

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

Article

Accepted Version

Kuhnle, G. G. C. ORCID: https://orcid.org/0000-0002-8081-8931 (2019) Stable isotope ratios - nutritional biomarkers of long-term intake? American Journal of Clinical Nutrition, 110 (6). pp. 1265-1267. ISSN 0002-9165 doi: https://doi.org/10.1093/ajcn/nqz239 Available at https://centaur.reading.ac.uk/86359/

It is advisable to refer to the publisher's version if you intend to cite from the work. See <u>Guidance on citing</u>.

To link to this article DOI: http://dx.doi.org/10.1093/ajcn/nqz239

Publisher: American Society for Nutrition

All outputs in CentAUR are protected by Intellectual Property Rights law, including copyright law. Copyright and IPR is retained by the creators or other copyright holders. Terms and conditions for use of this material are defined in the <u>End User Agreement</u>.

www.reading.ac.uk/centaur

## CentAUR

Central Archive at the University of Reading



Reading's research outputs online

## 1 Stable isotope ratios – nutritional biomarker and more

3 Department of Food and Nutritional Sciences
4 University of Reading, Reading, UK
5 email address: <u>g.g.kuhnle@reading.ac.uk</u>
6
7 Sources of financial support: none
8 The author has no conflict of interest to disclose.
9
10
11
12 The automatic support of the prior of the prior

2

Gunter G. C. Kuhnle

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

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 <sup>15</sup>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 35 Archaeology, for example to show the transition from fishing to farming during the Neolithic in 36 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 scarcely used in nutrition and nutritional epidemiology, and only very few studies have investigated 40 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 ( $\delta^{13}$ C and  $\delta^{15}$ C) can be used to identify the intake of animal 45 derived food. O'Brien and colleagues (13) could demonstrate that  $\delta^{15}$ 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 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.

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 between  $\delta^{15}$ N and cognition in patients with Alzheimer's disease (22). In a series of studies in the 61 Yup'Ik population in Alaska,  $\delta^{15}$ N was used as biomarker of marine food intake to investigate gene  $\times$ 62 diet interactions and DNA methylation patterns (8). In a case-cohort study of type 2 diabetes, Patel 63 and colleagues (23) found positive associations between  $\delta^{15}N$  and incident diabetes, but inverse 64 65 associations for  $\delta^{13}$ 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  $\delta^{15}N$  and the dietary intake of fish and seafood in a population with moderate 79 intake, and of a combination of  $\delta^{13}$ C and  $\delta^{15}$ N with total animal protein intake. Interestingly, no 80 associations between  $\delta^{13}$ C and added sugars or SSB were found, presumably due to the low intake in 81 the study population.

3

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) and changes in body mass (8). Lipids are generally depleted in <sup>13</sup>C due to the preferences of enzymes 87 in the biosynthetic pathways for <sup>12</sup>C (34), thus having a lower  $\delta^{13}$ C than other tissues. The results of 88 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 applications in nutrition research, for example  $\delta^{15}N$  and  $\delta^{18}O$  of nitrate (39) to elucidate the role of 102 103 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

109	Acknowledgements	
110	The so	ole author had responsibility for all parts of the manuscript.
111	Notes	
112	The author has no conflict of interest to disclose.	
113	References	
114		
115		
116		
117 118	1.	Freedman LS, Schatzkin A, Midthune D, Kipnis V. Dealing With Dietary Measurement Error in Nutritional Cohort Studies. J Natl Cancer Inst. 2011;103:1086–92.
119 120 121	2.	Subar AF, Freedman LS, Tooze JA, Kirkpatrick SI, Boushey C, Neuhouser ML, Thompson FE, Potischman N, Guenther PM, Tarasuk V, et al. Addressing Current Criticism Regarding the Value of Self-Report Dietary Data. J Nutr. 2015;145:2639–45.
122 123	3.	Kuhnle GGC. Nutrition epidemiology of flavan-3-ols: The known unknowns. Mol Aspects Med. 2018;61:2–11.
124 125	4.	Jenab M, Slimani N, Bictash M, Ferrari P, Bingham SA. Biomarkers in nutritional epidemiology: applications, needs and new horizons. Hum Genet. 2009;125:507–25.
126 127 128	5.	Lampe JW, Huang Y, Neuhouser ML, Tinker LF, Song X, Schoeller DA, Kim S, Raftery D, Di C, Zheng C, et al. Dietary biomarker evaluation in a controlled feeding study in women from the Women's Health Initiative cohort. Am J Clin Nutr. 2017;105:466–75.
129 130 131	б.	Ottaviani JI, Fong R, Kimball J, Ensunsa JL, Britten A, Lucarelli D, Luben R, Grace PB, Mawson DH, Tym A, et al. Evaluation at scale of microbiome-derived metabolites as biomarker of flavan-3-ol intake in epidemiological studies. Sci Rep. 2018;8:9859.
132 133 134 135	7.	Yun HY, Lampe JW, Tinker LF, Neuhouser ML, Beresford SAA, Niles KR, Mossavar- Rahmani Y, Snetselaar LG, Horn LV, Prentice RL, et al. Serum nitrogen and carbon stable isotope ratios meet biomarker criteria for fish and animal protein intake in a controlled feeding study of a Women's Health Initiative cohort. Journal of Nutrition. 2018.
136 137	8.	O'Brien DM. Stable Isotope Ratios as Biomarkers of Diet for Health Research. Annual Review of Nutrition. 2015;35:565–94.
138	9.	Isotope Fractionation: Why Aren't We What We Eat? 1999;26:667-73.
139 140	10.	Richards MP, Schulting RJ, Hedges R. Archaeology: sharp shift in diet at onset of Neolithic. Nature. 2003.
141 142	11.	van der Merwe NJ, Vogel J. 13C content of human collagen as a measure of prehistoric diet in woodland North America. Nature. 1978;276:815–6.

143 12. Petzke KJ, Boeing H, Klaus S, Metges CC. Carbon and nitrogen stable isotopic composition of 144 hair protein and amino acids can be used as biomarkers for animal-derived dietary protein 145 intake in humans. J Nutr. 2005;135:1515-20. 146 13. O'brien DM, Kristal AR, Jeannet MA, Wilkinson MJ, Bersamin A, Luick B. Red blood cell 147 delta15N: a novel biomarker of dietary eicosapentaenoic acid and docosahexaenoic acid 148 intake. 2009 ed. 2009;89:913-9. 149 14. Kuhnle GGC, Joosen AMCP, Kneale CJ, O'Connell TC. Carbon and nitrogen isotopic ratios of urine and faeces as novel nutritional biomarkers of meat and fish intake. Eur J Nutr. 2012 ed. 150 Springer-Verlag; 2013;52:389–95. 151 152 15. Huelsemann F, Flenker U, Koehler K, Schaenzer W. Effect of a controlled dietary change on 153 carbon and nitrogen stable isotope ratios of human hair. 2009;23:2448-54. Tasevska N. Urinary Sugars—A Biomarker of Total Sugars Intake. Nutrients. 154 16. 155 Multidisciplinary Digital Publishing Institute; 2015;7:5816–33. 156 17. Jahren AH, Saudek C, Yeung EH, Kao WH, Kraft RA, Caballero B. An isotopic method for 157 quantifying sweeteners derived from corn and sugar cane. Am J Clin Nutr. 2006;84:1380-4. 158 18. Davy BM, Jahren AH, Hedrick VE, Comber DL. Association of  $\delta^{13}$ C in fingerstick blood with 159 added-sugar and sugar-sweetened beverage intake. J Am Diet Assoc. 2011;111:874-8. 160 19. Nash SH, Kristal AR, Hopkins SE, Boyer BB, O'Brien DM. Stable Isotope Models of Sugar Intake Using Hair, Red Blood Cells, and Plasma, but Not Fasting Plasma Glucose, Predict 161 162 Sugar Intake in a Yup'ik Study Population. Journal of Nutrition. 2014;144:75-80. 20. 163 Cook CM, Alvig AL, Liu YQ, Schoeller DA. The Natural 13C Abundance of Plasma Glucose 164 Is a Useful Biomarker of Recent Dietary Caloric Sweetener Intake. Journal of Nutrition. 165 2010;140:333-7. 166 21. Kristal AR, Chov K, Nash SH, Hopkins S, Boyer BB, O'Brien DM. The carbon isotope ratio of alanine in red blood cells is a new candidate biomarker of sugar-sweetened beverage intake. 167 168 Journal of Nutrition. American Society for Nutrition; 2013;143:878-84. 22. Williams JH, O'Connell TC. Differential relations between cognition and 15N isotopic content 169 170 of hair in elderly people with dementia and controls. J Gerontol A Biol Sci Med Sci. 171 2002;57:M797-802. Patel PS, Cooper AJM, O'Connell TC, Kuhnle GGC, Kneale CK, Mulligan AM, Luben RN, 172 23. 173 Brage S, Khaw K-T, Wareham NJ, et al. Serum carbon and nitrogen stable isotopes as 174 potential biomarkers of dietary intake and their relation with incident type 2 diabetes: the 175 EPIC-Norfolk study. 2014;100:708-18. O'Connell TC, Kneale CJ, Tasevska N, Kuhnle GG. The diet-body offset in human nitrogen 176 24. 177 isotopic values: a controlled dietary study. American Journal of Physical Anthropology. 178 2012;149:426-34. 179 25. Kraft RA, Jahren AH, Saudek CD. Clinical-scale investigation of stable isotopes in human blood:  $\delta 13C$  and  $\delta 15N$  from 406 patients at the Johns Hopkins Medical Institutions. 180 181 2008;22:3683-92. Nash SH, Kristal AR, Boyer BB, King IB, Metzgar JS, O'Brien DM. Relation between stable 182 26. 183 isotope ratios in human red blood cells and hair: implications for using the nitrogen isotope

- ratio of hair as a biomarker of eicosapentaenoic acid and docosahexaenoic acid. American
  Society for Nutrition; 2009;90:1642–7.
- 186 27. O'Connell TC. Nitrogen balance and δ15N: why you're not what you eat during pregnancy.
   187 2004;18:2889–96.
- 188 28. O'Connell TC. Nitrogen balance and  $\delta 15$ N: why you're not what you eat during nutritional stress. 2005;19:2497–506.

190