.48 | -20.39 |
| **AZ115-T9** | 37 | -19.33 | -12.34 | -18.86 | -13.04 | -14.73 | -21.53 | -16.49 | -25.96 | -19.22 | -27.02 | -21.69 | -23.74 | -19.83 | -24.79 | -19.58 |
| **AZ115-T9** | 37.5 | -19.88 | -13.80 | -18.59 | -13.69 | -13.22 | -21.62 | -17.47 | -25.89 | -18.83 | -27.26 | -21.62 | -23.60 | -20.69 | -24.70 | -19.88 |
| **AZ115-T9** | 38 | -18.21 | -13.85 | -16.96 | -15.01 | -10.34 | -19.55 | -16.61 | -26.25 | -19.37 | -27.36 | -21.74 | -22.72 | -19.92 | -24.74 | -19.28 |
| **AZ115-T9** | 38.5 | -17.83 | -13.88 | -17.33 | -14.31 | -11.00 | -20.18 | -16.22 | -25.89 | -19.21 | -26.98 | -22.11 | -22.80 | -19.78 | -24.14 | -19.20 |
| **AZ115-T9** | 39 | -16.68 | -12.35 | -15.65 | -12.64 | -9.14 | -17.80 | -14.01 | -22.74 | -15.69 | -23.87 | -20.23 | -19.52 | -17.94 | -21.28 | -17.06 |
| **AZ115-T9** | 39.8 | -19.69 | -12.95 | -18.52 | -13.63 | -9.35 | -19.46 | -15.87 | -24.70 | -17.10 | -24.93 | -19.80 | -19.99 | -18.00 | -22.10 | -18.41 |
| **CAM9-T39** | 0.5 | -10.59 | -0.06 | -6.05 | -1.25 | -7.79 | -10.49 | -8.01 | -15.36 | -11.12 | -18.64 | -13.03 | -16.34 | -12.77 | -17.99 | -9.84 |
| **CAM9-T39** | 1 | -10.03 | -4.17 | -5.47 | -2.11 | -5.06 | -10.77 | -7.76 | -15.22 | -11.79 | -18.11 | -13.18 | -15.35 | -9.29 | -17.92 | -9.65 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **CAM9-T39** | 1.5 | -11.38 | -1.65 | -3.85 | -2.70 | -6.07 | -12.03 | -9.61 | -15.38 | -10.76 | -18.75 | -13.15 | -15.25 | -9.56 | -18.02 | -9.59 |
| **CAM9-T39** | 2 | -10.41 | -0.72 | -4.84 | -1.81 | -7.46 | -12.57 | -7.13 | -15.98 | -11.90 | -19.15 | -13.26 | -16.06 | -11.58 | -17.77 | -9.73 |
| **CAM9-T39** | 2.5 | -10.51 | -0.78 | -4.60 | -1.07 | -5.94 | -12.07 | -10.71 | -15.16 | -11.83 | -18.86 | -13.32 | -15.33 | -10.45 | -18.07 | -9.71 |
| **CAM9-T39** | 3 | -11.05 | -3.63 | -7.18 | -1.03 | -6.09 | -9.27 | -6.69 | -16.75 | -12.84 | -18.89 | -14.02 | -16.76 | -10.71 | -18.05 | -10.17 |
| **CAM9-T39** | 3.5 | -14.22 | -3.36 | -8.41 | -4.30 | -6.40 | -12.55 | -10.77 | -17.99 | -13.53 | -20.22 | -14.46 | -18.52 | -13.19 | -19.34 | -11.97 |
| **CAM9-T39** | 4 | -11.80 | -5.04 | -8.68 | -3.73 | -7.23 | -12.49 | -9.54 | -16.94 | -12.00 | -20.01 | -14.91 | -18.69 | -14.05 | -20.14 | -11.86 |
| **CAM9-T39** | 4.5 | -10.97 | -1.60 | -6.70 | -2.31 | -5.62 | -12.30 | -12.00 | -17.43 | -12.13 | -19.67 | -14.30 | -18.64 | -12.95 | -19.83 | -11.13 |
| **CAM9-T39** | 5 | -10.60 | -4.45 | -8.26 | -4.53 | -7.30 | -11.67 | -11.90 | -17.64 | -11.70 | -18.68 | -13.97 | -18.88 | -11.95 | -18.84 | -11.55 |
| **CAM9-T39** | 5.5 | -11.97 | -4.50 | -7.91 | -3.98 | -8.06 | -12.21 | -10.70 | -18.60 | -15.19 | -21.21 | -14.73 | -20.93 | -14.73 | -20.99 | -12.44 |
| **CAM9-T39** | 6 | -11.16 | -1.67 | -7.38 | -3.96 | -7.90 | -11.40 | -10.65 | -17.77 | -13.51 | -19.57 | -14.31 | -19.12 | -13.07 | -20.54 | -11.39 |
| **CAM9-T39** | 6.5 | -10.05 | -1.25 | -6.66 | -2.98 | -6.41 | -10.73 | -9.64 | -17.10 | -13.20 | -20.03 | -14.04 | -19.22 | -13.42 | -20.04 | -10.93 |
| **CAM9-T39** | 7 | -9.96 | -1.24 | -6.27 | -3.80 | -6.68 | -10.95 | -7.59 | -16.00 | -12.35 | -19.07 | -13.11 | -18.42 | -12.13 | -19.09 | -10.29 |
| **CAM9-T39** | 7.5 | -11.18 | -2.19 | -6.27 | -2.73 | -5.16 | -9.12 | -7.40 | -15.62 | -12.79 | -19.15 | -13.80 | -19.38 | -12.28 | -19.53 | -10.32 |
| **CAM9-T39** | 8 | -9.68 | -4.32 | -8.62 | -5.71 | -7.61 | -9.72 | -7.20 | -17.16 | -13.25 | -19.59 | -14.52 | -20.51 | -14.04 | -22.10 | -11.62 |
| **CAM9-T39** | 8.5 | -12.02 | -2.94 | -7.71 | -5.36 | -8.84 | -10.65 | -11.36 | -19.70 | -14.80 | -21.92 | -17.24 | -21.35 | -15.51 | -22.09 | -12.80 |
| **CAM9-T39** | 9 | -11.71 | -2.20 | -7.55 | -3.25 | -7.57 | -10.27 | -10.74 | -18.88 | -15.12 | -21.92 | -16.26 | -20.21 | -14.81 | -22.08 | -12.18 |
| **CAM9-T39** | 9.5 | -10.08 | -3.46 | -8.15 | -7.11 | -8.34 | -10.93 | -12.10 | -19.17 | -14.06 | -21.84 | -16.36 | -20.93 | -14.94 | -22.55 | -12.82 |
| **CAM9-T39** | 10 | -11.94 | -3.25 | -8.31 | -5.94 | -8.60 | -10.49 | -12.90 | -19.19 | -13.95 | -21.70 | -16.74 | -19.55 | -15.17 | -22.70 | -12.85 |
| **CAM9-T39** | 10.5 | -12.39 | -3.30 | -9.20 | -4.82 | -8.67 | -11.59 | -12.31 | -19.64 | -15.23 | -22.15 | -16.17 | -21.04 | -16.16 | -22.81 | -13.21 |
| **CAM9-T39** | 11 | -10.88 | -3.25 | -6.78 | -4.92 | -7.31 | -12.20 | -12.24 | -18.40 | -13.72 | -21.78 | -16.05 | -20.47 | -14.79 | -22.33 | -12.35 |
| **CAM9-T39** | 11.5 | -9.68 | -2.01 | -7.06 | -4.80 | -8.10 | -12.68 | -12.10 | -18.69 | -14.30 | -21.08 | -15.65 | -20.56 | -14.23 | -21.60 | -12.11 |
| **CAM9-T39** | 12 | -10.07 | -3.01 | -7.67 | -5.06 | -5.53 | -13.17 | -12.45 | -16.32 | -11.41 | -19.43 | -13.57 | -19.01 | -12.38 | -19.11 | -11.41 |

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **CAM9-T39** | 12.5 | -9.43 | -1.86 | -8.07 | -4.72 | -7.37 | -13.62 | -11.98 | -18.97 | -13.48 | -21.38 | -15.90 | -20.32 | -14.73 | -20.61 | -12.27 |
| **CAM9-T39** | 13 | -12.04 | -4.28 | -6.50 | -4.38 | -8.43 | -15.08 | -11.34 | -19.08 | -13.82 | -21.86 | -15.85 | -20.37 | -14.53 | -21.10 | -12.46 |
| **CAM9-T39** | 13.5 | -8.27 | -2.19 | -7.60 | -4.47 | -7.34 | -14.66 | -13.43 | -19.27 | -14.24 | -21.54 | -15.98 | -21.94 | -14.55 | -21.76 | -12.50 |
| **CAM9-T39** | 14 | -8.86 | -1.38 | -8.08 | -4.92 | -8.41 | -16.03 | -12.27 | -19.27 | -14.72 | -21.61 | -16.08 | -20.21 | -15.31 | -22.12 | -12.58 |
| **CAM9-T39** | 14.5 | -9.60 | -1.30 | -7.13 | -4.38 | -8.54 | -13.42 | -12.39 | -19.39 | -14.69 | -21.77 | -15.68 | -20.18 | -14.65 | -21.64 | -12.29 |
| **CAM9-T39** | 15 | -8.53 | -3.04 | -8.10 | -5.39 | -8.58 | -16.21 | -12.49 | -19.92 | -15.02 | -21.66 | -16.14 | -20.50 | -15.31 | -22.51 | -12.88 |
| **CAM9-T39** | 15.5 | -10.76 | -2.79 | -5.56 | -3.44 | -9.76 | -15.08 | -10.91 | -18.59 | -12.75 | -19.94 | -13.93 | -20.64 | -13.72 | -20.02 | -11.55 |
| **CAM9-T39** | 16 | -9.98 | -3.76 | -7.45 | -6.05 | -9.86 | -14.93 | -12.20 | -20.39 | -15.28 | -22.01 | -16.52 | -19.98 | -14.82 | -21.50 | -12.93 |
| **CAM9-T39** | 16.5 | -9.36 | -3.21 | -4.18 | -2.52 | -6.57 | -13.26 | -8.95 | -17.78 | -13.04 | -19.43 | -14.29 | -18.24 | -12.01 | -19.39 | -10.51 |
| **CAM9-T39** | 17 | -9.69 | -6.40 | -6.03 | -4.99 | -10.03 | -14.37 | -11.08 | -20.38 | -15.53 | -22.05 | -16.93 | -20.39 | -13.52 | -21.60 | -12.65 |
| **CAM9-T39** | 17.5 | -9.36 | -6.12 | -6.16 | -4.90 | -9.06 | -14.54 | -10.44 | -19.37 | -14.60 | -21.54 | -16.53 | -19.85 | -12.97 | -20.78 | -12.23 |
| **CAM9-T39** | 18 | -8.75 | -5.82 | -6.71 | -5.09 | -9.79 | -15.43 | -11.50 | -20.17 | -16.10 | -22.36 | -17.07 | -21.31 | -14.48 | -22.12 | -12.97 |
| **CAM9-T39** | 18.5 | -8.32 | -5.58 | -6.81 | -5.77 | -9.29 | -15.46 | -12.38 | -20.33 | -14.35 | -22.11 | -16.74 | -21.60 | -15.79 | -22.33 | -13.07 |
| **CAM9-T39** | 19 | -8.23 | -6.15 | -6.42 | -6.53 | -8.12 | -15.89 | -11.70 | -19.83 | -15.40 | -22.03 | -17.02 | -22.17 | -15.40 | -22.40 | -13.08 |

One single hair from each individual was analyzed, with the exception of CAM15A-T14, QUI7-T13 and ME-C2 individuals of which two single hairs [(I), (II)] were analyzed. Distance (Dist.) of hair segments from scalp is expressed in centimeters. Orientation and distance from scalp of ME-C2 (I) and (II) hairs were unknown due to the fragmented status of the sample. $\delta^{13}C$ Mass balance (‰) values have been calculated using keratin amino acid composition from Wolfram (2003).

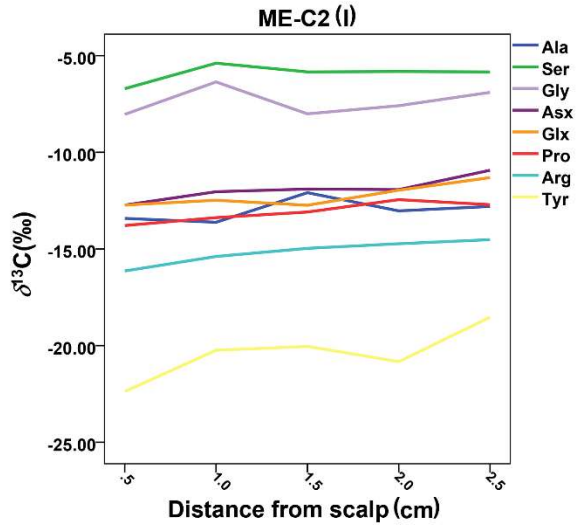
Three segments (1.5cm, 4cm, and 5cm) of CAM15A-T14 (I) hair have been detected as having altered AA compositions and rejected. The hydrolysis of the segment 6.5cm of QUI7-T13 (I) hair was unsuccessful.

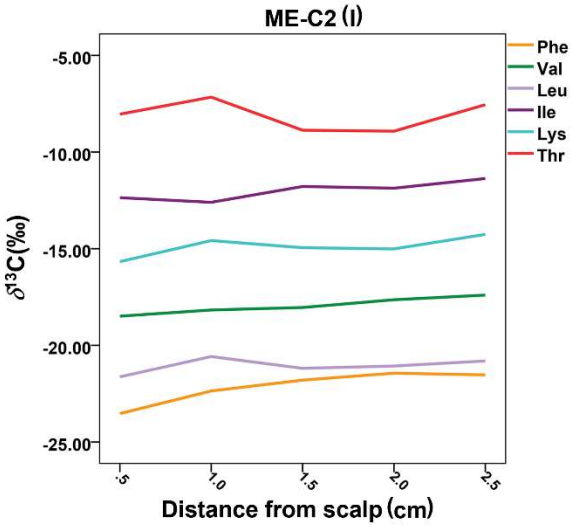
Amino acids are presented in order of elution: Aspartic acid (Asx), Serine (Ser), Glutamic acid (Glx), Threonine* (Thr*), Glycine (Gly), Alanine (Ala), Proline (Pro), Valine* (Val*), Isoleucine* (Ile*), Leucine* (Leu*), Lysine* (Lys*), Tyrosine (Tyr), Arginine (Arg), and Phenylalanine* (Phe*). Asterisk indicates essential amino acids (EAAs).

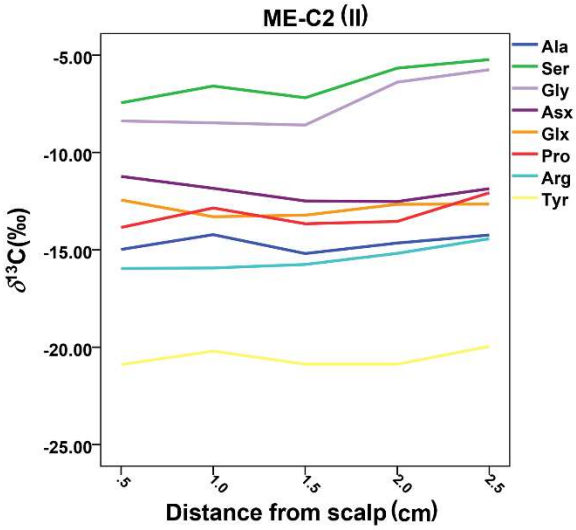
**References**

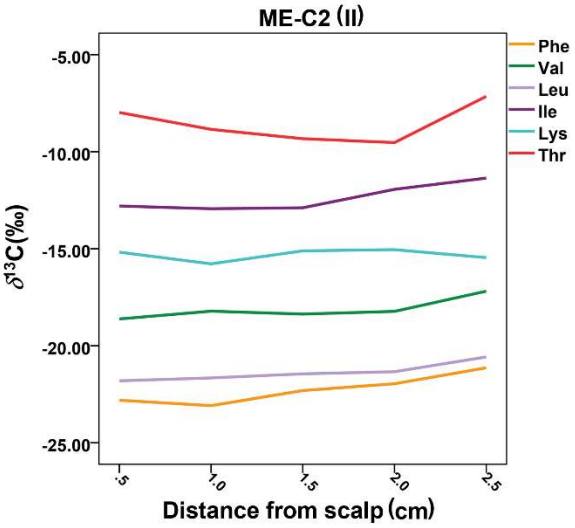
Wolfram, L.J., 2003. Human hair: A unique physicochemical composite. Journal of the American Academy of Dermatology 48, S106–S114.

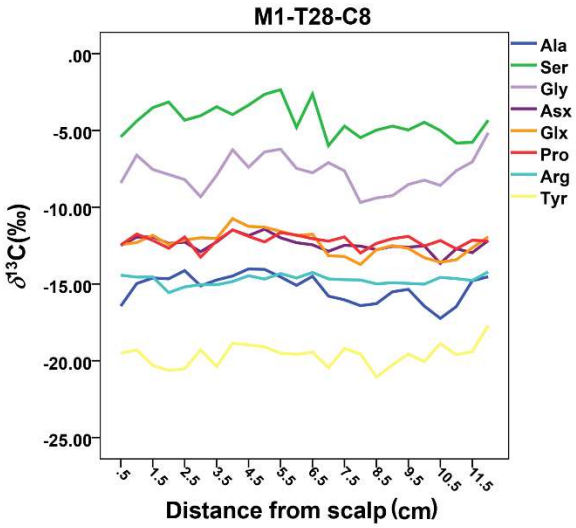
**Supporting Information 4.** Plots of $\delta^{13}$C amino acid values along the single hair fibers.

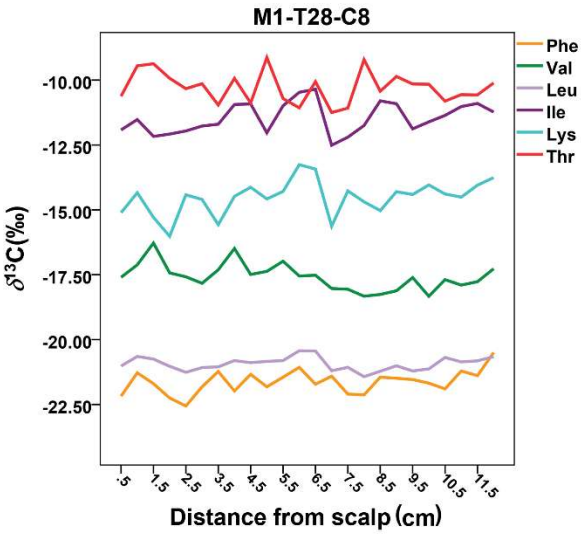


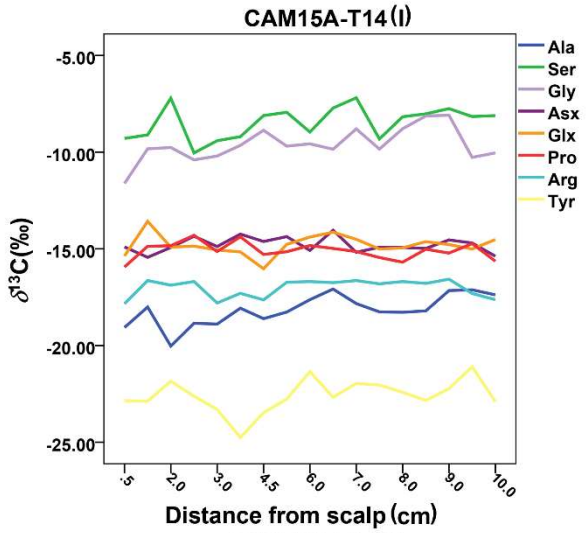



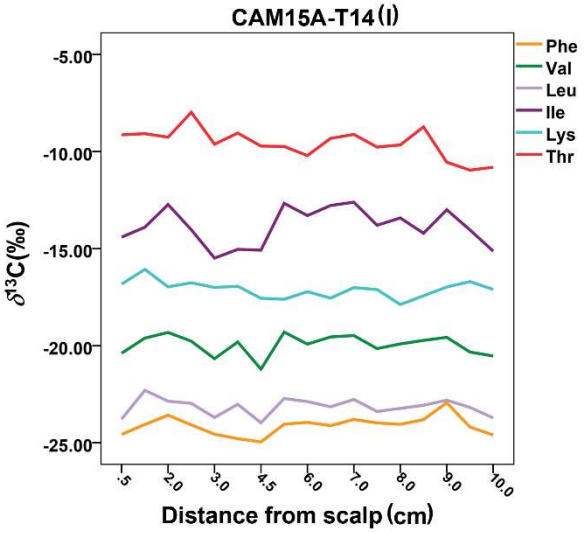



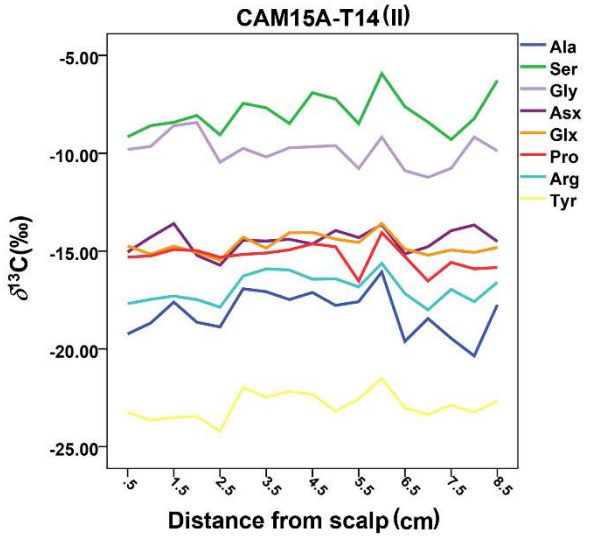



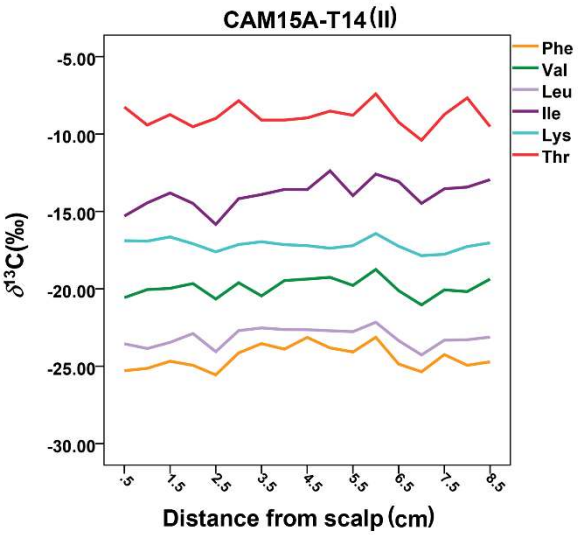



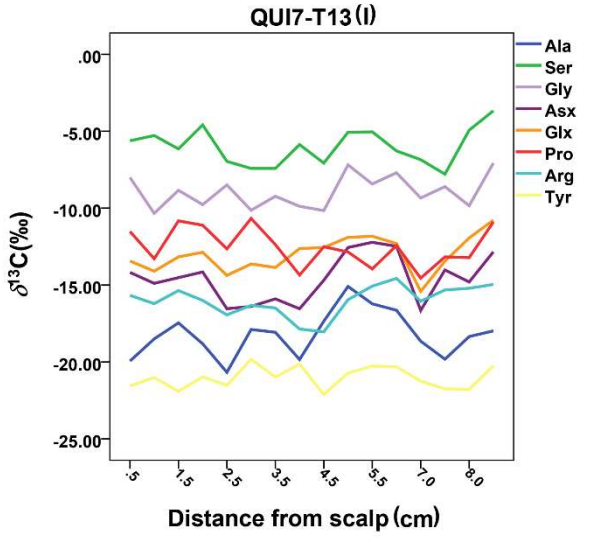



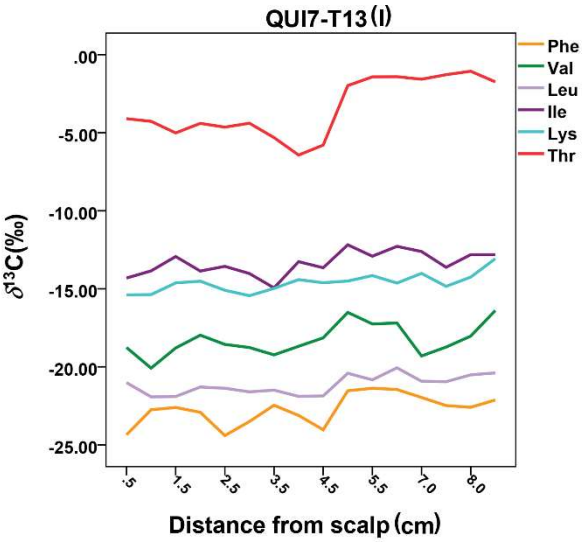

CAM15A-T14 (I)
Ala
Ser
Gly
Asx
Glx
Pro
Arg
Tyr
-5.00
-10.00
-15.00
-20.00
-25.00
δ13C(‰)
.5
2.0
3.0
4.5
6.0
7.0
8.0
9.0
10.0
Distance from scalp (cm)

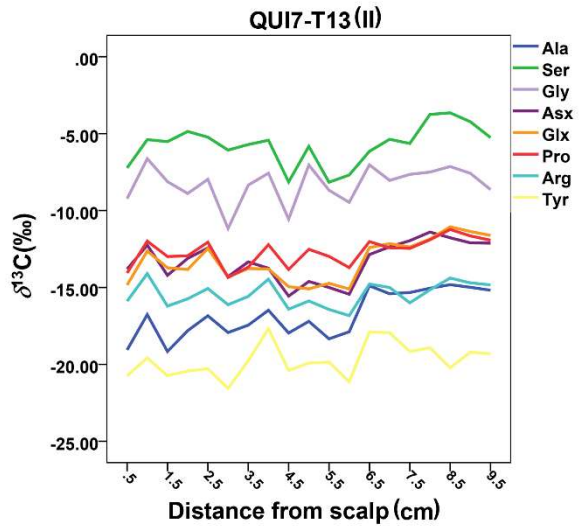

CAM15A-T14 (I)
Phe
Val
Leu
Ile
Lys
Thr
-5.00
-10.00
-15.00
-20.00
-25.00
δ13C(‰)
.5
2.0
3.0
4.5
6.0
7.0
8.0
9.0
10.0
Distance from scalp (cm)

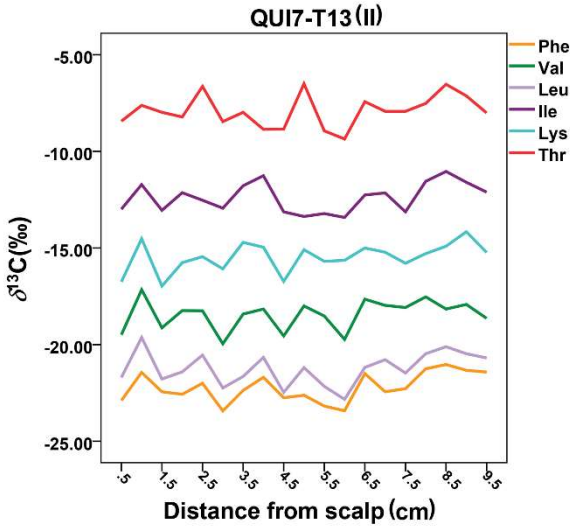

CAM15A-T14 (II)
Ala
Ser
Gly
Asx
Glx
Pro
Arg
Tyr
-5.00
-10.00
-15.00
-20.00
-25.00
δ13C(‰)
.5
1.5
2.5
3.5
4.5
5.5
6.5
7.5
8.5
Distance from scalp (cm)

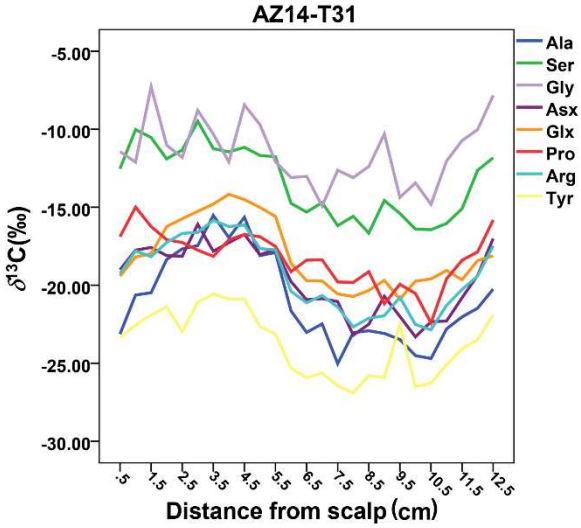

CAM15A-T14 (II)
Phe
Val
Leu
Ile
Lys
Thr
-5.00
-10.00
-15.00
-20.00
-25.00
-30.00
δ13C(‰)
.5
1.5
2.5
3.5
4.5
5.5
6.5
7.5
8.5
Distance from scalp (cm)

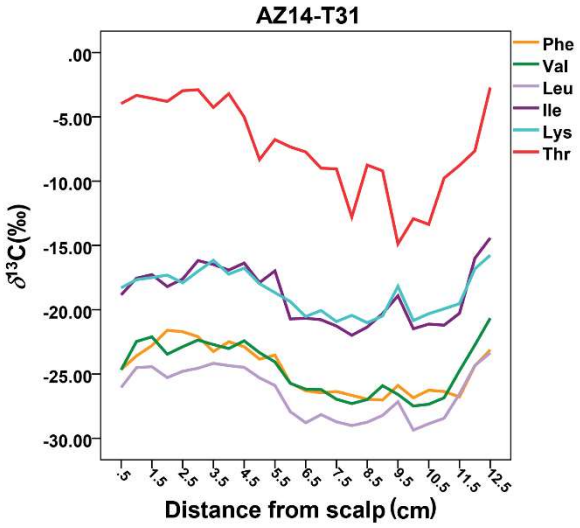

QUI7-T13 (I)
Ala
Ser
Gly
Asx
Glx
Pro
Arg
Tyr
.00
-5.00
-10.00
-15.00
-20.00
-25.00
δ13C(‰)
.5
1.5
2.5
3.5
4.5
5.5
7.0
8.0
Distance from scalp (cm)

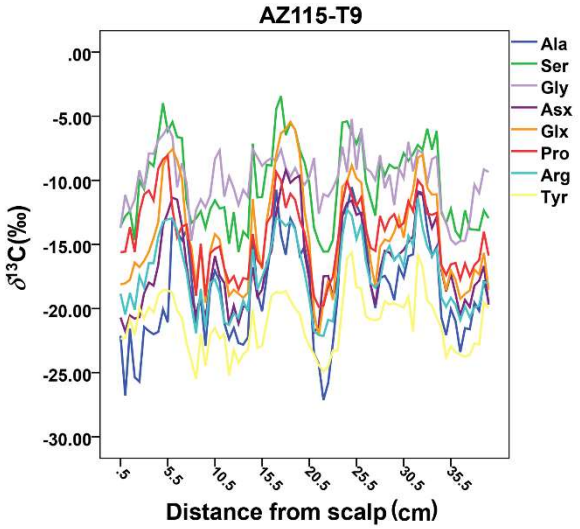

QUI7-T13 (I)
Phe
Val
Leu
Ile
Lys
Thr
.00
-5.00
-10.00
-15.00
-20.00
-25.00
δ13C(‰)
.5
1.5
2.5
3.5
4.5
5.5
7.0
8.0
Distance from scalp (cm)

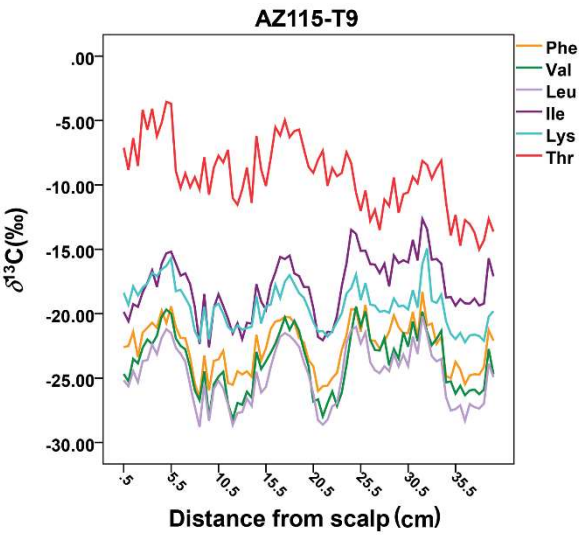

QUI7-T13 (II)
Ala
Ser
Gly
Asx
Glx
Pro
Arg
Tyr
δ13C(‰)
.00
-5.00
-10.00
-15.00
-20.00
-25.00
.5
1.5
2.5
3.5
4.5
5.5
6.5
7.5
8.5
9.5
Distance from scalp (cm)

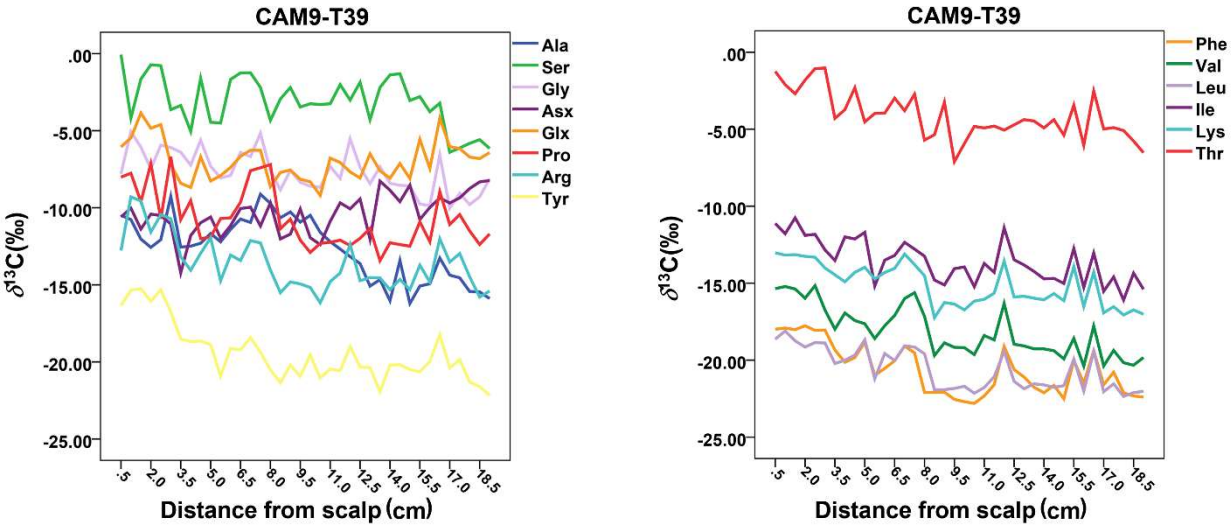

QUI7-T13 (II)
Phe
Val
Leu
Ile
Lys
Thr
δ13C(‰)
-5.00
-10.00
-15.00
-20.00
-25.00
.5
1.5
2.5
3.5
4.5
5.5
6.5
7.5
8.5
9.5
Distance from scalp (cm)


AZ14-T31
Ala
Ser
Gly
Asx
Glx
Pro
Arg
Tyr
δ13C(‰)
-5.00
-10.00
-15.00
-20.00
-25.00
-30.00
.5
1.5
2.5
3.5
4.5
5.5
6.5
7.5
8.5
9.5
10.5
11.5
12.5
Distance from scalp (cm)


AZ14-T31
Phe
Val
Leu
Ile
Lys
Thr
δ13C(‰)
.00
-5.00
-10.00
-15.00
-20.00
-25.00
-30.00
.5
1.5
2.5
3.5
4.5
5.5
6.5
7.5
8.5
9.5
10.5
11.5
12.5
Distance from scalp (cm)


AZ115-T9
Ala
Ser
Gly
Asx
Glx
Pro
Arg
Tyr
δ13C(‰)
.00
-5.00
-10.00
-15.00
-20.00
-25.00
-30.00
.5
5.5
10.5
15.5
20.5
25.5
30.5
35.5
Distance from scalp (cm)


AZ115-T9
Phe
Val
Leu
Ile
Lys
Thr
δ13C(‰)
.00
-5.00
-10.00
-15.00
-20.00
-25.00
-30.00
.5
5.5
10.5
15.5
20.5
25.5
30.5
35.5
Distance from scalp (cm)




The error associated with $\delta^{13}C$ amino acid values is ~ <±0.6‰.