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Global spatial distributions of nitrogen and carbon stable isotope ratios of modern human hair

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RATIONALE: Natural stable carbon ($\delta^{13}C$) and nitrogen isotope ratios ($\delta^{15}N$) of humans are related to individual dietary habits and environmental and physiological factors. In forensic science the stable isotope ratios of human remains such as hair and nail are used for geographical allocation. Thus, knowledge of the global spatial distribution of human $\delta^{13}C$ and $\delta^{15}N$ values is an essential component in the interpretation of stable isotope analytical results.
METHODS: No substantial global datasets of human stable isotope ratios are currently available, although the amount of available (published) data has increased within recent years. We have herein summarised the published data on human global $\delta^{13}C$ and $\delta^{15}N$ values (around 3600 samples) and added experimental values of more than 400 additional worldwide human hair and nail samples. In order to summarise isotope ratios for hair and nail samples correction factors were determined.
RESULTS: The current available dataset of human stable isotope ratios is biased towards Europe and North America with only limited data for countries in Africa, Central and South America and Southeast Asia. The global spatial distribution of carbon isotopes is related to latitude and supports the fact that human $\delta^{13}C$ values are dominated by the amount of C_4 plants in the diet, either due to direct ingestion as plant food, or by its use as animal feed. In contrast, the global spatial distribution of human $\delta^{15}N$ values is apparently not exclusively related to the amount of fish or meat ingested, but also to environmental factors that influence agricultural production.
CONCLUSIONS: There are still a large proportion of countries, especially in Africa, where there are no available data for human carbon and nitrogen isotope ratios. Although the interpretation of modern human carbon isotope ratios at the global scale is quite possible, and correlates with the latitude, the potential influences of extrinsic and/or intrinsic factors on human nitrogen isotope ratios have to be taken into consideration. Copyright © 2015 John Wiley & Sons, Ltd.

In forensic investigations natural stable carbon ($\delta^{13}C$) and nitrogen isotope ratios ($\delta^{15}N$) of human hair and nail are increasingly used – in combination with other elements – for the geographical allocation of individuals.[1–4] In principle, the carbon and nitrogen isotopic composition of an organism is characterised by the isotopic composition of its diet. However, human hair and nail are enriched in ^{13}C and ^{15}N compared with the individual's diet, because the lighter isotope is preferentially excreted during metabolism.[5] Dietary changes can be identified by retrospective segmental analysis of the carbon and nitrogen isotopic composition of human hair.[6] Because human dietary habits and nutritional sources are different all over the world, the $\delta^{13}C$ and $\delta^{15}N$ values of human hair and nail can provide information about the diet and thus about the geographical provenance of the individual.

Since the 1980s carbon and nitrogen isotope ratios of contemporary human hair have been analysed.[7,8] Due to the easy and non-invasive sampling, hair – and to a lesser extent, nail – became the favoured tissue for natural stable isotope analysis of modern humans. The $\delta^{13}C$ value of human hair, as well as of other tissues, is affected by the amount of C_3 and C_4 plants in the diet, and also by the amount of C_3 versus C_4 plants in the diet of the animals eaten by humans.[6–8] Because C_4 plants such as maize or sugar cane are significantly enriched in ^{13}C compared with C_3 plants such as wheat or rice, an increased consumption of C_4 plants leads to increased human $\delta^{13}C$ values.[6–8] On the other hand, human $\delta^{15}N$

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values are related to the $\delta^{15}N$ of the dietary intake and are probably determined by the amount of fish or meat and animal products consumed.[5,6] Differences in human hair $\delta^{15}N$ values have been reported for vegans, vegetarians and omnivores,[9–12] although the amount of meat intake cannot always be identified by stable isotope ratio analysis of human hair.[13] Nevertheless, geographically distinct patterns of meat consumption might be identifiable if the $\delta^{15}N$ values of the ingested meat are significantly different from those of plant-based food. Humans living in remote areas with specialised diets might show significant different $\delta^{15}N$ values from the average populations. For example, Inuit living in Greenland have highly ^{15}N enriched fingernails.[14]

Altered carbon and nitrogen isotope ratios of the diet are identifiable in human hair.[6,7,11,15] However, significant time must elapse (from days to weeks) before the isotopic signal can be detected in the tissue.[6,7,11,15] Conditions of physiology and health (e.g. eating disorders, pregnancy) are additional factors influencing natural stable isotope ratios, with a probably smaller effect on carbon, but a strong influence on nitrogen isotope ratios.[16] Therefore, changes in the carbon and nitrogen isotopic composition along a strand of hair cannot exclusively be related to dietary changes.

In order to be able to interpret $\delta^{13}C$ and $\delta^{15}N$ values of human hair, sufficient knowledge of the fundamental principles defining the isotopic composition of the human organism is necessary in addition to information about the global spatial distribution of carbon and nitrogen isotopes. It has been proposed that carbon and nitrogen isotope ratios – among others – can be used for the geographical allocation of human remains.[1–4,7,15] For some areas such as Europe comprehensive datasets are available,[4,17] whereas for other regions little or no data are available. The aim of this study was to compile literature and unpublished data on the carbon and nitrogen stable isotope ratios of worldwide-collected human hair and nail samples. This dataset might serve as a basis for the interpretation of stable isotope analysis in forensic investigations and should illustrate which regions require additional isotope data.

EXPERIMENTAL

Literature values

Stable isotope data of human hair and nail samples were obtained by scanning online literature databases including published data from 1982 through 2014. The selected publications dealt with the natural carbon and nitrogen stable isotope ratios of contemporary humans. Data for historical populations were not included, nor were data for children, pregnant women, and people with certain diseases or subjects with special diets such as a vegan lifestyle. From each publication those subsets of data representing clearly defined groups were included. Such groups were defined by geographical provenance or dietary characteristics. The arithmetic mean, the number of individual samples and, if available, the standard deviation were imported into the dataset for each group. Data presented only in graphical form were converted into numerical values using the image analysis program ImageJ (National Institute of Health (NIH)).[18]

Experimental values

New experimental data (406 samples for carbon, 415 samples for nitrogen) were obtained by analysing hair and nail from contemporary humans provided by different individuals from all over the world. Only samples with specified origin of the requested country were analysed. Prior to analysis, the hair and nail samples were washed using standard procedures.[11] The samples were processed and analysed by different laboratories, including: (1) Institute of Biochemistry, German Sport University Cologne, Germany; (2) Institute of Forensic Medicine, Ludwig-Maximilians-University Munich, Germany; (3) Forensic Science Institute, Federal Criminal Police Office, Unit Central Analytics II, Wiesbaden, Germany; (4) Department of Forensic and Investigative Science, West Virginia University, Morgantown, WV, USA; (5) LGC, Teddington, UK. The samples were analysed using different elemental analyser/isotope ratio mass spectrometry (EA/IRMS) systems in the different laboratories following recommended methods of stable isotope ratio measurements and reporting results thereof.[19] Detailed information about the analytical systems can be found in the following.

EA/IRMS analysis laboratory (1)

Hair samples (approximately 40 µg for carbon, 200 µg for nitrogen) were cut into approximately 1-mm segments, loaded into tin capsules and analysed by EA/IRMS using an Eurovector EA 3000 elemental analyser (Hekatech, Wegberg, Germany) equipped with a Zero Blank Revolver autosampler (Blisotec, Jülich, Germany) coupled to a Delta C continuous-flow isotope ratio mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). Carbon isotope ratios are expressed relative to VPDB, nitrogen isotope ratios are expressed relative to atmospheric nitrogen (AIR). Working standard gases (CO_2, purity 5.3; N_2, purity 5.0; both from Linde, Munich, Germany) and a working standard (creatine monohydrate, AlzChem, Trostberg, Germany) were scale calibrated using IAEA-CH-6 (–10.4 ‰) and IAEA-CH-7 (–31.8 ‰) for $\delta^{13}C$ and IAEA-N-1 (0.4 ‰) and IAEA-N-2 (20.3 ‰) for $\delta^{15}N$ (all from IAEA, Vienna, Austria). The standard deviation of three repeated measurements of the working standard was ±0.2 ‰ for $\delta^{13}C$ and $\delta^{15}N$. Isotope ratio analyses of the hair samples of each individual were carried out in different runs and in triplicate for both carbon and nitrogen. The analytical procedure was checked after every sixth analysis for the zero blank. The instrument stability was also checked by the analysis of the working standard after every sixth measurement.

EA/IRMS analysis laboratory (2)

For the analysis of carbon and nitrogen isotope ratios, between 1.8 and 2.0 mg of finely cut hair and nail samples were weighed into tin capsules in quadruplicate. Hair and nail samples were both cut into very small pieces, for hair less than 1 mm. The samples were analysed for carbon, nitrogen (and sulfur) simultaneously at isolab, Schweitenkirchen, Germany, using an Vario EL Cube elemental analyser (Elementar, Hanau, Germany) connected with an Isoprime mass spectrometer (Isoprime, Cheadle Hulme, UK). The internal standards used during the analysis were casein (Kremer Pigmente, Aichstätten, Germany) and two different

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horsetail hair samples (both from local suppliers). Scale calibrations were performed with IAEA-NO-3 (4.6 ‰, IAEA) and USGS25 (–30.41 ‰, USGS, Reston, VA, USA) for δ^{15}N and NBS 22 (–29.91 ‰, IAEA) and IRMM-BCR 657 (–10.76 ‰, IRMM, Geel, Belgium) for δ^{13}C. The analytical precisions using at least quadruplicate measurements were: ±0.1 ‰ (carbon), and ±0.2 ‰ (nitrogen). The data were drift corrected using repeated measurement of the laboratory standard after 30 samples.

EA/IRMS analysis laboratory (3)

For elemental analysis 0.2 mg of powdered hair or nail sample was weighed in tin capsules in triplicate. These were flash combusted on a Flash EA 1112 elemental analyser online coupled to a Delta V plus isotope ratio mass spectrometer via a ConFlo IV interface (all Thermo Fisher Scientific). The working standard gases and the in-house standard acetanilide were calibrated by using secondary IAEA standards. For nitrogen the standards IAEA-N1 (0.4 ‰), IAEA-N2 (20.3 ‰) and IAEA-NO3 (4.7 ‰) and for carbon the standards IAEA-CH6 (–10.45 ‰) and IAEA-CH7 (–32.15 ‰) were used. In addition to acetanilide, one hair and one nail sample were used as in-house standards to routinely check the accuracy and reproducibility of the system. The measurement uncertainty (standard deviation of repeated measurements) was determined to be ± 0.10 ‰ for δ^{13}C and ± 0.16 ‰ for δ^{15}N. The raw values were blank corrected and the weighted amount was adapted to the linearity range of the instrument. Drift was checked by measuring the in-house standard at the beginning, the end and throughout the sequence after nine unknown samples.

EA/IRMS analysis laboratory (4)

Bulk isotope measurements were made on samples of approximately 0.5 mg of powdered hair that were placed in tin capsules in a Flash HT Plus elemental analyser which was coupled via a ConFlo IV interface to a Delta V Advantage isotope ratio mass spectrometer (all Thermo Fisher Scientific). Data acquisition was carried out using Isodat 3.0 Software (Thermo Fisher Scientific). During EA analysis, high-purity gases from Airgas (Morgantown, WV, USA) were used. The results of the isotope ratio analyses are reported in per mill (‰) on the relative δ-scale. Normalisation to international isotope scales was accomplished through the use of a four-point calibration curve for δ^{13}C and a two-point calibration curve for δ^{15}N, after ^{17}O corrections using the standard Santrock algorithm. The standards used were L-SVEC (–46.60 ‰), NBS 19 (1.950 ‰, both from NIST, Gaithersburg, MD, USA), USGS40 (26.389 ‰), and USGS41 (37.626 ‰) for δ^{13}C, and USGS40 (–4.5 ‰), USGS41 (47.6 ‰, all from USGS, Reston, VA, USA) for δ^{15}N. An in-house working standard of sulfanilamide was used to monitor for any drift during the course of the data acquisition. Blanks were run periodically and were below threshold. The measurement uncertainty was determined to be ± 0.1 ‰ for δ^{13}C and ± 0.2 ‰ for δ^{15}N.

EA/IRMS analysis laboratory (5)

For the determination of carbon and nitrogen isotope ratios approximately 250 µg of finely cut hair samples were weighed into tin capsules in triplicate. IRMS analyses for carbon were carried out using a Finnigan MAT CN elemental analyser coupled to a MAT 252 isotope ratio mass spectrometer via a ConFlo III universal interface (all Thermo Fisher Scientific). All raw measurements were made relative to pulses of a CO_2 working gas. Data were analysed using Isodat 2.0 software (Thermo Fisher Scientific) using the Santrock ^{17}O correction algorithm. The raw δ^{13}C values obtained by the software were normalised to the VPDB scale using a multiple-point linear regression based upon certified secondary reference materials run during the same sequence, namely: NBS-22 (–30.031 ‰), IAEA-CH-6 (–10.449 ‰), USGS40 (–26.389 ‰) and USGS41 (37.626 ‰) (all from IAEA).

IRMS analyses for nitrogen were carried out using a Flash EA/HT in combustion mode with a MAS 200R autosampler with a 'NoBlank Device' coupled to a Delta V Advantage isotope ratio mass spectrometer via a ConFlo IV universal interface (all Thermo Fisher Scientific). All raw measurements were made relative to pulses of a N_2 working gas. Data were analysed using Isodat 3.0 software (Thermo Fisher Scientific). The raw δ^{15}N values were normalised to the AIR scale using a multiple-point linear regression based upon certified secondary reference materials run during the same sequence, namely: IAEA-N1 (0.43 ‰), IAEA-N2 (20.41 ‰), IAEA-NO3 (4.7 ‰), USGS40 (–4.52 ‰) and USGS41 (47.53 ‰) (all from IAEA).

The raw δ^{13}C values obtained for the hair samples were corrected for blank using a simple mass balance approach; however, as the nitrogen blank was too small to determine reliably, this correction was not applied to the raw δ^{15}N values. Neither drift nor area (linearity) corrections were applied. For quality control purposes, a similarly prepared hair sample previously analysed in-house was included in each sequence of analyses. Typical measurement uncertainties (combined uncertainty k = 1) were ±0.33 ‰ for carbon and ±0.43 ‰ for nitrogen.

Correction for nail samples

According to literature reports, human hair is enriched in ^{13}C and depleted in ^{15}N compared to nail.[20,21] We summarised the available literature data (12[20] + 3[21] pairs of corresponding hair and nail samples) and new experimental data (30 pairs of corresponding hair and nail samples, analysed by laboratory (3)) in order to allow for differences in the isotopic composition of the matrices. Correction factors for nail samples were calculated as the mean difference between the isotope ratios of hair and the corresponding nail sample. Factors were applied to the nail samples of the dataset where necessary and summarised with the hair data.

Calculation of country and 'country cluster' mean values

Mean values for a country were calculated if ten[a] or more different experimental samples in at least two (preferably minimal three) different laboratories had been analysed. If these criteria were not achieved for a single country, different (preferably neighbouring) countries were summarised in a

[a]The minimal number of samples (n) was estimated using $n \geq (z^2\ \sigma^2)/e^2$ with z = 1.67 (90 % probability), σ^2 = 0.25 ‰ (variance of human populations with n > 100), and e = 0.25 ‰ (mean uncertainty error of δ^{13}C and δ^{15}N measurements).

'country cluster'. Criteria for establishing a country cluster were also based on ethnology, culture or political history. For some country clusters, the minimum number of three laboratories was still not achieved; these countries are marked in the tables. The country or country cluster mean values were calculated as the weighted mean of all individual laboratory mean values reported. In order to compensate for inhomogeneous quantities of samples analysed by the different laboratories the crucial number of samples for each country or country cluster from one laboratory was limited. If more than ten samples had been analysed, the number was set to ten, if fewer than ten samples had been analysed the original number was used. For each mean value the biased weighted estimator of variance was calculated using the reported mean values, standard deviations, and number of samples analysed.

RESULTS AND DISCUSSION

Correction factor for nail samples

It has been reported that nail and hair from the same individual show different carbon and nitrogen isotope ratios.[20,21] In order to incorporate nail samples in the global dataset, correction factors for nail carbon and nitrogen isotope ratios were determined. Using paired samples of hair and nail from the same individuals the offsets between the hair and nail isotope ratios could be determined. A total number of 45 paired samples of hair and nail with both published and unpublished data were compared (Fig. 1). Hair is enriched in ^{13}C compared with nail, with a mean offset of +0.4 ‰, comparable with values which have been reported previously (+0.21 ‰[20] and +0.3 ‰[21]). Hair is depleted in ^{15}N compared to nail by –0.6 ‰ (literature: –0.65 ‰[20] and –0.9 ‰[21]). The estimated correction factors were implemented in the global data.

Global human isotope ratio dataset

Around 4000 individual samples were summarised for the global human isotope ratio dataset. New experimental data account for about 10 % of the data (for individual data, see Supporting Information). For carbon, data from 32, and for nitrogen, data from 30 different publications were incorporated, with between 4 and 763 individual samples analysed for a single dataset. Around one-third of the data result from the analysis of (finger-)nail samples, although these data derive from only four publications. Most of the publications and the unpublished datasets represent results from the analysis of human hair.

The majority of the samples originate from Europe and North America, providing together around 55–58 % of the total dataset (Table 1). About a quarter of the samples originate from the USA. In contrast, the amount of data for Asia and Africa is considerably less. Approximately 60 % of the world population is located in Asia, but only 17–18 % of the isotope data are from Asian people. The situation is the same for Africa, which provided only 1 % of the carbon samples and 2 % of the nitrogen samples, whereas 16 % of the world's population is from Africa – a much higher figure. Thus, the whole dataset is biased towards Europe and North America. In general, the amount of data from outside Europe and North America is poor for most of the countries, with a few exceptions like Brazil. This uneven distribution required the creation of country clusters that often cover huge regions. In order to summarise sufficient data for African countries only four country clusters were established, which stretch along huge parts of the whole continent. For forensic interpretations, such large areas are unsatisfying and highlight the fact that more samples from African, Central and South American residents are needed.

Global human carbon isotope ratios

Country mean carbon isotope ratios for contemporary human hair samples vary from –16.6 ±2.7 ‰ for Brazil to –21.6 ±0.7 ‰ for Finland and Sweden (Table 2). Humans living in northern Europe have the lowest $\delta^{13}C$ values worldwide, as has been reported previously.[17] In general, the $\delta^{13}C$ values are lower in Europe than in the rest of the world. The highest $\delta^{13}C$ values are found in South and North America as well as in Eastern and Southern Africa. Compared with the American country mean values all the European values are significantly different ($p < 0.05$) and most of the values from European countries are

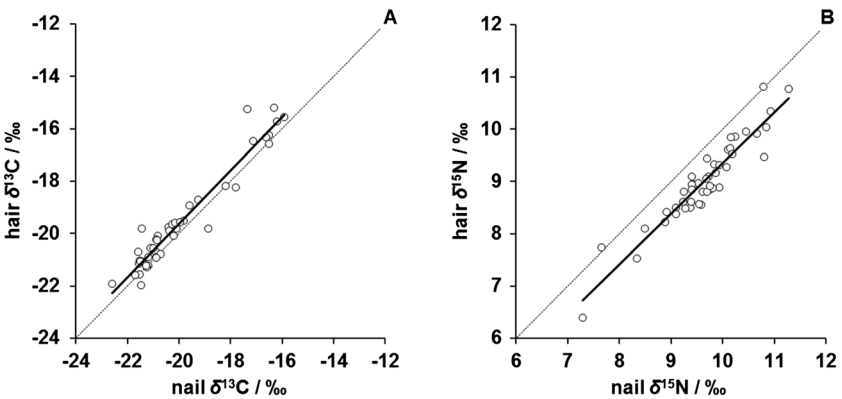

Figure 1. Comparison of hair and nail stable isotope ratios for carbon (A) and nitrogen (B) from the same subjects. Data points include carbon and nitrogen stable isotope values from literature[20,21] as well as unpublished data. The dotted line represents the 1:1 correlation, the black solid line represents the linear regression.

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Table 1. Continental distribution of available carbon and nitrogen isotope data for hair and nails

Continent	Population[a]	$\delta^{13}C$		$\delta^{15}N$	
	%	*n*	(%)	*n*	(%)
Europe	10	1220	(30)	1199	(29)
North America	8	1114	(28)	1082	(26)
South America	6	813	(20)	809	(20)
Asia	60	689	(17)	731	(18)
Africa	16	58	(1)	69	(2)
Australia	1	108	(3)	227	(6)
total		4002		4117	

[a]Population percentage calculated from United Nations data.[22]

different from the African mean values ($p < 0.05$). Within Europe, the country cluster Finland and Sweden with its extremely low $\delta^{13}C$ mean value is significantly different from the rest of the European countries ($p < 0.05$). Likewise, Portugal and Spain, ^{13}C enriched, are different from most of the other European countries ($p < 0.05$). Central European countries like Austria and Switzerland with a mean value of –20.7 ‰ are only significantly different from those European countries with extreme $\delta^{13}C$ values. Brazil is significantly different from most other countries worldwide ($p < 0.001$) except Southern Africa and Central/Northern South America. In Africa, the $\delta^{13}C$ values are different in Eastern and Southern Africa from Northern and Western Africa ($p < 0.001$ and $p < 0.05$). For most of the countries in Asia, the mean $\delta^{13}C$ values are in the middle range of global $\delta^{13}C$ values and only Japan and Korea samples are enriched in ^{13}C and significantly different from the European countries ($p < 0.01$). The mean $\delta^{13}C$ value from New Zealand is significantly different from that of Australia ($p < 0.001$) and comparable with North European $\delta^{13}C$ values.

Global human nitrogen isotope ratios

Global human hair $\delta^{15}N$ values range from 7.7 ±1.2 ‰ for Iran and Pakistan to 10.1 ±1.3 ‰ for Brazil (Table 3). The lowest $\delta^{15}N$ values are found in South Asia and Iran, but also for New Zealand inhabitants with values of 8.2 ±0.5 ‰, significantly different from most other countries ($p < 0.05$ to $p < 0.001$). The highest $\delta^{15}N$ values are spread worldwide in North/East Europe, Mongolia and Korea as well as in Brazil and Northern Africa. In Europe, the $\delta^{15}N$ values do not differ much, although North or Northeast European countries are different from most countries in Central or Southern Europe ($p < 0.05$). In the Americas, the $\delta^{15}N$ country mean value of Brazil is significantly higher than that for most of the other countries ($p < 0.001$), except Mexico, and the country cluster Colombia, Ecuador and Venezuela. In Asia, the highest $\delta^{15}N$ values are found for Central Asia including Mongolia, as well as for Japan and Korea. These samples are significantly enriched in ^{15}N ($p < 0.001$) compared with South Asian countries on the Indian subcontinent and Iran. The African country clusters do not differ significantly from each other.

Representativeness of the dataset

The traceability and comparability of isotope data between laboratories are key issues in the development of global databases.[45] In this work carbon and nitrogen isotope data from more than 25 different laboratories over a period of 30 years were summarised. As usual, all reported and incorporated isotope data are reported according to the IAEA-TECDOC 825.[46] However, inter-laboratory comparisons of EA/IRMS measurements of organic substances showed results that varied by up to ±1 ‰ for carbon and up to more than ±1 ‰ for nitrogen isotope ratios compared with the mean value.[47] Consequently, such variances in isotope ratios have to be assumed in the presented dataset and would lead to higher estimated over-all variances on the one hand and to potential biases in the dataset on the other. In addition, over the period of several decades different (secondary) isotopic reference materials have been used for normalisation of the measurement methods. Unfortunately, the chosen reference materials and the attributing isotopic ratios have not been mentioned in every publication. Nevertheless, all isotope ratios included in this work have been reported relative to VPDB (or PDB) for carbon and AIR for nitrogen. Therefore it is assumed that the included isotope data derived from publications in peer-reviewed journals are in accordance with prevailing analytical recommendations at the date of publication. As a consequence, the estimated variances of the reference values are associated not only with real-life intra-population variances, but also with analytical uncertainties due to the different analytical techniques and the reference materials used. In order to compensate for the potential effect of an analytical technique-based laboratory 'outlier', country or country mean values were calculated if data from at least three different laboratories (either published or unpublished data) had been analysed. However, due to limited available data, this could not be realised for all countries/country clusters (see Tables 2 and 3 for details).

The present dataset on global human hair and nail $\delta^{13}C$ and $\delta^{15}N$ values is weighted towards Europe and North America because most of the published data are from these regions. For other regions, especially Africa and Asia, only limited data are available. As can be seen in Figs. 2 and 3, the countries without a colour code (i.e. white) do not have any publically available information about contemporary human isotope data. Ideally, these white spaces should be filled with isotopic data in the future in order to be able to interpret isotopic analytical results in respect of human provenance and allocation successfully. Not every country shown in colour had a sufficient number of experimental samples (minimum of ten samples), which is why we created country clusters to be able to incorporate such samples into the dataset. For Africa, for example, so few samples have been analysed that only four country clusters were created, which stretch across huge regions. It is debatable if such large regions are reasonable for creating a geographically distinct dataset, but these were the smallest geographic areas that could be formed from the aggregate data to provide a reasonable degree of reliability. A similar situation is present for large countries like Russia, the USA, Canada, or China. There are large differences in the isotopic data of humans from different locations, and it is reasonable to assume that there are geospatial differences within these countries, too.

Table 2. Summary of worldwide human hair carbon isotope data (weighted mean value ± variance including number of samples), separated in continents and sorted by decreasing values. References in parentheses refer to laboratory numbers; references in brackets refer to literature

	Country/Country Cluster	$\delta^{13}C$/‰	*n*	References
Europe	Former Yugoslavia, Albania*	–19.8 ±0.6	(18)	(3);[4]
	Portugal, Spain	–19.9 ±0.6	(49)	(1,3);[4,17]
	Bulgaria, Moldova, Romania*	–20.0 ±0.5	(36)	(2,3);[4]
	Czech Rep., Hungary, Slovakia*	–20.1 ±0.8	(70)	[4,29]
	France	–20.3 ±0.6	(≥61)$^{+}$	(3);[4,17,26,36]
	Italy incl. Malta*	–20.3 ±0.5	(48)	[4,17]
	Russia, Ukraine	–20.3 ±0.6	(≥58)	(2,3);[4,36]
	Belgium, Luxembourg	–20.4 ±0.7	(≥19)$^{+}$	(2);[4,17,26,36]
	Germany	–20.5 ±0.6	(478)	(1,3);[4,6,7,9,12,13,17,36]
	Greece incl. Cyprus	–20.6 ±0.5	(10)	(3);[4,17]
	Netherlands	–20.6 ±0.4	(≥18)	(3);[4,17,36,37]
	Austria, Switzerland	–20.7 ±0.9	(58)	(2,3);[4,10,17]
	Denmark	–20.8 ±0.4	(57)$^{+}$	[4,14,17]
	Ireland incl. Northern Ireland	–21.0 ±0.7	(≥27)$^{+}$	(3);[4,26,36]
	Norway	–21.0 ±0.5	(≥13)	(2,3);[4,36]
	UK excl. Northern Ireland	–21.0 ±0.6	(≥134)	(3,5);[4,9,11,17,20,34,36]
	Poland, Lithuania	–21.2 ±0.5	(50)	(1–3);[4]
	Finland, Sweden	–21.6 ±0.7	(16)	(1–3);[4,17]
America	Brazil	–16.6 ±2.7	(740)$^{+}$	(1,3);[4,33,37,39,40]
	Colombia, Ecuador, Venezuela	–16.9 ±0.3	(47)	(2,3);[23]
	Costa Rica, Panama*	–17.3 ±0.9	(19)	(3);[4]
	Mexico	–17.5 ±1.8	(10)	(1,3);[4]
	USA	–17.6 ±1.2	(≥1021)$^{+}$	(3,4);[4,7–9,17,26–28,35,36,39,41,48]
	Bolivia, Peru*	–18.6 ±0.9	(16)	(3);[4]
	Canada	–18.7 ±1.1	(49)	[4,9,33]
	Caribbean*[a]	–18.8 ±1.1	(15)	(2,3)
	Argentina, Chile, Uruguay	–19.0 ±0.9	(10)	(1–3);[4,9]
Asia	Japan, Korea	–18.5 ±0.7	(113)	(1);[4,7,33,37,38]
	Eastern Medit., Arab. Peninsula[b]	–19.7 ±0.8	(116)	(2,3);[4,32,36]
	Southeast Asia[c]	–19.9 ±0.8	(36)	(1–3);[4]
	China	–20.1 ±1.3	(297)	(3,5);[4,24,37,42,43]
	Central Asia incl. Mongolia[d]	–20.3 ±0.7	(>29)	(2,3);[34,42]
	Iran, Pakistan	–20.4 ±0.4	(30)	(3);[4,42]
	India incl. Nepal, Sri Lanka	–20.4 ±0.9	(65)$^{+}$	(1–3);[4,24,26,33,42]
Africa	Southern Africa[e]	–16.2 ±0.8	(≥12)$^{+}$	(1–3);[26,36]
	Eastern Africa[f]	–17.2 ±1.6	(24)$^{+}$	(1–3);[4,26]
	Northern Africa[g]	–19.2 ±0.7	(≥10)$^{+}$	(1–3);[26,36]
	Western Africa[h]	–19.3 ±0.9	(12)	(1,3);[4]
Australasia	Melanesia*	–19.0 ±1.2	(69)	[31,43]
	Australia	–19.1 ±1.0	(27)	(2,3,5);[4,9,33]
	New Zealand*	–21.2 ±0.5	(12)	(1);[4]

* only data from two laboratories.
$^{+}$ including nail data (corrected).
[a] including: Cuba, Dominican Republic, Martinique, Trinidad and Tobago.
[b] including: Jordan, Lebanon, Saudi Arabia, Syria, Turkey, Palestinian territories, Yemen.
[c] including: Indonesia, Malaysia, Philippines, Thailand, Vietnam.
[d] including: Kazakhstan, Mongolia, Tajikistan.
[e] including: Namibia, South Africa.
[f] including: Ethiopia, Kenya, Malawi, Tanzania, Uganda.
[g] including: Egypt, Morocco, Sudan, Tunisia.
[h] including: Cape Verde, Ghana, Mauritania, Nigeria, Senegal.

For human identification or other purposes, human hair and nail samples are used for stable isotope analysis. In order to compare $\delta^{13}C$ and $\delta^{15}N$ analytical results for different matrices, isotopic differences between these matrices have to be taken into account. Our results confirm that the $\delta^{13}C$ values of human nail are lower than those of hair and that nail $\delta^{15}N$ values are higher than those of hair.[20,21] The differences in isotope ratios between hair and nail are attributed to differing amino acid compositions as well as to potentially different amino acid turnover in hair follicles and the nail matrix, although the exact mechanisms remain unclear.[20] Because most of the published carbon and

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Table 3. Summary of worldwide human hair nitrogen isotope data (weighted mean value ± variance including number of samples), separated in continents and sorted by decreasing values. References in parentheses refer to laboratory numbers; references in brackets refer to literature

	Country/Country Cluster	$\delta^{15}N$/‰	*n*	References
Europe	Former Yugoslavia, Albania*	8.7 ±0.5	(18)	(3);[4]
	Bulgaria, Moldova, Romania*	8.7 ±0.9	(36)	(2,3);[4]
	Austria, Switzerland	8.8 ±0.8	(58)	(2,3);[4,10,17]
	Italy incl. Malta*	8.8 ±0.7	(47)	[4,17]
	Germany	8.9 ±0.7	(463)	(1,3);[4,6,9,12,13,17,36]
	Czech Rep., Hungary, Slovakia*	8.9 ±1.0	(71)+	[4,29]
	Poland, Lithuania	9.1 ±0.5	(49)	(1,3);[4]
	Greece incl. Cyprus	9.1 ±0.5	(10)	(3);[4,17]
	France	9.2 ±0.7	(≥62)+	(3);[4,17,26,36]
	Ireland incl. Northern Ireland*	9.3 ±0.8	(≥27)+	(3);[4,26,36]
	UK excl. Northern Ireland	9.3 ±0.6	(≥128)	(3,5);[4,9,11,17,20,36]
	Netherlands	9.4 ±0.5	(≥18)	(3);[4,17,36,37]
	Belgium, Luxembourg	9.4 ±0.9	(≥19)+	(2);[4,17,26,36]
	Portugal, Spain	9.4 ±0.6	(49)	(1,3);[4,17]
	Norway	9.4 ±0.8	(≥13)	(2,3);[4,36]
	Finland, Sweden	9.5 ±0.5	(16)	(1–3);[4,17]
	Denmark	9.7 ±0.6	(57)+	[4,14,17]
	Russia, Ukraine	9.9 ±0.9	(≥58)	(2,3);[4,36]
America	Canada*	8.4 ±0.5	(16)	[4,9]
	Costa Rica, Panama*	8.8 ±0.4	(19)	(3);[4]
	Argentina, Chile, Uruguay	8.8 ±0.7	(10)	(1–3);[4,9]
	USA	8.9 ±0.9	(≥1022)+	(3,4);[4,8,9,17,26–28,30,36,39,41,48]
	Bolivia, Peru*	8.9 ±0.6	(16)	(3);[4]
	Caribbean*[a]	9.2 ±0.8	(15)	(2,3)
	Mexico	9.5 ±0.4	(10)	(1,3);[4]
	Colombia, Ecuador, Venezuela	9.8 ±0.5	(47)	(2,3);[23]
	Brazil	10.1 ±1.3	(736)+	(3);[4,37,39,40]
Asia	Iran, Pakistan	7.7 ±1.2	(30)	(3);[4,42]
	India incl. Nepal, Sri Lanka	8.1 ±1.3	(59)+	(1–3);[4,24,26,42]
	China	8.5 ±1.0	(297)	(3,5);[4,24,37,42,43]
	Eastern Medit., Arab. Peninsula[b]	8.6 ±0.6	(116)	(2,3);[4,32,36]
	Southeast Asia[c]	9.1 ±0.8	(36)	(1–3);[4]
	Japan, Korea	9.5 ±0.7	(161)	(1);[4,25,37,38]
	Central Asia incl. Mongolia[d]	9.9 ±1.0	(>29)	(1–3);[34,42]
Africa	Western Africa[h]	9.4 ±0.8	(13)	(1,3);[4]
	Eastern Africa[f]	9.4 ±1.1	(31)+	(1–3);[4,26]
	Southern Africa[e]	9.9 ±0.6	(≥14)+	(1–3);[26,36]
	Northern Africa[g]	9.9 ±0.7	(≥11)+	(1–3);[26,36]
Australasia	New Zealand*	8.2 ±0.5	(12)	(1);[4]
	Melanesia	9.2 ±0.9	(69)	[31,44]
	Australia	9.7 ±0.9	(20)	(1–3,5);[4,9]

* only data from two laboratories.
\+ including nail data (corrected).
[a] including: Cuba, Dominican Republic, Martinique, Trinidad and Tobago.
[b] including: Jordan, Lebanon, Saudi Arabia, Syria, Turkey, Palestinian territories, Yemen.
[c] including: Indonesia, Malaysia, Philippines, Thailand, Vietnam.
[d] including: Kazakhstan, Mongolia, Tajikistan.
[e] including: Namibia, South Africa.
[f] including: Ethiopia, Kenya, Malawi, Tanzania, Uganda.
[g] including: Egypt, Morocco, Sudan, Tunisia.
[h] including: Cape Verde, Ghana, Mauritania, Nigeria, Senegal.

nitrogen isotope data on contemporary ('alive') humans are from the analysis of hair the dataset of this study was referenced to human hair isotope ratios. Any nail isotope data was corrected by constant factors for inclusion. If this dataset were to be used for the interpretation of $\delta^{13}C$ and $\delta^{15}N$ values of other human matrices such as bone and blood cells, different correction factors would have to be applied.

Spatial distribution of isotope ratios

It has been reported for smaller datasets that modern human carbon isotope ratios change significantly with latitude,[17] which is related to the decrease in C_4 vegetation with increasing latitude.[49] This can be observed for our compiled dataset, wherein the $\delta^{13}C$ country mean values typically

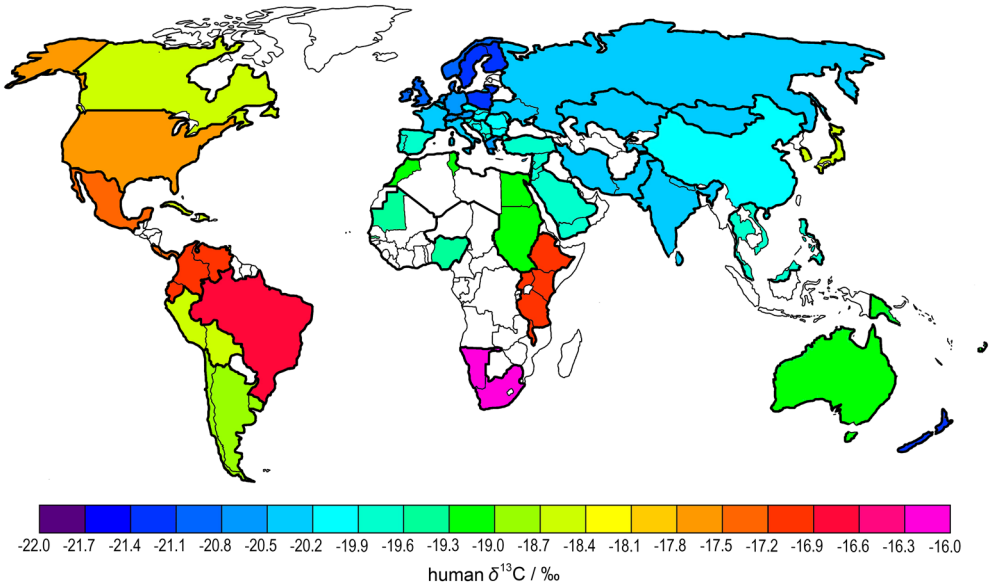

Figure 2. Global spatial distribution of natural stable carbon isotope ratios of contemporary human hair and nails. For countries marked white no data are available. Solid black country borders indicate individual countries and country cluster for which isotope data were summarised.

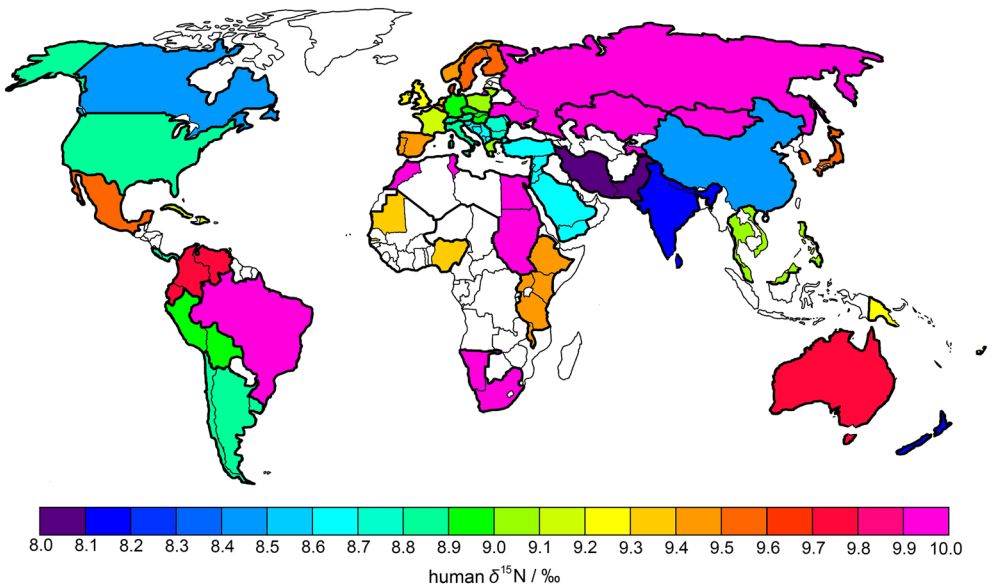

Figure 3. Global spatial distribution of natural stable nitrogen isotope ratios of contemporary human hair and nails. For countries marked white no data are available. Solid black country borders indicate individual countries and country cluster for which isotope data were summarised.

decrease (i.e. become more negative) with increasing distance to the equator (Fig. 4). Clearly, latitude alone is not the only variable for explaining the trend in modern human $\delta^{13}C$ values. Regions like the USA or Southern Africa show higher (less negative) $\delta^{13}C$ values than other countries on the same latitude. The highest $\delta^{13}C$ values are found for regions like Southern Africa and Brazil, which are influenced by the dominant use of C_4 plants as the dietary source for humans and animals. In addition to the influence of dietary C_4 plants the consumption of maritime food leads to increased $\delta^{13}C$ values in modern humans.[6,14,50] This has been demonstrated by the analysis of fingernails and hair of indigenous populations in Northern Greenland and Southwest Alaska who consume high amounts of maritime food.[14,50] However, such high amounts of maritime food in the modern human diet are exceptional and the observed spatial distribution of human $\delta^{13}C$ values in this dataset can primarily be attributed to the proportion of C_3 and C_4 plants in the human diet.

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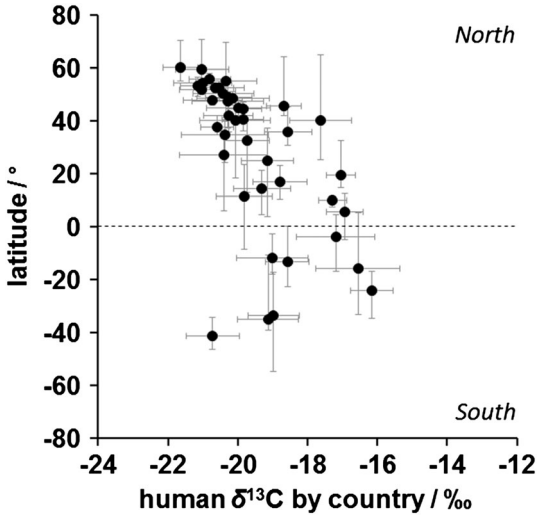

Figure 4. Latitudinal distribution of modern human hair and nail carbon isotope ratios. Each data point represents the mean $\delta^{13}C$ value (± estimate of variance) for a country (or country cluster) in relation to the capital's latitude. Vertical bars reflect the latitudinal extent of the certain country or country cluster.

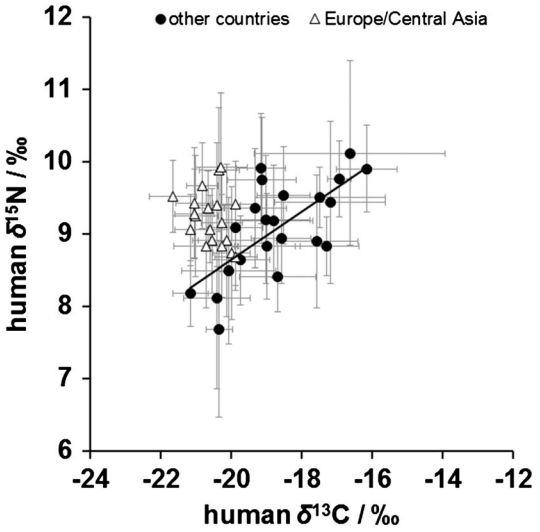

Figure 5. Carbon and nitrogen isotope ratios for human hair and nail. Each data point represents the mean value (± estimate of variance) for a country (or country cluster). Data for countries outside Europe and Central Asia/Mongolia (circles) are positively correlated ($r = 0.69$, $p < 0.001$), whereas data for Europe and Central Asia/Mongolia (triangles) show no correlation.

In contrast to Europe, the data coverage in Africa and Central and South America (except Brazil) is too sparse to identify intra-continental differences and variations in human $\delta^{13}C$ values. Because of the comparatively comprehensive data set within Europe, regional variances in the $\delta^{13}C$ values of human hair can be detected along transcontinental transects. One transect with continuously increasing $\delta^{13}C$ values runs from Portugal and Spain (–19.9 ‰) over France (–20.3 ‰), Belgium and Luxembourg (–20.4 ‰), Germany (–20.5 ‰), Denmark (–20.8 ‰) to Finland and Sweden (–21.6 ‰).

Several factors have been identified to influence human $\delta^{15}N$ values: the amount of marine food, terrestrial meat, and animal products in the diet,[6,9–14,25] physiological situations[27,28] and disease, illness or malnutrition.[30,51,52] As the $\delta^{15}N$ value of human hair might be influenced by such a multitude of factors, generalised assumptions explaining the spatial distribution of human nitrogen isotope ratios are difficult. There is no correlation between latitude and country $\delta^{15}N$ mean values; high $\delta^{15}N$ values are found near the equator as well as in higher latitudes. Small amounts of fish and meat and also the consumption of pulses (such as lentils or beans) might be the cause for the ^{15}N-depleted hair samples from the Indian subcontinent, including Iran and Pakistan. However, this is not the case for other countries in Africa, with typically scant meat and fish consumption but ^{15}N-enriched samples. Additional factors known to influence human dietary $\delta^{15}N$ values are climate and agricultural practices. Temperature and precipitation also have an influence on plant $\delta^{15}N$ values, with ^{15}N-enriched plants in hot and arid climates and ^{15}N-depleted plants in cold and humid regions.[53] Fertilization is another factor with a huge influence on plant $\delta^{15}N$ values: fertilization with animal-derived manure leads to highly ^{15}N-enriched plants and crops, whereas the use of synthetic fertiliser leads to ^{15}N-depleted agricultural products.[54,55] As a conclusion, human hair $\delta^{15}N$ values seem to be a result of different influences: dietary preferences and food supply, physiology and metabolism, climate and fertilisation methods. None of these influences has a clear spatial driver to explain the global nitrogen isotope ratio variation, unlike $\delta^{13}C$ values that vary with latitude.

Carbon and nitrogen isotope ratios for countries outside Europe and Central Asia/Mongolia show a positive correlation ($r = 0.69$ and $r = 0.64$, respectively, $p < 0.001$), whereas data for European and Central Asian countries/Mongolia do not show such a correlation (Fig. 5). The observed effect at lower latitudes might be caused by climatic factors: Increased $\delta^{13}C$ values are mainly due to increased amounts of C_4 plants in the diet, whereas the domination of C_4 plants over C_3 plants in the ecosystem is caused by climatic factors such as aridity and temperature.[49] Accordingly, temperature and precipitation have an effect on the $\delta^{15}N$ values of an ecosystem, leading to increased $\delta^{15}N$ values in more arid and hot environments.[56] In contrast, countries at higher latitudes than 46° north, which is mainly Northern and Central Europe as well as Central Asia/Mongolia, human hair and nail are enriched in ^{15}N in comparatively cold and humid environments. This is probably a consequence of the high consumption of animal-derived proteins, above all of meat and dairy products, partially produced by organic farming.

CONCLUSIONS

In this work, new experimental $\delta^{13}C$ and $\delta^{15}N$ data of more than 400 hair and nail samples of contemporary humans living all over the world were added to the present publicly accessible datasets. However, there is still a lack of data on modern humans for huge regions, especially Africa. Our data on human $\delta^{13}C$ values support the established interpretation that the global spatial distribution of human carbon isotopic composition is strongly related to the amount of C_4 plants in the diet. As $\delta^{13}C$ values are correlated with latitude the

influence of a marine carbon isotope signal on modern human isotope ratios seems to be lower. The global spatial distribution of human $\delta^{15}N$ values cannot be directly related to dietary or environmental factors. Nevertheless, the analysis of carbon – and to lesser extent nitrogen – isotope ratios of post-mortem human remains may help in geo-location and subsequent identification of human victims. In addition, the present dataset may help in the interpretation of carbon and nitrogen stable isotope results for modern or ancient humans in view of dietary preferences, geo-location or health issues.

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