The role of stable isotopes in human identification: a longitudinal study into the variability of isotopic signals in human hair and nails

I. Fraser*, W. Meier-Augenstein and R. M. Kalin

Environmental Forensics and Human Health, Environmental Engineering Research Centre, Queen's University Belfast, Belfast BT9 5AG, UK

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Recent natural catastrophes with large-scale loss of life have demonstrated the need for a new technique to provide information for disaster victim identification when DNA methods fail to yield the identification of an individual, or in other situations where authorities need to determine the recent geographical life history of people. The latter may be in relation to the identification of individuals detained on suspicion of terrorism or in relation to people-trafficking or smuggling. One proposed solution is the use of stable isotope profiling (SIP) using isotope ratio mass spectrometry (IRMS). Exploiting the link between the isotopic signal of dietary components and the isotopic composition of body tissue, the aim of this study was to refine a non-invasive method of analysing human material such as scalp hair and fingernails using SIP and to assess the degree of natural variability in these profiles. Scalp hair and fingernail samples were collected from British and non-British volunteers at Queen’s University Belfast every 2 weeks for a minimum of 8 months. Samples were analysed using IRMS to determine their isotopic composition for $^{13}$C, $^{15}$N, $^2$H and $^{18}$O. The results of this longitudinal study yielded information on the natural variability of the isotopic composition of these tissues. The data demonstrate the relatively low degree of natural variation in the $^{13}$C/$^{15}$N isotopic abundance of scalp hair and fingernails whilst greater variations were recorded in the hydrogen and oxygen values of the same samples. The $^{15}$N and $^{18}$O values of nail are noticeably more variable than that of scalp hair from the same subject. A hypothesis explaining this trend is put forward based on the faster rate of formation of hair than of nails. This means that there is less time for the compounds forming hair to be affected by biochemical processes that could alter their isotopic signature. Copyright © 2006 John Wiley & Sons, Ltd.

In light of recent mass disasters and an increase in cases of people-trafficking, there is an urgent need for a technique which is capable of identifying an individual's recent movements, their geographical origin and life history. For example, problems are encountered when human remains are badly decomposed or disfigured. In these cases it may not be possible to rely on traditional methods of identification from fingerprints or dental records. In many situations an insufficient quantity of preserved, and hence viable, DNA prevents the construction of a DNA profile for the individual. Even when this is possible it does not guarantee a match with a person in a DNA database. Hence, an alternative technique is required to provide additional or complementary information.

Stable isotope profiling (SIP), based on sample analysis by isotope ratio mass spectrometry (IRMS), is a new forensic technique that may aid with the source determination of compounds such as explosives and illicit drugs.1–4 Several studies have also demonstrated its potential as a tool for determining the place of origin of living humans, unidentified human remains and the identification of disaster victims.5–10 However, if this technique is to be implemented in police investigations and plans for disaster victim identification, a considerable amount of additional research is required.

Bulk stable isotope analysis of hair and nails can provide information concerning a person’s dietary intake and the geo-location in which a person has lived. Generally, the $^{13}$C and $^{15}$N found in human material reflects the $^{13}$C (and $^{15}$N) isotopic composition of the food consumed by an individual whereas the $^2$H (and to a degree $^{18}$O) content is a reflection of a person’s direct and indirect water intake. In addition, a proportion of $^2$H can be directly derived from food. Analysis of local water sources from different geographical locations will therefore be useful in the interpretation of an individual’s isotopic ratios.11–14
Analysis of hair and nails is preferable to the analysis of bone and teeth as hair and nail samples can be obtained in a non-invasive manner and because the technique can be applied to the living as well as the dead.

The majority of work carried out on human tissues has involved interpretation of isotopic data from archaeological human remains in a palaeoecological/palaeodietary context. Samples analysed date back as far as the Ptolemaic era (332 BCE – 30 BCE) and the Ice Age (5200 BP) – the Nubian Mummy and the Ice Man, respectively – and therefore data from this work is of limited use for a number of reasons. [BCE = Before Current Era; BP = Before Present.] First, the diet of pre-modern individuals was substantially more restricted than today. Secondly, ancient diets were possibly more seasonally diverse than modern diets as individuals relied heavily on locally produced crops and other products for food and availability of these foods was dependent on the season. Due to problems obtaining authentic human material for analysis the majority of experimental work has been carried out using proxy samples such as plant material and animal hair. However, given the differences between animals and humans with regard to size, growth, physiology and metabolism, it is doubtful whether these results can be used in the interpretation of specific human dietary situations. If the technique is to be applied to any kind of forensic work it is imperative that the experimental research is modelled on modern human material using a systematic approach.

In addition, the number of subjects assessed in earlier studies has been limited and samples were only collected on a ‘one-off’ basis. No information is therefore available on intra- and inter-individual natural variability of stable isotope signals recorded by scalp hair and fingernails, and the factors influencing this.

EXPERIMENTAL

Longitudinal study

After obtaining ethical approval from the Office for Research Ethics Committees Northern Ireland (ORECNI), 20 adult volunteers were recruited from Queen’s University Belfast (QUB) and their informed consent was obtained. All subjects included in the study had been living in Belfast for a minimum of 6 months, but were originally from different ethnic and geographical backgrounds. Subjects were asked to donate scalp hair and fingernail clippings (here after referred to as ‘hair’ and ‘nail’ unless otherwise specified) on a fortnightly basis during the period of May 2004 to January 2005 (where possible some candidates donated further samples but this data is not shown here). Where volunteers were unable to donate samples due to absence or insufficient sample no value was recorded. During the study subjects were asked to document their dietary intake, details of any recent travel, as well as standard personal details. A summary of these details can be found in Table 1, where ‘time in Belfast’ refers to the period of time resident in Belfast prior to the study.

Hair samples approximately 0.5 cm in length and 10–25 strands thick (to represent approximately 2 weeks growth) were cut from the nape of the neck close to the scalp. Subjects were asked to cut their nails at the start of the study to ensure that the cuttings collected subsequently represented 2 weeks growth. Nail cuttings were taken from each finger. All samples were stored in plastic self-seal bags, in keeping with current forensic and police practice. Typically, samples were analysed within 2 weeks of collection.

Geographical database

In order to construct a map depicting variations in isotopic signatures on a global scale, volunteer recruitment packages were sent to various locations across the world requesting the donation of hair and nail samples. Each volunteer package consisted of an information sheet, two self-seal collection bags, a questionnaire, and a consent form. In accordance with the ORECNI guidelines, samples could only be analysed if accompanied by a signed consent form. Each package was assigned a consecutive number to ensure the anonymity of volunteers. Samples were obtained from countries listed in Table 2.

Cleaning of samples

All samples were collected and cleaned according to standard procedure. In order to overcome problems caused by static electricity, once cleaned all samples were ground using a SPEX CertiPrep cryogenic freezer/mill using stainless steel vials and impactors. Samples were ground using the standard programme setting of a 15-min pre-cool followed by three grinding cycles of 2 min each at a rate of 10, where rate refers to the number of impacts per second. There was a 2-min cooling period between each grinding cycle.

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Table 1. Personal details of the subjects

<table>
<thead>
<tr>
<th>Subject</th>
<th>Male/Female</th>
<th>Ethnic origin</th>
<th>Time in Belfast</th>
<th>Recent travels</th>
<th>Vegan/vegetarian</th>
<th>Hair dye</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>British</td>
<td>12 months</td>
<td>Portugal 2 weeks Oct 2004</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>Indian</td>
<td>12 months</td>
<td>India 1 month Jan 2005</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>Ghanaian</td>
<td>6.5 months</td>
<td>Barbados 2 weeks July 2004</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>British</td>
<td>12 months</td>
<td>Portugal 2 weeks Oct 2004</td>
<td>No</td>
<td>Tint</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>British</td>
<td>25 years</td>
<td>Germany 2 weeks March 2004</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>German</td>
<td>15 months</td>
<td>Germany 2 weeks March 2004</td>
<td>Vegetarian</td>
<td>No</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>British</td>
<td>15 months</td>
<td>Periodic trips worldwide</td>
<td>Vegetarian</td>
<td>No</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>American</td>
<td>10 years</td>
<td>Periodic trips worldwide</td>
<td>Vegetarian</td>
<td>No</td>
</tr>
</tbody>
</table>

Table 2. Countries involved in the geographical database

<table>
<thead>
<tr>
<th>Country</th>
<th>N</th>
<th>Date of collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Belgium</td>
<td>6</td>
<td>20/12/04</td>
</tr>
<tr>
<td>France</td>
<td>6</td>
<td>09/09/04</td>
</tr>
<tr>
<td>The Netherlands</td>
<td>6</td>
<td>11/05/05</td>
</tr>
<tr>
<td>India</td>
<td>6</td>
<td>05/01/05</td>
</tr>
<tr>
<td>Ithaca, USA</td>
<td>10</td>
<td>21/07/05</td>
</tr>
<tr>
<td>Norway</td>
<td>6</td>
<td>10/09/05</td>
</tr>
<tr>
<td>Sudan</td>
<td>2</td>
<td>08/09/04</td>
</tr>
<tr>
<td>Syria</td>
<td>16</td>
<td>06/05</td>
</tr>
<tr>
<td>Tasmania, Australia</td>
<td>9</td>
<td>24/07/05</td>
</tr>
<tr>
<td>Utah, USA</td>
<td>3</td>
<td>27/08/04</td>
</tr>
</tbody>
</table>

Ground samples are easier to weigh out in small amounts into tin/silver capsules and also produce a more homogenous sample, producing more reproducible results with minimal sample loss (less than 10%). The cryogenic mill also allows the fine grinding of samples without the heat build up generated by conventional grinding methods – thus avoiding potential fractionation through temperature changes. A comparison of ground versus not-ground samples showed no significant difference in the isotopic values obtained but it was found that grinding the samples improved the reproducibility of triplicate analyses.

After grinding, samples were placed in glass vials, sealed and stored in an evacuated desiccator containing phosphorous pentoxide to remove residual moisture traces from the samples. This was particularly important when performing hydrogen and oxygen analysis as it is known that moisture exchange can have a significant effect on the measured isotope values of samples. To preclude this, all samples were exposed to the same ambient environment after collection so that labile H-atoms prone to exchange would were exposed to the same ambient environment after collection so that labile H-atoms prone to exchange would have the same value.

Instrumentation set-up and analyses were performed according to QUB standard laboratory procedure, as described by Farmer et al. A Carlo Erba elemental analyser (EA) (CN 1500) coupled to an ABCA isotope ratio mass spectrometer (SerCon Ltd., Crewe, UK) was used for 13C/12C and 18O/16O isotope ratio measurement. A ThermoFinnigan DeltaPlus XP high-temperature conversion elemental analyser (Thermo Electron Corp., Bremen, Germany) was used for H2/H and 15N/14N isotope ratio measurement of ground hair and nail samples.

Isotopic calibration and quality control of EA-IRMS measurements

Calibration and quality controls were again performed according to Farmer et al.

RESULTS AND DISCUSSION

Longitudinal study

A longitudinal study was carried out to assess the natural variability of isotopic abundance in people who have lived in the same location for a minimum of 6 months. Twenty healthy volunteers from QUB donated hair and nail samples on a fortnightly basis. Similar patterns were visible amongst all 20 volunteers studied; however, due to space constraints and to avoid repetition, only data from eight of the subjects are directly presented and discussed in this paper. Where no result is visible for an individual, no sample was collected for that particular date, either through absence or a lack of sample.

Data demonstrating the relatively low degree of natural variation in 13C and 15N isotopic abundance of hair and nails of several subjects are shown in Figs. 1(a) and 1(b). Inter-individual pooled mean δ13C and δ15N values of these nail samples were −21.14% and 10.06%, respectively. The 15N results ranged from 8.91% to 10.26%, demonstrating the relatively tight banding of isotopic values of individuals living in the same area with slightly differing dietary habits.

As this data represents several subjects from different ethnic backgrounds with different dietary intakes, the results do not assume a normal distribution per se. However, data falling within the stated confidence limits are indistinguishable. Values outside these boundaries demonstrate the natural variation of hair and nail in isotopic abundance due to the biological processes occurring within the body. It is this ‘biological noise’ which needs to be taken into consideration when interpreting the results of stable isotope analysis of human tissues.

Analysis of hair samples gave inter-individual pooled mean δ13C and δ15N values of −20.59% and 9.90%, with SDs.
of ±0.59%o and ±0.71%o, and 95% confidence intervals of ±0.15 and ±0.18%o, respectively. Again the results of the bulk analysis of hair were found to fall within distinct bands with intra-individual mean δ13C and δ15N values ranging from −20.82%o to −20.42%o and 9.45%o to 10.60%o, respectively.

Longitudinal 13C/15N data for both hair and nails displays a slight sigmoidal pattern over time. Although the reason for this pattern is unclear at this time, the data suggests that a constituent of hair and nail, which is isotopically different from the other constituents, is present in the samples in increasing amounts. At a certain point a limiting factor is

Figure 1. Longitudinal plots showing the isotopic composition of human nail samples collected from subjects living in Northern Ireland. (a) 13C/15N plot of nail, (b) 13C/15N plot of hair, (c) 2H/18O plot of nail, and (d) 2H/18O plot of hair. Each data point represents the mean of a triplicate analysis. The dashed line indicates the pooled mean value of all individual results per isotope. Due to the scale of the plot error bars are not visible. Standard deviations for 13C, 15N, 2H and 18O were no greater than 0.39, 0.71, 1.91 and 0.55%o, respectively.
encountered and the amount of this substance decreases. In
hair, this pattern could be attributed to the growth cycle and
the changes which occur as hair passes from one phase to
another. In the life cycle of hair, periods of growth are
followed by a transition stage, when the hair shaft becomes
fully keratinised, and finally a period of rest.24 Hair is formed
during the growth phase when cells of the matrix
differentiate, become keratinised, and die. After the transi-
tion, or resting stage, another growth phase begins in which a
new hair replaces the old hair. Although hairs grow at
different rates, at any given time, the majority of hair is in the
growth stage.25 During the growth cycle of hair the
requirements for key amino acids and co-enzymes necessary
for keratin biosynthesis vary.

Similar sigmoid patterns noted in previous studies have
been attributed to seasonal variations in food.9 However, as
an increasing amount of the food eaten in a country is now
imported from other countries or is grown in the artificial
environment of a polytunnel, many products are available
outside their usual time frame – this has led to less
discernible seasonal variations in food. Differences in diet,
however, may be more pronounced in less developed
countries where dietary intake depends on seasonal avail-
ability and the cost of locally produced food products.26 Only
8 months of data are presented in this paper although, where possible, subjects donated samples for a further 4 months. Twelve months of data would obviously provide more information regarding seasonal variation; however, this data is not yet available. Physiological differences are also associated with changing seasons, which may contribute to the observed trend. For example, nail growth is faster during warm summer months than in the winter. However, as mentioned by Bean, the use of central heating in the winter and air conditioning in the summer has diminished this effect to a certain degree.

The $^{15}$N values of nail are noticeably more variable than those of hair from the same subject. Unlike nail, hair has a very fast formation rate; i.e. the time taken to form hair is significantly less than the time taken to form nail. This means that there is less time for the compounds forming hair to be affected by biochemical processes that could alter their isotopic signature, thus providing good chronological resolution along the length of the hair shaft. For this reason, hair may have an isotopic composition more consistent with dietary uptake than is provided by nail. A similar trend can also be seen in the $^{18}$O data recorded for hair and nails (Figs. 1(c) and 1(d)).

Greater variations were found with hydrogen and oxygen values of both hair and nail samples. The inter-individual pooled mean $\delta^2$H and $\delta^{18}$O values of these nails were $-66.2\%$ and $14.7\%$, with SDs of $\pm 4.1\%$ and $\pm 1.7\%$ and 95% confidence intervals of $\pm 1.2$ and $\pm 0.5\%$, respectively. Intra-individual $\delta^{15}$N mean values of nail were found to lie between $14.4\%$ and $15.1\%$. Hydrogen values produced a greater spread with values ranging from $-69.9\%$ to $-63.8\%$. Although this spread appears relatively large it is not surprising given the circumstances of the subjects involved. Most volunteers are lecturers or PhD students recruited through QUB and they are occasionally required to travel abroad. Such changes in location are undoubtedly accompanied by changes in diet and, most significantly, in the water consumed.

Subjects typically took a 2-week annual holiday abroad in addition to minor trips within the UK. Where the time spent abroad was less than approximately 10 days or to a country with a similar diet and water values as the UK, the change is not visible in the longitudinal study. However, where the place of travel was a location with significantly different diet and water the change in isotopic signature is apparent. For example, in Fig. 1(d) the $\delta^2$H value of the female UK subject at week 18 is approximately 10% more enriched than the previous sample – indeed it is the most enriched sample analysed for that subject (indicated by an arrow on the chart). This particular value could be attributed to the fact that the subject spent 2 weeks in Barbados in July. The modelled annual precipitation $\delta^2$H value for the area is approximately $-0\%$, which would account for the subject’s hair samples becoming more positive immediately following this trip.

Analysis of hair samples produced inter-individual pooled mean $\delta^2$H and $\delta^{18}$O values of $-60.7\%$ and $13.1\%$, with SDs of $\pm 7.6\%$ and $\pm 1.5\%$ and 95% confidence intervals of $\pm 2.1\%$ and $\pm 0.5\%$, respectively. Again, intra-individual $\delta^2$H values were found to lie within a large band, ranging from $-51.1\%$ to $-70.0\%$. However, the $\delta^{15}$N values of hair fell within a significantly smaller bracket of $11.5\%$ to $14.2\%$. As previously mentioned, this is probably due to the fast growth rate of hair limiting the biochemical processes that can affect the isotopic composition of the hair.

When interpreting results regarding a subject’s origin and/or movement between different geographic locations, the observed variations discussed above must be taken into consideration.

Comparing bulk hair and nail isotope values it was noted that hair samples were more enriched in $^{13}$C, but depleted in $^{15}$N, than nail samples were. The samples analysed were collected at different times to ensure that the growth time approximately correlated. Since a whole nail is approximately equivalent to 6 months of growth, and hair grows at a rate of approximately half an inch every month, for time-matching purposes hair samples corresponding to the tip of nail samples should be collected approximately 3 inches from the scalp. Isotope data for hair and nails reported by other groups show a similar offset. At present an explanation for this observed offset cannot be given, although it may be linked to the different formation rates of hair and nail.

**Geographical database**

Hair and nail samples donated by anonymous subjects from various locations across the world have been analysed and their isotopic signatures were recorded in a geographical database for future reference. The objective of this project is to assess whether the differences, if any, that exist between residents of different countries, and indeed within countries such as America and Africa, could be used to discriminate between individuals of different geographical origin. As illustrated in Fig. 2(a), $\delta^{13}$C values range from $-15.78\%$ to $-21.49\%$ and $\delta^{15}$N values between $9.56\%$ and $11.76\%$.

![Figure 2](https://example.com/fig2.png)

**Figure 2.** Plots of measured isotopic composition of globally sourced human hair and nail samples. Each data point represents the mean of a triplicate analysis. (a) $^{13}$C/$^{15}$N plot of hair and nails and (b) $^2$H/$^{18}$O plot of nails. Standard deviations for $^{13}$C, $^{15}$N, $^2$H and $^{18}$O were no greater than 0.47, 0.34, 1.70 and 0.27%, respectively.
the value of around subjects from European countries are clustered together at a barley and rye. This trend can be seen in Fig. 2(a), where source.30 Therefore, the consumption of bottled water in an modern-day diet. Indeed a study carried out by Bowen imported foods or bottled water, which can be found in the individual, despite potential scatter from the intake of isotopic signature of local water that has been consumed by an attribution to the fact that the body can acquire oxygen from /C0 could, therefore, together with sugar derived from sugar can, contribute to the δ13C value of approx. –15‰.

Further discrimination is possible when considering the results of 2H and 18O analysis as shown in Fig. 2(b). The variability in δ2H values for different countries is significantly greater than that of the carbon and nitrogen results, ranging from –50.0‰ to –91.3‰. Similarly, there is a greater spread in the δ18O values, with results varying from 9.2‰ to 19.0‰. The plot shown in Fig. 3 seems to indicate that hair preserves the 2H signature of local water consumed directly and indirectly by an individual, as indicated by the equation of the regression analysis. For example, hair samples received from Ithaca, NY, USA, have a δ2H mean value of –72.2‰, which is in line with reported and modelled annual precipitation values for the area (–60 to –80‰).28,29 The offset seen for the 18O values compared with the meteoric water line (MWL) could be attributed to the fact that the body can acquire oxygen from sources other than water, e.g. hydroxylation of the amino acid proline uses atmospheric oxygen. Figure 3 suggests that the hydrogen isotopic signature of hair is a good reflection of the isotopic signature of local water that has been consumed by an individual, despite potential scatter from the intake of imported foods or bottled water, which can be found in the modern-day diet. Indeed a study carried out by Bowen et al. suggests that the isotopic value of locally produced bottled water does not vary greatly from the value of the local water source.30 Therefore, the consumption of bottled water in an individual’s diet should not greatly skew the isotopic signature of their tissues. Where the isotopic value of water from different countries is very similar, e.g. for countries within Europe, hydrogen isotopic data alone may not be sufficient to discriminate between countries irrespective of whether it is bottled water or not.

CONCLUSIONS

In order to correctly interpret the findings of isotopic data from the analyses of hair and nails, all the factors influencing the stable isotope profile of individuals must be fully investigated. We have determined variations associated with sample preparation and the technique itself; however, the natural variability or ‘biological noise’ has yet to be fully explained. The results of the longitudinal study quantified the degree of variation encountered in the isotopic composition of hair and nail samples from individuals residing in the same area. Whilst some of these fluctuations can be attributed to travel and the consumption of foods not locally sourced, it must be acknowledged that natural variability of isotope signatures will be affected by biological processes within the body such as different growth rates of tissues and differences in metabolic rates, resulting in the observed intra- and inter-individual variability in the isotopic composition of hair and nails. Further research into the isotopic composition and turnover of individual amino acids in keratin should assist in the interpretation and potential exploitation of these findings.

If stable isotope profiling (SIP) is to play a successful role in the identification of humans as well as human remains it is important to map intra- and inter-individual variations across the globe in order to determine significant cut-off values for geographic points of origin. This will help establish the range across which the technique can be used in a meaningful way. It is also important to determine if the variations in isotopic signatures seen within a given country are great enough to provide discrimination between different geographical locations within that particular country.

In addition, the creation of a database will help to provide reference points for the comparison of samples of unknown origin, which would undoubtedly aid in disaster victim identification as well as criminal investigations.

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