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**Commentary**

**See corresponding article on page 2764.**

# Rough Diamond: A Carbon Isotopic Biomarker of Added Sugar Intake

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What did I eat last month? Even as I write this in the monotony of Covid-19-lockdown, I am hard-pressed to recall accurately and quantitatively what I ate last week, let alone last month. Or last year. Yet so much of nutritional epidemiology relies on accurately answering the initial question for both individuals and cohorts. The inherent biases of recall methods and FFQs have driven a quest for objective biomarkers of nutritional exposure. A good biomarker gives an accurate and precise indication of the target intake, be that of macronutrients, energy, or particular foods, ideally with a useful exposure chronology.

In this issue of *The Journal of Nutrition*, Yun et al. (1) demonstrate that carbon isotope ratios of the amino acid alanine in blood serum ($\delta^{13}C_{alanine}$) correlate with added sugar intake in a controlled feeding study of postmenopausal women in the United States. Their study builds on work by the authors and others over the last decade documenting the correlation between consumers' carbon isotope ratios and the sugar that they eat (2–6). In such work, there has been iterative progress in the strength of the relation between target variable and proxy measure (from hair to blood to RBC to amino acid), moving from observational to mechanistic, from correlation to causation.

Consumption of added sugars and sugar-sweetened beverages has been linked with a range of chronic diseases that require public health interventions as well as clinical treatments. However, accurately quantifying sugar intake is difficult (7). The relation Yun et al. find between added sugar intake, serum $\delta^{13}C_{alanine}$, and participant characteristics was comparable to the performance of well-established recovery biomarkers in the same cohort (8), offering the potential of a biomarker of long-term added sugar intake in free-living populations whose sugar is derived from cane and not beet.

Like doubly labeled water (DLW), the "gold-standard" biomarker for energy intake, the biomarker measured here is an isotope ratio: the relative proportion of the rarer heavy version of an element compared with the more common lighter form. Stable isotope ratios are ideal biomarkers in many ways: the ratios are intrinsic at the atomic (elemental) level, so the patterning can be tracked as molecules transformed through metabolic processes; the consumer is unaffected by the mass differences between isotopes—it makes no difference to them what their isotope ratio is; and the isotopes (and ratios) are stable, thus their use raises no concern about radioactive harm and they can be retrospectively measured in archived samples (9).

Unlike DLW, the ratio measured here is at "natural abundance"—the signal is derived from isotopic discrimination that occurs during physical, chemical, and biochemical processes in the biosphere, rather than an artificially enriched ratio with the heavy isotope at a higher concentration than normal. DLW and other isotopic studies in physiology frequently use a labeled isotopic marker as a *tracer*, following it through metabolic processes—an approach with a long and distinguished history in the study of dynamic biological systems (10). In the work by Yun et al., carbon isotope ratios at natural abundance are used rather as a *signal* representative of integrated dietary intake over the longer term, an approach common in ecology and archeology (11). Such longer-term information is valuable in assessing people's quotidian diet, smoothing out short-term dietary variation, but is typically beyond the scope of most nutritional biomarkers.

There are inherent concerns of sensitivity and specificity in this approach. The naturally occurring carbon isotopic range in global foodstuffs is small (*c*.25‰), giving little room for maneuver. The measured signal is not specific to the target foodstuff (sugar) but is simultaneously broader (all plants that use the $C_4$ photosynthetic pathway) and narrower (not all sugar is $C_4$: cane sugar is, but sugar beet uses $C_3$ photosynthesis and thus has a significantly lower carbon isotope ratio). Furthermore, the technique is analytically complex and time-consuming. I see the potential of this approach as worth the effort, but to my mind there are 2 prevailing challenges.

The first challenge concerns our understanding of the causal mechanism. The use of $\delta^{13}C_{alanine}$ as a biomarker passes the test of biochemical plausibility (12), because $C_4$ sugars have a distinctive carbon isotope ratio, and serum alanine is linked to glucose via the glucose–alanine cycle. But there is no straightforward one-to-one mapping of the proxy ($\delta^{13}C_{alanine}$) to the target variable (added sugar) because the relation has proximal and distal causes, and there are varying degrees of "fuzziness," or fidelity, along the chain of inference.

The distal cause of the biomarker signal—plant carbon isotope ratios reflecting the photosynthetic pathway—is well understood at a mechanistic level (13). The proximal cause—the link between the carbon isotope ratios of glucose and alanine—is less well constrained. Around 40% of the alanine in blood derives from glucose in the postabsorptive state, but this proportion may vary considerably with other factors

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(e.g., obesity, growth, fasting, dietary composition) (14), which hints at why $\delta^{13}C_{alanine}$ captures only 37% of the added sugar intake. It would be interesting to explore a multivariate amino acid approach (15) because we know that nontrivial proportions of other amino acids are derived from glucose (*c.*13% of glutamine). Alanine carbon isotope ratios in blood serum are an integration of the myriad metabolic processes that occur between food consumption and amino acid synthesis, and clearly more experimental work is needed here.

The second challenge concerns the process of validation. The validity of any potential nutritional biomarker is traditionally determined by comparison with the current best reference method that provides a good measure of the true exposure (12). Yun et al. demonstrate a good correlation between $\delta^{13}C_{alanine}$ and known added sugar intake over a fortnight's controlled feeding. A significant potential advantage of $\delta^{13}C_{alanine}$ as a biomarker is in estimating habitual added sugar intake because of the biochemical and isotopic half-life of blood serum (6). Yet because there is no other longer-term accepted method of gauging sugar intake, they have little choice but to validate their approach by comparison with a shorter-term measure. I see this as an unsatisfactory compromise. This is not a criticism of the authors who provide the best justification for this within their power, including only those study participants deemed to be in isotopic equilibrium with their controlled diet. But a mismatch between the exposure chronology recorded in new biomarkers and accepted reference methods is a challenge for the field as a whole. One should not confuse analytical and biological validity. Unless a way to reconcile this tension can be found, the process of validating long-term biomarkers may not be meaningful.

I welcome this article, as someone who has been perplexed by the slow take-up of isotopic biomarkers in nutrition, in contrast to other research fields focused on dietary intake. Yun et al.'s work is an exciting step in the development of a long-term biomarker of added sugar intake. The method is something of a rough diamond at the moment, and it requires some polishing. Then we must find appropriate and robust ways to judge its worth, not by comparison to fundamentally different measures, "gold-standard" or not.


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## References


1. Yun HY, Tinker LF, Neuhouser ML, Schoeller DA, Mossavar-Rahmani Y, Snetselaar LG, Van Horn LV, Eaton CB, Prentice RL, Lampe JW, et al. The carbon isotope ratios of serum amino acids in combination with participant characteristics can be used to estimate added sugar intake in a controlled feeding study of US postmenopausal women. J Nutr 2020;150(10):2764–71.
2. Cook CM, Alvig AL, Liu YQ, Schoeller DA. The natural $^{13}C$ abundance of plasma glucose is a useful biomarker of recent dietary caloric sweetener intake. J Nutr 2009;140:333–7.
3. Fakhouri THI, Jahren AH, Appel LJ, Chen L, Alavi R, Anderson CAM. Serum carbon isotope values change in adults in response to changes in sugar-sweetened beverage intake. J Nutr 2014;144:902–5.
4. Nash SH, Kristal AR, Bersamin A, Hopkins SE, Boyer BB, O'Brien DM. Carbon and nitrogen stable isotope ratios predict intake of sweeteners in a Yup'ik study population. J Nutr 2013;143:161–5.
5. Choy K, Nash SH, Kristal AR, 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. J Nutr 2013;143:878–84.
6. Votruba SB, Shaw PA, Oh EJ, Venti CA, Bonfiglio S, Krakoff J, O'Brien DM. Associations of plasma, RBCs, and hair carbon and nitrogen isotope ratios with fish, meat, and sugar-sweetened beverage intake in a 12-wk inpatient feeding study. Am J Clin Nutr 2019;110:1306–15.
7. Tasevska N. Urinary sugars—a biomarker of total sugars intake. Nutrients 2015;7:5816–33.
8. 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.
9. O'Brien DM. Stable isotope ratios as biomarkers of diet for health research. Annu Rev Nutr 2015;35:565–94.
10. Schoenheimer R, Rittenberg D. The application of isotopes to the study of intermediary metabolism. Science 1938;87:221–6.
11. Boecklen WJ, Yarnes CT, Cook BA, James AC. On the use of stable isotopes in trophic ecology. Annu Rev Ecol Evol Syst 2011;42:411–40.
12. Dragsted LO, Gao Q, Scalbert A, Vergères G, Kolehmainen M, Manach C, Brennan L, Afman LA, Wishart DS, Andres Lacueva C, et al. Validation of biomarkers of food intake—critical assessment of candidate biomarkers. Genes Nutr 2018;13:14.
13. Cernusak LA, Ubierna N, Winter K, Holtum JA, Marshall JD, Farquhar GD. Environmental and physiological determinants of carbon isotope discrimination in terrestrial plants. New Phytol 2013;200:950–65.
14. Perriello G, Jorde R, Nurjhan N, Stumvoll M, Dailey G, Jenssen T, Bier DM, Gerich JE. Estimation of glucose-alanine-lactate-glutamine cycles in postabsorptive humans: role of skeletal muscle. Am J Physiol 1995;269:E443–E50.
15. Larsen T, Ventura M, Andersen N, O'Brien DM, Piatkowski U, McCarthy MD. Tracing carbon sources through aquatic and terrestrial food webs using amino acid stable isotope fingerprinting. PLoS One 2013;8:e73441.