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Association of $\delta^{13}\text{C}$ in Fingerstick Blood with Added Sugars 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 co-morbidities; 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 (SSB) are the primary dietary source, may be reflected in the $\delta^{13}\text{C}$ value of blood. Fingerstick blood represents an ideal substrate for bioassay due to its ease of acquisition. The objective of this investigation was to determine if the $\delta^{13}\text{C}$ value of fingerstick blood is a potential biomarker of added sugar and SSB intake. Individuals aged ≥ 21 years ($n=60$) 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 [BEVQ], sequence two: four-day food intake record [FIR]). Sequence one participants completed an FIR at visit two, and non-fasting blood samples were obtained via routine finger sticks at visits one and three. Sequence two participants completed a BEVQ at visit two, and provided fingerstick blood samples at visits two and three. Samples were analyzed for $\delta^{13}\text{C}$ value using natural abundance stable isotope mass spectrometry. $\delta^{13}\text{C}$ 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 $66 \pm 5 \text{ g/day}$, and SSB consumption was $330 \pm 53 \text{ g/day}$ and $134 \pm 25 \text{ kcal/day}$. Mean fingerstick $\delta^{13}\text{C}$ value was $-19.94 \pm 0.10\text{‰}$, which differed by BMI status. $\delta^{13}\text{C}$ 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 SSB (g, $r=0.28$; kcal, $r=0.35$). The $\delta^{13}\text{C}$ value in the lowest and the highest added sugar intake tertiles were significantly different (mean difference = -0.48‰ , $p=0.028$). Even though there are several potential dietary sources for blood carbon, the $\delta^{13}\text{C}$ value of fingerstick blood shows promise as a non-invasive biomarker of added sugar and SSB intake based on these findings.

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Keywords

Biomarker; Sugar; Sweetener; Diet Assessment; Isotope; Sugar-sweetened beverages

Introduction

Consumption of energy-containing added sugars and in particular, sugar-sweetened beverages (SSB), have been suggested as contributors to weight gain (1–3). Although recognized by major health organization (4), the role of added sugars and their primary food source, SSB, in the development and progression of obesity and related co-morbidities 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 (e.g., high fructose corn syrup), and sugar cane and its derivatives which includes molasses, plain cane sugar, brown cane sugar, and powdered cane sugar. Because these plants employ the C₄ photosynthetic pathway, their sugars contain a high natural concentration of ¹³C, a naturally-occurring stable carbon isotope (11). For these reasons, a high $\delta^{13}\text{C}$ value of human blood may reflect a high $\delta^{13}\text{C}$ value of diet (12). Other workers have found an association between the $\delta^{15}\text{N}$ value of red blood cells and dietary eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) intake (13), and between the $\delta^{13}\text{C}$ value of serum retinol and dietary provitamin A (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 might be used as a less invasive measure of EPA and DHA intake over red blood cells (15). Stable isotopes in hair samples, although non-invasively obtained, have been shown to lag dietary change by more than four weeks (16). Our objective was to determine if fingerstick blood (i.e., a non-invasively sampled tissue, uniquely feasible for large-scale clinical and field studies) is a potential biomarker of added sugar and SSB intake, and to determine if fingerstick blood $\delta^{13}\text{C}$ 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, 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 prior to 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 two-week period, all within the hours of 12 pm to 5 pm; visits were completed in one of two randomly assigned visit sequences. Visit sequences were randomized so as to avoid the possibility of an order effect in dietary outcome 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 four-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 Figure 1.

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 weight (kg)/height (m²). Anthropometric measures were assessed once, at the initial visit. Participants also provided information on their general health status (medications usage, medical history, etc). On two laboratory visits (depending upon visit sequence), non-fasting blood samples were obtained via routine finger sticks (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 $\delta^{13}\text{C}$ value using natural abundance stable isotope mass spectrometry (NA-SIMS). To measure $\delta^{13}\text{C}$ value, samples were quantitatively combusted to CO₂ 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, United Kingdom). The reporting standard is Vienna Pee Dee Belemnite (VPDB) characterized by the International Atomic Energy Agency in Vienna. The value of $^{13}\text{C}/^{12}\text{C}$ in VPDB is independently fixed; high sample $^{13}\text{C}/^{12}\text{C}$ value corresponds to a high sample $\delta^{13}\text{C}$ value (in units of permil, designated "‰"). 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 $< \pm 0.1\text{‰}$ is associated with each sample measurement, resulting in an intra-assay coefficient of variation of 0.1‰. Two internal laboratory standards referenced to VPDB were used, for a two-point calibration that encompassed the range of $\delta^{13}\text{C}$ values present in our samples (-22.9‰ to -15.6‰).

Usual dietary intake was assessed with four-day food intake records (FIR). Participants were instructed to complete the four-day FIR either on Sunday through Wednesday or Wednesday through Saturday in order to capture both weekend and weekday dietary habits; FIR were reviewed for completeness upon return, and analyzed using nutritional analysis software (Nutrition Data System for Research[NDS-R], University of Minnesota, Minneapolis, MN). Variables derived from the FIR included total dietary added sugars from all foods and beverages consumed. Participants also completed a beverage intake questionnaire (BEVQ) (17), which is a quantitative food frequency questionnaire assessing habitual beverage consumption in the past month. The BEVQ assesses 19 beverage categories, including the following variables: grams and energy (kcal) of sugar sweetened beverages (SSB; 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 v. 12.0 for Windows, SPSS Inc, Chicago, IL). Descriptive statistics (mean $\pm$ SEM; 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 (ANOVA) were used to assess associations among variables, group differences, and differences in assessment methods.

Finally, to determine the ability of $\delta^{13}\text{C}$ measurement to detect differences among reported sugar consumption levels, the sample was divided into tertiles based upon total added sugar (g) intake; group differences in $\delta^{13}\text{C}$ were assessed between the top third of the sample, who represented high added sugar consumers (“Hi”, mean intake=108±10 g), and the bottom third of the sample, who represented low added sugar consumers (“Lo”, mean intake=32±3 g).

Results and Discussion

All 60 participants completed the three study sessions. The sample was reasonably balanced with respect to gender (25 males, 35 females), and 90% Caucasian (6% Asian, 2% African American, 2% other). Age ranged from 21–89 years (mean age 43±2 years). Mean BMI status (26.7±0.9 kg/m²) was in the overweight range (25–29.9 kg/m²). Among males and females in this sample, reported total daily added sugar intake was 76±11g and 58±26g, respectively, which is similar to that reported by middle-aged adults (males: 19 tsp/d, ~76g; females: 14 tsp/d, ~56g) in large US population-based studies (18). Reported mean daily consumption of SSB, determined by the BEVQ, was 330±53 g and 134±25 kcal. No significant differences in outcomes according to visit sequence were found.

Mean $\delta^{13}\text{C}$ values at time one and time two, respectively, were $-19.88\pm0.09\text{‰}$ (range: -22.09 to -18.87‰) and $-19.99\pm0.11\text{‰}$ (range: -22.91 to -18.68‰); these values are comparable to those reported in a prior investigation which used venipuncture to obtain serum samples (12). The $\delta^{13}\text{C}$ measurements were correlated across visits ($r=0.873$, $p<0.001$), and for subsequent analyses, the mean $\delta^{13}\text{C}$ value was used (sample mean: $-19.94\pm0.10\text{‰}$). No difference in $\delta^{13}\text{C}$ values was detected across age groups ($p=0.370$), however there were significant differences according to BMI and gender. The $\delta^{13}\text{C}$ value differed between normal weight (18.5–24.9 kg/m²) and overweight (25–29.9 kg/m²) individuals (mean difference = $-0.60\pm0.20\text{‰}$, $p=0.005$), and between normal weight and obese (≥ 30 kg/m²) individuals (mean difference = $-0.68\pm0.29\text{‰}$, $p=0.028$), but not between overweight or obese weight status categories. Significant correlations were noted between $\delta^{13}\text{C}$ values and BMI ($r=0.343$, $p=0.007$). The $\delta^{13}\text{C}$ value was higher in males compared with females (mean difference = $0.40\pm0.19\text{‰}$, $p=0.043$), which is consistent with previous reports on large populations (12). However, this sex difference may be attributed to body mass differences or to differences in added sugar consumption, as opposed to sex differences; $\delta^{13}\text{C}$ value was not different when overweight and obese men were compared to overweight and obese women ($-19.67\pm0.19\text{‰}$ vs $-19.64\pm0.12\text{‰}$, respectively; $p=0.907$).

Significant correlations were noted between $\delta^{13}\text{C}$ values and dietary intake variables. The $\delta^{13}\text{C}$ 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 kcal and gram of soft drinks ($r=0.270$ and $r=0.258$, $p<0.05$; respectively) and total SSB ($r=0.345$, $p<0.01$ and $r=0.284$, $p<0.05$; respectively) determined by the BEVQ. After controlling for BMI, associations between $\delta^{13}\text{C}$ values and these dietary variables remained significant (added sugar g: $r=0.279$, $p=0.033$; SSB kcal: $r=0.353$, $p=0.006$; SSB g: $r=0.288$, $p=0.027$). When evaluating nutritional biomarkers compared to reported dietary intake, correlations have been reported to range 0.03 to 0.73, with a mean of ~0.39 (20). 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 (20). Although correlations of $\delta^{13}\text{C}$ value with self-reported dietary intake in the present investigation are slightly below this level ($r\sim 0.3\text{--}0.4$), 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 a significant and controversial public health issue: added sugar intake and health (4–6, 21).

Others have shown that the $\delta^{13}\text{C}$ value of plasma glucose is a valid biomarker for C_4 sugars consumed in the previous meal (19); a more integrative tissue such as blood, if at all sensitive to sweetener intake may 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/SSB intake in epidemiologic or cross-sectional studies, or to assess changes in intake during intervention studies. In addition, findings related to self-reported SSB intake suggests that the brief self-reported BEVQ tool (17) may be used to rapidly assess SSB and soft drink intake, when more resource-intensive dietary assessment tools are not feasible.

As presented in Table 1, the lowest and the highest added sugar intake tertiles demonstrated significant differences in $\delta^{13}\text{C}$ value (mean difference = -0.48% , $p=0.028$). These groups were not significantly different with respect to age or BMI (Table 1). As intended, the groups were different with respect to reported consumption of total added sugars (kcal, g), SSB energy, and soft drinks (kcal, g). The significant association of the $\delta^{13}\text{C}$ value of fingerstick blood with the consumption of total added sugars, SSB energy, and soft drinks bodes well for the development of a $\delta^{13}\text{C}$ -assay on low-invasive fingerstick samples that may be able to identify high- *versus* 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 thus do not carry a conspicuous $\delta^{13}\text{C}$ value: specifically beet sugar, honey, and maple syrups. However, these represent a small fraction of total sweetener consumption (e.g., U.S. per capita corn sweetener availability ~ 69 lbs/yr; honey and edible syrups is ~ 1 lb/yr) in the U.S. (22). A second limitation is that $\delta^{13}\text{C}$ value in whole blood (unlike plasma glucose; (19)) 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 $\delta^{13}\text{C}$ value of fingerstick blood shows promise as a non-invasive biomarker of added sugar and SSB intake. Future research should be conducted to determine if body mass and composition influence $\delta^{13}\text{C}$ 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 if the technique may be used in children or adolescents. Future work is also warranted to refine this technique; specifically to determine the extent to which corn consumption (*versus* sugar consumption) impacts the body's $\delta^{13}\text{C}$ value pool, and if 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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Figure 1.
Study procedures: Association of $\delta^{13}\text{C}$ in Fingerstick Blood with Added Sugars and Sugar-sweetened Beverage Intake*

*Abbreviations Used: BEVQ, Beverage Intake Questionnaire; FIR, Food Intake Record.

Table 1

Group Characteristics of Low (Lo) and High (Hi) Added Sugar Consumers: Investigation of $\delta^{13}\text{C}$ as a Potential Biomarker of Added Sugars and Sugar-sweetened Beverage Intake

Participant Characteristics	Lo Group	Hi 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	7 (35)	4 (20)
Mean, yrs [*]	47.8±4.1	38.1±3.8
Body mass index (BMI) status, n (%)		
Underweight (<18.5 kg/m ²)	0 (0)	1 (5)
Normal weight (18.5–24.9 kg/m ²)	8 (40)	6 (30)
Overweight (25–29.9 kg/m ²)	9 (45)	7 (35)
Obese (≥30 kg/m ²)	3 (15)	6 (30)
Mean BMI, kg/m ² [*]	26.4±0.9	29.1±2.5
$\delta^{13}\text{C}$, ‰ [*]	−20.06±0.17	−19.59±0.13 ^a
Added Sugar, [*]		
kcal	128±44	432±184 ^b
g	32±3	108±10 ^b
Beverage Intake Questionnaire (BEVQ)		
Sugar-sweetened beverage consumption [*]	98±16	236±63 ^a
kcal	308±58	511±128
g		
BEVQ Soft drink consumption [*]		
kcal	7±3	67±26 ^a
g	33±19	152±58 ^a

^{*} Mean±Standard error of the mean.

^a Significant group difference, $p < 0.05$.

^b Significant group difference, $p < 0.001$.