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Stable isotope composition of human fingernails from Slovakia

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HIGHLIGHTS

- This study deals with stable isotope analyses of fingernails from Slovak volunteers.
- $\delta^{13}C$ and $\delta^{15}N$ values of vegetarian and omnivore fingernails were compared.
- Influence of sex, diet and smoking was studied.

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ABSTRACT

Stable isotope composition of human fingernails has proven to be useful for documenting human dietary information and geographical patterns in archeological, forensic, anthropological and biological studies. Therefore, it is of interest to detect all factors influencing the stable isotopic composition in the certain regions in the world.

Carbon and nitrogen isotope data of human fingernail keratin from 52 individuals from Slovakia were reported in this study. The online combustion and continuous flow isotope-ratio mass spectrometer Delta V Advantage was used for $\delta^{13}C$ and $\delta^{15}N$ analysis of fingernail keratin samples from 24 vegetarian and 28 omnivorous individuals. A group of people with frequent meat consumption showed enrichment in ^{13}C and ^{15}N isotopes in fingernails. A similar trend was observed with increasing seafood in an individual's diet. Moreover a significant difference was revealed between smokers and nonsmokers for both $\delta^{13}C$ and $\delta^{15}N$ values. These data were compared to previously published $\delta^{13}C$ and $\delta^{15}N$ fingernail values from across the globe.

This study brings new information on the stable isotope signature of individuals from Slovakia and characterizes the Central European region for the first time. The stable isotope composition of fingernails is influenced by the frequency of meat and seafood consumption as well as smoking.

1. Introduction

The following paper is focused on issues of the stable isotope composition of human fingernails. Stable isotopes are a good marker of different processes in many fields of study. Only the applications of the stable isotopes on biological tissues will be highlighted. Stable isotope ratios of nitrogen and carbon in tissues have proven to be useful for documenting human dietary information (Bol and Pflieger, 2002; Buchardt et al., 2007; Caut et al., 2008; Huelsemann et al., 2009; McCullagh et al., 2005; Nardoto et al., 2006, 2011). This approach is based on the principle that isotopic composition of a tissue depends on the origin and type of compounds incorporated into the tissue during its synthesis. This fact can be useful, especially in archeological studies (Williams and Katzenberg, 2012; Linderholm and Kjellström, 2011; Gil et al., 2011).

Biological systems are complex and therefore it is difficult to determine what influences isotopic composition of tissue and how. Generally, there are two main factors that affect isotopic ratios of tissue. The first factor is food, its entire isotopic and nutritional composition. The second factor is the physiology and metabolism of each individual which includes chemical and biological processes. Further, it can be assumed that the metabolism of healthy individuals is similar and determines the isotopic composition of tissue in a predictable way (Petzke et al., 2005).

The relative tissue enrichment in ^{15}N and also in ^{13}C during the process of assimilation is called trophic shift (Caut et al., 2008; Macavoy et al., 2005). Tissue of consumers showed enrichment in ^{15}N of about 3–5‰ and in ^{13}C of about 1‰ relative to food (Caut et al., 2008; Macavoy et al., 2005; Poupin et al., 2011).

It was found that isotope ratios of C and N in consumer tissues correlates well with the food consumed (Buchardt et al., 2007; Nardoto et al.,

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2006; Petzke et al., 2005). In addition, carbon isotopes can be used to discriminate between C_3 and C_4 plant intakes. C_3 and C_4 plants have different photosynthetic pathways that lead to their distinguishable isotopic composition ($\delta^{13}C = -25.7$ for C_3 plants, $\delta^{13}C = -12.3$ for C_4 plants) (Huelsemann et al., 2009). Stable isotopes were successfully used in many archeological studies for reconstruction of human dietary habits in ancient times (Ambrose et al., 1997; Hu et al., 2007; Lightfoot et al., 2012). Many of these studies were performed on bones found on archeological sites. However, there are several studies that have tested keratinized tissues (hair and fingernails) for their ability to represent the isotopic composition of the whole body (Bol and Pflieger, 2002; Buchardt et al., 2007; Huelsemann et al., 2009; Nardoto et al., 2006, 2011; O'Connell and Hedges, 1999, 2001; Petzke et al., 2005).

Fingernails are suitable tissue for stable isotope studies as they can be acquired non-invasively. They are commonly available and relatively metabolically inert. Despite these advantages, there is a limited number of isotopic studies where fingernails were used as a sample (Buchardt et al., 2007; Nardoto et al., 2006, 2011). Nardoto et al. (2011) found significant differences in the δ-values of nitrogen and carbon in people living in urban and rural settings in the Brazilian Amazon region. Likewise, Buchardt et al. (2007) compared δ-values of carbon, nitrogen and sulfur in a modern community of Inuit Greenlanders and Danes. The conclusion of this study was that fingernails of Inuits were significantly enriched in ^{13}C, ^{15}N, and ^{34}S relative to Danish fingernails. Nardoto et al. (2006) also found significant differences in δ-values of carbon and nitrogen between Brazilian and American populations. These studies have shown that the $\delta^{13}C$ and $\delta^{15}N$ composition of fingernails records information on dietary habits of the local region, despite the increasing globalization and expansion of supermarket food that should homogenize isotope dietary patterns. Finally, there are few studies of stable isotope composition of fingernails comparing humans on different diets. According to the δ-values of carbon and nitrogen in Danish fingernails, omnivores (consumption of meat) are enriched in ^{13}C, ^{15}N, and ^{34}S relative to vegetarians (consumption of only secondary animal protein such as dairy products and eggs) and vegans (consumption of no animal proteins) (Buchardt et al., 2007). They found that values of $\delta^{13}C$ were $-21.0‰ \pm 0.5‰$ for omnivores, $-21.1‰ \pm 0.3‰$ for vegetarians and $-21.7‰ \pm 0.1‰$ for vegans. Isotope values of $\delta^{15}N$ were more different: $10.4‰ \pm 0.6‰$ for omnivores, $9.7‰ \pm 0.3‰$ for vegetarians and $8.6‰ \pm 0.2‰$ for vegans (Buchardt et al., 2007). In Nardoto's et al. (2006) study, analogous results in omnivore and vegetarian groups from Brazil and USA have been discovered. It can be assumed that isotope analysis can be helpful in the qualitative evaluation of the proportion of animal protein in food in nutritional studies.

The aim of this study was to detect isotopic patterns in fingernails of Slovak volunteers in order to evaluate their nutritional status. We assumed that fingernails of individuals with a higher proportion of animal protein in their diets are isotopically enriched relatively to those with the lower proportion. The reason for this type of research is that these kinds of analyses have never been done on individuals from middle- and eastern-European geographic regions. Furthermore, we expect that our results will be helpful for dietary and geographic studies.

2. Experimental

2.1. Sample preparation

Samples of human fingernails were collected from 52 volunteers between ages 22 and 47 years (average age was 28.2 ± 5.1 years). All individuals were from Slovakia and gave spoken consent to their participation in this research. They were asked about details of their diet in a questionnaire. The additional information about drinking habits, smoking, and liver diseases was also requested. The first group consisted of 24 individuals following special vegetarian diets: 20 OLV ovo-lacto-vegetarians (15 females and 5 males), 2 LV lacto-vegetarians (1 female and 1 male) and 2 OV ovo-vegetarians (2 females). People in the vegetarian group did not eat meat or meat products for a long time, from 1 to 26 years. Because of the low number of individuals in OV and LV groups, all vegetarian groups (OLV, LV, OV) were analyzed together as one combined group. The control group included 28 omnivores (17 females and 11 males).

All samples were collected by clipping the distal edge of fingernails that were prepared according to the standard procedure (O'Connell and Hedges, 2001). The surface contamination was removed by rubbing using a diamond abrasive wheel. Samples were then rinsed twice in the mixture of methanol and chloroform (2:1, v/v), the first time for 60 min and then for 30 min. Another 20 min of rinsing in deionized water was followed by the second wash in deionized water for 60 min in an ultrasonic bath. Samples were dried overnight at ambient temperature, then they were cut into small pieces (300 μg) and wrapped in tin capsules in triplicate, ready for $\delta^{13}C$ and $\delta^{15}N$ analysis using an elemental analyzer connected to an isotope ratio mass spectrometer.

2.2. Stable isotope ratio analysis

Isotopic analyses in this study were performed in the continuous flow mode using Delta V Advantage mass spectrometer (Thermo Fischer Scientific, Bremen) interfaced with an elemental analyzer Flash HT 2000 (Thermo Fischer Scientific, Bremen). Samples were introduced into the elemental analyzer using a solid autosampler flushed by helium. The elemental analyzer Flash HT 2000 allowed combined C/N analyses using a combustion glassy reactor filled with chromium oxide, reduced copper and silver cobaltous-cobaltic oxide held at temperature of 1020 °C. The post-reactor gas chromatography (GC) column at the temperature of 40 °C provided the separation of evolved N_2 and CO_2. The data were processed using Isodat 3.0 software. The stable isotope values were measured relative to international standards and reported using delta notation. Results are expressed in δ-notation as $\delta\ (‰) = [R_{sample} / R_{standard} - 1] \times 1000$, where R is the molar ratio of the heavy to the light isotope in the sample and standard. Where R represents $^{13}C/^{12}C$ for $\delta^{13}C$ and $^{15}N/^{14}N$ for $\delta^{15}N$. Stable isotope values are expressed in units of per mil and $\delta^{13}C$ is reported relative to the international standard VPDB and $\delta^{15}N$ relative to AIR. Fingernail samples were measured together with following reference materials: USGS 35 (IAEA, Vienna; $\delta^{15}N_{AIR} = 2.7‰$), IAEA–N2 (IAEA, Vienna; $\delta^{15}N_{AIR} = 20.3‰$), IA-R022 (Iso-analytical, UK; $\delta^{13}C_{VPDB} = -28.63‰$), IAEA-CH-6 (IAEA, Vienna; $\delta^{13}C_{VPDB} = -10.45‰$), IAEA C1 (IAEA, Vienna; $\delta^{13}C_{VPDB} = 2.4‰$) and laboratory working standard materials urea ($\delta^{13}C_{VPDB} = -40.06‰$; $\delta^{15}N_{AIR} = 1.56‰$) and Carrara marble ($\delta^{13}C_{VPDB} = 2.4‰$). The stable isotope values were also corrected for instrumental drift and blanks.

2.3. Statistical tools

All statistical analyses listed below in the text were carried out using statistical software StatsDirect. First, the data in every analyzed group were checked for normal distribution by the Shapiro–Wilk test. Subsequently, an appropriate parametric (two sided unpaired Student's t-test or one-way ANOVA with post-hoc Tukey–Kramer test) or non-parametric (Mann–Whitney test as nonparametric analogy of two sided unpaired Student's t-test, or Kruskal–Wallis test — nonparametric one-way ANOVA followed by Conover–Inman test) test was applied. The Levene test for equality of variances was also performed before using ANOVA. In our study, this test evaluates homogeneity of variances in all tested groups. The same threshold level of significance $\alpha = 0.05$ was used for all statistical tests. The descriptive analysis including the basic statistical characteristics was performed for the measured data. All graphical box results are reported as means ± quartile and the whiskers demonstrate the minimal and maximal values.

Table 1 summarizes the $\delta^{13}C$ and $\delta^{15}N$ mean values of different groups within the omnivorous and vegetarian data. In the next sections