Stable isotope ratios - nutritional biomarkers of long-term intake?

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Stable isotope ratios – nutritional biomarker and more

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The accurate assessment of dietary intake is one of the biggest challenges in nutrition research. While there have been considerable advances in the development of new methods (1,2), many of the fundamental problems remain – in particular the bias introduced by misreporting and the limitations of food composition data (3). Nutritional biomarkers can address many of these problems as they measure actual intake and do not rely on self-reporting or food composition data to estimate intake (4). However, there is still a paucity of biomarkers, especially biomarkers that have been evaluated in controlled dietary intervention studies with known intakes.

Many biomarkers are based directly on the compounds of interest, such as micronutrients or fatty acids (5), or their metabolites (6). The inter-individual variability in metabolism, and more importantly the huge variability in food composition, make them generally unsuitable to estimate intake of foods or dietary patterns. They are also unable to distinguish between different sources of the self-same compound, for example added sugars from intrinsic sugars or different sources of fatty acids.

Yun and colleagues (7) have investigated a very different type of nutritional biomarker – natural abundance stable isotope ratios – which can be used to estimate intake of foods or dietary patterns. They are well established in ecological and archaeological research, where they are used to

reconstruct diet and food-web patterns (8). While it is often assumed that different isotopes behave in the same way, the small differences in the masses of the nuclides, as well as differences in quadrupole and magnetic moment (9), can result in a discrimination between different isotopes (*isotope effect*). While this isotopic fractionation is usually small (measured in *per mille* differences from a defined standard), it can be measured very reliably in bulk material such as blood or urine, or in individual compounds. The isotopic composition reflects the history of a molecule (9): increasing trophic levels for example result in an enrichment of ^{15}N, and the differences in photosynthesis between C3 and C4 plants result in differences in the enrichment of ^{13}C (11). This has been used extensively in Archaeology, for example to show the transition from fishing to farming during the Neolithic in Europe (10), investigate long-term dietary trends (13) or the introduction of maize in North America (11).

Despite the common use of stable isotope ratios in Archaeology and Ecology, they have been scarcely used in nutrition and nutritional epidemiology, and only very few studies have investigated their suitability as nutritional biomarker. The most common application so far has been the identification of dietary patterns, in particular the intakes of animal-derived foods like meat and fish. Petzke and colleagues (12) have used samples from a German nutritional survey (VERA) to show that carbon and nitrogen stable isotope ratios ($\delta^{13}C$ and $\delta^{15}C$) can be used to identify the intake of animal derived food. O'Brien and colleagues (13) could demonstrate that $\delta^{15}N$ is a marker of fish and fish-derived fatty acid (EPA and DHA) intake and can therefore provide an alternative to laborious and expensive fatty acid analysis. In a small-scale feeding study, we could show that carbon and nitrogen stable isotope ratios can be used to distinguish between a vegan or vegetarian diet, and high meat or fish intake (14). However, many of these studies relied on extremes of intake and Hülsemann and colleagues showed that they are less sensitive to smaller changes in intake (15).

Another application is the use as biomarker of sugars intake, which is very difficult to assess from dietary data alone (16). In North America, the majority of sugars, especially in sugar-sweetened beverages (SSB), are derived from C4 plants, corn and sugar cane, whereas most other plants in the food supply are C3 plants. Foods containing sweeteners derived from corn or sugar cane therefore

have a distinct range of $\delta^{13}C$ (17) and their consumption affects serum (21), whole blood (18) and hair stable isotope ratios (19) sufficiently to identify consumers. This method has been refined by using $\delta^{13}C$ of glucose (20) and alanine (21) in blood, which is more specific for sugars and sugar-sweetened beverages.

There have been only few applications of stable isotope ratios to investigate associations between diet and health. In one study, Williams and O'Connell could show a positive association between $\delta^{15}N$ and cognition in patients with Alzheimer's disease (22). In a series of studies in the Yup'Ik population in Alaska, $\delta^{15}N$ was used as biomarker of marine food intake to investigate gene × diet interactions and DNA methylation patterns (8). In a case-cohort study of type 2 diabetes, Patel and colleagues (23) found positive associations between $\delta^{15}N$ and incident diabetes, but inverse associations for $\delta^{13}C$. In particular the results of the last study highlight the need for a better evaluation of stable isotope ratios as nutritional biomarkers, as they can not only be affected by diet but also other factors.

The study by Yun and colleagues (7) in this issue is therefore very topical and provides important data for the evaluation of stable isotope ratios as nutritional biomarkers, not only because of the study size, but in particular because of the study design. The evaluation of nutritional biomarkers requires reliable data of actual intake, which cannot be obtained from self-reported dietary data. Yet despite the limitations of self-reporting, many studies rely on it to estimate actual intake. However, without reliable data on actual intake, the evaluation of a candidate biomarker is not possible, and any observed associations are likely to be biased. In this study, like in previous studies in the Women's Health Initiative (5), participants were therefore provided with their habitual diet, ensuring that the actual diet consumed was known. This should be the standard study design for biomarker evaluation, as it is the only method to obtain reliable data. The results of this evaluation clearly show an association between $\delta^{15}N$ and the dietary intake of fish and seafood in a population with moderate intake, and of a combination of $\delta^{13}C$ and $\delta^{15}N$ with total animal protein intake. Interestingly, no associations between $\delta^{13}C$ and added sugars or SSB were found, presumably due to the low intake in the study population.

The results also highlight one of the main challenges of using stable isotope ratios as nutritional biomarkers. Isotopic fractionation does not end when the food is consumed, but continues (24), and there are considerable differences between different tissues and thus the samples used for analysis (19,25,26). Isotopic fractionation of nitrogen depends on the availability of dietary nitrogen, especially from protein, and can be affected by factors such as pregnancy (27), nutritional stress (28) and changes in body mass (8). Lipids are generally depleted in ^{13}C due to the preferences of enzymes in the biosynthetic pathways for ^{12}C (34), thus having a lower $\delta^{13}C$ than other tissues. The results of the study by Yun and colleagues (7), as well as data from the case-cohort study in EPIC Norfolk (23), show that other factors such as physical activity, BMI, smoking status and sex can also affect stable isotope ratios. They therefore not only provide information about dietary data, but also metabolic processes and subsequently the fate of dietary constituents.

The intricate nature of stable isotope ratios makes them a promising tool for future nutritional research. Yun and colleagues have demonstrated that the comparatively inexpensive bulk analysis of stable isotope ratios can be used as nutritional biomarker of fish/seafood and animal protein intake. Compound specific isotope ratio analysis can provide considerably more information: serum fatty acids reflect dietary fatty acid intake (35), and it is possible to distinguish between dairy and adipose fat of different animals using isotope ratio analysis of individual fatty acids (36). It is also possible to distinguish between endogenously and exogenously formed compounds, such as PUFAs (37), or to infer information on protein sources by determining the stable isotope ratios of individual amino acids (38). Many other methods have been developed for the analysis of specific compounds with applications in nutrition research, for example $\delta^{15}N$ and $\delta^{18}O$ of nitrate (39) to elucidate the role of exogenous and endogenous NO.

Stable isotope ratios have been used very successfully in other disciplines but have been underused in nutritional research for too long. The study by Yun and colleagues provides more evidence that this technique has an important place in nutritional research and should be used much more commonly.

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Notes

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