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ORIGINAL ARTICLE

Assignment of unknown persons to their geographical origin by determination of stable isotopes in hair samples

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Abstract Analysing the isotope ratio of light elements in human tissue of an unknown person helps to reconstruct the life history with regard to geographical origin and/or typical food supply. In this study the isotope ratios of the bio-elements in hair samples of 111 persons from 13 different countries all over the world were measured with the aim of provenance determination. The results indicated that individuals from Costa Rica and Brazil can be differentiated from typical European individuals by $\delta^{13}C$, Australians by $\delta^{34}S$ and $\delta^{2}H$ in hair samples. The combination and evaluation of the data by multivariate statistical analysis considerably improved origin assignment. Investigation of hair samples from a number of individuals from one particular region (southern Germany) yielded remarkable variation of isotopic values indicating different nutritional habits. The possibilities and limitations of this method in its current state are demonstrated and discussed.

Keywords Hair · Geographic origin · Stable isotope · Bio-elements · Multivariate analysis

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Introduction

Hydrogen (H), oxygen (O), carbon (C), nitrogen (N) and sulphur (S) occur in nature as mixtures of stable isotopes with variable isotope ratios. Isotopic signatures of the local environment are transported into plants and animals via soil, water and air, and are passed along the food chain. Multi-element isotope analysis is used to determine the origin and authenticity of dairy products, wine and beef [1–6] and to implicate human palaeodiets and residence mobility [7].

Isotope ratios of light elements in human tissue provide information about nutrition ($\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$), and the geographic and climatic conditions ($\delta^{2}H$, $\delta^{18}O$) of the habitat [8–11].

Over the past years the Institute of Forensic Medicine in Munich, the Department of Geo- and Environmental Science, the Bavarian State Collection in Munich and Isolab GmbH, Schweitenkirchen, Germany, have cooperated in investigation of tissues of recent human bodies to assign the geographical origin of persons by determining stable isotope ratios of light and heavy elements, and not only to the country of birth, such as DNA investigations could do [12–17], but also to other countries where a person has lived during their lifetime.

Origin assignment of unidentified bodies using stable isotopes is based on the comparison of analytical data with reference samples from humans of known provenance. Therefore a reference database of human tissues and environmental samples was established at the Institute of Forensic Medicine, Munich, in cooperation with and through technical and financial support of the Munich Police Department.

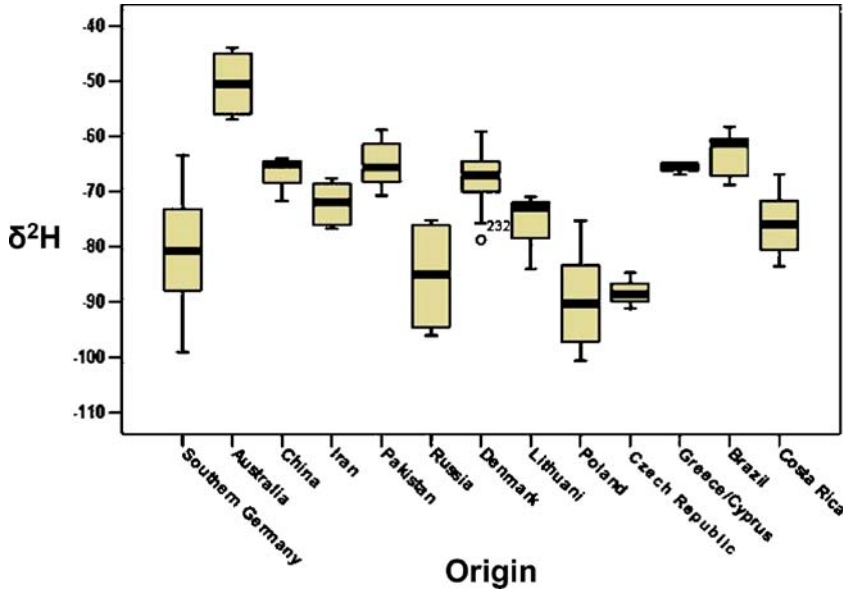

Fig. 1 δ^2H-values of hair samples from different geographical origins, illustrated as *box plots* with minimum, maximum and mean values. An outlier exists for Denmark

The aim of this paper is to demonstrate the potential of provenance determination of humans by investigating hair samples for isotope ratios of light elements (H, C, N, S).

Materials and methods

Scalp hair samples from 111 residents (people permanently living in the relevant region during the growth of their hair) of 13 different countries from around the world were collected and analysed. From each hair sample 50 mg was homogenised by cutting into small fragments and incubated overnight with petrol ether. For determination of δ^{13}C, δ^{15}N and δ^{34}S, 2 mg dry mass was placed into tin capsules, for determination of δ^2H, 3 mg dry mass of hair sample.

Determination of stable isotope ratios of H, C, N, and S followed internationally accepted methods described in detail elsewhere [2, 18]. Measurement of the light elements was carried out using an elemental analyzer isotope ratio mass spectrometer (EA-IRMS). All samples were run in triplicate. Results of H-, C-, N- and S-isotopic ratios are referred to international standards: V-SMOW, V-PDB, air-nitrogen (ATM), and CDT. The analytical uncertainties (precision and accuracy) were: δ^2H ±3‰/V-SMOW, δ^{13}C ±0.1‰/V-PDB, δ^{15}N ±0.2‰/ATM and δ^{34}S ±0.3‰/CDT.

Stable isotopes occur with relatively mean abundance in nature. The isotope content of samples is described as an isotope ratio, the relationship of the heavy to the light isotopes from one element. For instance, C has two stable isotopes, ^{12}C and ^{13}C. In practice the relationship is given as δ^{13}C value,

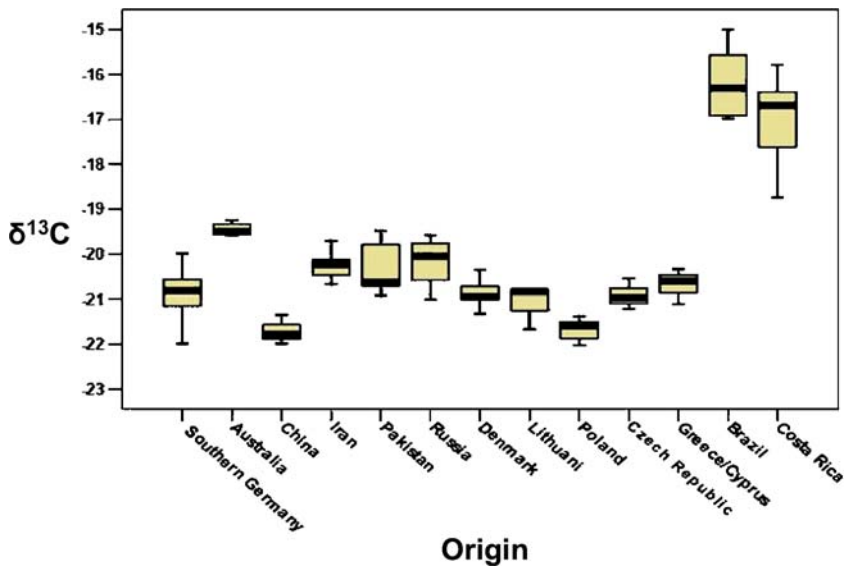

Fig. 2 δ^{13}C-values of hair samples from different geographical origins, illustrated as *box plots* with minimum, maximum and mean values

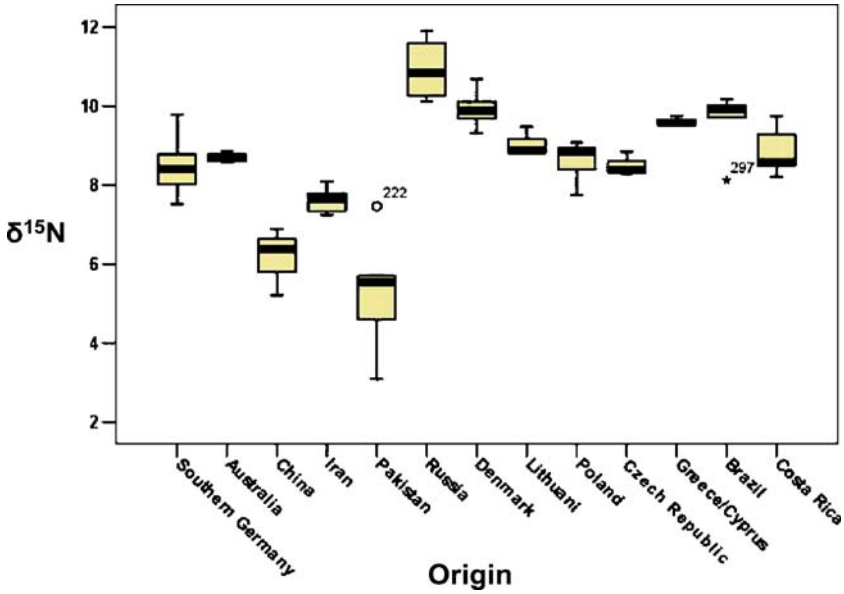

Fig. 3 δ^{15}N-values of hair samples from different geographical origins, illustrated as *box plots* with minimum, maximum and mean values. An outlier exists for Pakistan and Brazil

which is calculated from the difference of the isotopic ratio (R) of the sample and an international reference material, divided by the ratio of the reference material and multiplied by 1,000 according to the formula:

$$\delta\text{-value} = [(R_{sample} - R_{standard})/R_{standard}] \times 1000\ (‰)$$

Isotope ratios of light elements (δ values) in human hair samples from different origins were statistically analysed and the data illustrated as box-plots (Figs. 1, 2, 3 and 4).

The number of samples from each country is described in Table 1.

Additionally, data were evaluated by multivariate statistical analysis and discriminant analysis was performed by SPSS program 13.0 for Windows.

Results

The δ^2H values show a considerable overlap with the exception of the Australian data (Fig. 1). Hair samples from

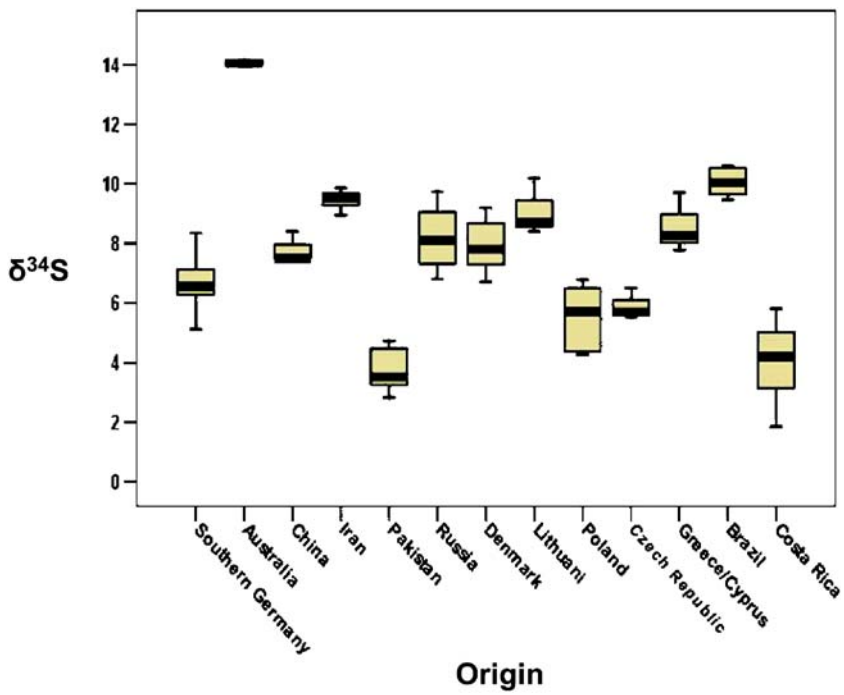

Fig. 4 δ^{34}S-values of hair samples from different geographical origins, illustrated as *box plots* with minimum, maximum and mean values

Table 1 Countries of origin for scalp hair samples

Country	Number of samples
Southern Germany	39
Australia (Darwin)	4
China (Huxian)	3
Iran (Teheran)	5
Pakistan (Peshawar)	6
Russia	4
Denmark	13
Lithuania	3
Poland	7
Czech Republic	3
Greece/Cyprus	3
Brazil (Sao Paulo)	6
Costa Rica (San Josè)	15
Total	111

Brazil and Costa Rica presented the highest $\delta^{13}C$ values and the mean values were approximately 4‰ higher compared to the other countries (Fig. 2), where the $\delta^{13}C$ values showed overlap. More discrimination between the countries is demonstrated by the $\delta^{15}N$ values (Fig. 3). For instance, Russia can be differentiated from Pakistan or southern Germany, or Lithuania from Iran. $\delta^{34}S$ values of the origins however showed many overlaps, except for Australia (Fig. 4).

Multivariate analysis of the isotope data was applied to allocate the hair samples from different countries of origin into the correct group using isotopic parameters. For this, the four variables ($\delta^{2}H$, $\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$) were evaluated by discriminant analysis. The structure matrix shows that $\delta^{13}C$ and $\delta^{34}S$ have the highest absolute correlation coefficient of the four elements, thus accounting for the discrepancy between the countries. Figure 5 describes the assignment of the light elements relating to the countries. For instance Australia, Brazil and Costa Rica are separated completely from the other groups.

To qualify the criterion of the evaluated discriminant analysis, the hair samples were reassigned to groups via the determined discriminance function. Nevertheless, about 91% of the originally grouped hair samples were correctly assigned to the country of origin by consideration of the numbers of the group samples.

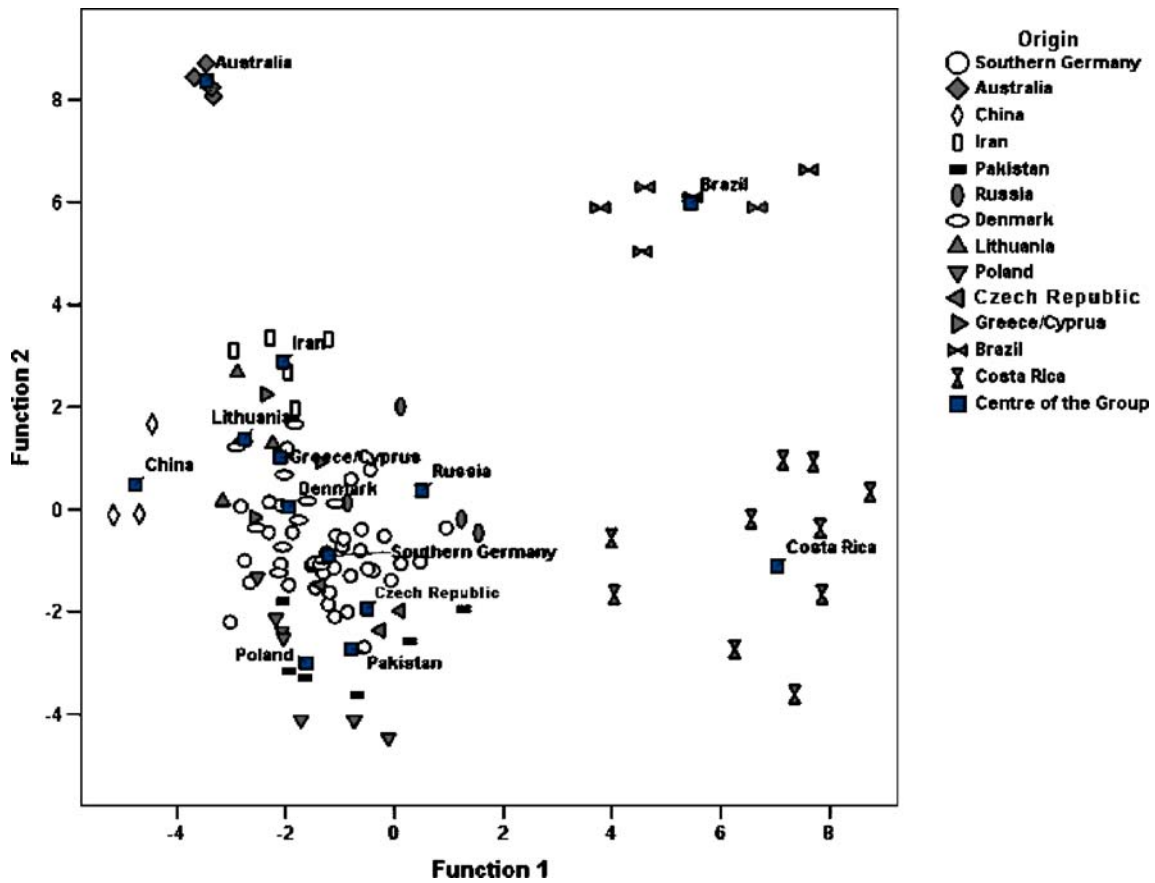

Fig. 5 Isotope ratio data of the light elements ($\delta^{2}H$, $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$) of hair samples, evaluated by discriminant analysis. Function 1 and Function 2 describe the individual discriminant scores for the two discriminant functions. Group means are centroids, demonstrated as *black squares* (centre of the group)

Discussion

The H isotopic signatures of human tissues are mainly determined by the isotopic ratio of drinking water and by water and organic hydrogen in the diet. The δ^2H values of the single countries correlate with different climate zones and altitude. For instance very low mean δ^2H values of hair samples from southern Germany, Russia, Poland and the Czech Republic may be due to a common moderate continental climate, whereas the higher mean δ^2H value for Denmark reflects different conditions due to the place of origin near the sea (hair donors are from Copenhagen and Vejle). In contrast, Mediterranean and tropical climates show rather high δ^2H values (e.g. Brazil, Sao Paulo, Greece/Cyprus; www.naweb.iaea.org). If the climate is dry and hot, but the δ^2H value is relatively low, then the altitude of the region of origin could be high above sea level (Teheran, Costa Rica, San Josè; www.waterisotopes.org).

$\delta^{13}C$ values reflect the composition of the food and indicate whether an individual eats C4 and/or C3 plants. European nutrition is based primarily on C3 crops (range −24‰ to −32‰), for example potatoes, rice and cereals [19]. High proportions of C4 plants (range −10‰ to −16‰) like maize and/or cane products prevail, for instance, in Costa Rica and Brazil.

$\delta^{15}N$ values of the investigated hair samples differed, depending on the origin of the samples and known trophic fractionation (~+3‰ from a trophic level to the next higher level) [8]. The higher the $\delta^{15}N$ values of hair samples are, the more animal products were consumed and conversely, low $\delta^{15}N$ values are due to a diet containing less animal products, consumption of legumes or poultry fed with Soya protein (Brazil, Costa Rica, Pakistan, China, Iran). $\delta^{15}N$ values of hair samples from southern Germany varied widely, because the relatively high number of samples may reflect various individual nutritional habits (eg. inclusion of vegetarians). The variety of values from the European countries is lower, suggesting similar nutritional habits with regard to protein (nitrogen) sources.

Together with high $\delta^{15}N$ values, high $\delta^{34}S$ values are the result of fish and seafood consumption. Particularly high $\delta^{34}S$ values have also been detected in meat and milk samples from the same area, which may be due to sea spray and special geological conditions [20, 21]. High $\delta^{34}S$ values have also been observed both for Brazilian fruit juices and Brazilian hair samples [22]. Low $\delta^{34}S$ values in hair samples are basically unspecific, but may be related, for instance, to the influence of volcanic sulphur (Costa Rica; private communication from S. Rummel, Bavarian State Collection of Paleontology and Geology, Munich).

It is usually impossible to discriminate between different countries of origin when applying only stable isotope data from one single element. Therefore it is necessary to take into account the information of four elements using multivariate analysis. Based on their structure matrix, $\delta^{13}C$ and $\delta^{34}S$ add the highest contribution for origin discrimination of hair samples.

Conclusion

As only of a limited number of samples have been investigated from several countries, and the highest number of samples originates from southern Germany, the present results can be used only as a feasibility test. The large number of hair samples from Germany probably represents a wide spectrum of nutritional habits and different geographical conditions. Since the number of collected and analysed hair samples from other countries of origin is very small, they probably represent only a portion of individual nutritional habits and in a very small part of the country.

We expect that when more regions of a country are included in the reference data base for stable isotope values, the better the possibility to assign a sample to its origin. In addition it enables a better understanding of the main factors that influence multi-element stable isotope patterns of human hair to be obtained. Therefore, our current results should be regarded as a first indication of the potential of this method.

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