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ORIGINAL ARTICLE

Multi-element stable isotope analysis of H, C, N and S in hair and nails of contemporary human remains

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Abstract This paper presents a comparison of the isotopic ratios of hydrogen, carbon, nitrogen and sulphur of three pairings of hair and nail tissue taken from contemporary human remains. Our aim was to examine the possibility of a direct comparison of isotopic data in hair with that of nail tissue for forensic purposes. The results indicate that stable isotope ratios of the elements were best comparable between human scalp hair longer than 3 cm and the distal end of the nails. There were no distinct variations between finger and toenails. Our isotopic data for bulk hair and nail confirmed that hair samples were slightly enriched in ^{13}C but depleted in ^{15}N compared to nail samples. Furthermore, our data reveal that $\delta^{34}S$ values in nail samples were more variable than in hair samples. Direct comparison of the corresponding segments of hair and nail samples may be difficult due to individual differences especially for $\delta^{15}N$ and $\delta^{2}H$. Hair may have an isotopic composition more consistent with the ingested food within a specific time than is provided by nail. It can be concluded that once a hair is formed, no further metabolic changes of the isotopic pattern should occur. Nevertheless, our data suggest that there was a change in isotope ratios particularly for $\delta^{2}H$ along the hair shaft. Interpretation of the isotope data in corresponding segments of hair and nail for forensic purposes must consider particular variations, especially for chronological considerations.

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Introduction

Geographic origin determination of unknown people is a relevant application of stable isotope investigations in the field of forensics. For several years, multi-element stable isotope analysis has been used in food research for origin determination or detection of adulteration [1–4]. As food and drinking water have isotope signatures derived from their geographical provenance region, these isotope signatures are transferred into human tissues over the food chain. Consequently, knowing the location of plants and animals used for nutrition and the geographic origin of drinking water, one can conclude the consumer's whereabouts. Although there is a tendency for a 'global supermarket' diet, isotope signatures in human tissues hold dietary information directly related to both food sources and dietary practices in a region [5–7]. Several studies have demonstrated the potential of multi-element stable isotope analysis as a tool for determining the place of origin of living humans, unidentified humans and the identification of disaster victims [8–12].

Description of regional differences is possible using stable isotope ratios of bio- (H, C, N, O and S) and geo-elements (Sr and Pb). 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 recently [12–14]. Generally, the ^{13}C, ^{15}N and ^{34}S in human material reflects the isotopic composition of the food consumed by an individual whereas ^{2}H content is a reflection of a person's direct and indirect water intake via drinking water and food [6, 15–17]. In addition, a proportion of organically bound ^{2}H can be directly derived from food.

The majority of work carried out on human tissues involved interpretation of isotopic data from archaeological human remains in a palaeo-dietary and palaeoecological context [18]. Data of this work are of limited usefulness for forensic investigations for a number of reasons. Unlike today, the diet of pre-modern individuals was substantially more restricted and less variable than today [18–20]. Ancient diets were possibly more seasonally diverse than modern diets as individuals relied predominately on locally produced crops and other food products, and availability of these foods was dependent on the season. Furthermore, stable isotope studies on archaeological human remains mostly deal with diet reconstruction by C, N and O isotopes [18]. Nowadays, new analytical techniques enable stable isotope determination of additional elements in human remains, like H and S, which contain important geographical information. Isotope signatures of at least the four elements H (or O), C, N and S are necessary for forensic purposes to provide differentiated evaluation and more precise origin assignment [11, 12, 21, 33]. Our database at the Institute of Forensic Medicine in Munich comes up with the isotope data of H, C, N and S of worldwide collected hair samples, and geographical origin determination of unknown individuals is carried out by discriminant analysis of the isotope parameters of the four elements.

Several papers have dealt with isotope analysis of contemporary human hair [6–17, 21, 22, 23, 26–32 34, 35] and also of nail [5, 8–10, 22, 36]. Since isotopic composition of hair and nail keratin reflects that of the diet and water intake, which are connected to geographical location [18, 23–25, 37–40], both tissues can be used for geographical origin determination of individuals in a forensic context. In contrast to other body proteins, hair and nail keratin are not subjected to metabolic turnover, and thus preserve the signature of dietary habits at the time of their formation.

The isotope ratios of bio-elements of proteins with a slow turnover rate, such as bone collagen, reflect an average of the body's dietary protein intake over several years or decades [41, 42]. Isotopic values in hair approximate to a linear record of the most recent diet along the hair shaft length. With a monthly growth rate of approximately 1 cm [43, 49], a fairly short length of e.g. 6 cm covers the diet of the last 6 months. Like hair, nail tissue is built from keratin, with a growth rate of about 1.5 to 3 mm per month [44, 50, 51] and a length of about 2 cm. Under these circumstances, nail tissue contains dietary and environmental information of up to 1 year.

The two different keratins that comprise hair and nails are both classified as ‘hard’ keratins; they have similar amino acid profiles, varying with narrow limits [22]. Since the term keratin refers to a group of approximately 100 different proteins [45] with some diversity in their amino acid sequence, we have to investigate if there are isotopic distinctions in keratins of hair and nail.

Regarding application of isotope analysis on human tissues for determination of geographical origin and whereabouts of unknown dead bodies in cases of legal medicine, it may be assumed that investigation of nail tissue may hold the same information and can be used instead of scalp hair. For unidentified persons, special circumstances in reality might lead to the fact in some cases that the hair or the nail tissue is absent. For example, scalp hair of unknown bodies may not be available because of different reasons, e.g. a clean-shaven head, concealing the scalp of a murder victim, or through burning. Fingernails could be absent for example through removal of fingertips to prevent identification of an individual. Such being the case, only one of these two tissues is present and can be investigated by stable isotope analysis.

Substitutions of hair with nail and vice versa leads to the following questions: (1) are isotope signatures of the bio-elements comparable in both tissues, (2) could different segments be assigned to specific lifetimes of an individual, and (3) could nail tissue be used instead of hair and vice versa?

This paper provides an investigation of H, C, N and S isotopes in hair and nail segments taken from three individuals to assess whether both types of tissues have comparable isotopic information.

Experimental

Origin and preparation of hair and nail samples

Scalp hair and nail samples were taken from three persons who died in 2006 and 2007. As the circumstances of their death and also their identity remained unclear, autopsies were performed at the Institute of Legal Medicine in Munich. Samples of body tissues, including hair and nail, were collected in order to deliver an expert opinion on determination of the person's geographic origin by stable isotope analysis.

1. Person ‘BG’: Middle-aged male, grey scalp hair with a length of approximately 6.5 cm and thumb nail with a length of 1.7 cm. Three small hair strands were taken directly at the scalp and cut into three segments with a length of 2.0 cm each (0.3–2.3 cm, 2.3–4.3 cm, 4.3–6.3 cm). A thumb nail was dissected and cut into 11 segments of 1.5 mm each. Three segments of the hair strand (distance to scalp=1–2 cm, 3–4 cm, 5–6 cm) and six segments of the nail (distance to nail root=0–0.2 mm, 0.35–0.5 mm, 0.65–0.8 mm, 0.95–1.1 mm, 1.25–1.4 mm, 1.55–1.7 mm) were taken for analysis.

2. Person 'DW': 20- to 30-year-old female, blond scalp hair with a length of 30 cm and a big toenail with a length of 1.8 cm. A single hair strand was taken directly at the scalp and cut into segments of 1 cm each. The hair samples were cut with scissors into very small pieces. The big toenail was dissected and cut into 12 segments of 1.5 mm. Seven segments of the hair strand (distance to scalp=0–1 cm, 1–2 cm, 2–3 cm, 3–4 cm, 4–5 cm, 5–6 cm, 6–10 cm) and six segments of the nail (distance to nail root=0.15–0.3 mm, 0.45–0.6 mm, 0.75–0.9 mm, 1.05–1.2 mm, 1.35–1.5 mm, 1.65–1.8 mm) were taken for analysis.
3. Person 'TH': 20- to 30-year-old male of Asian appearance, black scalp hair with a maximum length of 6.0 cm and a big toenail with a length of 1.8 cm. A bundle of scalp hair was taken directly at the scalp and cut into six segments of 1.0 cm each. A big toenail was dissected and cut into eight sections of 2.0 mm each. Six hair segments (distance to scalp=0–1 cm, 1–2 cm, 2–3 cm, 3–4 cm, 4–5 cm, 5–6 cm) and eight nail segments (distance to nail root=2–4 mm, 4–6 mm, 6–8 mm, 8–10 mm, 10–12 mm, 12–14 mm, 14–16 mm, 16–18 mm) were taken for analysis.

The hair and nail segments were cleaned with a mild hair shampoo in an ultrasonic bath, rinsed several times with distilled water, once with methanol/chloroform (2:1), rinsed again with distilled water and dried at room temperature. The hair and nail segments were cut with scissors into very small pieces. The chopped material was analysed for δ^2H, δ^{13}C, δ^{15}N and δ^{34}S.

Stable isotope analysis

For stable isotope analysis of δ^2H, the chopped hair and nail samples were analysed according to the comparative equilibration method [46]. The tissue samples and working standards were stored under identical conditions for several days before analysis to enable exchange with hydrogen from ambient air moisture. After equilibration, 150 μg of the samples and the reference materials (casein, two samples of horse tail hair with δ^2H values of −56‰ and −84‰) were weighed into tin capsules in triplicate and dried under vacuum for at least 24 h to remove all adhering humidity. Samples and the working standards were then loaded onto the helium flushed autosampler of the elemental analyser. Hydrogen gas produced by high temperature conversion at 1,400°C was analysed isotopically. A correction was performed by normalisation of values for working standards to assigned results obtained after inter-laboratory calibration of these materials and correcting the sample data accordingly. International reference material was NIST-22 with the assigned value of −120‰ vs. V-SMOW.

For multi-element isotope analysis of C, N and S isotopes, 2.0 mg of the chopped hair and nail samples was weighed into tin capsules in quadruple. Internal standards were casein and horse tail hair. Determination of stable isotope ratios of H, C, N, and S followed internationally accepted methods and is described in detail elsewhere [1, 47]. Measurement of the light elements was carried out using an Elemental Analyzer–Isotope Ratio Mass Spectrometer (EA–IRMS). The analytical precision using at least triplicate measurements 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‰/V-CDT.

During the process of multi-element isotope analysis, gases such as SO_2 and CO_2 were adsorbed on specific columns and sequentially released; N_2 passes without being retained. Thereby the elemental content of carbon, nitrogen and sulphur can be determined by the elemental analyser. In contrast to that, for δ^2H analysis the sample is pyrolysed, whereby different gases are generated and no specific adsorption of hydrogen occurs. Consequently, determination of the elemental content of hydrogen is not reliably possible.

Results and discussion

General remarks

The stable isotope profiles of human hair and nail samples are determined by the diet and water consumed by an individual, the metabolic and physiological processes that occur within the human body, and the climate of the region in which an individual lives [48]. By investigating different segments of hair and nail, we wanted to determine whether isotope values of H, C, N and S in contemporaneously grown tissues could be compared directly and allocated to definite growth times.

It is known that 1 cm of scalp hair represents approximately 1 month's growth (rate of 0.35 mm/day [43, 49]). Changes in nutrition are shown in the hair with a 14-day delay for carbon isotope ratios of food components [23]. Taking this delay into consideration, the 'age' for the 'oldest' hair segment from 'TH' was 180 days, for 'BG' 175 days and for 'DW' 250 days prior to death.

Nail growth rate is usually considered to be one third of the average hair growth rate. The average growth rate of all fingernails is 0.6 mm/week [44]. For toenails, the growth rates are significantly lower than for fingernails [50, 51]. Therefore, we applied a growth rate of the thumb nail collected from 'BG' of 3.0 mm per month. A growth rate of 1.5 mm per month was assumed for the toenails collected from 'TH' and 'DW'.

Taking the same assumption of a 14-day delay for isotopic incorporation of food elements into nails, the

'oldest' segments of the investigated thumb nail ('BG') were grown 165 days and of the toenails ('TH' and 'DW') 250 days prior to death.

Hydrogen

Our results for δ^2H in hair and nail tissue show values between −55‰ and −87‰, whereas the highest variations occur in the youngest tissue samples (Fig. 1). In hair and nail tissue older than 200 days, δ^2H values varied only between −73‰ and −76‰, which is in the range of δ^2H values analysed in hair samples from residents of South Germany. Every hair segment was enriched in ^2H compared to the corresponding nail segment. The mean differences in $\Delta_{hair-nail}$ ranged from 7‰ to 14‰ (Table 1). Conspicuously 'older' hair segments (period of formation longer than 6 months ago) were enriched in ^2H by 2‰ to 5‰, whereas in the most recent hair segments, which were grown during the last month before death, δ^2H values were distinctly enriched by 10‰ to 27‰ compared to nail tissue of the same individual. Since both finger and toenail tissue of each individual had almost the same δ^2H value over the whole growth time, δ^2H values along the hair strand changed distinctly with a remarkable enrichment in younger hair segments. An enrichment of ^2H values in human hair compared to nail tissue by a mean value of 6‰ can also be seen in the results of Fraser and Meier-Augenstein [9]. Contrary to our study, the authors took hair samples directly from the scalp, which were compared with nail samples clipped off at the distal edge of the nail. They assumed that the shift in ^2H abundance to more depleted values in nails is related to a slower formation rate of nail tissue and different metabolic processes referred to amino acid incorporations from food and drink.

To verify the observation that the most recent hair samples are enriched in ^2H, we conduct a specific study devoted to H isotopes along hair strands taken from two male ('VW', 'VR') and one female ('CL') persons. The results indicated a shift in δ^2H values along every hair strand; the older ones were depleted in ^2H at within the range of 4‰ to 10‰ compared to the most recent hair segments (Fig. 2), whereas the highest extend of depletion occurred at the first 3 cm of the hair strand. Further observations in hair samples of several female and male bodies, which were investigated for forensic purposes, also showed a depletion of ^2H along their hair strands up to 18‰, depending on the hair length (data not shown). However, the origins of the bodies under investigation were unknown and the δ^2H value may have been influenced by individual changes in their living conditions, or hair dye products. Contrary to the latter individuals, the results of the persons investigated ('CL', 'VW', 'VR') were residents of South Bavaria who declared that they had no changes in their living circumstances or nutrition behaviours (with the exception that 'VW' spent 2 weeks in Texas 60 to 70 days before the hair was cut). Furthermore, only 'CL' used herbal hair dye, which may perhaps influence δ^2H values, but 'VW' and 'VR' did not use any hair treatment products. It is known that C/N ratios and also $\delta^{13}C$ and $\delta^{15}N$ values of hair are slightly affected by dyes and bleaching, and the amino composition can be altered [18, 35, 52], but possible effects on δ^2H are unknown.

Nevertheless, the decrease in ^2H content from the scalp to the hair tip is striking because hair shaft is thought to be unaltered after leaving the hair root. However, the reason for the alteration of δ^2H along the hair shaft may be the result of physiological changes taken place by time. The hair shaft may be influenced by mechanical or physical exposure (e.g. hair brushing, leaching by sun) and forced by hair treatment with shampoo, other hair products, dyeing and environmental conditions. Exposure to ultraviolet rays on hair leads to modifications of hair keratin, cleavage of

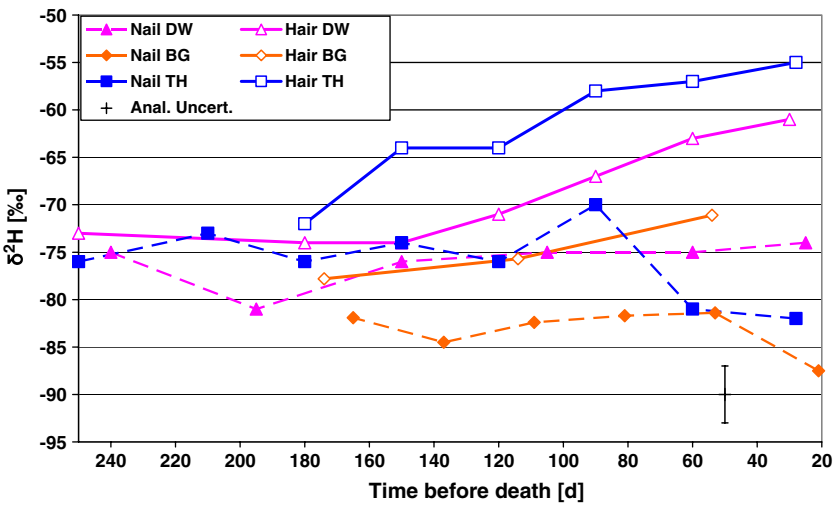

Fig. 1 δ^2H values in hair and nail segments taken from different individuals: 'TH', 'BG' and 'DW'

Table 1 Mean isotope values (mean value±standard deviation) of hydrogen, carbon, nitrogen and sulphur in hair and nail tissue taken from different individuals: 'TH', 'BG' and 'DW'

Person	Tissue	δ^2H (‰)	δ^{13}C (‰)	δ^{15}N (‰)	δ^{34}S (‰)
BG	Hair	−75±3	−20.9±0.1	8.8±0.2	6.6±0.2
	Fingernail	−83±2	−21.2±0.1	9.7±0.1	7.6±0.4
	$\Delta_{hair-nail}$	8	0.3	−0.9	−1.0
DW	Hair	−69±5	−21.1±0.1	8.1±0.1	6.5±0.1
	Toenail	−76±3	−21.4±0.1	8.5±0.3	6.3±0.1
	$\Delta_{hair-nail}$	7	0.3	−0.4	0.2
TH	Hair	−62±6	−20.1±0.3	9.1±0.1	6.7±0.1
	Toenail	−76±4	−20.2±0.1	9.4±0.1	6.1±0.6
	$\Delta_{hair-nail}$	14	−0.1	−0.3	0.6

peptide and disulphide bonds and oxidation of amino acids, and the incorporation of water and oxygen molecules into the hair filament is relieved [53]. Furthermore, it is known that between 9% and 16% of the structural hydrogen in keratin is freely exchangeable [54]. If changes in the tertiary and secondary structure of hair keratin occur, e.g. under mechanical stress (esp. hair grinding) or an 'ageing' process e.g. split ends, this could be associated with the enhanced accessibility of hydrogen-exchange sites. Since δ^2H value of atmospheric water vapour is depleted by 70–90‰ compared to precipitation [55], redundant structural hydrogen atoms in keratin could be replaced with isotopically 'depleted' hydrogen atoms from the air moisture. Consequently, especially for longer hair, δ^2H values along the hair strand decrease, whereby the extent of the shift in δ^2H values along the hair strand could vary individually depending on hair length, different treatment habits and hair structure.

Sigmoidal patterns in δ^2H values along the hair strand noted in previous studies due to seasonal variations in food [17] cannot be concluded from our data. At least for the most industrialised regions, availability of almost all food all the year and the fact that drinking water is kept in large water reservoirs make it unlikely that seasonal variation patterns occur in hair and nails.

Carbon

The δ^{13}C values of hair and nail samples range from −21.5‰ to −19.9‰ (Fig. 3). Person 'TH' had the highest values (mean value −20.1‰), whereas δ^{13}C values for 'DW' and 'BG' were between −21.5‰ and −20.8‰. Also, δ^{13}C values in tissues from 'TH' are enriched compared to those from 'BG' and 'DW' by a mean of 1.0‰. This can be due to a special Asian-influenced diet, which agrees with data from our database that the mean δ^{13}C value in Asian hair samples is about 1‰ higher than in hair samples taken from German residents.

Hair samples generally were enriched in ^{13}C compared to nail samples (Table 1); the mean differences for $^{13}C_{hair-nail}$ for 'BG' and 'DW' were 0.3‰. These results agree with the slight enrichment of ^{13}C in hair keratin compared to nail keratin by 0.2±0.4‰ observed by O'Connell et al. [22]. Basically, the δ^{13}C pattern along the hair shaft was slightly more variable than along the nail plate. For 'TH', two hair samples were depleted in δ^{13}C compared to nail. Noticeable is the change of δ^{13}C in hair and nail tissue of 'TH' in opposite directions. Whereas δ^{13}C along the hair strand increased by 0.7‰, the values in the corresponding nail segments indicated a slight decline in ^{13}C values of about

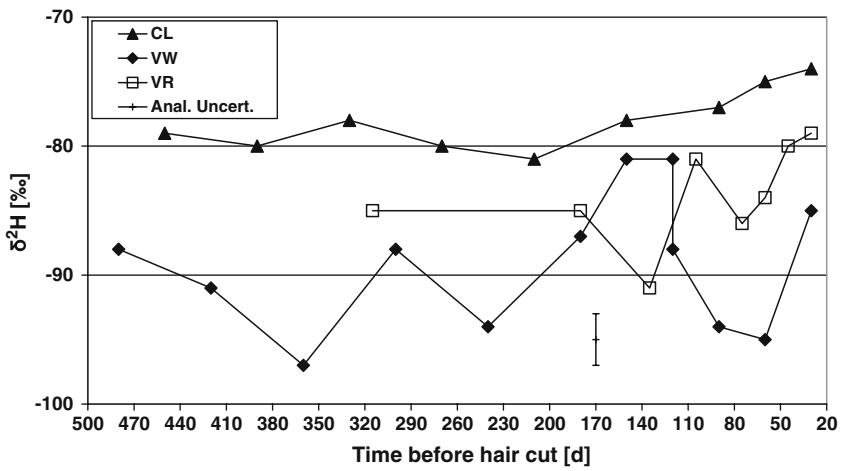

Fig. 2 δ^2H values in scalp hair segments taken from different individuals: 'CL', 'VW' and 'VR'

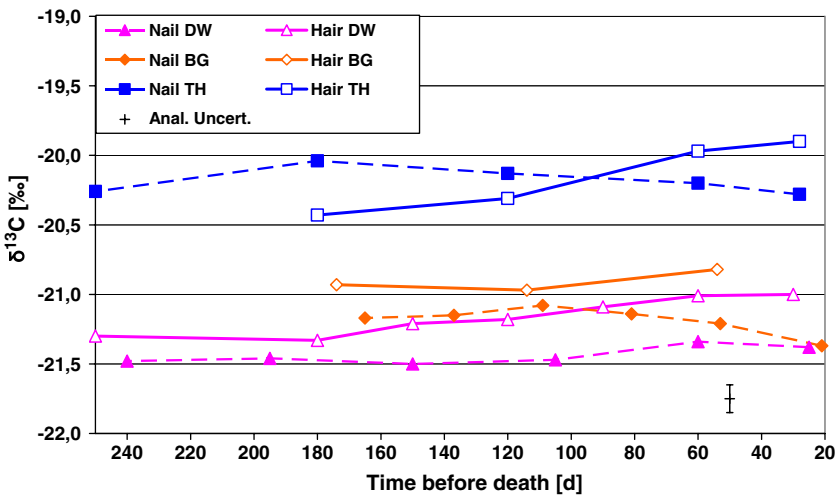

Fig. 3 $\delta^{13}C$ values in hair and nail segments taken from different individuals: 'TH', 'BG' and 'DW'

0.3‰. A similar but less distinct pattern is indicated for hair and nail segments of 'BG'. Otherwise, both hair and nail segments of 'DW' showed a uniform slight increase of $\delta^{13}C$ in younger segments. This can lead to the assumption that 'TH' and, to a lesser extent, 'BG' may have changed their whereabouts and nutrition behaviours during the last month of their life.

Carbon elemental content in hair and nail tissue varied from 41.8% to 49.2% (Fig. 4 and Table 2) without any difference depending on the kind of tissue or age of the segment. Tissues of 'TH', the young man with the Asian appearance, had the highest and 'DW', the lowest carbon contents. Noticeable is the observation, especially in tissues of 'TH', but also for the other individuals, that changes in carbon contents in hair and nail are associated with changes in $\delta^{13}C$ values. The results indicate that enrichment in $\delta^{13}C$ values is associated with a decrease in carbon contents and vice versa. This may be caused by unknown physiological parameters, whereby nutrition may have an influence.

Nitrogen

$\delta^{15}N$ values in hair and nail tissue varied between 7.9‰ and 9.8‰ (Fig. 5). The highest $\delta^{15}N$ values could be found in nail samples from 'BG', the lowest in hair samples of 'DW'. $\delta^{15}N$ values in nail segments generally were enriched compared to hair segments on average by 0.5± 0.3‰ (Table 1). These data agree with the significant enrichment of ^{15}N in human nail keratin compared to hair by a mean value of 0.65±0.2‰ [22]. In addition, our results show a slight depletion of ^{15}N in toenails from root to top and in the fingernail vice versa.

Nitrogen contents in the toenails were distinctly higher than in the fingernail, and the total nitrogen content of hair samples were in between (Fig. 6 and Table 2). The results indicate that $\delta^{15}N$ value seems to be associated with nitrogen content. Especially for nail tissue of the individuals, increased nitrogen content resulted in a depletion of ^{15}N. For example, in nail segments of 'DW', a depletion of ^{15}N by 0.5‰ is associated with a change in nitrogen

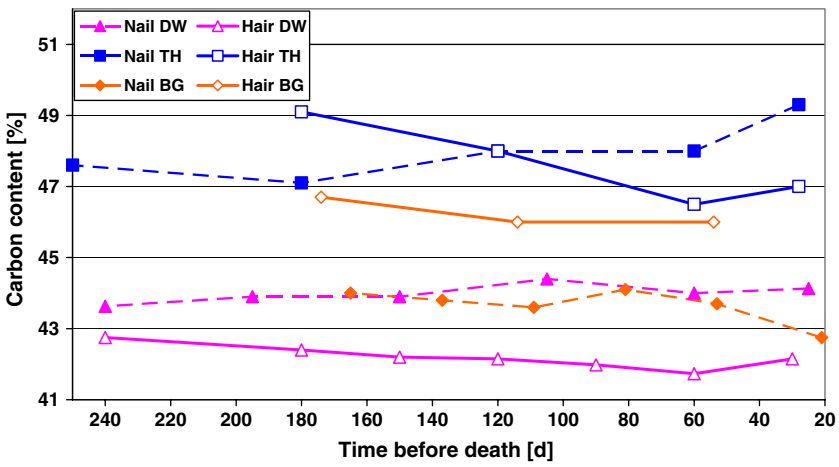

Fig. 4 Carbon content in hair and nail segments taken from different individuals: 'TH', 'BG' and 'DW'

Table 2 Mean elemental contents (mean value±standard deviation) of carbon, nitrogen and sulphur in hair and nail tissue taken from different individuals: 'TH', 'BG' and 'DW'

Person	Tissue	C (%)	N (%)	S (%)	Mol. C/N	Mol. C/S
BG	Hair	46.3±0.3	15.2±0.1	5.2±0.1	3.56	25.0
	Fingernail	43.7±0.4	14.1±0.2	3.0±0.4	3.62	41.6
	$\Delta_{hair-nail}$	2.6	1.1	2.2		
DW	Hair	42.2±0.3	14.8±0.3	4.3±0.2	3.32	28.0
	Toenail	44.0±0.2	16.0±0.3	2.0±0.3	3.22	62.3
	$\Delta_{hair-nail}$	−1.8	−1.2	2.3		
TH	Hair	47.7±1.0	14.8±0.5	4.5±0.2	3.75	30.3
	Toenail	48.0±0.7	15.5±0.1	2.1±0.6	3.61	65.4
	$\Delta_{hair-nail}$	−0.3	−0.7	2.4		

content from 15.4% to 16.3%. For continuous alteration of $\delta^{15}N$ values and nitrogen contents along the nail plate, incorporation of different amino acids and/or transamination processes during nail formation may be responsible. Different types of keratin with varying compositions of basic amino acids (arginine, histidine and lysine) may be involved in the nail structure and incorporated along the nail bed. Our results are supported by the assumption of O'Connell et al. [22] that variable nitrogen isotope ratios in human hair, nails and collagen may be due to different amino acid compositions or transamination processes [22], the latter in general results in a decrease of $\delta^{15}N$ [56]. Also, intracellular turnover of amino acids, which are connected to transamination processes and therefore related to isotopic fractionation, could be different in the hair follicle and the nail matrix.

Sulphur

$\delta^{34}S$ in the hair segments remained constant along the hair shaft (with regard to analytical precision of $\delta^{34}S$ measurements), whereas the results of $\delta^{34}S$ in nail tissue, particularly in more recent nail segments, differed distinctly between the three individuals (Fig. 7). ^{34}S in nail was enriched ('BG'), depleted ('TH') or the same ('DW') compared to hair. Basically, $\delta^{34}S$ in the hair correlated well with $\delta^{34}S$ in the 'oldest' nail segment from the distal end.

Contents of sulphur in hair were constant along the hair shaft, but different between the individuals (Fig. 8 and Table 2), whereas hair segments had distinctly higher contents of sulphur than nail segments, and for nail it increased from the root to the distal end. Sulphur content in the fingernail was higher than in the toenails. According to our results, a large spread in cysteine content between hair and nail tissue and a high variability from 11.9% to 18.1% of total amino acid exist, with also lower cysteine contents in nail [22].

Considering that the content of sulphur in nails increased from the root to the distal end, it seems likely that keratinisation occurs over the whole nail plate and has not already been completed at the visible lower end of the nail. As sulphur content is connected to keratinisation processes, it can be stated that keratinisation of nails continues during the continuous production of nail tissue by the bed [57]. This can be supported by the fact that keratins have a common mode of synthesis in which there is a primary

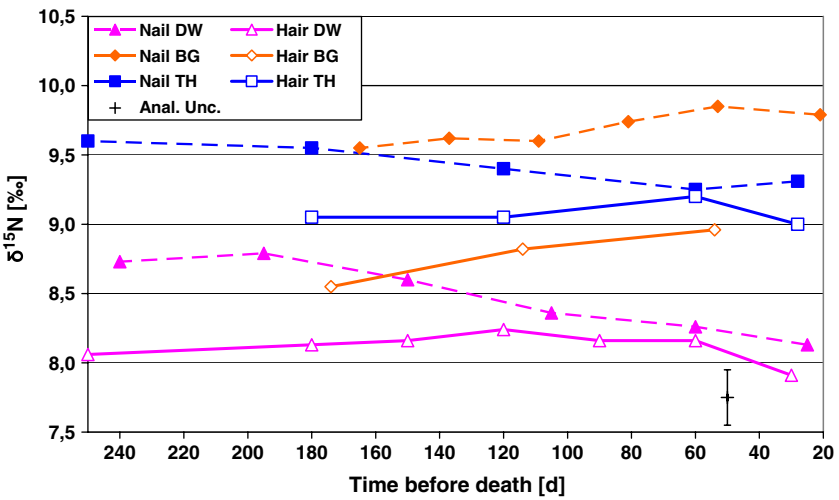

Fig. 5 $\delta^{15}N$ values in hair and nail segments taken from different individuals: 'TH', 'BG' and 'DW'

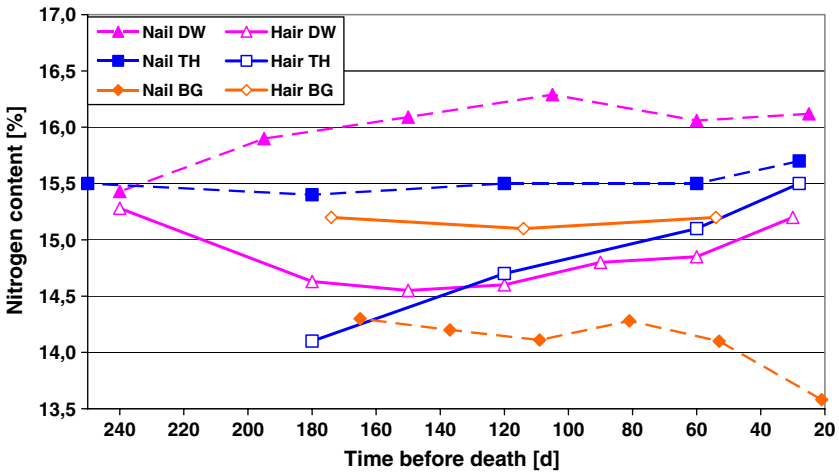

Fig. 6 Nitrogen content in hair and nail segments taken from different individuals: 'TH', 'BG' and 'DW'

synthesis of 'low-sulphur' proteins (as α-filaments), and afterwards, probably at a later stage during nail development, the 'high-sulphur' proteins are produced in a secondary synthesis and are inserted between the filaments as an amorphous matrix [58]. In contrast to nail, keratinisation processes in hair are finalised when it is visible at the scalp and afterwards is unchangeable by physiological alterations.

Obviously, there is no uniform pattern for the change of ^{34}S along the nail plate. There are several factors which may be responsible for the distinct $\delta^{34}S$ values along the nail bed. Basically, $\delta^{34}S$ values in animal tissues are dependent on the diet. The results of Richards et al. [59] show that horse hair samples nearly had the same $\delta^{34}S$ than diet, if they had an optimal protein supply. Under protein deficiency, hair got enriched in ^{34}S by 4‰ compared to diet. Results of Tanz and Schmidt [60] indicate an enrichment of ^{34}S up to 4‰ in hair, hooves and horn of herbivores relative to diet. They conclude that $\delta^{34}S$ values of animal samples basically reflect the diet but show small ^{34}S enrichments relative to it, depending on the animals' nutrition status, the trophic level and the individual tissue [60]. Additionally, as in metabolism methionine generally is depleted in ^{34}S compared to cysteine [60], alteration in the concentration of methionine respectively cysteine can result in changes $\delta^{34}S$ values along the nail plate. Therefore, tissues with high cysteine contents should have enriched $\delta^{34}S$ values compared to tissue with high methionine contents. If it is the fact, rise of sulphur content and enrichment of ^{34}S along the nail plate must be due to incorporation of cysteine. These considerations agree with the data of 'TH' and 'DW', but opposed to this, $\delta^{34}S$ values along the nail plate of 'BG' decrease while sulphur contents increase. The observation of various patterns of ^{34}S incorporation during nail plate formation allows the assumption that sulphur-containing metabolites from more than one pool with different $\delta^{34}S$ values were incorporated into the nail tissue. Therefore, we conclude that along the nail plate, sulphur metabolites from different pools, which were supplied by diets with different $\delta^{34}S$ values, were

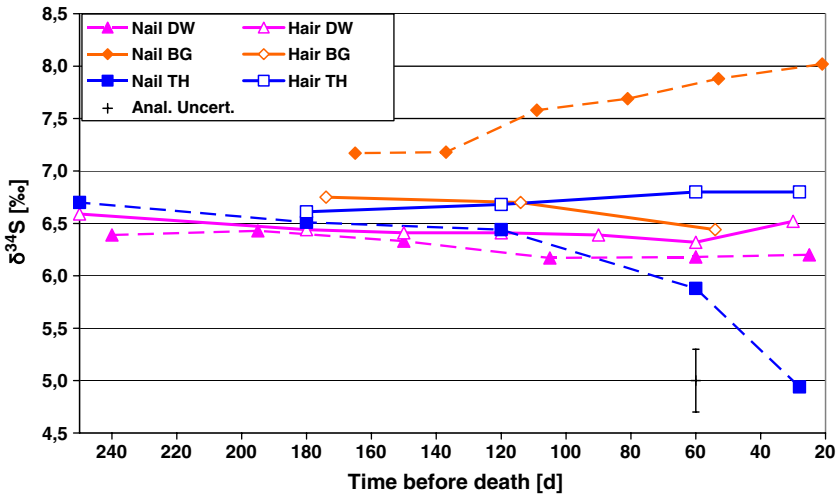

Fig. 7 $\delta^{34}S$ values in hair and nail segments taken from different individuals: 'TH', 'BG' and 'DW'

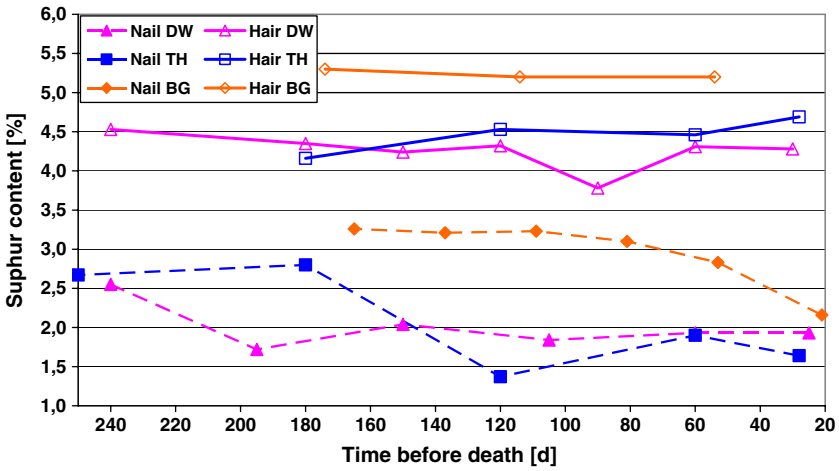

Fig. 8 Sulphur content in hair and nail segments taken from different individuals: 'TH', 'BG' and 'DW'

evident. Our results permit the suggestion that 'BG' had been living in a region with marine influence for a time period of more than 6 months before his death, which can only be seen in nail tissue but not in the hair samples. Furthermore, we assume that the $\delta^{34}S$ value of each pool mainly depends on the ^{34}S content of the metabolites, which were supplied by the ingested food within a specific time and, after different retention times, ultimately incorporated into the nail plate during its growth time. Each pool may have different turnover rates for sulphur. Additionally, results of Bahar et al. [61] show that the half-life of sulphur in bovine skeletal muscle was 219 days, which was about 30% longer than for C and N. This suggests the assumption that sulphur in nail tissue could stem from food which was ingested more than 6 months before death. Consequently, if a person had changed its whereabouts with an effect on $\delta^{34}S$ values of the consumed diet, it probably takes a longer time until this change can be seen in hair for ^{34}S than for ^{13}C, and in nail this effect additionally is thought to be influenced by different physiological aspects.

According to these considerations, if an individual did not undergo any changes of $\delta^{34}S$ values in diet during growth time of the tissues, $\delta^{34}S$ values could be compared between corresponding segments of hair and the distal end of the nail, whereas in the lower parts of the nail the $\delta^{34}S$ value could be very different from that in the hair.

Conclusions

Referring to question 1 in the 'Introduction', if isotope signatures of the bio-elements are comparable in both tissues, our data suggested that stable isotope ratios of the elements (H, C, N and S) were best comparable between human scalp hair and the distal end of the nails. There were no distinct variations between finger and toenails. Our data for bulk hair and nail isotope investigations confirmed that hair samples were slightly enriched in ^{13}C (mean value 0.2± 0.2‰) but depleted in ^{15}N (mean value −0.5±0.3‰) compared to nail samples. Furthermore, the data indicated that $\delta^{34}S$ values in nail samples were more variable than in hair samples. Variations in amino acid composition of keratins, different incorporation times and the abundance of several sulphur pools with different $\delta^{34}S$ values may be responsible for some of the differences in isotopic values of the investigated element; above all, cysteine concentration differs in major proteins in human hair and in nail.

Direct comparison of the corresponding hair and nail segments may be difficult due to individual differences of the two tissues especially for $\delta^{15}N$ and $\delta^{2}H$. For $\delta^{2}H$, there was a decline in $\delta^{2}H$ in scalp hair strands from the root to the hair tip between 7‰ and 17‰, whereas $\delta^{2}H$ in nail showed marginal variation. This consideration was supported by the observation that scalp hair of other individuals investigated for a study denoted to H isotopes in hair also had a general decrease of $\delta^{2}H$ along their hair strands. This fact is probably due to changes in physical properties of the hair strands through mechanical or chemical stress, which give rise to an exposition of more exchangeable hydrogen atoms, which can be replaced by isotopically 'lighter' hydrogen derived from the air moisture respectively atmospheric water vapour. Comparing isotope data from our hair isotope databank, the mean value of scalp hair from German male individuals was −72.5±7.9‰ (N=84), which is significantly different to German females with a mean value of −80.5±8.9‰ (N=47). Reference hair samples for the database usually were taken from the tip of the hair strand, and it can be assumed that females in general have longer hair than males. The differences in $\delta^{2}H$ between men and women, respectively between short or long hair, should be taken into account for geographic determination of individuals for forensic purposes. This must be considered when hair samples were either taken directly from the scalp or from the end of the hair shaft.

With respect to question 2 in the 'Introduction', if different segments could be assigned to specific lifetimes of an individual, especially the results of $\delta^{13}C$ and $\delta^{34}S$ in hair and nail particularly support the assumption that nail tissue reacts more slowly than hair, and is less sensitive to short-term changes of diet and environmental conditions. The shift in $\delta^{13}C$ of corresponding hair and nail segments in reverse for 'TH' may be due to a time delay of the nutritional elements incorporated in the nail. Incorporation of metabolic intermediates into the nail tissue occurs during the whole growth time of the nail plate. Therefore, one segment of nail tissue contains isotopic information over a longer time period than the corresponding hair. Formation of the nail is more complicated than formation of hair due to different generating matrixes for the dorsal, intermediate and ventral nail plate, as it can be seen especially for ^{34}S. Unlike nail, hair has a very fast formation rate, i.e. the time taken to form hair is significantly shorter than the time taken to form nail. There is less time for the metabolites 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 hair, it is valid that once it has been formed, no further metabolic changes at the isotopic pattern should occur. Therefore, we conclude that hair has an isotopic composition which is more consistent with dietary uptake than is provided by nail. Our data otherwise indicate that exposition of exchangeable hydrogen atoms due to mechanical stress could be the reason for the observed change in $\delta^{2}H$ along the hair shaft.

Taking into account question 3 of the 'Introduction', if nail tissue could be used instead of hair and vice versa for forensic purposes, we conclude that nail segments contain less distinct information than hair segments with regard to changes in the individual's migration patterns or nutrition behaviour. Comparing corresponding segments of hair and nail, our results indicate that isotope ratios of different elements must be interpreted very carefully regarding the above-mentioned particular variations, at least for chronological considerations.

When interpreting results regarding a person's origin and/or movement between different geographic regions, the observed variations as discussed above must be taken into consideration.

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