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Short Communication

Distinguishing vegan-, vegetarian-, and omnivorous diets by hair isotopic analysis

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SUMMARY

Background: Dietary risks contribute heavily to disability-adjusted life years (DALYs), being more important than hypertension, obesity, or smoking. To measure dietary exposure remains a challenge in nutrition research.
Aims: The aim of the present study was to test the hypothesis that isotope ratios of ^{15}N and ^{13}C in human hair could distinguish between subjects adhering to different habitual diets.
Methods: 20 male and 29 female subjects average 31 years old (range 19–53), with stable dietary habits volunteered. Diets were vegan, vegetarian and omnivorous. Hair samples were processed on an elemental analyser coupled to isotope-ratio mass spectrometry.
Results: $\delta^{15}N$ differed between vegan, vegetarian and omnivorous diets, $p < 0.05$ for all. $\delta^{13}C$ differed between vegan and omnivorous diets, $p < 0.05$, but neither of these diets were separated from the vegetarian diet.
Conclusion: Elemental Analysis of $\delta^{13}C$ and especially $\delta^{15}N$ with isotope ratio mass spectrometry seems to be a promising, non-invasive and objective way to distinguish groups of subjects on different habitual diets, at least if n=>10.

1. Introduction

In 2015, a report demonstrated that dietary risks contributed to the largest proportion of disability-adjusted life years (DALYs), being more important than hypertension, obesity, or smoking [1].

The challenge to measure dietary exposure remains a limiting step and thus constitutes one urgent problem in nutrition research [2,3]. Unfortunately, commonly used subjective methods such as food frequency questionnaires (FFQ) and 24-h recalls are associated with substantial measurement errors [4]. Too few objective methods using quantitative biomarkers exist [5], limited to measuring total energy, protein, potassium and a few vitamins.

Hair-sampling is non-invasive and hair measured by isotope-ratio mass spectrometry (IRMS) is representative of the body protein pool [6], where hair closest to the scalp will represent the last month's intake. Thus, hair can possibly be used for longer-term dietary intake analysis.

Analysis of the stable isotopes carbon-13 (^{13}C) and nitrogen-15 (^{15}N) in human hair has been used to analyse historical diets [7], determine nutritional and metabolic status [6], and has been proposed to evaluate adherence to different diets and thus validate subjective dietary assessment methods [8].

The aim of the present study was to test the hypothesis that isotope ratios of ^{15}N and ^{13}C in human hair by IRMS could distinguish between subjects adhering to different habitual diets. The study is an add-in to a larger project to test if metabolomics by nuclear magnetic resonance spectroscopy (NMR-S) could distinguish between subjects adhering to different habitual diets.

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2. Methods

2.1. Study design and ethics

Initially three different habitual diets were specified; 1: vegan (strictly vegetarian, no food or drink of animal origin) 2: vegetarian (including dairy products and eggs), 3: mixed diet (omnivorous) (diet including fish and meat). Dietary habits are all self-reported, but subjects were interviewed, and they completed a 4-day diet record, which was checked before inclusion.

Subjects were recruited by advertisement at the Sahlgrenska Academy, University of Gothenburg, and through vegan- and vegetarian societies in Gothenburg. Inclusion criteria were age 18–65 years, body mass index (BMI) 18.0–30.0, apparently healthy and with normal clinical chemistry test results, including haemoglobin, CRP, glucose, electrolytes, liver enzymes and bilirubin, vitamin B12 and folate, thyroid status and plasma lipids. Exclusion criteria were regular medication (except anti-conception drugs), nicotine, dietary supplements, or herbal tea used less than 2 weeks before study, pregnancy, lactation, or vaccination less than one month before study.

For the study of hair, 20 male and 29 female subjects volunteered, average 31 years old (range 19–53), mean (BMI) 21.6 (range 18.0–28.9), with stable dietary habits for the last 2 months and without regular medication.

The regional ethics board in Gothenburg, Sweden approved the overall metabolomics project (561-12), including the present hair study (T 087-13). The project was registered with ClinicalTrials.gov (NCT020395596). All participants were informed about study protocol and signed an informed consent sheet.

2.2. Sample preparation and isotope-ratio mass spectrometry

Hair was collected by cutting ~5–10 hairs from the back of the head as close to the scalp as possible. The 2 cm hair closest to the scalp, i.e. corresponding to the last two months hair growth, was analysed. External contaminants such as lipids were removed from each hair sample by soaking in acetone for 30 min and sonicating twice in Milli-Q water. The samples were left 24 h to dry at 37 °C. Isotopic analysis of hair samples was conducted on an elemental analyser (EA) coupled to an IRMS (Sercon Ltd, Crewe, UK). For this purpose, the hair samples were individually packed in tin capsules prior to combustion. As internal standard, wheat flour was used and included after about every tenth sample. The standard has certified isotope values of $\delta^{15}N$ of 2.47 (±0.08) ‰ and $\delta^{13}C$ of −26.29 (±0.3) ‰).

2.3. Statistics

Results are presented as mean ± SD if not stated otherwise. Stable isotope ratios are expressed as parts per mil ‰ by the delta (δ) notation, relative to standards. Ambient Inhalable Reservoir (AIR) is standard for nitrogen and Vienna Pee Dee Belemite (VDPB) for carbon. Differences between diets were analysed by one-way ANOVA with Tukey post hoc correction. Calculations were performed in IBM SPSS version 24.

3. Results

The IRMS data from one female vegan subject was discarded because of technical errors. Five subjects adhered to a vegetarian diet including fish and were excluded due to low numbers. Clinical data and isotope ratios are presented in Table 1.

Table 1
Background data and stable isotope abundances in 43 subjects with different habitual diets.

	All	Vegan	Vegetarian	Omni-vorous
	n = 43	n = 16	n = 10	n = 17
Men/women	18/25	8/8	1/9	9/8
Age, year	31 ± 7	29 ± 6	31 ± 6	32 ± 8
BMI	22 ± 2	21 ± 2	22 ± 3	22 ± 2
$\delta^{15}N$ (dAIR)	7.3 ± 1.7	5.6 ± 1.2[a]	7.6 ± 1.0[b]	8.9 ± 0.9[c]
$\delta^{13}C$ (dVPDB)	−21.0 ± 0.5	−21.3 ± 0.4[a]	−21.0 ± 0.4	−20.9 ± 0.5[c]

Data presented as mean ± SD.
BMI = body mass index.
AIR = reference for nitrogen-15.
VPDB = reference for carbon −13.
a/b/c = columns with different letters differ (P < 0.05) by one-way ANOVA with Tukey post hoc correction.
$\delta^{15}N$ and $\delta^{13}C$ did not differ between male and female subjects, neither in the whole group nor in the vegan or omnivorous groups. $\delta^{15}N$ differed between vegan, vegetarian and omnivorous diets, p < 0.05 for all (Fig. 1).

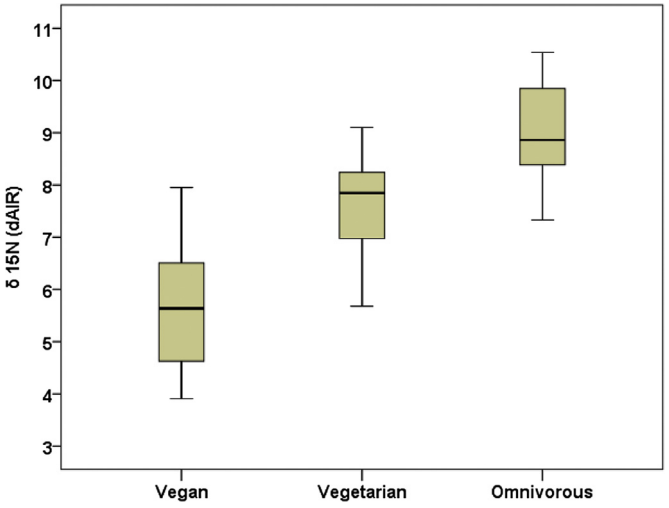

Fig. 1. Differences in δ ^{15}N (dAIR) by IRMS in 43 subjects on three different habitual diets: vegan (n = 16), vegetarian (n = 10), and omnivorous (n = 17). p < 0.05 between all diets by ANOVA with Tukey post hoc correction. $\delta^{13}C$ differed between vegan and omnivorous diets, p < 0.05, but neither of these diets were separated from the vegetarian diet (Fig. 2).

4. Discussion

We found elemental analysis by isotope-ratio mass spectrometry (IRMS) able to distinguish between vegan and omnivorous subjects by their hair $\delta^{13}C$ values, and to distinguish between vegan, vegetarian and omnivorous subjects by their $\delta^{15}N$ values.

The $\delta^{15}N$ values in omnivorous populations in Northern Europe have been reported in two studies to be on average 9.3 [9] and 9.2 respectively [10], indicating somewhat higher values than ours at 8.9. The average $\delta^{13}C$ values in omnivores in those studies were −21.1 [9] and −21.0 respectively [10], indicating very similar values as ours at −20.9.

Petzke et al. reported on hair isotope values to reflect protein intake in vegetarians and omnivores in Germany [8], with similar $\delta^{15}N$ values in vegetarians compared to our study (7.7 vs 7.6). In vegans $\delta^{15}N$ was 0.6‰ lower (5.6 vs 6.2), and in omnivorous subjects 1.0‰ lower (8.9) in Swedish vs (9.9) in German subjects. This value is higher than reported for the general population in Germany by others [9,10].

The $\delta^{13}C$ values in our vegan subjects were 0.4‰ lower while the values in our vegetarian subjects were 0.8‰ lower, and in omnivorous subjects 1.3‰ lower in Swedish subjects compared to German

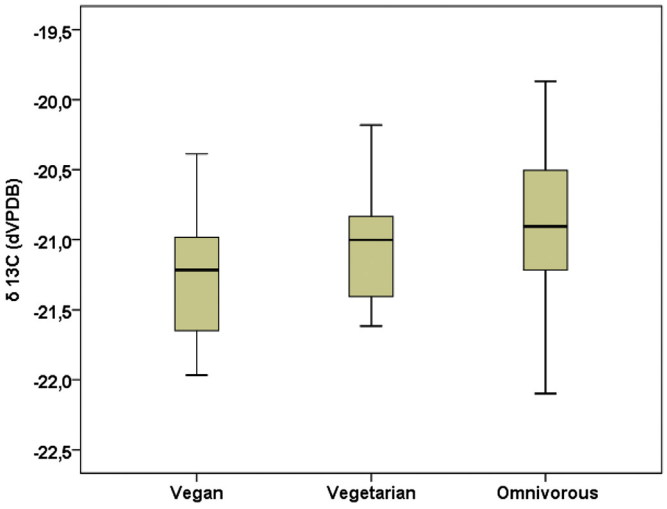

Fig. 2. Differences in δ ^{13}C (dVPDB) by IRMS in 43 subjects on three different habitual diets: vegan (n = 16), vegetarian (n = 10), and omnivorous (n = 17). $p < 0.05$ between vegan and omnivorous diets by ANOVA with Tukey post hoc correction.

subjects [8]. The differences in δ^{13}C values could in part be explained by more of the C4-crop corn, fed to livestock in Germany with a green maize production 576 times greater than in Sweden, while the number of cattle and pigs are only 8.7 and 18.6 times greater, where most livestock are brought up on C3 crops such as oats and barley (Eurostat; http://ec.europa.eu/eurostat/data/database).

Differences between the diet groups were similar in our study compared to those reported by Petzke [8], (2.0‰ δ^{15}N and 0.3‰ δ^{13}C) between Swedish vegan and vegetarian subjects and (1.5 and 0.7) in German subjects. Differences between vegetarian and omnivorous subjects were lower in Swedish (1.3‰ δ^{15}N and 0.1‰ δ^{13}C) compared to (2.2 and 0.6) in German subjects.

It was not possible to separate the vegetarians from either vegans or omnivores by the ^{13}C, although vegans and omnivores could be separated in our study. As vegetarian diets mix animal and vegetable foods, this would be expected to result in isotope values somewhere in between. With a guaranteed IRMS precision of 0.2‰ for ^{13}C, such small differences is not possible to measure with enough precision.

Using δ^{15}N, with better precision, these different diets were possible to distinguish, also in a small population, by a non-invasive, convenient and objective method. This implicates that hair isotopic analysis could be a useful addition to conventional dietary assessment methods.

4.1. Strengths and limitations

This work was an add-in study to test the possibility to distinguish groups with different habitual diets in addition to existing tools such as dietary surveys, conventional biomarkers such as urinary nitrogen excretion, and metabolomics. Thus, the number of subjects in each dietary group was unequal, as was the proportion between genders. Still, the number of vegan and vegetarian subjects were of the same magnitude as in previous studies on differences between diets by EA analysis [8–10].

The analyses did not point at any gender differences, which further improves the usefulness, since gender differences in serum analysis is common for many biomarkers and complicates the data analysis.

The guaranteed precision of the IRMS is 0.2‰ for ^{13}C. This implies that the δ^{13}C differences between the groups are within the instrumental uncertainty, thus these results have to be interpreted cautiously.

5. Conclusion

Elemental Analysis of δ^{13}C and especially δ^{15}N with isotope ratio mass spectrometry seems to be a promising, non-invasive and objective way to distinguish groups of subjects on different habitual diets, at least if n=>10.

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Contributors

LE: designed the study, analysed and interpreted the data, draughted & revised the manuscript, and approved the final version.

TA: initialized the study, analysed and interpreted the data, revised the manuscript and approved the final version.

TR: processed samples, analysed and interpreted the data, revised the manuscript and approved the final version.

PHJ: processed samples, revised the manuscript, and approved the final version.

HL: designed the study, interpreted the data, and approved the final version.

AW: designed the study, analysed and interpreted the data, and approved the final version.

Conflict of interest

All authors declare they have no conflict of interest.

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