



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

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1 Stable isotope ratios – nutritional biomarker and more

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

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

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

28 reconstruct diet and food-web patterns (8). While it is often assumed that different isotopes behave in
29 the same way, the small differences in the masses of the nuclides, as well as differences in quadrupole
30 and magnetic moment (9), can result in a discrimination between different isotopes (*isotope effect*).
31 While this isotopic fractionation is usually small (measured in *per mille* differences from a defined
32 standard), it can be measured very reliably in bulk material such as blood or urine, or in individual
33 compounds. The isotopic composition reflects the history of a molecule (9): increasing trophic levels
34 for example result in an enrichment of ^{15}N , and the differences in photosynthesis between C3 and C4
35 plants result in differences in the enrichment of ^{13}C (11). This has been used extensively in
36 Archaeology, for example to show the transition from fishing to farming during the Neolithic in
37 Europe (10), investigate long-term dietary trends (13) or the introduction of maize in North America
38 (11).

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

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

55 have a distinct range of $\delta^{13}\text{C}$ (17) and their consumption affects serum (21), whole blood (18) and hair
56 stable isotope ratios (19) sufficiently to identify consumers. This method has been refined by using
57 $\delta^{13}\text{C}$ of glucose (20) and alanine (21) in blood, which is more specific for sugars and sugar-sweetened
58 beverages.

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

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

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

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

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

108

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