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Association of $\delta^{13}C$ in Fingerstick Blood with Added-Sugar and Sugar-Sweetened Beverage Intake

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ABSTRACT

A reliance on self-reported dietary intake measures is a common research limitation, thus the need for dietary biomarkers. Added-sugar intake may play a role in the development and progression of obesity and related comorbidities; common sweeteners include corn and sugar cane derivatives. These plants contain a high amount of ^{13}C, a naturally occurring stable carbon isotope. Consumption of these sweeteners, of which sugar-sweetened beverages are the primary dietary source, might be reflected in the $\delta^{13}C$ value of blood. Fingerstick blood represents an ideal substrate for bioassay because of its ease of acquisition. The objective of this investigation was to determine if the $\delta^{13}C$ value of fingerstick blood is a potential biomarker of added-sugar and sugar-sweetened beverage intake. Individuals aged 21 years and older (n=60) were recruited to attend three laboratory visits; assessments completed at each visit depended upon a randomly assigned sequence (sequence one or two). The initial visit included assessment of height, weight, and dietary intake (sequence one: beverage intake questionnaire, sequence two: 4-day food intake record). Sequence one participants completed a food intake record at visit two, and nonfasting blood samples were obtained via routine fingersticks at visits one and three. Sequence two participants completed a beverage intake questionnaire at visit two, and provided fingerstick blood samples at visits two and three. Samples were analyzed for $\delta^{13}C$ value using natural abundance stable isotope mass spectrometry. $\delta^{13}C$ value was compared to dietary outcomes in all participants, as well as among those in the highest and lowest tertile of added-sugar intake. Reported mean added-sugar consumption was 66±5 g/day, and sugar-sweetened beverage consumption was 330±53 g/day and 134±25 kcal/day. Mean fingerstick $\delta^{13}C$ value was −19.94‰±0.10‰, which differed by body mass index status. $\delta^{13}C$ value was associated (all $P<0.05$) with intake of total added sugars (g, $r=0.37$; kcal, $r=0.37$), soft drinks (g, $r=0.26$; kcal, $r=0.27$), and total sugar-sweetened beverage (g, $r=0.28$; kcal, $r=0.35$). The $\delta^{13}C$ value in the lowest and the highest added-sugar intake tertiles were significantly different (mean difference=−0.48‰; $P=0.028$). Although there are several potential dietary sources for blood carbon, the $\delta^{13}C$ value of fingerstick blood shows promise as a noninvasive biomarker of added-sugar and sugar-sweetened beverage intake based on these findings.

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Consumption of energy-containing added sugars and, in particular, sugar-sweetened beverages, has been suggested as a contributor to weight gain (1-3). Although recognized by major health organizations (4), the role of added sugars and their primary food source, sugar-sweetened beverages, in the development and progression of obesity and related comorbidities, remains controversial (5,6). Added sugars refer to "sugars and syrups added to foods during processing or preparation, and includes sugars and syrups added at the table" (4). A common limitation of research in this area is a reliance on self-reported measures of habitual dietary intake (7). Thus, the need for novel methods to objectively assess dietary intake, such as biomarkers of food or nutrient intake, has been recognized (8-10).

Common sources of added sweeteners include corn derivatives, such as corn starch and corn syrup (eg, high-fructose corn syrup) and sugar cane and its derivatives, which include molasses, plain cane sugar, brown cane sugar, and powdered cane sugar. Because these plants use the C_4 photosynthetic pathway, their sugars contain a high natural concentration of ^{13}C, a naturally occurring stable carbon isotope (11). For these reasons, a high $\delta^{13}C$ value of human blood can reflect a high $\delta^{13}C$ value of diet (12). Others have found an association between the $\delta^{15}N$ value of red blood cells and dietary eicosapentaenoic acid and docosahexaenoic acid intake (13), and between the $\delta^{13}C$ value of serum retinol and dietary provitamin A

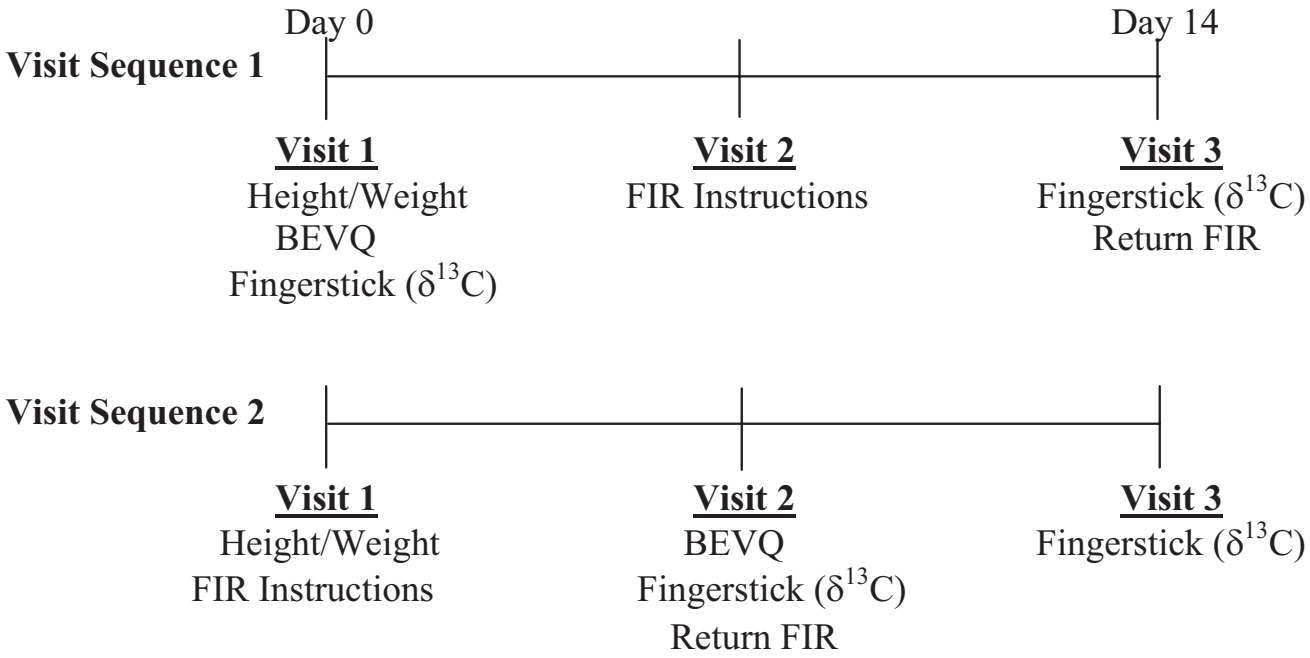

Figure. Study procedures: Association of δ^{13}C in fingerstick blood with added-sugar and sugar-sweetened beverage intake. BEVQ=beverage intake questionnaire; FIR=food intake record.

(14), and have suggested biomarkers based on these results.

Limitations of biomarker techniques often include cost and degree of invasiveness (7). For example, nitrogen stable isotopes in hair can be used as a less invasive measure of eicosapentaenoic acid and docosahexaenoic acid intake than red blood cells (15). Stable isotopes in hair samples, although noninvasively obtained, have been shown to lag dietary change by more than 4 weeks (16). Our objective was to determine whether fingerstick blood (ie, a noninvasively sampled tissue, uniquely feasible for large-scale clinical and field studies) is a potential biomarker of added-sugar and sugar-sweetened beverage intake, and to determine whether fingerstick blood δ^{13}C value is associated with sweetened-beverage intake assessed by a newly developed brief beverage intake questionnaire (17).

METHODS

Subjects and Design

Sixty healthy adults, aged 21 years and older, were recruited for this investigation from a local university community between June 2008 and June 2009. The Virginia Tech Institutional Review Board approved the study protocol. All participants provided written informed consent before their participation. However, they were not aware of the specific purpose of the study; they were informed that the purpose of the study was to evaluate a new dietary questionnaire. Participation entailed three laboratory visits within a 2-week period, all between the hours of 12 PM and 5 PM; visits were completed in one of two randomly assigned visit sequences. Visit sequences were randomized to avoid the possibility of an order effect in dietary outcomes variables. For example, completion of the beverage intake questionnaire at the first session could heighten awareness of beverage consumption in the subsequent session, when the 4-day food intake record was completed, and produce changes in beverage intake patterns or greater accuracy in recording beverage intake. Thus, two randomly assigned visit sequences were used to minimize this possibility. An overview of the study design, including the measurements at each laboratory visit according to visit sequence, is depicted in the Figure.

Procedures

On the initial visit, height was measured in meters without shoes using a scale-mounted stadiometer, body weight was measured in light street clothing without shoes to the nearest 0.2 kg using a physician's balance scale (Seca, Hanover, MD), and body mass index (BMI) was calculated as kg/m^2. Anthropometric measures were assessed once, at the initial visit. Participants also provided information on their general health status (eg, medication usage, medical history, etc). On two laboratory visits (depending on visit sequence), nonfasting blood samples were obtained via routine fingersticks (One Touch Fine Point Lancet, Lifescan; Johnson & Johnson Company, New Brunswick, NJ). Blood samples were blotted onto sterilized binder-free glass microfiber filters (Whatman, type GF/D, 2.5-cm diameter; Whatman, Inc, Piscataway, NJ), air-dried, then analyzed for δ^{13}C value using natural abundance stable isotope mass spectrometry. To measure δ^{13}C value, samples were quantitatively combusted to CO_2 in a Eurovector elemental analyzer (EURO.EA3000; Euro Vector Instruments and Software,

Milan, Italy) configured with a continuous-flow stable isotope ratio mass spectrometer (Isoprime; Micromass UK Ltd, Manchester, UK). The reporting standard is Vienna Pee Dee Belemnite characterized by the International Atomic Energy Agency in Vienna. The value of $^{13}C/^{12}C$ in Vienna Pee Dee Belemnite is independently fixed; a high sample $^{13}C/^{12}C$ value corresponds to a high sample $\delta^{13}C$ value (in units of permil [‰]). Each sample was analyzed in triplicate and the mean value was used in statistical analysis. Total variability across the three measurements never exceeded 0.1‰. An analytical uncertainty of $<\pm 0.1$‰ is associated with each sample measurement, resulting in an intra-assay coefficient of variation of 0.1‰. Two internal laboratory standards referenced to Vienna Pee Dee Belemnite were used for a two-point calibration that encompassed the range of $\delta^{13}C$ values present in our samples (−22.9‰ to −15.6‰).

Usual dietary intake was assessed with 4-day food intake records. Participants were instructed to complete the 4-day food intake record either on Sunday through Wednesday or Wednesday through Saturday in order to capture both weekend and weekday dietary habits; food intake records were reviewed for completeness upon return, and analyzed using nutritional analysis software (Nutrition Data System for Research, University of Minnesota, Minneapolis). Variables derived from the food intake record included total dietary added sugars from all foods and beverages consumed. Participants also completed a beverage intake questionnaire (17), which is a quantitative food frequency questionnaire assessing habitual beverage consumption in the past month. The beverage intake questionnaire assesses 19 beverage categories, including the following variables: grams and energy (kcal) of sugar-sweetened beverages (ie, sweetened juice beverages/drinks, regular soft drinks, sweet tea, sweetened coffee, energy drinks, mixed alcoholic drinks, meal replacement beverages), and grams and energy (kcal) of regular soft drinks. Participants were compensated $10 upon completion of all three study visits.

Data Analysis

Statistical analyses were performed using statistical analysis software (SPSS version 12.0 for Windows, SPSS Inc, Chicago, IL). Descriptive statistics (mean±standard error of mean, frequencies) are reported for subject demographic characteristics and beverage intake variables (total g, kcal; total dietary added sugar g, kcal). Simple and bivariate correlations, paired sample *t* tests, independent sample *t* tests, and one-way analysis of variance were used to assess associations among variables, group differences, and differences in assessment methods. Finally, to determine the ability of $\delta^{13}C$ measurement to detect differences among reported sugar consumption levels, the sample was divided into tertiles based on total added-sugar (g) intake; group differences in $\delta^{13}C$ were assessed between the top third of the sample, who represented high added-sugar consumers (mean intake=108±10 g), and the bottom third of the sample, who represented low added-sugar consumers (mean intake=32±3 g).

RESULTS AND DISCUSSION

All 60 participants completed the three study sessions. The sample was reasonably balanced with respect to sex (25 males, 35 females), and 90% were white (6% were Asian, 2% were African American, and 2% were other). Age ranged from 21 to 89 years (mean age=43±2 years). Mean BMI status (26.7±0.9) was in the overweight range (ie, 25 to 29.9). Among males and females in this sample, reported total daily added-sugar intake was 76±11 g and 58±26 g, respectively, which is similar to that reported by middle-aged adults (males: 19 tsp/day, ~76 g; females: 14 tsp/day, ~56 g) in large US population-based studies (18). Reported mean daily consumption of sugar-sweetened beverages, determined by the beverage intake questionnaire, was 330±53 g and 134±25 kcal. No significant differences in outcomes according to visit sequence were found (all $P>0.05$).

Mean $\delta^{13}C$ values at time one and time two, respectively, were −19.88‰±0.09‰ (range=−22.09‰ to −18.87‰) and −19.99‰±0.11‰ (range=−22.91‰ to −18.68‰); these values are comparable to those reported in an earlier investigation that used venipuncture to obtain serum samples (12). The $\delta^{13}C$ measurements were correlated across visits ($r=0.873$; $P<0.001$), and for subsequent analyses, the mean $\delta^{13}C$ value was used (sample mean=−19.94‰±0.10‰). No difference in $\delta^{13}C$ values was detected across age groups ($P=0.370$); however, there were significant differences according to BMI and sex. The $\delta^{13}C$ value differed between normal weight (range=18.5 to 24.9) and overweight (range=25 to 29.9) individuals (mean difference=−0.60‰±0.20‰; $P=0.005$), and between normal weight and obese (≥30) individuals (mean difference=−0.68‰±0.29‰; $P=0.028$), but not between overweight or obese weight status categories. Significant correlations were noted between $\delta^{13}C$ values and BMI ($r=0.343$; $P=0.007$). The $\delta^{13}C$ value was higher in males compared with females (mean difference=0.40‰±0.19‰; $P=0.043$), which is consistent with previous reports on large populations (12). However, this sex difference can be attributed to body mass differences or to differences in added-sugar consumption, as opposed to sex differences; $\delta^{13}C$ value was not different when overweight and obese men were compared to overweight and obese women (−19.67‰±0.19‰ vs −19.64‰±0.12‰, respectively; $P=0.907$).

Significant correlations were noted between $\delta^{13}C$ values and dietary intake variables. The $\delta^{13}C$ value is significantly correlated with total added sugars (g, kcal) from all food and beverages ($r=0.365$; $P<0.01$ for both g and kcal), and also with the kilocalories and grams of soft drinks ($r=0.270$ and $r=0.258$, respectively; $P<0.05$) and total sugar-sweetened beverage ($r=0.345$; $P<0.01$ and $r=0.284$; $P<0.05$; respectively) determined by the beverage intake questionnaire. After controlling for BMI, associations between $\delta^{13}C$ values and these dietary variables remained significant (added sugar g: $r=0.279$; $P=0.033$; sugar-sweetened beverage kcal: $r=0.353$; $P=0.006$; sugar-sweetened beverage g: $r=0.288$; $P=0.027$). When evaluating nutritional biomarkers compared to reported dietary intake, correlations have been reported to range from 0.03 to 0.73, with a mean of ~0.39 (19). However, these correlations may underestimate biomarker validity due to under-reporting of dietary intake, and correlations of 0.5 to 0.7 are considered acceptable

Table. Group characteristics of low and high added-sugar consumers: Investigation of $\delta^{13}C$ as a potential biomarker of added-sugar and sugar-sweetened beverage intake

Participant characteristics	Low group	High group
Total number of participants, n	20	20
Male, n (%)	8 (40)	10 (50)
Female, n (%)	12 (60)	10 (50)
Age, n (%)		
21-39 y	8 (40)	12 (60)
40-59 y	5 (25)	4 (20)
60 y or older	7 (35)	4 (20)
Age (y), mean±SEM[a]	47.8±4.1	38.1±3.8
BMI[b] status, n (%)		
Underweight (<18.5)	0 (0)	1 (5)
Normal weight (18.5-24.9)	8 (40)	6 (30)
Overweight (25-29.9)	9 (45)	7 (35)
Obese (≥ 30)	3 (15)	6 (30)
BMI, mean ±SEM	26.4±0.9	29.1±2.5
$\delta^{13}C$ (‰), mean±SEM	−20.06±0.17	−19.59±0.13[c]
Added sugar, mean±SEM		
kcal	128±44	432±184[d]
g	32±3	108±10[d]
BEVQ[e] sugar-sweetened beverage consumption, mean±SEM		
kcal	98±16	236±63[c]
g	308±58	511±128
BEVQ soft drink consumption, mean±SEM		
kcal	7±3	67±26[c]
g	33±19	152±58[c]

[a]SEM=standard error of the mean.
[b]BMI=body mass index (calculated as kg/m2).
[c]Significant group difference, P<0.05.
[d]Significant group difference, P<0.001.
[e]Beverage Intake Questionnaire.

(19). Although correlations of $\delta^{13}C$ value with self-reported dietary intake in the present investigation are slightly below this level (r=~0.3 to 0.4), this technique warrants further investigation given the limited sample size of this study, the early stage of development of this technique, and its potential to address an important and controversial public health issue, which is added-sugar intake and health (4-6,20).

Others have shown that the $\delta^{13}C$ value of plasma glucose is a valid biomarker for C_4 sugars consumed in the previous meal (21); a more integrative tissue such as blood, if at all sensitive to sweetener intake, might allow for prediction of longer-term intake. Such a biomarker has a number of potential applications: it could be used in large-scale field studies to support or validate self-reported dietary intake measures, to objectively assess total sugar/sugar-sweetened beverage intake in epidemiologic or cross-sectional studies, or to assess changes in intake during intervention studies. In addition, findings related to self-reported sugar-sweetened beverage intake suggest that the brief self-reported beverage intake questionnaire tool (17) may be used to rapidly assess sugar-sweetened beverage and soft drink intake, when more resource-intensive dietary assessment tools are not feasible.

As presented in the Table, the lowest and the highest added-sugar intake tertiles demonstrated significant differences in $\delta^{13}C$ value (mean difference=−0.48‰; P=0.028). These groups were not significantly different with respect to age or BMI (Table). As intended, the groups were different with respect to reported consumption of total added sugars (kcal, g), sugar-sweetened beverage energy, and soft drinks (kcal, g). The significant association of the $\delta^{13}C$ value of fingerstick blood with the consumption of total added sugars, sugar-sweetened beverage energy, and soft drinks bodes well for the development of a $\delta^{13}C$-assay on low-invasive fingerstick samples that might be able to identify high- vs low-end consumers of sweeteners.

There are several limitations that should be acknowledged. There are many sweeteners that are produced by the C_3 photosynthetic pathway and do not carry a conspicuous $\delta^{13}C$ value: specifically beet sugar, honey, and maple syrups. However, these represent a small fraction of total sweetener consumption (eg, US per capita corn sweetener availability ~69 lb/year; honey and edible syrups is ~1 lb/year) in the United States (22). A second limitation is that $\delta^{13}C$ value in whole blood [unlike plasma glucose (21)] does not fully distinguish between corn consumption and corn derivatives (23), thus it may be a less accurate indicator of sweetener consumption in populations consuming large amounts of corn products; this issue warrants additional study. Finally, livestock consuming corn products as feed, whose meat is then ingested by humans, are included within an individual's ^{13}C pool (24); however, it may be possible to refine this technique using fingerstick measurements of a second stable isotope, ^{15}N, to correct for animal protein consumption (12).

CONCLUSIONS

The $\delta^{13}C$ value of fingerstick blood shows promise as a noninvasive biomarker of added-sugar and sugar-sweetened beverage intake. Future research should be conducted to determine whether body mass and composition influence $\delta^{13}C$ value at a given added-sugar consumption level, to determine the sensitivity of the technique using larger samples sizes and controlled feeding designs to manipulate added-sugar intake, and to determine whether the technique can be used in children or adolescents. Future work is also warranted to refine this technique; specifically to determine the extent to which corn consumption (vs sugar consumption) impacts the body's $\delta^{13}C$ value pool, and whether adjustments should be made to account for a potential secondary corn signature imparted by dietary meat from livestock raised on corn.

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