



Original Contribution

Evaluation of a Novel Isotope Biomarker for Dietary Consumption of Sweets

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Carbon isotopic signatures (“ $\delta^{13}\text{C}$ ”) might reflect consumption of corn- and cane-based sweeteners. The authors hypothesized that the $\delta^{13}\text{C}$ value of human serum is higher for individuals with high versus low intakes of corn- and cane-based sweeteners (measured as sweetened beverage intake). They conducted a cross-sectional study within the Atherosclerosis Risk in Communities Magnetic Resonance Imaging study (Maryland, 2005–2006). Diet was assessed by food frequency questionnaire, and blinded serum samples were assayed by natural abundance stable isotope mass spectroscopy. Studied were 186 participants (53% male; mean age, 71 years; mean body mass index, 30 kg/m²). Serum $\delta^{13}\text{C}$ values for individuals with high sweetened beverage intakes were significantly higher than for those with low intakes (–19.15‰ vs. –19.47‰, $P < 0.001$). Serum $\delta^{13}\text{C}$ value increased 0.20‰ for every serving/day of sweetened beverages ($P < 0.01$). The association between sweetened beverages and serum $\delta^{13}\text{C}$ value remained significant after adjustment for confounding by corn-based product intake ($P < 0.001$). Serum $\delta^{13}\text{C}$ values were also associated with waist circumference, body mass index, and waist-to-hip ratio. This study provides the first known evidence that the $\delta^{13}\text{C}$ value of human serum differs between persons consuming low and high amounts of sweets. Within the proper framework, serum $\delta^{13}\text{C}$ value could be developed into an objective biomarker promoting more reliable assessment of dietary sweets intake.

biological markers; body mass index; diet; isotope labeling; sweetening agents

Abbreviations: BMI, body mass index; FFQ, food frequency questionnaire; $\delta^{13}\text{C}$, ratio of isotope carbon 13 (¹³C) to isotope carbon 12 (¹²C).

Dietary assessment is a central feature of many epidemiologic studies. A strong temporal relation has been demonstrated between national trends in increased consumption of corn-based sweeteners (i.e., high fructose corn syrup) and type 2 diabetes (1) and obesity (2). These and other epidemiologic assessments are limited in their ability to evaluate the effects of foods with corn-based sweeteners because of measurement error in self-reported dietary assessment techniques and limited information in food composition databases about the amount of these sweeteners present in foods and beverages (3, 4). A biomarker that objectively reflects intake of sweets would allow for more accurate evaluation of whether corn-based sweeteners affect health, and whether interventions to reduce intake of sweeteners are effective.

The naturally abundant isotope carbon 13 (¹³C) is a potential biomarker of dietary sweets intake. Both *zea* (corn) and *saccharum* (cane) species are known as C4 plants because they use the enzyme phosphoenolpyruvate carboxylase during photosynthesis (this enzyme is not used by the more common C3 plants) (5). Phosphoenolpyruvate carboxylase imparts an enrichment in ¹³C relative to isotope carbon 12 (¹²C) in C4 plant tissues (6), leading us to hypothesize that a diet rich in sweetened foods could be detected as a high ¹³C:¹²C ratio within human tissues (7). Many studies have quantified the carbon isotope composition (ratio of ¹³C to ¹²C, denoted as the $\delta^{13}\text{C}$ value) of tissues of animals with a known diet (8–14) and upon a change in animal diet (15–24). However, although surveys of the carbon and

nitrogen isotope composition of human blood (25–29), serum retinol (30), and other human tissues (31–38) are available, we know of no studies that specifically quantify a correlation between human sweetener consumption and serum $\delta^{13}\text{C}$ value.

This study tested the hypothesis that the $\delta^{13}\text{C}$ value of human serum reflects intake of food products containing corn- and cane-based sweeteners (measured as sweetened beverage intake). The secondary aim of the study was to assess associations of serum $\delta^{13}\text{C}$ value with other corn-related dietary components and anthropometric measures.

MATERIALS AND METHODS

Study population

The study population consisted of Atherosclerosis Risk in Communities (ARIC) Magnetic Resonance Imaging study participants, aged 60–80 years, who lived in Washington County, Maryland. All participants were Caucasian. Each had dietary data and serum collected during their 2005/2006 study visit. Of the total 573 Washington County participants, we excluded those who reported an implausible caloric intake (i.e., $<1,000$ kcal/day ($n = 88$) or $>4,000$ kcal/day ($n = 4$)), those with diagnosed diabetes who may have had dietary restrictions ($n = 89$), and those for whom the amount of serum archived was insufficient for analysis ($n = 14$). Of the remaining 378 individuals, participants were selected for either high or low sweetened beverage consumption according to their responses to food frequency questionnaire (FFQ) items. We sampled all participants who responded that they never drink sweetened beverages ($n = 42$) and those who responded that they drink 5 or more servings per week ($n = 60$). We assayed 84 additional samples from a moderate consumption category (1–4 servings/week). Within the moderate consumption category, we sampled all individuals from quintiles 1 and 2 of total sugar intake as well as all individuals from quintiles 4 and 5 of total sugar intake. The final sample size was 186 participants.

Assessment of demographic and anthropometric factors

Methods of collecting data for the Atherosclerosis Risk in Communities study have been previously described (39). Age, gender, height, weight, hip circumference, and waist circumference were measured during the clinic visit by trained personnel using a standardized protocol. Both waist and hip circumferences were measured with a tape measure to the nearest centimeter (rounding down). Hip circumference was taken at the maximal protrusion of the gluteal muscles and waist circumference at the level of the umbilicus. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared.

Assessment of sweetened beverage consumption

Complete dietary intake was measured using the previously validated Willett semiquantitative FFQ (40). Partici-

pants received the FFQ in the mail for self-administration prior to the clinical examination. At the study visit, clinic staff reviewed participant responses and queried participants if the questionnaire was incomplete.

Because sweetened beverages contribute the largest share of corn-based sweeteners (i.e., high fructose corn syrup) intake (2, 41), we used sweetened beverage consumption, characterized by FFQ, as a proxy for corn-based sweeteners intake. The following 4 FFQ items about sweetened beverages were used: 1) “Coke, Pepsi, or other soda with sugar”; 2) “caffeine-free Coke, Pepsi, or other cola with sugar”; 3) “other carbonated beverages with sugar (e.g., 7-Up)”; and 4) “Hawaiian Punch, lemonade, or other noncarbonated fruit drinks.” The frequency and amount of each type of sweetened beverage were determined. For frequency, there were 9 possible responses: never, <1 /month, 1–3/month, 2–4/week, 5–6/week, 1/day, 2–3/day, 4–5/day, ≥ 6 /day. Amounts were given as one glass or one bottle/can. Total servings per day were derived from responses to all 4 items summed for each participant. We categorized participants by low (≤ 1 /week), moderate (2–4/week), and high (≥ 5 /week) consumption. Although not specifically validated for the Atherosclerosis Risk in Communities Magnetic Resonance Imaging study cohort, similar self-reports of beverage intake from FFQs have been validated in other populations, with high correlation between FFQ data and data from daily diaries (42, 43).

Assessment of other dietary factors

Total intake of calories, carbohydrate, protein, animal protein, fat, animal fat, and different types of sugars (i.e., glucose, fructose) is presented in Table 1 and was calculated from FFQ data using the Harvard Nutrient Database (40). Macronutrient intakes (i.e., carbohydrate, fat, protein) are presented as a percentage of total caloric intake. Total meat consumption was derived from combined reports of intake of bacon, meat sandwiches, hamburger, hot dogs, processed meats, liver, and meat as a main or mixed dish. Red meat intake included hamburger, sandwiches, hot dogs, or main dishes containing beef, pork or lamb, and liver. Seafood consumption was considered canned tuna, breaded fish cakes/sticks, dark meat fish, or other fish, as well as shrimp, lobster, scallops, or clams as a main dish. Corn consumption from foods other than corn-based sweeteners consisted of whole corn (44) and foods such as corn chips and tortillas.

Laboratory methods

Blood was drawn after an 8-hour fast into silica-coated serum tubes free of any carbon-containing additives. Serum was stored at -70°C after collection and was thawed after approximately 2 years for measurement in 2007–2008. To measure $\delta^{13}\text{C}$ value, serum samples were quantitatively combusted to carbon monoxide in a EuroVector elemental analyzer (EA3000; EuroVector, Milan, Italy) configured with a continuous-flow stable isotope ratio mass spectrometer (Isoprime; Micromass UK Ltd., Manchester, United Kingdom). The reporting standard is Vienna Pee Dee

Table 1. Participant Characteristics^a at the 2005–2006 ARIC-MRI Study Visit by Sweetened Beverage Consumption (Servings/Week), Washington County, Maryland

	Servings/Week			
	Total	≤1	2–4	≥5
No. of participants	186	87	39	60
Age, years	71 (5)	70 (5)	72 (5)	72 (5)
Male gender, no. (%)	99 (53)	30 (34)	28 (71)	41 (68)
Weight, kg	82.4 (17)	81 (18)	86 (17)	82 (15)
Body mass index, kg/m ²	30 (6)	30 (6)	30 (5)	29 (5)
Waist, cm	104 (15)	104 (15)	106 (15)	103 (13)
Waist-to-hip ratio	0.96 (0.07)	0.95 (0.06)	0.97 (0.08)	0.97 (0.07)
Total calories, kcal	1,783 (594)	1,454 (385)	2,032 (558)	2,099 (622)
Protein, % kcal	16.5 (3)	18.1 (3)	16.1 (3)	14.6 (2)
Carbohydrates, % kcal	50.5 (8)	48.6 (9)	50.9 (7)	53.0 (6)
Total fat, % kcal	33.9 (6)	34.4 (7)	34.6 (6)	32.9 (5)
Animal fat, % kcal	16.4 (5)	16.8 (5)	16.2 (5)	16.0 (5)

Abbreviation: ARIC-MRI, Atherosclerosis Risk in Communities Magnetic Resonance Imaging.

^a All values are expressed as mean (standard deviation), unless indicated otherwise.

Belemnite, characterized by the International Atomic Energy Agency in Vienna, Austria. The value of $^{13}\text{C}/^{12}\text{C}$ in Vienna Pee Dee Belemnite is independently fixed; a high sample $^{13}\text{C}/^{12}\text{C}$ value corresponds to a high sample $\delta^{13}\text{C}$ value (in units of per mill, designated ‰). Organic standards were introduced every 15 samples, and blanks were introduced every 12 samples.

Each sample was analyzed in triplicate, and the mean value was used in statistical analysis. Total variability across the 3 measurements never exceeded 0.1‰. An analytical uncertainty of $\pm 0.1\%$ is associated with each sample measurement, resulting in an intraassay coefficient of variation of 0.1‰. All samples were analyzed, with blinding to sample source, in the Jähren laboratory at the Krieger School of Arts and Sciences, The Johns Hopkins University. Two internal laboratory standards referenced to Vienna Pee Dee Belemnite were used, for a 2-point calibration that encompassed the range of $\delta^{13}\text{C}$ values present in our samples (−22.9‰ to −15.6‰).

Statistical methods

Data were checked for outliers, and distributions of $\delta^{13}\text{C}$ values and other variables were assessed for normality. A *t* test was conducted of the difference in serum $\delta^{13}\text{C}$ value between those consuming high versus low amounts of sweetened beverages. Multiple linear regression was used to examine the association between serum $\delta^{13}\text{C}$ value and sweetened beverage intake, adjusting for potential confounders. Partial Spearman correlations and multiple linear regression were used to characterize dietary macronutrients (i.e., protein and fat intake) associated with $\delta^{13}\text{C}$ value as well as its relation with anthropometric variables (i.e., BMI and waist circumference). All analyses were carried out using Stata version 9.2 software (Stata Corporation, College Station, Texas). All 2-sided tests of significance were set at a type 1 error (alpha) level of 0.05.

RESULTS

Table 1 shows participant characteristics by low (≤ 1 /week), moderate (2–4/week), and high (≥ 5 /week) sweetened beverage consumption. Consumers of low amounts of sweetened beverages were predominantly female (66%), whereas consumers of medium and high amounts were predominantly male. No significant association between sweetened beverage consumption and anthropometric measures was found. The $\delta^{13}\text{C}$ distribution for all participants is shown in Figure 1. Mean $\delta^{13}\text{C}$ value was −19.34‰ (range, −17.21 to −21.72).

Association between serum $\delta^{13}\text{C}$ value and sweetened beverage consumption

Figure 2 shows mean $\delta^{13}\text{C}$ values stratified by the 3 levels of sweetened beverage consumption. The a priori aim of the study was to determine whether dichotomized sweetened beverage consumption was associated with serum $\delta^{13}\text{C}$ value by testing the mean difference for low (≤ 1 /week) versus high (≥ 5 /week) levels of consumption. The mean serum $\delta^{13}\text{C}$ value was lower for consumers of low (−19.47‰) compared with high (−19.15‰) amounts of sweetened beverages. This difference of 0.32‰ was statistically significant ($P < 0.01$). Combining those with moderate (2–4/week) intake with those with high intake decreased this difference (−0.24‰), but it remained significant ($P < 0.01$).

Association between $\delta^{13}\text{C}$ value and other dietary factors

Given the potential for association of $\delta^{13}\text{C}$ with other food sources of corn and cane, we report in Table 2 the Spearman correlations between serum $\delta^{13}\text{C}$ value and various dietary variables adjusted for gender. As expected,

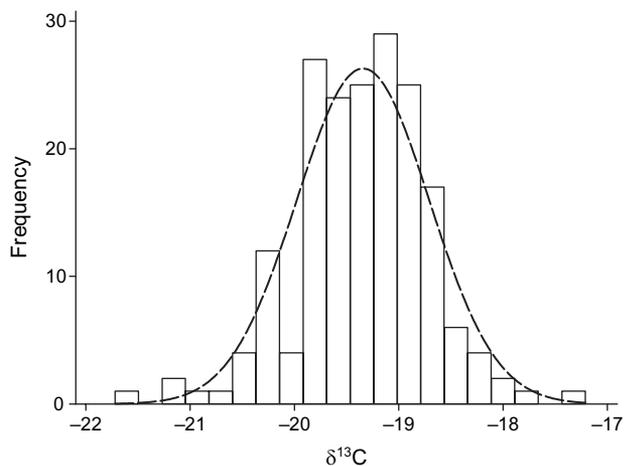


Figure 1. Distribution of serum ratio of isotope carbon 13 to isotope carbon 12 ($\delta^{13}\text{C}$) values among 186 participants in the Atherosclerosis Risk in Communities Magnetic Resonance Imaging (ARIC-MRI) study superimposed upon a normal distribution. Mean serum $\delta^{13}\text{C}$ value was -19.34‰ (range, -17.21 to -21.72). Values were considered “low $\delta^{13}\text{C}$ ” if they measured on the upper end of the range; values were considered “high $\delta^{13}\text{C}$ ” if they were on the low end of the range. Participants were sampled from the Washington County, Maryland, site at the 2005–2006 ARIC-MRI study visit.

whole-corn consumption was associated with higher serum $\delta^{13}\text{C}$ value, although the correlation was very weak ($r = 0.15$, $P = 0.04$). Significant correlations were found between serum $\delta^{13}\text{C}$ value and animal sources of protein ($r = 0.28$, $P < 0.001$) and fat ($r = 0.37$, $P < 0.001$). Further-

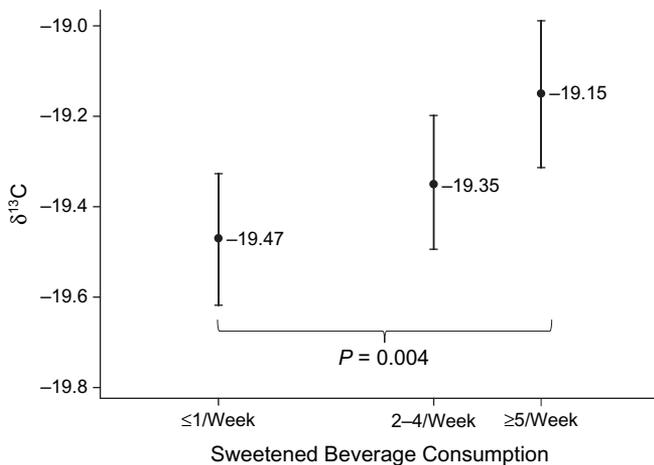


Figure 2. Mean (95% confidence interval) serum ratio of isotope carbon 13 to isotope carbon 12 ($\delta^{13}\text{C}$) values stratified by 3 levels of sweetened beverage consumption in the Atherosclerosis Risk in Communities Magnetic Resonance Imaging study, Washington County, Maryland, 2005–2006. Mean serum $\delta^{13}\text{C}$ value was significantly lower for those consuming sweetened beverages infrequently ($\leq 1/\text{week}$) compared with those consuming such beverages at a high frequency ($\geq 5/\text{week}$, $P = 0.004$ by t test of difference). Mean serum $\delta^{13}\text{C}$ value of those reporting moderately frequent intake (2–4/week) was in between that for the infrequent and extremely frequent consumers.

Table 2. Partial Spearman Correlations Between Serum $\delta^{13}\text{C}$ Value and Dietary Factors Adjusted for Gender, Atherosclerosis Risk in Communities Magnetic Resonance Imaging Study, 2005–2006 Visit

	Gender Adjusted	P Value
Sweetened beverages, servings/day	0.18	0.01
Whole corn, servings/day	0.15	0.04
Total calories, kcal	0.08	0.26
Total protein, % calories	0.11	0.12
Animal protein, % calories	0.28	<0.001
Total fat, % calories	0.10	0.19
Animal fat, % calories	0.37	<0.001
Carbohydrate, % calories	-0.21	0.004
Glycemic index	0.06	0.40
Glycemic load	-0.003	0.97
Total sugar, g	0.11	0.13
Glucose, g	0.06	0.39
Sucrose, g	0.06	0.41
Fructose, g	0.06	0.42

Abbreviation: $\delta^{13}\text{C}$, ratio of isotope carbon 13 to isotope carbon 12.

more, these correlations were similar even among individuals consuming low amounts of sweetened beverages ($r = 0.28$ ($P < 0.01$) for protein and $r = 0.31$ ($P < 0.01$) for fat). Total meat consumption ($r = 0.22$, $P = 0.003$) was less correlated with serum $\delta^{13}\text{C}$ value than red meat consumption ($r = 0.33$, $P < 0.001$). No correlations were found with seafood ($P = 0.76$) (data not shown). These patterns were expected given the widespread use of corn as feed for animals butchered for red meat, while not part of the diet of fish, for example. Total carbohydrate consumption was negatively associated with serum $\delta^{13}\text{C}$ value initially ($r = -0.21$), but this finding was due to its strong negative correlation with animal fat ($r = -0.57$) such that adjustment for animal fat intake completely eliminated the correlation ($r = -0.02$, $P = 0.79$). Total sugar, glucose, sucrose, and fructose intake was not correlated with serum $\delta^{13}\text{C}$ value after adjusting for gender.

We also tested whether the linear association between sweetened beverage consumption and $\delta^{13}\text{C}$ was independent of corn and animal protein/fat intake (Table 3). Adjustment for dietary factors was conducted by adding factors consecutively. Because of the strong correlation between animal fat and animal protein ($r = 0.81$), only animal fat was included to avoid overadjustment. The fully adjusted model (Table 3, model 4) shows that mean serum $\delta^{13}\text{C}$ values continued to be associated with increased sweetened beverage consumption, even after accounting for animal fat consumption ($\beta = 0.18\text{‰}$, $P < 0.01$). Table 4 displays the coefficients of the full model, where animal fat remained significantly associated with serum $\delta^{13}\text{C}$ value, with an increase of 0.05‰ ($P < 0.001$) observed with each additional percentage of calories of animal fat consumed. The variance in serum $\delta^{13}\text{C}$ value explained by the full model reached 29%.

Table 3. Beta Coefficient (95% Confidence Interval) of Association Between Serum $\delta^{13}\text{C}$ Values and Sweetened Beverage Consumption, Atherosclerosis Risk in Communities Magnetic Resonance Imaging Study, 2005–2006 Visit

Model	β Coefficient for Sweetened Beverage Consumption	95% CI	P Value	Adjusted <i>r</i> -Squared Value
1: Univariate	0.21	0.09, 0.32	<0.001	0.06
2: + Corn consumption (≥ 1 /week)	0.20	0.08, 0.31	0.001	0.08
3: + Male gender	0.13	0.02, 0.25	0.02	0.13
4: + Animal fat (% total calories/day)	0.18	0.08, 0.29	0.001	0.29

Abbreviations: CI, confidence interval; $\delta^{13}\text{C}$, ratio of isotope carbon 13 to isotope carbon 12.

Association between $\delta^{13}\text{C}$ and anthropometric measures

Univariate associations between serum $\delta^{13}\text{C}$ value and the anthropometric variables of weight, BMI, waist, and waist-to-hip ratio were examined (data not shown). The associations were in the hypothesized direction in that higher serum $\delta^{13}\text{C}$ values corresponded to higher waist circumference, BMI, and waist-to-hip ratio. All anthropometric variables were positively associated with serum $\delta^{13}\text{C}$ value in linear regression after adjustment for gender and intake of corn, sweetened beverages, and animal fat. On the other hand, the FFQ-derived measurements of sweetened beverage, total sugar (BMI, $P = 0.60$), or animal fat (BMI, $P = 0.10$) intake were not associated cross-sectionally with these anthropometric measures when the same regression models were used.

DISCUSSION

In this proof-of-principle approach, we confirmed our hypothesis that $\delta^{13}\text{C}$ values are higher in individuals with higher intakes of sweetened beverages. The significant association between serum $\delta^{13}\text{C}$ value and self-reported consumption of sweetened beverages was independent of

gender, corn consumption, and animal fat intake. Furthermore, serum $\delta^{13}\text{C}$ value was associated with adiposity, particularly waist circumference, suggesting that serum $\delta^{13}\text{C}$ measurements could be useful in studying aspects of the modern diet that may contribute to diabetes and obesity. Although this technique is novel and promising, several issues have not been resolved, and further studies are needed to decipher its specificity to intake of sweets and its kinetic properties.

To our knowledge, this is the first evidence that the $\delta^{13}\text{C}$ value of human serum is different for individuals who drink different amounts of sweetened beverages. Recent studies have investigated aspects of dietary intake on the $\delta^{13}\text{C}$ value of various biologic samples including hair protein (37, 38), plasma protein (37), plasma glucose (29), serum retinol (30), and red blood cells (26). Our results are in line with those from recent studies showing a correlation between the $\delta^{13}\text{C}$ value of serum retinol and specific nutritional components (i.e., provitamin A increase) (30) and long-term animal protein intake and hair $\delta^{13}\text{C}$ value (37). It was also recently proposed that urinary sucrose and fructose excretion be used as biomarkers for sugar consumption (45), and urinary sugar excretion (46) has been shown to reflect immediate prior intake even in people without diabetes. The kinetics of $\delta^{13}\text{C}$ in serum are not known, so we are unsure of the amount of time it would take for a change in diet to be reflected in a change in $\delta^{13}\text{C}$.

A recent cross-over feeding study measured plasma glucose $\delta^{13}\text{C}$ values in 5 individuals fed differing amounts of C4 sugars (5%, 16%, or 32%) over 3 periods lasting 1 week each (29). The investigators found no differences in fasting plasma glucose $\delta^{13}\text{C}$ values at the end of each period and concluded that fasting levels cannot be a marker of habitual intake. Nevertheless, important points must be raised in accessing this evidence, including the small number of participants ($n = 5$), which might not have enabled precise differences to be tested, the short period of feeding (7 days), and the different biologic media (plasma glucose).

To our knowledge, the distribution of $\delta^{13}\text{C}$ in components of serum has not been specifically studied, although it is likely to reflect predominantly carbon in amino acids and in fatty acids. These presumably are in equilibrium with large body stores, not simply reflecting immediate dietary intake. The long half-life of serum components (e.g.,

Table 4. Results of Linear Regression With Serum $\delta^{13}\text{C}$ Value as the Dependent Variable and Covariates From a Model Including Corn Consumption, Male Gender, and Animal Fat, Atherosclerosis Risk in Communities Magnetic Resonance Imaging Study, 2005–2006 Visit

	β Coefficient	95% CI	P Value
Sweetened beverages, servings/day	0.18	0.08, 0.29	0.001
Corn consumption (≥ 1 /week)	0.18	0.03, 0.34	0.02
Male gender	0.24	0.10, 0.42	0.004
Animal fat (% total calories/day)	0.05	0.03, 0.06	<0.001

Abbreviations: CI, confidence interval; $\delta^{13}\text{C}$, ratio of isotope carbon 13 to isotope carbon 12.

albumin) suggests that serum $\delta^{13}\text{C}$ represents a time-integrated reflection of carbon intake. This is distinct from urinary sugar excretion, which presumably reflects blood glucose over antecedent hours. Experiments expressly designed to measure turnover time of $\delta^{13}\text{C}$ in specific human tissues are needed to fully determine the integrated intake period reflected by serum $\delta^{13}\text{C}$ value (24).

Our data suggest that $\delta^{13}\text{C}$, when confirmed by further research, could be extremely useful in epidemiologic and public health settings. Dietary assessment is intrinsically cumbersome to administer and inaccurate, being dependent on self-report alone. The FFQ is subject to measurement error and may be systematically associated with factors such as BMI (e.g., overweight people underreporting their sweets intake), introducing error and biasing associations between subgroups. Even in the case of “nondifferential” misclassification, the resulting error introduces bias and underestimates diet-disease associations.

The measurement of serum $\delta^{13}\text{C}$ value, as an objective laboratory measurement, could supplement or replace self-report of consumption of sweetened foods and beverages in epidemiologic studies. Presumably, clinical use as a laboratory assessment of individuals will require refinements to improve accuracy.

$\delta^{13}\text{C}$ as a biomarker could address long-standing questions such as whether nutritively sweetened beverage consumption is in fact a cause of excessive weight gain. The relation between nutritively sweetened beverage consumption, obesity, and diabetes remains unclear despite a number of studies that have demonstrated an association between sweetened beverages and development of type 2 diabetes (47–51). Likewise, it is unclear whether changing the availability of sweetened beverages affects total caloric consumption, and how a change in sweets intake impacts glycemic control in diabetes.

The limitations of our work include our inability to capture and adjust for consumption of other forms of sugar (i.e., beet sugar) and potential confounding from other dietary sources of corn. Because beets do not share the conspicuously high $^{13}\text{C}:^{12}\text{C}$ value of corn and cane plants (i.e., beet is a “C3” plant), serum $\delta^{13}\text{C}$ value does not capture the consumption of sugar from beets, which accounts for a nontrivial amount of the raw sugar used in the United States (48). It is unlikely that the source of raw sugar differed much between individuals in this study, all of whom resided in Washington County, Maryland, but the extent to which beet versus cane sugar contributes to caloric content globally would need to be studied. Less prominent sources of high fructose corn syrup such as certain types of breads, cereals, grains, and artificially sweetened dairy products could also conceivably affect serum $\delta^{13}\text{C}$; however, FFQs also cannot determine high fructose corn syrup intake from these sources. Another limitation of our work is the limitation of the comparator: if the FFQ is not in fact an accurate “gold standard,” then it is possible that the $\delta^{13}\text{C}$ measurement is actually closer to “reality” than the FFQ. Lastly, because of the cross-sectional design of the study, we were unable to determine causal associations. Future longitudinal studies are needed to determine whether serum $\delta^{13}\text{C}$ value reliably reflects long-term intake and whether the small, but signif-

icant difference in $\delta^{13}\text{C}$ value detected here reflects an important biologic difference in metabolic risk.

The magnitude of the difference we detected (Figure 2) is small compared with the $\delta^{13}\text{C}$ variability in serum across a large anonymous population (25), suggesting that other factors influence the overall carbon isotope composition of human blood. Dietary carbon comes not only from ingested carbohydrates such as corn, sugar cane, and other plants but also from fat and protein in the meat of animals that themselves consume corn or cane-based foods (44). Because livestock or chicken are fed corn, for example, the high $\delta^{13}\text{C}$ value is incorporated into their tissues, and presumably into the humans who consume this meat. Our finding that $\delta^{13}\text{C}$ was associated with animal fat and animal protein, particularly from beef, is consistent with the animals having been at least partially corn-fed. Because the serum $\delta^{13}\text{C}$ value of our participants was affected by corn and animal fat intake, serum $\delta^{13}\text{C}$ value cannot be considered specific to dietary sweets. Nevertheless, the highly significant association of $\delta^{13}\text{C}$ with sweetened beverage intake, after adjustment for these known confounding factors, showed that differences in corn or animal fat intake did not eliminate the sweetened beverage intake signal. Controlled trials are needed to fully tease apart the contributions of each food item, but several studies suggest that consumption of sweetened beverages and high amounts of animal fat are each part of an unhealthy dietary pattern associated with obesity and type 2 diabetes (45, 49).

We utilized whole plasma samples in this study, but it is possible to target specific components in plasma, such as amino acid, lipid, or carbohydrate molecules. Measuring $\delta^{13}\text{C}$ in one component of plasma may be a way to better distinguish the carbon source from animal (e.g., via protein $\delta^{13}\text{C}$) (37) versus plant (e.g., via glucose $\delta^{13}\text{C}$) sources (29). Evaluating the individual kinetics of different plasma components, each of which presumably reflects the contribution of a different, larger, whole body pool, will require more research.

Extensive archives of blood serum exist from large national and international epidemiologic studies. These archives can be used, as in this work, to further evaluate the relation of diet and disease. Less than 1 μL of serum is required for $\delta^{13}\text{C}$ analysis, and we have shown that serum $\delta^{13}\text{C}$ value is not affected by the archiving process or by preservation or de clotting additives (25). Thus, the $\delta^{13}\text{C}$ value in serum could easily be determined with existing serum archives or incorporated into the planning of epidemiologic studies.

In summary, these data provide the first known evidence that serum $\delta^{13}\text{C}$ value is associated, to a highly statistically significant degree, with dietary exposure to sweetened beverage intake. Further research is needed to better characterize the contribution of individual nutritional components that affect serum $\delta^{13}\text{C}$ value, such as animal fat. Research is also needed to elucidate the time course over which changes in diet are reflected in serum $\delta^{13}\text{C}$ value. With further confirmation, serum $\delta^{13}\text{C}$ value could become an objective, sensitive measure of sweets intake, promoting more reliable studies of diet on obesity, type 2 diabetes, and cardiovascular disease.

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