A Novel Carbon Isotope Biomarker for Dietary Sugar

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Abstract

Light element isotope signatures have been used to study food webs in many ecological niches, including those of ancient humans, but to a far lesser extent in modern humans. A recent paper presented results from a pilot study testing the utility of carbon isotope ratio analysis of alanine to measure an individual’s sugar intake in the United States. A strong correlation was found between the enrichment of $^{13}$C in alanine and sugar-sweetened beverage consumption. This candidate biomarker of sugar consumption deserves further consideration as an objective marker for use in the study of the relationship between sugar intake and health. J. Nutr. 143: 763–765, 2013.

“Does she or doesn’t she?” was a question asked in a hair color advertisement some 50 y ago when Clairol introduced a line of home hair coloring products that were advertised as virtually undetectable by visual inspection. So it is with the human diet. With the exception of nutrient deficiencies, little can be determined about the consumption of a specific food from outward appearances of the human body. Despite this difficulty, there has been scientific interest in the relationship between diet and health for more than a millennium. The science of dietary analysis, as performed today, however, has a more recent history. Some of this history fell into my hands when I moved into an office previously occupied by Dr. Dorothy Pringle. In the back of a filing cabinet, I found the Proceedings of the Conference on Methods for Evaluating Nutritional Status of Mothers, Infants and Children held in Detroit on February 21–22, 1947. In the introduction, Chairwomen Icie Macy-Hoobler wrote that the Research Laboratory of Children’s Fund of Michigan had demonstrated that eating the right foods could improve both maternal and infant outcomes, but that their methods of “making, laboriously, meticulous collections of foods and analyzing dietary composites” was slow and limited their research endeavors. This conference gathered 21 U.S. scientists for the purpose of evaluating and standardizing “short-cut” methods for dietary analysis that research groups were beginning to use in large-scale studies. This short-cut involved the use of self-report surveys and food composition tables that have been the prime tools used in the development of the current large literature base on diet and disease during the past 60 y.

During that technology conference held in Detroit, which may have been the first dietary methods meeting focused on dietary survey methods, attendees discussed the utilization of the short-cut methods, and there was confidence that the methods were accurate but that precision was lost compared with duplicate diet tray analysis. In the decades since that technology conference, scientific attention, especially in the US and other industrialized countries, has moved away from emphasis on nutrient deficiencies to one focusing on nutritional excesses. In doing so, investigators began to compare self-reported dietary energy intake with measured energy expenditure. Unfortunately, it was found, repeatedly, that the survey data on energy intake were not only imprecise but also inaccurate. Self-reported survey methods consistently underestimated weight maintenance energy requirements based on subjectively measured energy expenditure and that negative, or under-reporting, bias increased with increasing obesity (1). Thus, it became clear to many that these short-cut survey instruments could not be used to investigate the role of total energy intake in the development and maintenance of the obese state (2).

Survey instruments have not, however, lost favor, but are instead continuing to be employed in the study of the role of diet in obesity. In most cases, attention has been directed away from the problematic assessment of total energy intake toward studies of the role of dietary patterns, specific nutrients, or specific foods in obesity (3,4). Perhaps the largest current debate is whether sugar or high fructose corn syrup consumption is a major contributor to obesity, for which evidence is mixed (5,6). Like energy intake, however, there is a need for a dietary biomarker to assess self-reported dietary intake that might cut through the controversies.

In a paper in this issue, Choy et al. (7) ask “Does s/he or doesn’t s/he consume sugar or high fructose corn syrup?” They do so using an objective stable isotope method. These authors pilot tested a sugar intake biomarker that takes advantage of a well-known difference in the natural abundance of $^{13}$C that occurs during photosynthesis. Most food crops utilize the Calvin-Benson or $C_3$ photosynthetic route to fix carbon (8). During this process, CO$_2$ and ribulose-bisphosphate are converted to 2 molecules of 3-phosphoglycerate, a reaction catalyzed by ribulose-1,5- bisphosphate carboxylase oxygenase. This route of carbon fixation discriminations against $^{13}$C compared with $^{12}$C and thus the resulting plant mass carbon will have a lower $^{13}$C:$^{12}$C ratio

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than the atmospheric carbon from which it is derived. The difference is small, as the fixed carbon will have a $^{13}$C/$^{12}$C ratio of $-0.01096$ compared with $0.01115$ for atmospheric CO$_2$. Not all plants, however, fix carbon via the Calvin pathway. About 5% of plants fix carbon via the Hatch-Slack pathway. This pathway is almost nondiscriminating against $^{13}$C, and the resulting plant mass will have a $^{13}$C/$^{12}$C ratio of $-0.01112$. These differences are so small that one might think they cannot be measured; however, they are easily measured using isotope ratio MS or cavity ring-down carbon spectroscopy. The expression of these as ratios, however, is quite cumbersome, so they are instead expressed in del per mil units ($\delta^{13}$C) relative to a blemnite limestone from Pee Dee County, South Carolina, where:

$$\delta^{13}$C = \left[ \frac{^{13}$C/^{12}$C_{sample}}{^{13}$C/^{12}$C_{PDB}} - 1 \right] \times 1000,$$

which leads to the values of $-8^\circ/o$ for atmospheric CO$_2$, $-25^\circ/o$ for C$_3$ plant mass, and $-10^\circ/o$ for C$_4$ plant mass.

These carbon isotope abundances are generally robust and thus provide an isotopic signature of the photosynthetic route through which the carbon was fixed. These signatures have been used by ecologists, zoologists, and entomologists, among others to study food webs, because the signatures are largely preserved as carbon is assimilated, metabolized, and stored. They provide information about which species is eating which and in what proportions, information that is difficult or impossible to obtain by other means (9). The use of stable isotope signatures has even provided information about the diet of Ozi (aka The Iceman), the 5000-y-old natural mummy found in the Alps in 1991 (10).

Moving to a contemporary setting, Choy et al. (7), took advantage of this carbon isotope signature to demonstrate that they could detect and measure the quantity of caloric sweeteners in the population of several villages in Alaska. They could do so because there are only 2 major C$_4$ crops in the U.S. agricultural system. These are sugar cane and corn, the sources for most caloric sweeteners. In addition, sorghum and millet are also C$_4$ production crops but are minor sources of carbon mass in the U.S. diet. The other U.S. crops that contribute to the human food chain are C$_3$ plants. Others have attempted to use these isotopic signatures to measure caloric sweetener intake but with less success. This potential has been noted by others who proposed the use of total blood protein, but the carbon signature signal was quite small (<1$/oo$), presumably because of extensive dilution from C$_3$ carbon in amino acids in the diet (11). We also noted this opportunity provided by the carbon isotope signature and proposed using plasma glucose to preserve the isotopic signature, but the contribution of de novo gluconeogenesis obscured the signal except for the period shortly after a meal (12).

Given the weak outcomes of these prior attempts at using the carbon isotope signature, the work of Choy et al. (8) represents a breakthrough in the study of dietary sugar consumption. They identified a molecule with far better qualities for use as a biomarker for measurement of caloric sweeteners than did others. They isolated alanine from the protein of either hair or RBCs. Alanine, a dispensable amino acid, can be synthesized from other carbon moieties, of which pyruvate is the major pathway, and hence much of the carbon for synthesis comes from carbohydrate. Circulating alanine has a high rate of turnover and thus the carbon backbone is unlikely to be preserved from dietary alanine but rather largely replaced by synthesis in vivo. Finally, because it is recovered from protein, it represents an integrated sample over days to a few weeks (hair collected from near the skin) or a few months for RBC protein. These qualities of alanine create an exciting potential tool for objectively measuring intake of sugar and high fructose corn syrup. The tool is not without drawbacks. Carbon isotope methodology is not a common methodology, but it should prove to be a benchmark against which other estimates of caloric sweetener intake can be validated in order to determine the accuracy and precision of those estimates or as a critical biomarker in well-funded intervention studies. This should be a major step toward resolving the controversy over the role of caloric sweetener intake in the development of obesity.

In addition to the methodological limitation, there are several other issues yet be resolved in validating the alanine carbon isotope abundance biomarker. For one, the carbon signature is not absolutely specific for dietary sugar. Rather it is specific for C$_4$ macronutrients that are metabolized to pyruvate. In the US, these are mostly cane sugar and high fructose corn syrup, but direct consumption of corn will probably also be detected as a caloric sweetener. This could introduce artifacts during Midwestern sweet corn season or in individuals who regularly consume corn tortillas or other corn-derived snack foods. Outside the US, cultures that obtain large portions of their dietary energy from millet will also be subject to overestimating caloric sweetener consumption using the isotopic biomarker, because millet is also a C$_4$ plant. Thus, it will be important to further test this biomarker for artifacts from other C$_4$ sources such as corn-fed meat consumption as well as the influence of high C$_4$ carbohydrate consumption. In addition, the carbon isotope abundance of alanine should be that of circulating glucose, which in turn should be a function of the ratio of added C$_4$ sugars to total dietary carbohydrate (C$_4$ in that population). The more C$_3$ sources in total carbohydrate, the more dilution of the added sugar in plasma glucose and the lower the C$^{13}$ abundance. To their credit, the authors did test for these potential artifacts and the results were very favorable. The population they studied, however, was one that had a high traditional diet consumption that was low in carbohydrate and corn-fed meats and thus would have been subject to a smaller artifact than many other populations. As such, the potential influences of artifacts from corn-fed meats and dilution from C$_4$ carbohydrate have been tested only under favorable conditions. Additional pilot studies are suggested in different population groups to test the general applicability of the carbon isotope biomarker in other U.S. populations.

In addition, the candidate biomarker is subject to negative artifacts from beet sugar. Beet sugar is the other of the 2 major sources of table sugar in the world. The sugar beet is a C$_3$ plant and thus, unlike cane sugar, beet sugar is not enriched in $^{13}$C. It would go undetected as a caloric sweetener using this isotopic biomarker. USDA estimates are that one-fourth of caloric sweeteners consumed in the US are derived from beet sugar and therefore the isotopic biomarker marker will slightly underestimate caloric sweeteners in the US; but in European counties and Japan, which rely on beet sugar, the error could be large. Less problematic in most populations is the consumption of honey (generally mostly C$_4$), maple syrup (C$_4$), or other minor sources of caloric C$_3$, which are minor (<2%).

An additional limitation of the pilot study presented by Choy et al. (7) is that findings were validated against self-reported dietary intake. As discussed above, self-report is thought to be less precise than the isotopic method. As such, additional validations are needed to fully understand the advantages and disadvantages of this method so that its use can be expanded. It is suggested that such validations include controlled feeding
studies. The results of this pilot study, however, were highly encouraging and justify further development and application of this isotopic method.

The work of Choy et al. (7) represents an exciting breakthrough in the development of a biomarker for caloric sweetener intake over a period of time that is sufficient to represent habitual intake. The use of this biomarker should be invaluable in research attempting to end the current controversies regarding the relationship between glucose and/or fructose consumption and obesity and will hopefully bring clarity to this field of investigation.

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Literature Cited


