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RCM

Choice of dietary protein of vegetarians and omnivores is reflected in their hair protein ^{13}C and ^{15}N abundance†

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Stable isotopic (^{15}N, ^{13}C) composition of tissues depends on isotopic pattern of food sources. We investigated whether the isotopic compositions of human hair protein and amino acids reflect the habitual dietary protein intake. Hair samples were analyzed from 100 omnivores (selected randomly out of the 1987–1988 German nutrition survey VERA), and from 15 ovo-lacto-vegetarians (OLV), and from 6 vegans recruited separately. Hair bulk and amino acid specific isotopic compositions were analyzed by isotope-ratio mass spectrometry (EA/IRMS and GC/C/IRMS, respectively) and the results were correlated with data of the 7 day dietary records. Hair bulk ^{15}N and ^{13}C abundances clearly reflect the particular eating habits. Vegans can be distinguished from OLV and both are significantly distinct from omnivores in both ^{15}N and ^{13}C abundances. ^{15}N and ^{13}C abundances rose with a higher proportion of animal to total protein intake (PAPI). Individual proportions of animal protein consumption (IPAP) were calculated using isotopic abundances and a linear regression model using animal protein consumption data of vegans (PAPI = 0) and omnivores (mean PAPI = 0.639). IPAP values positively correlated with the intake of protein, meat, meat products, and animal protein. Distinct patterns for hair amino acid specific ^{15}N and ^{13}C abundances were measured but with lower resolution between food preference groups compared with bulk values. In conclusion, hair ^{13}C and ^{15}N values both reflected the extent of animal protein consumption. Bulk isotopic abundance of hair can be tested for future use in the validation of dietary assessment methods. Copyright © 2005 John Wiley & Sons, Ltd.

Epidemiological evidence suggests that a high percentage of cancers of the digestive tract as well as breast and prostate tumors are due to dietary factors. Eating a healthy diet, plus staying physically active and maintaining an optimum weight, can cut cancer risk by 30–40%.[1] Meat is suspected to be one of the possible risk factors for colorectal cancers in humans. In several studies consumption of red and processed meats was proved to have a significant positive association with colorectal cancer and coronary artery disease[2] whereas other studies failed to show such correlation.[3] Further, there are indications that the excessive intake of dietary proteins might also be a risk factor for metabolic disorders in adolescents and adults.[4,5] However, there is a need for objective parameters which reflect chronic dietary intake and reliably describe the possible risk of an inadequate protein intake for nutritionally related diseases.[6,7]

Human hair is easily accessible and has been used to reveal information about prehistoric as well as modern diet and about geographical location based on stable isotope signatures.[8–16] This is because body proteins, including hair keratin, of animals and humans reflect the abundance ratio of stable isotopes of their dietary sources. In contrast to other body proteins hair is not turned over, and thus preserves a signature of dietary habits. Animal-derived food proteins are enriched in ^{15}N relative to plant-derived food proteins due to trophic shifts,[10,17–19] and the natural ^{15}N abundance of tissue proteins has been used to differentiate between food protein preference groups, although not unequivocally.[10,16]

We have shown that the ^{15}N abundance in individual human plasma of either free or protein-bound amino acids differs. For example, threonine and lysine are more depleted in ^{15}N and their ^{15}N values appear to be more stable in various populations than is seen in branched chain amino acids, or glutamic acid.[20,21] It is assumed that this is due to known differences in the degree of transamination of different amino acids.[21] Thus we hypothesize that individual amino acids with a higher degree of transamination, such as alanine or glutamic acid, strongly reflect the ^{15}N-isotopic values of dietary protein sources, because they mirror trophic shift and N-metabolic alterations in animal tissue and derived food more than the bulk ^{15}N value. The natural ^{13}C signature of indispensable in human nutrition amino acids might also be a good reflection of their plant or animal origin. The reason for this lies in the indispensability of the amino acid carbon

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skeleton which cannot be synthesized by mammalian enzymes.

Thus, we investigated whether the bulk nitrogen and carbon stable isotopic composition of human hair can be used to reflect different food preference groups. We further tested if the individual amino acid ^{13}C and/or ^{15}N values can reflect the habitual protein intake. To answer these questions we studied hair samples from volunteers who participated in a large German nutritional survey in which detailed dietary intake information was collected, and from a group of vegetarians living in Berlin. For comparative purposes, dietary proteins of animal and plant origin as well as complete test meals containing common foodstuffs and different amounts of animal proteins were analyzed for their bulk ^{13}C- and ^{15}N-isotopic composition.

EXPERIMENTAL

Subjects

Hair samples of subjects who participated in a population-based cross-sectional study in Germany 1987–1988 (VERA, Verbundstudie Ernährungserhebung und Risikofaktoren-Analytik, Nutrition Survey and Risk Factor Analysis Study)[22] were studied. A total of 1988 non-institutionalized adults aged 18 to 88 years (y) participated in this study. They attended selected laboratory clinics in which biological materials and anthropometric measures were taken. Small strands of hairs were clipped close to the scalp. Samples were collected in small plastic bags and stored in liquid N_2 until analysis. Personal identifiers were completely removed for anonymity after collection of the material and destroyed at the end of the study. All subjects gave a written informed consent. The complete study data and results of laboratory analysis are available as 'Public Use File' on compact disc. The results of the VERA project were published in several volumes between 1992 and 1995.[23]

For the present study we randomly selected 50 men and 50 women between the ages of 20 and 50 out of the complete study population (VERA-O, VERA study subgroup of omnivores). The general characteristics are summarized in Table 1. Stored hair samples of the selected subjects were used for carbon and nitrogen stable isotope analysis.

In order to check the validity of the hypothesis that the stable isotopic composition of hair protein amino acids depends on the consumption of animal- and/or plant-derived dietary proteins in 2001, we also collected hair samples from a group of vegetarians living in Berlin, Germany. They had been contacted via the German Vegetarian Association (Deutscher Vegetarierbund). After explaining the purpose of the study they agreed to donate hair samples which were clipped in the same manner as in the VERA study. They also answered questions on when they had changed from an omnivorous to a vegetarian diet and whether they regularly consumed certain plant-derived products such as cereals, corn, soybeans, potatoes, nuts, fruits and vegetables—in a semiquantitative manner indicating a high, an intermediate, a low or no consumption of the respective products. According to the interviews, the vegetarians had been following their special diet for several years prior to the study (Table 1). The vegetarians comprised 6 vegans (exclusively plant food) and 15 ovo-lacto-vegetarians (OLV) (plant food, milk, eggs, and dairy products). Their mean ages were 47 y (vegans) and 45 y (OLV).

Dietary data

Dietary data was collected using extended questionnaires, a 7 day (d) dietary record using typical household measures. The 7 d dietary records were analyzed for nutrients intake using the German Food Code and Nutrient Base BLS II.3 which allows the calculation of the intake of specific amino acids.[24] Original results of the survey as well as summary figures are available on 'Public Use File'.

Sample collection and preparation

Hair samples (5 mg, from up to 1 cm above the scalp—this approximately corresponds to the growth of hair during the

Table 1. General characteristics of the study participants

	Mean ± SD	Range	Mean ± SD	Range
VERA-O	Men ($n = 50$)		Women ($n = 50$)	
Age (y)	36 ± 9	20–50	33 ± 9	20–50
Weight (kg)	79 ± 11	55–122	62 ± 12[a]	45–104
BMI (kg/m^2)	25.0 ± 3.4	19.0–35.6	22.6 ± 3.6[a]	18.3–38.2
OVL	Men ($n = 5$)		Women ($n = 10$)	
Age (y)	51 ± 14	35–68	42 ± 16	17–68
Weight (kg)	75 ± 12	65–96	60 ± 10	47–74
BMI (kg/m^2)	22.0 ± 2.0	20.0–24.5	21.9 ± 3.5	17.9–26.9
Duration of diet (y)	14 ± 8	8–25	14 ± 7	4–22
Vegans	Men ($n = 5$)		Women ($n = 1$)	
Age (y)	49 ± 11	36–62	35	—
Weight (kg)	74 ± 11	60–86	53	—
BMI (kg/m^2)	23.1 ± 3.4	19.3–27.1	17.3	—
Duration of diet (y)	13 ± 10	5–23	6	—

[a]Significantly different from male subjects, $P < 0.05$.

last 4 weeks before collection and the time of dietary records) were clipped from the specific specimen and cleaned using a chloroform/methanol/acetone/ether (1:1:1:1) solution and agitated for about 30 min to remove any lipid or shampoo residue. Solvents were removed by filtration (folded filters, Machery-Nagel GmbH & Co. KG, Düren, Germany) and hair samples were subsequently air-dried at room temperature. Cleaned hair samples (~0.3 mg) were placed in tin capsules (4 × 6 mm, IVA Analysentechnik, Meerbusch, Germany) for determination of bulk ^{13}C and ^{15}N abundances.

Selected vegetable or animal protein sources were purchased in local health food stores or supermarkets. Some selected isolated and purified proteins of animal and vegetable origin (Sigma-Aldrich Chemie GmbH, Deisenhofen, Germany) were also analyzed. Grains or grain products were ground (analysis mill A 11 basic, IKA-Werke GmbH & Co. KG, Staufen, Germany) before isotope analysis. Bulk ^{13}C- and ^{15}N-isotopic compositions were also analyzed in samples of complete pooled daily meals (breakfast + dinner + lunch + snacks) providing either about 1 (adequate protein meals) or 2.5 g protein/(kg · d) (high protein meals). Target mean energy, fat and carbohydrate contents were calculated to provide 8000 kJ/d, 1.1 g/(kg · d), and 4.6 g/(kg · d) (adequate protein meals) or 3.6 g/(kg · d) (high protein meals), respectively. All foodstuffs used for the meals were purchased from local supermarkets. The high protein, isoenergetic meals were generated by adding dietary protein of animal origin (meat, low-fat sausages, and low-fat curd cheese) to substitute for carbohydrates. The calculated ratio of animal to total protein was 0.6 and 0.7 in adequate and in high protein meals, respectively. Meals were homogenized (Ultra-Turrax T25, IKA-Werke GmbH & Co. KG) and lyophilized (Alpha 1-4, Christ GmbH, Osterode, Germany).

Isotopic and amino acid analysis

The bulk ^{13}C and ^{15}N abundances in either food, protein or hair samples were determined using an elemental analyzer (EA 1108, Fisons Instruments, Rodano, Italy) coupled online via a conflo interface to an isotope-ratio mass spectrometer (EA/IRMS; Delta C, Thermo Electron Corp., Bremen, Germany). The combustion furnace was maintained at 1020°C and flash combustion occurred by injecting a pulse of O_2 at the time of sample drop. Helium was used as carrier gas. NO_x species were reduced to N_2 in a reduction furnace at 650°C. Water was removed by phosphorus pentoxide in a water trap and CO_2 was separated from N_2 using a gas chromatographic column (2 m length, 4 mm i.d., Poropak-QS (80–100 mesh), Fisons Instruments) operated isothermally at 40°C.

For isotopic analysis of individual amino acids another aliquot of cleaned hair (1.5 mg) was hydrolyzed in 2 mL distilled 6 N HCl for 24 h in PTFE-capped 16 × 100 mm vials at 110°C. Hydrolysates were dried under nitrogen at 60°C and dissolved in 0.1 N HCl.[20,25] ^{13}C and ^{15}N analysis of individual amino acids was performed by gas chromatography/combustion/isotope-ratio mass spectrometry (GC/C/IRMS; Thermo Electron Corp., Bremen, Germany) after derivatization to their N-pivaloyl-*i*-propyl esters (NPP) as described previously.[20] The ^{13}C abundances of amino acids measured by GC/C/IRMS were corrected by individual empirical correction factors due to the extra carbon that had been introduced during derivatization and the reproducible isotopic fractionation introduced by the derivatization process.[26]

Isotopic compositions of carbon and nitrogen are reported in the conventional delta per mill notation in the range of natural abundance and can be expressed in parts per thousand (‰) as defined by Craig.[27] The $\delta^{13}C$ and $\delta^{15}N$ values have been assigned a δ value of 0.0‰ relative to the international standards Peedee Belemnite Limestone carbonate (PDB) and atmospheric nitrogen (AIR), respectively, and can be calculated by using the following equation:

$$\delta\,\mathrm{ISO}(‰) = [R_{sample}/R_{standard} - 1] \times 10^3$$

where ISO is either ^{13}C or ^{15}N. Isotope ratios are $R_{standard} = [^{13}C]/[^{12}C] = 0.0112372$, whereas, for nitrogen, $R_{standard} = [^{15}N]/[^{14}N] = 0.0036765$. $^{13}C/^{12}C$ ratios are derived from the ratios of the mass spectrometer ion currents ranging from m/z 44 to m/z 46 and for $^{15}N/^{14}N$ from the ratios of the ion currents for m/z 29 to m/z 28. A substance with an isotope ratio larger than that of the standard has a positive δ value, and is thus enriched in the heavy isotope relative to the standard.

The bulk and amino acid specific ^{15}N and ^{13}C abundances as determined by EA/IRMS or GC/C/IRMS, respectively, were measured against laboratory standard gases N_2 and CO_2 (Linde AG, Leuna, Germany), which had been calibrated against the international standards AIR and PDB, respectively, using reference materials (sucrose RM 8542 ANU, -10.47 ± 0.13‰ $\delta^{13}C$, National Institute of Standards and Technology, Gaithersburg, MD, USA; ammonium sulfate, IAEA 305A, 39.8‰ $\delta^{15}N$, International Atomic Energy Agency, Vienna, Austria). Unmodified wheat starch (Sigma Chemicals Co., St. Louis, MO, USA, lot 84H0311, −23.7‰ $\delta^{13}C$) was used as the working standard for $\delta^{13}C$ measurements and acetanilide (Fisons Instruments, Cod. 33836700, −9.9‰ $\delta^{15}N$) for $\delta^{15}N$ measurements, respectively. Typical replicate measurement errors (±1 SD) for hair samples were ±0.2‰ for bulk carbon and nitrogen isotope abundances by EA/IRMS. In individual amino acids they were 0.3‰ and 0.5‰ using GC/C/IRMS and the NPP derivatives for ^{13}C and ^{15}N, respectively.

Data processing including the correction for ^{17}O moiety determining $\delta^{13}C$ values was performed by vendor-provided software ISODAT (Thermo Electron Corp., Bremen, Germany). During the GC/C/IRMS experiment the threshold slope (slope sensitivity) for peak start and stop definitions was set to be 0.2 and 0.4 mV/s for $\delta^{15}N$, and 0.6 and 0.6 mV/s for $\delta^{13}C$, respectively. Integration time was 0.25 s.[20] For GC/C/IRMS each sample was derivatized and analyzed in duplicate and for EA/IRMS the measurement of each sample was repeated 5 times.

Hair amino acid composition was analyzed after acid hydrolysis. Samples of about 1 mg of cleaned hair underwent an acid hydrolysis as described above. Amino acids were analyzed by ion-exchange chromatography (high efficiency column, 3 × 150 mm, Pickering Laboratories Inc., Mountain View, CA, USA) with a step-change elution method using lithium citrate buffers (Onken Laborservice, Gründau, Germany) and HPLC units (Beckman Instruments GmbH,

Munich Germany) and a postcolumn ninhydrin detection (TRIONE ninhydrin reagent, Pickering Laboratories) as reported previously.[28] For calculation of the amino acid concentration, norleucine was used as an internal standard added prior to hydrolysis. Laboratory and amino acid specific correction factors were determined using equal hydrolysis conditions and a standard amino acid mixture.

Calculations and statistics

The proportion of animal protein to total protein intake (PAPI) was computed based on 7 d dietary record data using the German Food Code and Nutrient Base BLS II.3. The individual proportion of animal protein consumption (IPAP) was calculated by linear regression using the mean PAPI value of omnivores of the VERA-O subgroup computed to be 0.639 and of vegans assumed to be zero and the respective mean $\delta^{13}C$ ($IPAP_{13C}$) and $\delta^{15}N$ ($IPAP_{15N}$) values of hair. $IPAP_{13C15N}$ is the mean from both $IPAP_{13C}$ and $IPAP_{15N}$ of each individual (for details. see Results section). Data are reported as means ±1 SD and means with 95% confidence intervals (CIs). Comparison of means was performed with two-sided unpaired Student's t-test. Significance was set at $P = 0.05$. The correlation between stable isotope abundance and reported intake values was given by Pearson's correlation coefficients. WinSTAT® (version 1999.2, R. Fitch software, Staufen, Germany) was used to compute statistical analyses.

RESULTS

General characteristics of the subjects studied are presented in Table 1. The body mass index (BMI, in kg/m^2) was significantly higher in men than in women of the VERA study population of omnivores. The PAPI values did not differ significantly between both sexes. The vegetarians had practiced their lifestyle for between 5 and 25 years.

Bulk stable isotopic composition of hair

Results of isotopic analysis of hair samples of groups with different food habits are presented in Table 2 and Fig. 1. The bulk stable isotope abundances of ^{15}N and ^{13}C in hair clearly reflect the particular eating habits. Vegans can be distinguished from OLV and both are distinct from omnivores. The δ values rise with a higher PAPI value for both $\delta^{15}N$ and $\delta^{13}C$ in omnivores. Mean $\delta^{15}N$ and $\delta^{13}C$ values differ significantly between vegans, OLV, and omnivores (VERA). On average the hair of vegans and OLV is, respectively, 3.6‰ and 2.1‰ lower in $\delta^{15}N$ than that from VERA subjects. Similarly, the hair from vegans and OLV is 1.3‰ and 0.6‰ lower in $\delta^{13}C$, respectively, than that from VERA-O omnivores. There is a strong correlation between $\delta^{15}N$ and $\delta^{13}C$ values in hair of omnivores and vegetarians ($r = 0.599$, $P = 2.42 \times 10^{-13}$, valid cases = 120). This correlation is of borderline significance in the group of VERA-O alone ($r = 0.159$, $P = 0.058$, valid cases = 99).

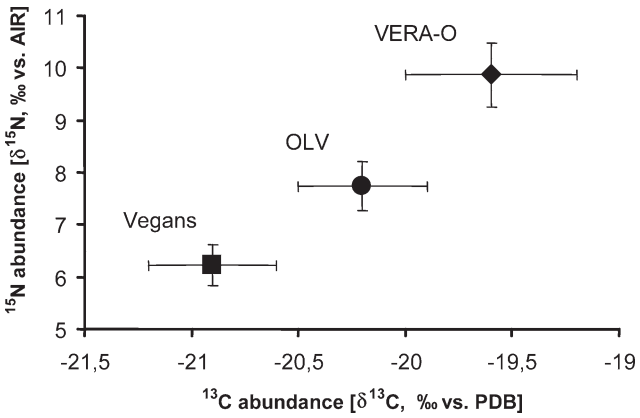

Figure 1. Plots of natural bulk ^{15}N versus ^{13}C abundances of human hair derived from omnivores of the nutrition survey-risk factor analysis study (VERA-O, $n = 99$), ovo-lacto-vegetarians (OLV, $n = 15$), and vegans ($n = 6$) measured by EA/IRMS. For details, see Experimental section. Values are means ± SD (each sample was determined 5 times).

Proportion of animal protein consumption

A value of individual proportion of animal protein consumption (IPAP) can be assessed using the mean bulk stable isotope abundances of ^{15}N and ^{13}C of hair samples of vegans and of the VERA-O omnivores and the mean PAPI of omnivores as calculated from 7 d dietary records (0.639 ± 0.105, $n = 100$). The proportion of animal protein consumption is 0.657 ± 0.090 ($n = 1988$) for the total VERA study group which indicates that the subsample selected for this study is representative for the habitual dietary consumption as assessed by the VERA study in Germany. The mean $\delta^{15}N_{hair}$ and $\delta^{13}C_{hair}$ values of vegans and VERA-O omnivores of our study are assumed to correspond to an IPAP of 0.0 and 0.639, respectively. Therefore, the IPAP can be calculated for every individual based on $\delta^{15}N_{hair}$ ($IPAP_{15N}$) and/or the $\delta^{13}C_{hair}$ values ($IPAP_{13C}$) assuming a linear relationship. The equations are arranged as shown in Fig. 2. From both $IPAP_{13C}$ and $IPAP_{15N}$ a mean $IPAP_{13C15N}$ can be calculated. The IPAP values are presented for 6 vegans and 15 OLV (Table 3). Four individuals out of the VERA-O subgroup of omnivores showed

Table 2. Bulk ^{15}N and ^{13}C abundances in human hair measured by EA/IRMS (‰ vs. international standard) and calculated IPAP values in comparison with data of protein intake based on 7 d dietary records[a,b]

	Number of subjects	$\delta^{15}N$ (‰ vs. AIR)	$\delta^{13}C$ (‰ vs. PDB)	$IPAP_{15N}$	$IPAP_{13C}$	$IPAP_{15N13C}$	Protein (g/d)	Meat (g/d)	PAPI
VERA-O	99	9.9 ± 0.6	-19.6 ± 0.4	0.639 ± 0.108	0.638 ± 0.197	0.639 ± 0.120	88 ± 28	81 ± 28	0.639 ± 0.105
OLV	15	7.7 ± 0.5[d]	-20.2 ± 0.3[d]	0.265 ± 0.081	0.372 ± 0.145	0.319 ± 0.088[d]	nd[c]	nd	nd
Vegans	6	6.2 ± 0.4[d,e]	-20.9 ± 0.3[d,e]	0.000 ± 0.070	0.002 ± 0.167	0.001 ± 0.056[d,e]	nd	nd	nd

[a] Values are means ± SD.
[b] For details, see Experimental section.
[c] nd, not determined.
[d] Value is different from that one of VERA-O, $P \ll 0.001$.
[e] Value is different from that one of OLV, $P < 0.001$.

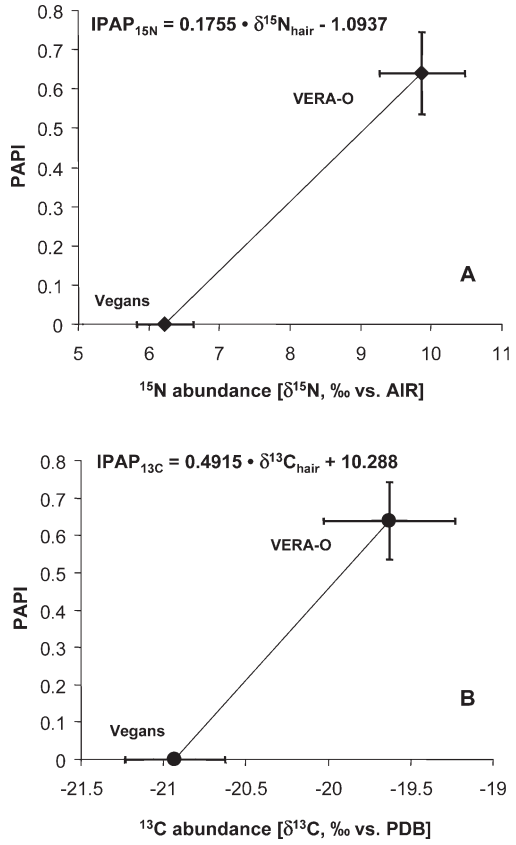

Figure 2. Plots of the mean (±SD) PAPI values (proportions of animal to total protein intake based on 7 d dietary record) of omnivores of the nutrition survey-risk factor analysis study (VERA-O, $n = 99$) and of vegans (set to be zero, $n = 6$) versus mean abundances of ^{15}N (A) and ^{13}C (B) and linear regression equations for the calculations of IPAP values (individual proportions of animal protein consumption based on isotopic abundances).

an animal protein to total protein ratio of 0.9 or more based on isotopic values (Fig. 3). Here a highly significant correlation ($P = 1.27 \times 10^{-6}$) was computed between IPAP values of the VERA study omnivores based on either 7 d dietary records or isotopic analysis ($IPAP_{13C15N}$). In addition, the $IPAP_{13C15N}$ values positively correlated with the daily intake of protein ($P = 0.05$), meat ($P = 0.002$), meat products and sausages ($P = 0.02$), and animal protein ($P < 0.001$) and negatively with plant protein ($P = 0.03$), confectionery and jam ($P = 0.03$), and not ($P > 0.05$) with milk and dairy products, and fish (data not shown).

Amino acid specific stable isotopic composition of hair

Mean ^{15}N and ^{13}C abundances in individual amino acids were not significantly different between vegans and OLV. Therefore, mean ^{15}N and ^{13}C abundances in individual amino acids of vegans and OLV were combined (vegetarian group) and compared with those from omnivores of the VERA study population (VERA-O) (Tables 4 and 5). The mean ^{15}N and ^{13}C abundances in most individual hair amino acids were found to be significantly lower in vegetarians than in those from VERA-O omnivores. With some exceptions the significance values were far below $P = 0.001$. No significant differences ($P > 0.05$) were obtained for $\delta^{15}N$ values of threonine, phenylalanine and histidine and for $\delta^{13}C$ values of glycine between vegetarians and VERA-O omnivores. The plot of the amino acid specific stable isotope abundance of ^{15}N and ^{13}C also reflects the particular eating habits and allows a differentiation of vegetarians and VERA-O omnivores (plots not shown). This particularly applies to the most abundant amino acids glutamic acid, serine, proline and leucine.

Threonine is the amino acid with the lowest $\delta^{15}N$ value whereas proline, glutamic acid and valine show the highest ^{15}N abundance in all subjects, irrespective of food preference group. $\delta^{15}N$ values for phenylalanine, lysine and histidine were determined to be higher than those for threonine but were considerably lower than those for proline, glutamic acid and valine. The difference between lowest and highest mean $\delta^{15}N$ values of the individual amino acids threonine and proline was about 25‰. Glycine is the amino acid with the highest $\delta^{13}C$ value whereas threonine shows the lowest $\delta^{13}C$ abundance. The difference between lowest and highest mean $\delta^{13}C$ values of the individual amino acids threonine and glycine is about 30‰ for VERA-O omnivores and up to about 40‰ for vegetarians. Moreover, the mean $\delta^{15}N$ and $\delta^{13}C$ values differ by about 2.3‰ (range between 1.7‰ for serine and 4.9‰ for isoleucine) and 8.0‰ (range between 2.0‰ for tyrosine and about 15‰ for threonine and serine) between VERA-O omnivores and vegetarians, respectively, when the amino acids with insignificantly different mean values are ignored. Results are not presented for cysteine and methionine since these amino acids are largely destroyed under conditions of acidic protein hydrolysis and subsequent derivatization.[20,25] Glutamine and asparagine are completely converted into glutamic acid and aspartic acid, respectively. Therefore, results for stable isotopic abundance of glutamic acid and aspartic acid represent only the α-amino nitrogen or total carbon of both glutamine and glutamic acid and asparagine and aspartic acid, respectively.

DISCUSSION

The results show that both hair bulk and amino acid specific ^{13}C and ^{15}N abundances are significantly related to intake levels of animal- or vegetable-derived dietary proteins. Looking at diet preference groups, vegans have lower bulk $\delta^{15}N$ and $\delta^{13}C$ values than OLV and both have lower values than omnivores. An estimation of the proportion of animal protein intake based on isotopic values positively correlated with the daily intake of protein, meat, animal protein and negatively with plant protein calculated on the basis of 7 d dietary records in omnivores. This is caused by the lower ^{15}N and ^{13}C content of vegetable proteins than of animal proteins, as shown in previous studies,[11,14,15,29,30] and confirmed for selected animal and plant proteins (Table 6). Measurements of bulk ^{15}N- and ^{13}C-isotopic compositions of proteins and foodstuffs have shown that on average animal-derived protein sources are higher in their ^{15}N values (~2–3‰) and $\delta^{13}C$ values (~10‰) than are plant-derived protein sources. Moreover, we have shown that in mixed meals the mean $\delta^{15}N$ values were significantly ($P < 0.001$) higher when the protein content was increased by adding animal proteins

Table 3. Individual bulk ^{15}N and ^{13}C abundances in human hair derived from vegetarians (n = 21) measured by EA/IRMS and calculated IPAP values.[a–c]

Duration of diet (y)	Gender	BMI (kg/m^2)	$\delta^{15}N$ (‰ vs. AIR)	$\delta^{13}C$ (‰ vs. PDB)	$IPAP_{15N}$	$IPAP_{13C}$	$IPAP_{15N13C}$
OLV							
25	m	20.3	7.1	−20.6	0.15	0.16	0.16
18	f	19.5	7.5	−20.6	0.23	0.14	0.18
10	f	24.8	7.8	−20.6	0.27	0.18	0.22
20	m	24.5	8.0	−20.4	0.30	0.27	0.28
10	f	19.7	7.9	−20.3	0.29	0.31	0.30
10	f	25.7	7.7	−20.2	0.26	0.36	0.31
20	f	25.7	8.1	−20.2	0.33	0.34	0.33
8	m	21.6	7.4	−20.0	0.20	0.45	0.33
4	f	19.1	7.8	−20.1	0.28	0.39	0.33
21	f	26.9	8.2	−20.3	0.34	0.33	0.33
10	m	23.4	7.3	−20.0	0.19	0.48	0.34
22	f	20.1	7.1	−19.7	0.16	0.59	0.37
5	f	19.1	7.7	−19.9	0.25	0.51	0.38
15	f	17.9	7.7	−19.9	0.25	0.51	0.38
8	m	20.0	9.0	−19.8	0.48	0.57	0.52
Vegans							
23	m	24.5	6.5	−21.4	0.05	−0.21	−0.08
23	m	24.8	6.4	−21.1	0.02	−0.06	−0.02
6	f	17.3	6.0	−21.0	−0.04	−0.01	−0.02
7	m	19.3	6.5	−21.0	0.04	−0.05	−0.01
5	m	20.0	6.5	−20.9	0.04	0.04	0.04
5	m	27.1	5.5	−20.3	−0.13	0.30	0.09

[a] Data are presented individually, sorted by $IPAP_{15N13C}$ values, and grouped in OLV ($n = 15$) and vegans ($n = 6$).
[b] Values of $\delta^{15}N$ and $\delta^{13}C$ (in ‰ vs. international standard) are means of each sample each determined 5 times.
[c] For details, see Experimental section.

(exclusively meat, low-fat sausages and curd cheese). The mean $\delta^{15}N$ value of meals with an adequate protein content ($n = 20$; protein content = 11%; PAPI = 0.6) was 0.1 ± 1.7 whereas those of high protein meals ($n = 20$; protein content = 26%; PAPI = 0.7) was 2.2 ± 1.4.

Results of two recent studies performed in the Okehampton area (southwestern England)[16] and with Oxford (UK) residents[10] indicate a relation between dietary behavior and hair ^{13}C and ^{15}N abundances. Whereas O'Connell and Hedges[10] were not able to distinguish OLV from omnivores in their $\delta^{15}N$ values and vegans from both OLV and omnivores in their $\delta^{13}C$ values, Bol and Pflieger[16] could differentiate among all three food preference groups for both bulk $\delta^{15}N$ and $\delta^{13}C$ values. In the first study[10] the mean (±SD) δ values were 6.9 ± 0.5, 8.7 ± 0.5, and 8.8 ± 0.6 for ^{15}N and -20.9 ± 0.8, -21.0 ± 0.3, and -20.2 ± 0.7 for ^{13}C of vegans, OLV, and omnivores, respectively. In the second study[16] the mean δ values were 6.7 ± 0.7, 8.5 ± 0.6, and 9.8 ± 0.6 of ^{15}N and -21.7 ± 0.1, -21.2 ± 0.3, and -20.8 ± 0.4 of ^{13}C for vegans, OLV, and omnivores, respectively. Further, the mean $\delta^{15}N$ values were higher (0.8‰) in OLV and the $\delta^{13}C$ values were lower (0.8–1.2‰) in all food preference groups in the study of Bol and Pflieger[16] than in our results (Table 2). These small but distinct differences are thought to be due to different isotopic values of food proteins produced and consumed in different geographical areas like the UK and Germany.[11,15] In addition, German adults generally consume less meat, fish, and milk but more meat products, eggs, cheese, fruit, and vegetables than British adults[31] which can slightly influence isotopic values in hair. However, the proportion of animal to total protein consumption seems to be comparable between British (63%, cited by O'Connell and Hedges[10]) and German adults (63.9% in the VERA-O subgroup of omnivores).

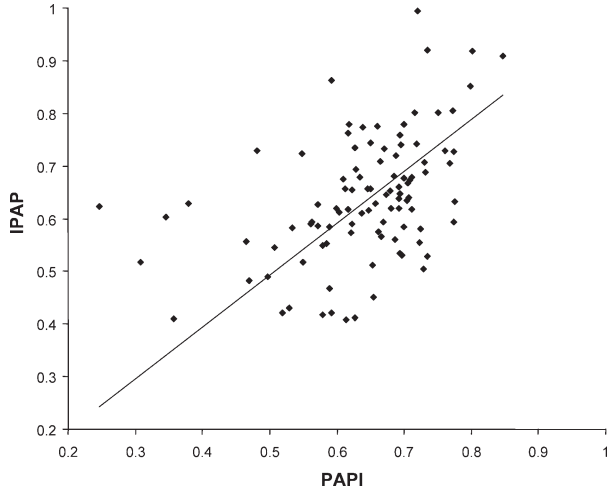

Figure 3. Plots of PAPI values (proportions of animal to total protein intake based on 7 d dietary records) versus IPAP values (individual proportions of animal protein consumption based on isotopic abundances of ^{13}C and ^{15}N ($IPAP_{15N13C}$) in hair of omnivores of the nutrition survey-risk factor analysis study (VERA-O, $n = 99$).

The range of −21.4‰ to −18.7‰ $\delta^{13}C$ observed in the hair of our study population is characteristic for a preferential consumption of C_3 plant based protein sources. This is typical for northwestern Europe and quite similar to that described previously for the UK (−21.7 to −20.0;[16] −22.6 to −19.0[10]). The ^{13}C abundance in plant biomass is dependent on the type of carbon fixation reaction during photosynthesis.[32]

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Table 4. Comparison of natural ^{15}N abundances of individual amino acids in human hair measured by GC/C/IRMS of the VERA study subgroup of omnivores (VERA-O, $n = 99$) and vegetarians ($n = 21$, 15 OVL and 6 vegans)[a]

	$\delta^{15}N$ (‰ vs. AIR)				
	VERA-O		Vegetarians		
	Mean ± SD (n)	CI	Mean ± SD (n)	CI	$P<$
Histidine	3.5 ± 2.2 (35)	0.7	1.5 ± 3.0 (3)	3.4	ns[b]
Isoleucine	9.5 ± 4.8 (69)	1.1	4.7 ± 4.0 (19)	1.8	0.001
Leucine	11.0 ± 1.6 (96)	0.3	9.2 ± 1.0 (21)	0.4	0.001
Lysine	2.9 ± 1.5 (95)	0.3	0.5 ± 2.0 (21)	0.8	0.001
Phenylalanine	2.4 ± 4.0 (95)	0.8	3.1 ± 1.6 (21)	0.7	ns
Threonine	−9.3 ± 3.3 (68)	0.8	−8.1 ± 3.6 (20)	1.6	ns
Valine	14.4 ± 2.0 (96)	0.4	12.1 ± 1.8 (21)	0.8	0.001
Alanine	10.3 ± 1.9 (96)	0.4	8.8 ± 1.9 (21)	0.8	0.01
Aspartic acid	9.5 ± 1.6 (73)	0.4	7.0 ± 0.9 (20)	0.4	0.001
Glutamic acid	14.8 ± 1.2 (95)	0.2	12.1 ± 1.8 (21)	0.8	0.001
Glycine	7.3 ± 1.2 (96)	0.2	5.2 ± 1.6 (21)	0.7	0.001
Proline	15.9 ± 1.4 (96)	0.3	14.4 ± 0.9 (21)	0.4	0.001
Serine	9.1 ± 1.3 (96)	0.3	7.5 ± 1.0 (21)	0.4	0.001
Tyrosine	4.7 ± 2.2 (85)	0.5	3.0 ± 2.2 (21)	1.0	0.01

[a] Sample numbers $n < 99$ for VERA-O and $n < 21$ for vegetarians are due to methodological reasons (e.g. poor resolution, low signal).
[b] ns, $P > 0.05$.

Consequently, different groups of plants important for human nutrition are distinguished. These are C_3 plants (wheat, barley, soy, potatoes, fruits, vegetables) with distinctly lower natural ^{13}C abundance than the C_4 plants (corn, sorghum, millet, sugar cane). The C_3 and C_4 designations refer to the number of carbon atoms in the first metabolites formed during photosynthesis, i.e. phosphoglyceric acid and oxalacetic acid, respectively. C_3 plants display ^{13}C abundances with ranges between −32‰ and −23‰ $\delta^{13}C$ in contrast to C_4 plants which have values between −15‰ and −11‰ $\delta^{13}C$.[18] Because the consumption of C_4 plants by Europeans is still relatively low as compared with individuals living in North America, the $\delta^{13}C$ values of tissue components and of human hair are also lower in individuals living in Europe than in America.[11,15] Therefore, in order to assess dietary animal protein intake from the ^{13}C- and ^{15}N-isotopic signature in hair it is important to consider the isotopic values of locally available dietary proteins. However, due to today's globalization of the market one can expect relatively similar conditions within large areas such as northwestern Europe.

In our study bulk ^{13}C values of hair turned out to be as predictive for the proportion of animal protein consumption as bulk ^{15}N values. This was not expected because previously a lower trophic level shift for ^{13}C abundances was observed than for ^{15}N abundances. In general the bodies of animals appear to be enriched in ^{13}C and ^{15}N relative to the diet by about 1‰ and 3‰, respectively.[33,34] However, the relatively

Table 5. Comparison of natural ^{13}C abundances of individual amino acids in human hair measured by GC/C/IRMS of the VERA study subgroup of omnivores (VERA-O, $n = 99$) and vegetarians ($n = 21$, 15 OVL and 6 vegans)[a]

	$\delta^{13}C$ (‰ vs. PDB)				
	VERA-O		Vegetarians		
	Mean ± SD (n)	CI	Mean ± SD (n)	CI	$P<$
Histidine	−18.1 ± 7.1 (63)	1.8	−22.4 ± 5.5 (15)	2.8	0.05
Isoleucine	−26.1 ± 4.7 (98)	0.9	−34.6 ± 4.8 (21)	2.1	0.001
Leucine	−30.3 ± 5.3 (99)	1.0	−39.3 ± 3.9 (21)	1.7	0.001
Lysine	−24.2 ± 9.5 (99)	1.9	−32.5 ± 7.4 (21)	3.1	0.001
Phenylalanine	−26.0 ± 11.6 (99)	2.3	−33.2 ± 3.5 (21)	1.5	0.001
Threonine	−28.2 ± 10.1 (99)	2.0	−43.7 ± 4.7 (21)	2.0	0.001
Valine	−24.1 ± 15.8 (99)	0.8	−32.0 ± 4.3 (21)	1.9	0.001
Alanine	−15.2 ± 7.7 (96)	1.5	−20.5 ± 4.8 (21)	2.1	0.001
Aspartic acid	−15.3 ± 3.4 (99)	0.7	−20.7 ± 4.4 (21)	1.9	0.001
Glutamic acid	−18.0 ± 3.2 (99)	0.6	−25.7 ± 5.0 (21)	2.1	0.001
Glycine	0.7 ± 6.3 (99)	1.2	−0.6 ± 4.2 (21)	1.8	ns[b]
Proline	−21.1 ± 5.5 (99)	1.1	−29.4 ± 3.8 (21)	1.6	0.001
Serine	−23.9 ± 9.2 (99)	1.8	−38.3 ± 5.1 (21)	2.2	0.001
Tyrosine	−17.2 ± 4.2 (99)	0.8	−19.2 ± 1.1 (21)	0.5	0.001

[a] Sample numbers $n < 99$ for VERA-O and $n < 21$ for vegetarians are due to methodological reasons (e.g. poor resolution, low signal).
[b] ns, $P > 0.05$.

Table 6. Comparison of bulk ^{15}N and ^{13}C abundances measured by EA/IRMS of selected isolated proteins and of dietary protein sources of animal and plant origin purchased in German supermarkets or organic food stores[a]

Protein source	$\delta^{15}N$ (‰ vs. AIR)	$\delta^{13}C$ (‰ vs. PDB)
Isolated animal proteins		
Sarkoplasma proteins, bovine[b]	7.5 ± 0.7	−22.0 ± 0.1
Albumin, bovine[c,d]	7.1 ± 0.2	−10.3 ± 0.1
Whey protein[b]	4.7 ± 0.5	−18.9 ± 0.1
Albumin, porcine serum[c]	6.5 ± 0.1	−16.3 ± 0.1
Globulin, pig[c]	7.2 ± 0.4	−13.6 ± 0.0
Gelatin, porcine skin[c]	4.1 ± 0.3	−15.8 ± 0.4
Animal dietary constituents		
Meat, bovine	6.6 ± 0.2	−20.0 ± 0.2
Meat, porcine	3.9 ± 0.1	−24.3 ± 0.2
Meat, turkey	2.3 ± 0.3	−24.0 ± 0.3
Sour milk cheese	6.4 ± 0.2	−21.1 ± 0.1
Camembert cheese	5.4 ± 0.3	−21.3 ± 0.2
Hen's egg, natural extract (Degussa, Hamburg, Germany)	4.8 ± 0.1	−22.0 ± 0.1
Isolated plant proteins		
Gliadin[c]	2.9 ± 0.4	−25.9 ± 0.1
Wheat gluten (Kröner Stärke GmbH, Ibbenbühren, Germany)	2.6 ± 0.4	−26.7 ± 0.2
Glycinin, Soybean[b]	0.4 ± 0.4	−25.2 ± 0.1
Pea protein[b]	−0.2 ± 0.2	−26.6 ± 0.2
Potato protein[b]	2.6 ± 0.2	−26.1 ± 0.1
Zein, from corn (C_4)[c]	3.4 ± 0.1	−13.0 ± 0.1
Plant dietary constituents		
Rye (Germany)	−2.7 ± 1.7	−25.0 ± 0.4
Wheat (Germany)	1.4 ± 0.8	−26.0 ± 0.2
Rolled oats (Germany)	0.1 ± 1.7	−27.3 ± 0.2
Soya flower (Degussa)	−1.7 ± 0.6	−24.4 ± 0.1
White beans (Turkey)	−1.2 ± 1.3	−25.9 ± 0.2
Cornflakes (C_4)	1.9 ± 2.8	−10.7 ± 0.0

[a] Values are means ± SD of each sample each determined 5 times.
[b] Isolated and provided by Dr. H. Rawel (Univ. of Potsdam, Dept. Food Chemistry).
[c] Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany.
[d] Possibly of animals fed with corn food.

high proportion of meat and meat products in the German diet[35] together with the use of corn products in animal production might explain the high predictive value of hair $\delta^{13}C$ values for animal protein intake. It can be assumed that an indirect consumption of amino acids from corn via meat and meat products may contribute to higher $\delta^{13}C$ values when the proportion of animal protein in the diet is high. As shown in Table 6, some of the animal-derived dietary proteins are much more positive in their $\delta^{13}C$ values than those from plant sources. This can probably be explained by the fact that the isolated protein sources are of American origin in some cases. This difference can be up to 16‰ $\delta^{13}C$ comparing wheat gluten with bovine albumin.

Regarding the bulk ^{15}N values of hair our results confirm their highly predictive value for the proportion of animal protein in the diet. As shown for example in Table 6, the animal-derived protein sources are likewise more positive in their $\delta^{15}N$ values than those from plant protein sources with the consequence that an increase in the content of animal proteins also increases the $\delta^{15}N$ value in meals. These results can be explained on the basis of the relatively high trophic level shift of ^{15}N. Although mechanisms for isotopic discrimination that result in this trophic level shift have not yet been described in detail, amino acid transamination may be involved.[18,36,37] It has been shown in rats that hepatic nitrogen metabolism causes a discrimination of ^{15}N isotope against ^{14}N resulting in a relative enrichment of ^{15}N in body proteins and depletion in urinary urea and ammonia of up to 10‰.[37]

Finally, using the ^{13}C and ^{15}N abundances of hair, it seems possible to estimate the proportion of animal protein to total protein consumption of each individual (IPAP, Tables 2 and 3) assuming a linear relationship between the δ values of vegans and omnivores and the consumption of dietary proteins derived from animals.

Only a few reports on the ^{15}N and ^{13}C abundance in amino acids of different tissues and proteins at the natural abundance level have been published to date. This is surprising because isotopic analysis of single amino acids could potentially allow a more detailed investigation of diet and body protein interrelationships than those from bulk isotopic values. We have earlier investigated biological samples using GC/C/IRMS to study the isotopic signature of individual amino acids.[20,26] It was shown that hair amino acids have a characteristic pattern for $\delta^{15}N$ and $\delta^{13}C$ values comparable with that in other tissue proteins of humans or animals.[20,25,38] A relative depletion, e.g., in the $\delta^{15}N$ value of threonine and an enrichment in the $\delta^{13}C$ value of glycine were also reported by others, the meaning of which deserves further investigation.[39,40] We first hypothesized that ^{15}N and ^{13}C abundances in individual amino acids could better predict the level of animal protein consumption than those from bulk isotopic values of hair. We were not, however, able to find significant differences between vegans and OLV in stable isotopic compositions in individual amino acids, although, notwithstanding some exceptions, there are highly significant differences between groups of individuals consuming a vegetable-based widely meat-free diet and omnivores (Tables 4 and 5). The reason for a somewhat lower resolution between diet preference groups in isotopic values of individual amino acids can be partially caused by a slightly lower precision of GC/C/IRMS than of EA/IRMS. Finally, the amino acid composition of hair between different food preference groups was determined to be identical (data not shown). Therefore, it is possible to exclude the possibility that the bulk $\delta^{15}N$ and $\delta^{13}C$ values of hair were simply the result of a different amino acid composition due to an influence of different isotopic abundances of individual amino acids.

CONCLUSIONS

Bulk analysis of hair ^{15}N and ^{13}C abundance by EA/IRMS seem to be suitable for distinguishing food preference groups and for estimating the level of animal protein consumption. Therefore, the bulk ^{15}N- and ^{13}C-isotopic values of hair can possibly be used to describe the habitual protein intake when cultural particularities of food choice and isotopic composition are considered. It remains to be confirmed in a long-term controlled dietary intervention study that the correlation between hair ^{13}C and ^{15}N abundances and dietary protein intake holds true.

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REFERENCES

1. World Cancer Research Fund and American Institute for Cancer Research. *Food, Nutrition and the Prevention of Cancer: A Global Perspective*. American Institute for Cancer Research: Washington, DC, 1997.
2. Fung TT, Hu FB. *Am. J. Clin. Nutr.* 2003; **78**: 357.
3. Flood A, Velie EM, Sinha R, Chaterjee N, Lacey JV, Schairer C, Schatzkin A. *Am. J. Epidemiol.* 2003; **158**: 59.
4. Metges CC, Barth CA. *J. Nutr.* 2000; **130**: 886.
5. Rolland-Cachera MF, Bellisle F, Deheeger M. *Eur. J. Clin. Nutr.* 2000; **54** (Suppl. 1): S41.
6. Kroke A, Klipstein-Grobusch K, Voss S, Möseneder J, Thielecke F, Noack R, Boeing H. *Am. J. Clin. Nutr.* 1999; **70**: 439.
7. McKoewn NM, Day NE, Welch AA, Runswick SA, Luben RN, Mulligan AA, McTaggart A, Bingham SA. *Am. J. Clin. Nutr.* 2002; **74**: 188.
8. Macko SA, Engel MH, Andrusevich V, Lubec G, O'Connell TC, Hedges RE. *Phil. Trans. R. Soc. Lond. B* 1999; **354**: 65.
9. Macko SA, Lubec G, Teschler-Nicola M, Andrusevich V, Engel MH. *FASEB J.* 1999; **13**: 559.
10. O'Connell TC, Hedges REM. *Am. J. Phys. Anthropol.* 1999; **108**: 409.
11. Nakamura K, Schoeller DA, Winkler FJ, Schmidt HL. *Biomed. Mass Spectrom.* 1982; **9**: 390.
12. Yoshinaga J, Minagawa M, Suzuki T, Ohtsuka R, Kawabe T, Inaoka T, Akimichi T. *Am. J. Phys. Anthropol.* 1996; **100**: 23.
13. Tokui N, Minari Y, Kusunoki K, Yoshimura T, Yamamoto T, Minagawa M. *J. UOEH* 2000; **22**: 219.
14. Minagawa M. *Appl. Geochem.* 1992; **7**: 145.
15. Schoeller DA, Minagawa M, Slater R, Kaplan I. *Ecol. Food Nutr.* 1986; **18**: 159.
16. Bol R, Pflieger C. *Rapid Commun. Mass Spectrom.* 2002; **16**: 2195.
17. Minagawa M, Wada E. *Geochim. Cosmochim. Acta* 1984; **48**: 1135.
18. Schoeller DA. *J. Archaeol. Sci.* 1999; **26**: 667.
19. Focken U. *Isotopes Environ. Health Stud.* 2001; **37**: 199.
20. Metges CC, Petzke KJ. *Anal. Biochem.* 1997; **247**: 158.
21. Petzke KJ, Korkushko OV, Semesko TM, Metges CC. *Isotopes Environ. Health Stud.* 1997; **33**: 267.
22. Heseker H, Adolf T, Eberhardt W, Hartmann S, Herwig A, Kübler W, Matiaske B, Moch K, Nitsche A, Schneider R, Zipp A. *Lebensmittel- und Nährstoffaufnahme Erwachsener in der Bundesrepublik Deutschland (Food and nutrient intake of adults in the Federal Republic of Germany)*. VERA-Schriftenreihe Bd. III, Wissenschaftlicher Fachverlag Dr. Fleck: Niederkleen, Germany, 1994.
23. VERA-Schriftenreihe Bd. I-XIV. Wissenschaftlicher Fachverlag Dr. Fleck: Niederkleen, Germany, 1992–1995.
24. Federal Institute for Risk Assessment (BfR). *The German Food Code and Nutrient Base (BLS II.3). Conception, Structure and Documentation of the Data-Base blsdat*. BgVV-Heft 10. Federal Institute for Risk Assessment: Berlin, Germany, 1999.
25. Metges CC, Petzke KJ, Hennig U. *J. Mass Spectrom.* 1996; **31**: 367.
26. Metges CC, Daenzer M. *Anal. Biochem.* 2000; **278**: 156.
27. Craig H. *Geochim. Cosmochim. Acta* 1953; **3**: 53.
28. Petzke KJ, Proll J, Elsner A, Thielecke F, Metges CC. *J. Nutr.* 2000; **130**: 2889.
29. Wada E, Mizutani H, Minagawa M. *Crit. Rev. Food Sci. Nutr.* 1991; **30**: 361.
30. Schoeller DA, Klein PD, Warkins JB, Heim T, MacLean WC. *Am. J. Clin. Nutr.* 1980; **33**: 2375.
31. Schulze A, Karg G, Steinel M. *Z. Ernährungswiss.* 1995; **34**: 190.
32. O'Leary MH. *BioScience* 1988; **38**: 328.
33. DeNiro MJ, Epstein S. *Geochim. Cosmochim. Acta* 1978; **42**: 495.
34. DeNiro MJ, Epstein S. *Geochim. Cosmochim. Acta* 1981; **45**: 342.
35. Mensink G, Burger M, Beitz R, Henschel Y, Hintzpeter B. *Beiträge zur Gesundheitsberichterstattung des Bundes. Was essen wir heute? Ernährungsverhalten in Deutschland. (Contributions to the health report refund of the federation. What do we eat today? Diet behavior in Germany)*. Robert Koch-Institut: Berlin, Germany, 2002.
36. Macko SA, Estep MLF, Engel MH, Hare PE. *Geochim. Cosmochim. Acta* 1986; **50**: 2143.
37. Sick H, Roos N, Saggau E, Haas K, Meyn V, Walch B, Trugo N. *Z. Ernährungswiss.* 1997; **36**: 340.
38. Petzke KJ, Grigorov JG, Korkushko OV, Kovalenko NK, Semesko TG, Metges CC. *Z. Ernährungswiss.* 1998; **37**: 368.
39. O'Connell TC, Hedges REM, Healey MA, Simpson AHRW. *J. Archaeol. Sci.* 2001; **28**: 1247.
40. Hare PE, Fogel ML, Stafford TW, Mitchell AD, Hoering TC. *J. Archaeol. Sci.* 1991; **18**: 277.