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Prediction of human dietary $\delta^{15}N$ intake from standardized food records: Validity and precision of single meal and 24-h diet data

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Supplemental data for this article follows the **References.**

Abstract

Natural stable isotope ratios ($\delta^{15}N$) of humans can be used for nutritional analyses and dietary reconstruction of modern and historic individuals and populations. Information about an individual's metabolic state can be obtained by comparison of tissue and dietary $\delta^{15}N$. Different methods have been used to estimate dietary $\delta^{15}N$ in the past; however, the validity of such predictions has not been compared to experimental values. For a total of 56 meals and 21 samples of 24-h diets, predicted and experimental $\delta^{15}N$ values were compared. The $\delta^{15}N$ values were predicted from self-recorded food intake and compared with experimental $\delta^{15}N$ values. Predicted and experimental $\delta^{15}N$ values were in good agreement for meals and preparations ($r = 0.89$, $p < .001$) as well as for the 24-h diets ($r = 0.76$, $p < .001$). Dietary $\delta^{15}N$ was mainly determined by the amount of fish, whereas the contribution of meat to dietary $\delta^{15}N$ values was less pronounced. Prediction of human dietary $\delta^{15}N$ values using standardized food records and representative $\delta^{15}N$ data sets yields reliable data for dietary $\delta^{15}N$ intake. A differentiated analysis of the primary protein sources is necessary when relating the proportion of animal-derived protein in the diet by $\delta^{15}N$ analysis.

Keywords: Diet study, food, human, nitrogen-15, protein

1. Introduction

There are multiple applications for natural nitrogen stable isotope ratio ($\delta^{15}N$) analysis of human hair, nail, blood and excreta. Analysis of $\delta^{15}N$ is frequently used for both nutritional analysis and dietary reconstruction of individuals and populations [1–3]. Additionally, human $\delta^{15}N$ values serve as biomarkers for the dietary intake of meat, fish or sweeteners [4–14].

In principle, the $\delta^{15}N$ value of an organism is characterized by the isotopic composition of its diet. However, as the lighter isotope is preferentially metabolized and excreted, organisms become enriched in ^{15}N compared to their diet [15]. Different dietary practices are known to affect human $\delta^{15}N$ values (for an overview see [16]). Especially marine fish is enriched in ^{15}N with $\delta^{15}N$ values around +12‰ [17], leading to increased $\delta^{15}N$ values in individuals who consume marine fish when compared to their non-fish-consuming counterparts [5, 7, 9, 12, 18, 19]. Terrestrial meat is less enriched in ^{15}N, and only beef and lamb exhibit elevated $\delta^{15}N$ when compared to plants [17]. Increased meat consumption might be identified by analysis of $\delta^{15}N$ of human tissue, as differing nitrogen isotopic compositions of hair of vegans, vegetarians and omnivores have been reported [8, 20–23]. Despite these interpretations, the influence of increased meat consumption on $\delta^{15}N$ in human hair, blood or urine in experimental studies has not always led to clearly identifiable differences [2, 24]. The $\delta^{15}N$ of red blood cells has also been used to represent the dietary intake of long-chain polyunsaturated fatty acids from fish [7] or as a correction factor for the amount of sweeteners in the diet [6].

Aside from the influence of the diet, several metabolic factors also affect human $\delta^{15}N$ values, such as pregnancy [25], malnutrition and starvation [26–29], liver cirrhosis [30] and diabetes [31]. The proposed effect of malnutrition and starvation on $\delta^{15}N$ values is explained by "recycling" of endogenous nitrogen, which is enriched in ^{15}N when compared to the diet. Such an effect was proposed for increasing $\delta^{15}N$ values along a hair shaft towards the point of death [27] or during a period of nutritional stress with increased physical activity [32]. In contrast, the opposite effect of protein anabolism, that is, an increased incorporation of dietary nitrogen, which is depleted in ^{15}N, has been described in pregnant women [25] and in patients recovering from anorexia nervosa [28]. The identification of these various metabolic states requires the comparison of $\delta^{15}N$ in a biological specimen with the dietary $\delta^{15}N$ intake. However, despite an increasing number of studies using human $\delta^{15}N$ data for the interpretation and reconstruction of dietary habits, the fundamental principles of ^{15}N enrichment in the human body are still not fully understood. In addition, only a handful of studies have attempted to estimate $\delta^{15}N$ values for human diets in order to determine diet–body offsets [1, 19, 33–37]. In order to differentiate dietary and physiological factors, the correct assessment of $\delta^{15}N$ dietary intake is essential, even though laborious and time-consuming. The most accurate assessment of dietary $\delta^{15}N$ is obtained via analysis of duplicate diets [4, 33]. Another, less accurate, approach is the prediction of human dietary $\delta^{15}N$ by means of combining self-reported diets with reference data sets of $\delta^{15}N$ values of "typical" food items consumed by the participants [see 19, 34, 35]. In recent years, different approaches have been used for the estimation of human $\delta^{15}N$ dietary intakes (Table 1), spanning from the use of average country-specific diet compositions [1, 36], household food consumption surveys [35] and self-reported dietary records or food frequency questionnaires [19, 34, 37] to the use of the duplicate meal method [4, 33]. Dietary $\delta^{15}N$ intakes have been estimated and interpreted both on the group level as well as on the individual level, even though information about accuracy and uncertainty of these estimations are lacking.

Common dietary analyses utilize substantial databases [e.g. 38] and are frequently validated against reference methods [e.g. 39]. To our knowledge, no attempts have been made to validate the prediction of the $\delta^{15}N$ of human diets. The purpose of this study was to validate the prediction of dietary $\delta^{15}N$ by comparing predicted $\delta^{15}N$ for (1) single meals and diet preparations and (2) for 24-h diets against $\delta^{15}N$ as measured by elemental analysis–isotope ratio mass spectrometry (EA-IRMS). The secondary, exploratory goal of our study was to estimate

Table 1. Studies analyzing modern human $\delta^{15}N$ values including estimation of dietary $\delta^{15}N$ intake.

Population	*n*	Method	Ref
USA	9	National food consumption statistics	[36]
Japan	42	National food consumption statistics	[1]
Several*	65	National food consumption statistics (?)	[43]
Papua New Guinea	49	14-day household food consumption survey	[35]
Fiji	20	14-day self-reported dietary record	[34]
Germany	3	3-day self-reported dietary record	[19]
UK	11	Duplicate meal method	[33]
Papua New Guinea	115	7-day food frequency questionnaire	[37]

* Including Japan, Korea, Brazil, Netherlands and China.

the impact of different protein sources on the predicted food item's $\delta^{15}N$ values as the analysis of human $\delta^{15}N$ values can provide information about the consumer's preferred source of protein. This is based on the fact that $\delta^{15}N$ values (of food items) increase with increasing position in the food web. However, a distinct interpretation of the underlying principles is necessary. Natural stable nitrogen isotopes from animal proteins have to be divided into different categories due to their different $\delta^{15}N$ values, as a sole differentiation between omnivorous, vegetarian or vegan diets is inadequate.

2. Materials and methods

The present study involved the comparison of predicted dietary $\delta^{15}N$, which was estimated for samples of single meals and preparations as well as for 24-h diets, against dietary $\delta^{15}N$ as measured by EA-IRMS.

2.1. Diets, meals and preparations

Samples of single meals and preparations (n = 56), which contain more than one primary protein source (Table 2) were collected between 2003 and 2011 as part of various studies conducted in our labs [17,19]. All food was readily prepared and suitable for consumption. A small aliquot (approximately 10 g) was sampled from each meal or preparation. The aliquot was diluted with three- to five-fold amount of demineralized water and homogenized using a food processor. About 10 ml of the suspension were separated, finely homogenized and dried *in vacuo* at room temperature. Dried samples were stored at room temperature in a dry area that was protected from sunlight.

2.2. Duplicate diets

Complete 24-h diets were collected during two different studies: For study (I), 14 authentic 24-h diets were randomly chosen from a nutritional study with small children in Germany (Ruhr and Steinfurt District, North Rhine-Westphalia) in 1998, which were collected using the duplicate method [40]. Collected diets were homogenized, lyophilized and stored at – 80 °C until stable isotope ratio analysis. Collection of these duplicate meals was accompanied by a food record completed by the parents [40]. Parents recorded the type and amount of their children's intake using household measures (cup, plate, spoon, etc.), units (slices, pieces, etc.) and portion sizes (small, medium and large). For the present analysis, food records were analyzed

Table 2. Products and meals tested including protein content.

Main protein source from	Description	Protein content (g/100 g)	n[a]	$\delta^{15}N$ (‰) experimental	calculated
Cereals	Whole-grain bread	7	2	2.3 ± 0.3	2.9 ± 1.4
	White bread	8	2	2.7 ± 0.2	2.9 ± 1.4
	Rye bread	10	2	3.1 ± 0.1	2.8 ± 1.4
	Crispbread	10	3	3.2 ± 0.6	3.2 ± 1.4
	Croissant	8	4	3.1 ± 0.3	3.6 ± 1.1
	Cake	6	4	4.7 ± 0.2	4.5 ± 1.1
	Nut cake	10	4	3.5 ± 0.1	3.6 ± 1.0
	Biscuits	6	3	4.3 ± 0.3	3.7 ± 1.3
	Chocolate biscuit	8	4	3.8 ± 0.2	4.7 ± 1.1
	Chocolate cereal bar	2	3	3.0 ± 0.2	3.4 ± 1.5
	Pasta	5	2	3.0 ± 0.2	3.6 ± 1.3
	Pretzel sticks	11	3	4.4 ± 0.3	3.9 ± 1.2
Vegetables	Green leaf salad with dressing	4	2	7.2 ± 0.2	4.2 ± 1.8
	Brown sauce	1	2	1.3 ± 0.4	4.4 ± 1.6
	Mashed potatoes	2	2	4.5 ± 0.4	4.0 ± 2.2
	Fried mashed potatoes	1	3	2.4 ± 0.3	4.2 ± 1.5
	Gnocchi	4	4	3.5 ± 0.2	3.6 ± 1.7
	Polenta	8	3	1.4 ± 0.3	3.7 ± 1.7
	Lentil stew with sausage	5	4	2.1 ± 0.2	3.9 ± 1.3
	Millet with vegetables	7	2	2.9 ± 0.3	3.2 ± 2.7
	Pizza	11	4	4.7 ± 0.3	4.0 ± 1.0
	Soya Bolognese	16	2	2.4 ± 0.2	1.3 ± 1.6
	Vegetarian Pilaf from millet	13	3	2.7 ± 0.2	3.1 ± 1.4
Meat	Breaded pork cutlet	19	3	4.2 ± 0.1	4.5 ± 1.7
	Meat loaf	20	4	4.3 ± 0.2	5.4 ± 1.2
	Beef roulade	15	4	7.4 ± 0.3	6.4 ± 2.1
	Beef with pasta and vegetables	29	3	6.8 ± 0.1	5.7 ± 1.7
	Cheeseburger	20	4	6.2 ± 0.1	5.1 ± 1.0
	Minced meat sauce	4	3	5.3 ± 0.3	5.3 ± 1.3
	Pizza Bolognese	9	4	6.3 ± 0.6	5.4 ± 1.3
Dairy	Chocolate	9	2	3.8 ± 0.3	5.6 ± 1.6
	Chocolate milk	3	2	6.5 ± 0.2	5.3 ± 1.7
	Chocolate with almonds	9	3	4.4 ± 0.3	4.9 ± 1.3
	Chocolate–hazelnut spread	1	3	5.4 ± 0.1	3.4 ± 1.7
	Omelet	10	2	5.1 ± 0.4	5.1 ± 1.7
	Yogurt dressing	1	2	6.2 ± 0.4	5.2 ± 1.8
	Yogurt low-fat with fruits	4	2	6.0 ± 0.3	5.0 ± 1.5
	Protein bar	18	5	6.6 ± 0.1	4.8 ± 1.4
	Rice pudding	3	2	4.6 ± 0.4	5.2 ± 1.6
Fish	Calamari, omelet, vegetables	17	4	8.4 ± 0.2	8.8 ± 2.9
	Herring salad	16	4	9.6 ± 0.4	10.9 ± 3.5
	Fish ball	14	4	5.8 ± 0.4	11.4 ± 3.8
	Fish fingers	13	3	11.8 ± 0.3	11.6 ± 3.9
	Breaded fillet of fish	15	3	11.9 ± 0.3	11.6 ± 3.9
	Tuna with peppers, corn pasta	15	3	12.6 ± 0.1	10.5 ± 3.5
	Tuna ragout with corn pasta	15	5	10.5 ± 0.2	10.2 ± 3.2
	Tuna with vegetables, polenta	12	3	12.5 ± 0.4	11.3 ± 3.8

a. Number of protein sources used for calculation.

for protein content of each single food item based on the nutrient database LEBTAB, which is based on common food tables and contains additional data of commercially produced foods calculated from simulated recipes using labelled ingredients and nutrients [41].

For study (II), seven 24-h duplicate diets were collected in 2010 from seven adults (three males and four females) in Cologne (North Rhine-Westphalia, Germany). Each 24-h diet was homogenized as an aqueous suspension and an aliquot of approximately 10 ml was dried over phosphorous pentoxide. The dried samples were finely ground and stored at room temperature until stable isotope ratio analysis. Collecting of duplicate diets was accompanied by completion of a validated food record [39]. Food records were analyzed for protein content using commercial software (EBISpro 7.0, University of Hohenheim, Germany) based on the present German food code [38].

2.3. Stable isotope ratio measurements

Samples (approximately 200 µg) were loaded into tin capsules and analyzed by EAIRMS using an elemental analyzer Eurovektor EA 3000 (Hekatech, Wegberg, Germany) coupled to a continuous-flow isotope ratio mass spectrometer Delta C (Thermo Fisher Scientific, Bremen, Germany). Nitrogen isotope ratios are expressed relative to atmospheric nitrogen (AIR). Working standard gas (N^2, purity 5.0; Linde, Munich, Germany) and a working standard (creatine-monohydrate, AlzChem, Trostberg, Germany) were scale calibrated using IAEA-N-1 (+0.4‰) and IAEA-N-2 (+20.3 ‰) (both from IAEA, Vienna, Austria). The standard deviation of three repeated measurements of the working standard was ±0.2‰. Analysis of the samples were carried out in triplicate and checked every sixth measurement for zero blank analysis. Instrument stability was checked accordingly by analysis of the working standard every sixth measurement.

2.4. Calculation of predicted $\delta^{15}N$ for meals and diets

$\delta^{15}N$ values were predicted using a recently published comprehensive data set for $\delta^{15}N$ values of contemporary human food items [17]. Data are representative for the so-called glocal[1] supermarket present in today's Western communities. All $\delta^{15}N$ values for food items and preparations in this work were derived from this data set. Diets, meals and preparations were analyzed for their primary constituents such as cereals, vegetables, kind of meat, etc. as specified by the nutritional software. If meals and preparations were not specified in the database, typical recipes and compositions from cookbooks or online databases were used. Each meal or diet was analyzed for the protein content of each food item, which were classified in food categories characterized by distinct $\delta^{15}N$ values [17]. In principle, there is a differentiation between vegetable, meat and terrestrial animal products and fish. However, there are still further differentiations due to their stable nitrogen isotopic compositions, for instance between legumes (pulses) and fruits, cereals, etc. [9]. Predicted stable nitrogen isotope ratios of meals and diets ($\delta^{15}N^{diet}$) were calculated based on the proportion of protein from n different food categories (P^i) over the total protein of the meal or diet (P), which was multiplied with the reference value of the nitrogen isotopic composition of the respective food category (δ_i):

$$\delta^{15}N_{diet(predicted)} = (P_1\delta_1 + P_2\delta_2 + \dots)/P \quad (1)$$

1. *Glocal* is a portmanteau of *global* and *local*.

2.5. Estimation of measurement and prediction uncertainties

Uncertainties of experimental and predicted $\delta^{15}N$ values of meals and diets were estimated as follows: due to methodological and natural variations, the published $\delta^{15}N$ values for the different food categories are associated with uncertainties between ±0.8 and ±3.4‰ (1σ) [17]. The amount of protein in human food items varies typically around ±7 %, as estimated from a reference food composition table [42]. In this study, the uncertainties of the estimated $\delta^{15}N$ values of meals and diets (u_{diet}) were calculated combining the reported uncertainties for $\delta^{15}N$ values ($u_{\delta i}$) and a constant value of 0.07 for the protein content (u_P) of the different food items (for calculation see Supplementary Information):

$$u_{diet} = \sqrt{(\delta_1{}^2 + \delta_2{}^2 + \dots)u_P{}^2 + ((P_1 u_{\delta 1})^2 + (P_2 u_{\delta 2})^2 + \dots)} \quad (2)$$

2.6. Data analysis

For both data sets, the regression coefficient and the standard error of estimate (SEE) were calculated, which were obtained by simple linear regression between predicted and experimental values. In addition, Bland–Altman analysis was performed to estimate the 95 % limits of agreement between the methods and to identify potential bias. Statistical significance was set at $p < .05$. Unless otherwise stated, values are reported as means ± standard deviations (s). All statistical analyses were performed using R version 3.0.2 and Microsoft Excel 2013.

3. Results and discussion

3.1. Meals and preparations

A total of 47 meals and preparations were analyzed which contained components from more than one food category as specified in the $\delta^{15}N$database [17]. Fifteen meals and preparations contained 2 different sources, 16 samples 3, 14 samples 4 sources and 2 preparations were composed out of 5 sources. Experimental $\delta^{15}N$ values were between +1.3 and +12.5‰ (Table 2). The lowest experimental $\delta^{15}N$ values were found in vegetarian or legumes-based meals and preparations. The highest $\delta^{15}N$-values meals were found in meals and preparations with fish as main protein source. Predicted values for the different samples ranged between +1.3 and +11.6‰. Estimated uncertainties (1σ) for these preparations and meals were between ±1.0 and ±3.8‰, with a mean of ±1.6‰.

There was a high degree of correlation between predicted and experimental $\delta^{15}N$ values for these meals and preparations ($r = 0.89$, $p < .001$, Figure 1), and the SEE was ±1.3‰. Bland–Altman analysis revealed almost no difference between experimental and calculated $\delta^{15}N$ values (mean bias: +0.1‰), but very wide limits of agreement (+2.6 to –2.7‰). There was no significant proportional bias ($p = .21$, Figure 1).

3.2. 24-h diets

A total number of 21 experimental 24-h samples were analyzed. The mean predicted $\delta^{15}N$ value across all 24-h samples was identical to the mean experimental $\delta^{15}N$ value (both +4.3 ± 0.7‰). Predicted $\delta^{15}N$ values ranged between +3.4 and +6.1‰, and likewise, experimental

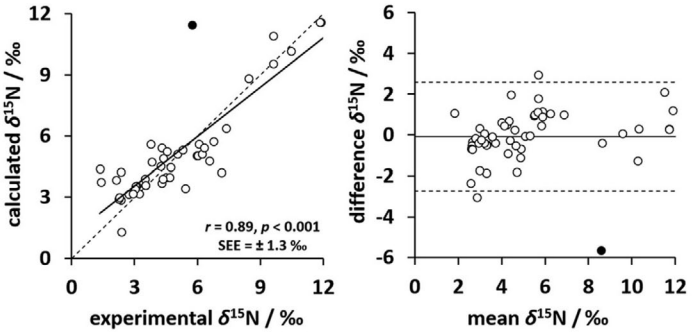

Figure 1. Correlation analysis (left) and Bland–Altman plot of predicted and experimental $\delta^{15}N$ values for meals and preparations. The dotted line represents the perfect agreement among methods. The solid horizontal line on the right represents the mean difference between predicted and experimental values, and the dashed horizontal represent the 95 % limit of agreement. The solid black data point represents an outlier deemed as 'fish cake' by the research subject (see discussion for interpretation).

$\delta^{15}N$ values ranged between +3.2 and +6.3‰. Predicted and experimental $\delta^{15}N$ values were highly significantly correlated with each other (r = 0.76, p < .001, Figure 2). The SEE was ±0.5‰. Bland–Altman analysis revealed no significant difference among predicted and experimental $\delta^{15}N$, and limits of agreement between the two methods were –1.0 to +1.0‰. There was no significant proportional bias (p = .29, Figure 2).

3.3. Influence of protein choice

In order to analyze the effect of different plant and animal protein sources on the $\delta^{15}N$ values of typical human meals, we predicted $\delta^{15}N$ values for the food items and meals specified in a routinely used food record. The used food record lists food items, which represent foods frequently consumed in Germany and specifically used by athletes (for details see [39]). Most of the items listed contain protein (181). Additionally, 166 food items were

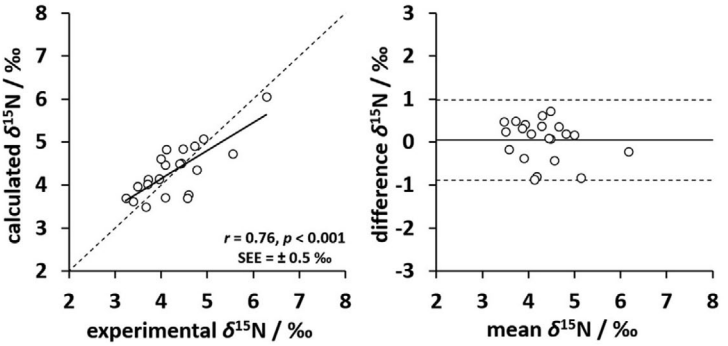

Figure 2. Correlation analysis (left) and Bland–Altman plot of predicted and experimental $\delta^{15}N$ values for 24-h diets. The dotted line represents the perfect agreement among methods. The solid horizontal line on the right represents the mean difference between predicted and experimental values, and the dashed horizontal represent the 95 % limit of agreement.

added, which not had been initially listed and were noted by subjects during their completion of the food record. Out of this compilation, 192 items and meals contained more than 1 protein source. For these 192 items and meals $\delta^{15}N$ values were calculated and they were analyzed for their main protein source: either legumes ($\delta^{15}N$ of +1.1‰), plants (+3.3‰, excluding legumes), pork and poultry (+4.1‰), dairy and eggs (+5.2 ‰), beef and lamb (+6.5‰) or fish (+12.4‰) [17].

4. Discussion

4.1. Validation results

The main purpose of our study was to assess the validity of $\delta^{15}N$ values predicted using a food record combined with nutritional and nitrogen stable isotope data sets when compared to experimental $\delta^{15}N$ values obtained via EA-IRMS. The duplicate method represents the most accurate possibility to assess human dietary $\delta^{15}N$ intake. However, the duplicate method's disadvantage is the high amount of work necessary for collecting and processing the samples. Therefore in practice, the use of food records and representative data sets for calculation of dietary $\delta^{15}N$ values seems to be appropriate. The duplicate meal method's disadvantage of potential alteration of the subject's habitual diet is negligible for our purposes. Overall, we found a satisfying accuracy and precision between the predicted dietary $\delta^{15}N$ values for both single meals and preparations as well as for 24-h diets. The estimation of $\delta^{15}N$ for single foods and meals based on food records and previously published $\delta^{15}N$ values is associated with a relative high uncertainty, which is due to the variation of the natural abundances of $\delta^{15}N$ of food items. For some meals and preparations, large differences between experimental and calculated mean values were observed. A difference between experimental and calculated $\delta^{15}N$ values in the range of ±3‰ most likely results from inadequate prediction of $\delta^{15}N$ dietary values rather than from errors in the measurement of $\delta^{15}N$, which are around ±0.2‰. The classification and identification of a certain meal or preparation was conducted by the subject collecting the sample. If such a classification is incorrect due to missing information or false identification, it might lead to erroneous $\delta^{15}N$ prediction. One of our samples with a predicted $\delta^{15}N$ of +11.4‰ had an experimental $\delta^{15}N$ of +5.8‰. This sample was classified as "fish cake." For the theoretical calculation, the $\delta^{15}N$ value for marine fish was used, which was probably wrong. Even if the $\delta^{15}N$ value for freshwater fish would be used, which is approximately 2‰ lower [17], theoretical and experimental $\delta^{15}N$ values would differ. It is possible that this sample was misclassified as "fish cake" by the subject, or that the proportion of fish in the meal was lower than accounted for by our nutritional software. Secondly, differences between experimental and calculated $\delta^{15}N$ values may originate from differing protein sources. We have previously shown that some modern commercial fish species exhibit $\delta^{15}N$ values that deviate significantly from naturally occurring $\delta^{15}N$ values for marine fish (+12.4‰) [17]. For instance, commercial shark catfish ("pangasius"), a popular food in Germany, has an experimental $\delta^{15}N$ of +5.1‰ [17]. Using this value for calculation of the "theoretical" $\delta^{15}N$ value for fish cake, the result would be in accordance with the experimental values.

These findings suggest that it is inappropriate to estimate the $\delta^{15}N$ value for a single or few food items or preparations in terms of a "snapshot," but a good opportunity to estimate the dietary $\delta^{15}N$ intake for a population or a mean value for an individual over a longer period.

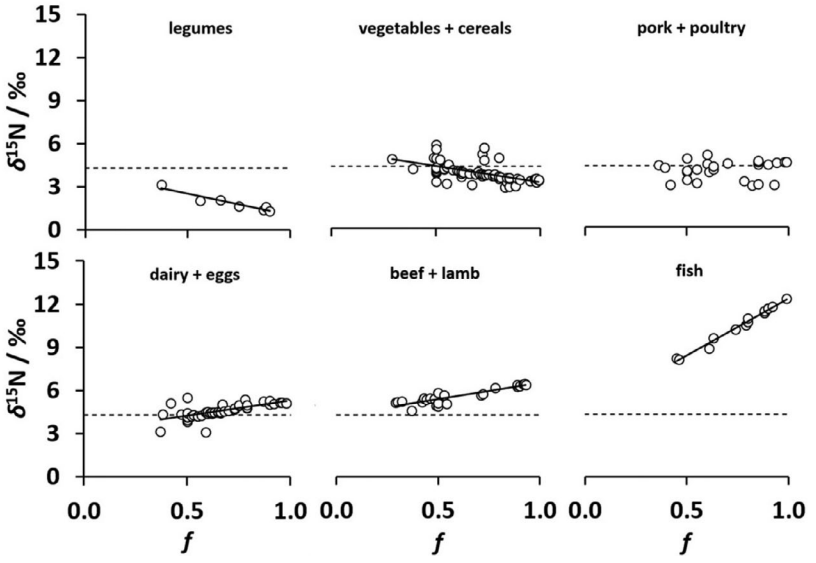

Figure 3. Predicted stable nitrogen isotope ratios for meals and food based on items used in a food frequency questionnaire [39] versus the fractional protein content of the main protein source in a food item. Each data point represents one food item or meal consisting of more than one protein source. *f* = 1: all protein in the food item or meal derives from the certain food source, *f* = 0: no protein derives from the certain source. Dashed horizontal line represents the mean stable nitrogen isotopic composition of German diets as calculated in the study.

4.2. Influence of protein choice

Dietary $\delta^{15}N$ values are reflective of the $\delta^{15}N$ values of the main protein source(s) in the diet. If a diet is rich in fish, $\delta^{15}N$ values are elevated, and a diet high in legumes will result in lower $\delta^{15}N$ values. Using the present data set of representative $\delta^{15}N$ values [17] as well as country nutrition statistics, the mean $\delta^{15}N$ value for the typical German diet would be around +4.3‰, which is consistent with our experimental results for the 24-h diets. Consequently, an increased proportion of protein sources with $\delta^{15}N$ values lower than +4.3‰ (especially legumes) would result in lower dietary $\delta^{15}N$ (Figure 3). On the contrary, an increased proportion of food items with $\delta^{15}N$ values higher than +4.3‰ (especially fish, but also beef, lamb, dairy and eggs) would result in higher dietary $\delta^{15}N$ values. Whereas changes in the proportion of pork or poultry protein in the diet will not change the overall dietary $\delta^{15}N$, a fact that has been recognized in an experimental study [24].

In principal "you are what we eat – (plus a few per mil)" [44], and despite physiological or pathophysiological metabolic conditions, human $\delta^{15}N$ values are strongly controlled by the dietary $\delta^{15}N$. There is no doubt that a high proportion of fish or sea mammals in the diet can be identified by $\delta^{15}N$ analysis of human hair [14, 19], nails [18] or blood [5, 7]. However, our results suggest that it is difficult to conclude by analysis of $\delta^{15}N$ of human tissue or excreta on the amount of dietary meat, as the influence of increased dietary meat proportion does not lead to such increased $\delta^{15}N$ values for a diet as it was shown for fish. Likewise,

differentiating between omnivores and ovo–lacto vegetarians is likely impossible based solely on $\delta^{15}N$, whereas $\delta^{15}N$ analysis can be used to identify individuals who consume a vegan diet or a diet rich in legumes. To summarize, there is need for more intervention studies looking at the influence of dietary or metabolic changes on human (or animal) $\delta^{15}N$ values, if the information apparently included in tissue or metabolite $\delta^{15}N$ values shall be used for answering archaeological, forensic, nutritional or physiological questions.

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Supplementary information

Calculation of the uncertainty of estimation of dietary δ^{15}N

The δ^{15}N of a diet is calculated by the proportional amount of protein in the different protein sources (P_1, P_2 …) relative total protein (P) multiplied by the different isotope ratios (δ_1, δ_2 …):

$$\delta_{\text{diet}} = (P_1\,\delta_1 + P_2\,\delta_2 + \ldots)/P \tag{1}$$

$$\Leftrightarrow \quad \delta_{\text{diet}} = \frac{P_1}{P}\delta_1 + \frac{P_2}{P}\delta_2 + \ldots \tag{2}$$

$$\Leftrightarrow \quad \delta_{\text{diet}} = \sum \frac{P_i}{P}\delta_i \tag{3}$$

The uncertainty of the diet (u_{diet}) is calculated using the uncertainty of the δ^{15}N value for each food item ($u_{\delta i}$) as well as of the mean uncertainty of the proportional protein content estimation (u_p) multiplied with the partial derivatives of δ_{diet}:

$$u_{diet} = \sqrt{\sum\left(\left(\frac{\partial \delta_{\text{diet}}}{\partial P_i}u_p\right)^2 + \left(\frac{\partial \delta_{\text{diet}}}{\partial \delta_i}u_{\delta_i}\right)^2\right)} \tag{4}$$

$$\Rightarrow \quad u_{diet} = \sqrt{\sum\left(\left(\frac{\delta_i}{P}u_p\right)^2 + \left(\frac{P_i}{P}u_{\delta_i}\right)^2\right)} \tag{5}$$

$$\Leftrightarrow \quad u_{diet} = \sqrt{\left(\frac{\delta_1}{P}u_p\right)^2 + \left(\frac{P_1}{P}u_{\delta_1}\right)^2 + \left(\frac{\delta_2}{P}u_p\right)^2 + \left(\frac{P_2}{P}u_{\delta_2}\right)^2 + \ldots} \tag{6}$$

$$\Leftrightarrow \quad u_{diet} = \sqrt{\left(\frac{\delta_1}{P}u_p\right)^2 + \left(\frac{\delta_2}{P}u_p\right)^2 + \ldots + \left(\frac{P_1}{P}u_{\delta_1}\right)^2 + \left(\frac{P_2}{P}u_{\delta_2}\right)^2 + \ldots} \tag{7}$$

If P and P_i are expressed as relative amounts it is $P = 1$ and thus P can be deleted leading to:

$$\Rightarrow \quad u_{diet} = \sqrt{(\delta_1 u_p)^2 + (\delta_2 u_p)^2 + \ldots + (P_1 u_{\delta_1})^2 + (P_2 u_{\delta_2})^2 + \ldots} \tag{8}$$

$$\Leftrightarrow \quad u_{diet} = \sqrt{(\delta_1^2 + \delta_2^2 + \ldots)u_P^2 + \left((P_1 u_{\delta_1})^2 + (P_2 u_{\delta_2})^2 + \ldots\right)} \tag{9}$$