The $\delta^{13}$C Value of Fingerstick Blood Is a Valid, Reliable, and Sensitive Biomarker of Sugar-Sweetened Beverage Intake in Children and Adolescents

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Abstract

Background: Reliance on self-reported dietary intake methods is a commonly cited research limitation, and dietary misreporting is a particular problem in children and adolescents. Objective indicators of dietary intake, such as dietary biomarkers, are needed to overcome this research limitation. The added sugar (AS) biomarker $\delta^{13}$C, which measures the relative abundance of $^{13}$C to $^{12}$C, has demonstrated preliminary validity in adults.

Objective: The purpose of this investigation was to determine the comparative validity, test-retest reliability, and sensitivity of the $\delta^{13}$C biomarker to detect AS and sugarsweetened beverage (SSB) intake using fingerstick blood samples in children and adolescents.

Methods: Children (aged 6–11 y, $n = 126$, 56% male, mean ± SD age: 9 ± 2 y) and adolescents (aged 12–18 y, $n = 200$, 44% male, mean ± SD age: 15 ± 2 y) completed 4 testing sessions within a 3-wk period. Participants’ height, weight, demographic characteristics, and health history were determined at the first session; 24-h recalls were obtained at each visit and fingerstick blood samples were collected at visits 1 and 3. Samples were analyzed for $\delta^{13}$C value using natural abundance stable isotope mass spectrometry. $\delta^{13}$C value was compared with dietary outcomes in the full sample, and in child and adolescent subgroups. t Tests and correlational analyses were used to assess biomarker validity and reliability, whereas logistic regression and area under the receiver-operator characteristic curve (AUC) were used to evaluate sensitivity.

Results: Reported mean ± SD AS consumption was 82.2 ± 35.8 g/d and 329 ± 143 kcal/d, and SSB consumption was 222 ± 243 mL/d and 98 ± 103 kcal/d. Mean $\delta^{13}$C value was −19.65 ± 0.69‰, and was lower in children than in adolescents (−19.80 ± 0.67‰ compared with −19.56 ± 0.67‰, $P = 0.002$). $\delta^{13}$C values were similar across sessions (visit 1: $r = 0.90$, $P < 0.001$) and were associated ($P < 0.001$) with intake of total AS (grams, kilocalories: $r = 0.29$) and SSB (milliliters, kilocalories: $r = 0.35$). The biomarker was able to better discriminate between high and low SSB consumers than high and low AS consumers, as demonstrated by the AUC (0.75 and 0.62, respectively).

Conclusions: The $\delta^{13}$C biomarker is a promising, minimally invasive, objective biomarker of SSB intake in children and adolescents. Further evaluation using controlled feeding designs is warranted. Registered at clinicaltrials.gov as NCT02455388.


Keywords: dietary assessment, obesity, sugarsweetened beverages, children and adolescents, corn and cane sugar, added sugar

Introduction

Childhood obesity has become a major public health concern, with 17% of children and adolescents (in the US) now considered obese (1). Excessive consumption of added sugar (AS) has been suggested as a contributor to this public health problem (2–5). Despite recommendations from major health organizations (6–8), children and adolescents consume AS in excess of the 10% intake level currently recommended (9), with children consuming ~16% of their total calories from AS (7).
Sugar-sweetened beverages (SSBs) represent the primary source of AS, accounting for 8% of total caloric intake (10). Excessive AS intake, particularly in the form of SSBs, has demonstrated an association with obesity (11, 12). High AS consumption is also associated with an increased risk for dental caries (13), chronic diseases such as diabetes, hypertension, ischemic heart disease and stroke (6, 14, 15), stunted growth and development (9), and an inadequate micronutrient intake (8, 9, 16, 17).

Although research findings have suggested a relation between AS and SSB intake and several chronic diseases (6, 14, 15, 18–22), a causal relation is difficult to determine due to the limitations of self-reported dietary assessment methodologies (23). Misreporting is more pronounced in younger populations who may have difficulty estimating portion sizes, conceptualizing foods, understanding food preparation, and recalling all foods and beverages consumed (23–25). Furthermore, it is common for people of all ages to underreport foods deemed socially undesirable (e.g., sugar-rich foods) and those that are consumed as snacks (25), which makes the relation between dietary intake and disease prevention or progression challenging to assess. The Institute of Medicine and others have highlighted the need to develop and validate dietary biomarkers in order to objectively assess dietary intake (26, 27). Currently, there are no validated predictive dietary biomarkers of AS intake in children and adolescents—a population that consumes even greater amounts of AS than adults (28).

Naturally occurring variations in stable isotope ratios, including \(^{13}\)C:\(^{12}\)C (reported as \(\delta^{13}\)C), have been used as objective measures of diet in modern humans (29–34). The \(\delta^{13}\)C value of a given plant sugar reflects the biochemical pathways used by the plant during photosynthesis. "C4" plants, such as corn and sugar cane, give rise to sugar with high \(\delta^{13}\)C value, while "C3" plants, which include the majority of fruits and vegetables, give rise to sugars with much lower \(\delta^{13}\)C values (33, 38). Therefore, the consumption of corn and cane sugars and their derivatives is reflected in the \(\delta^{13}\)C value of adult human tissues using a variety of biological sample types, including a minimally invasive fingerstick blood sample (29, 35). The \(\delta^{13}\)C AS biomarker has demonstrated promising preliminary evidence of validity and reliability in adults (26, 29, 31–33, 35). However, tissue turnover times may vary according to growth stage, which could impact biomarker values (35, 36). Thus, the purpose of this investigation was to determine the comparative validity and test-retest reliability of the \(\delta^{13}\)C biomarker of AS intake, particularly in the form of SSBs, using fingerstick blood samples in children and adolescents aged 6–18 y.

**Methods**

**Subjects.** Three-hundred sixty-four children (aged 6–11 y) and adolescents (aged 12–18 y) were screened online from a local university community in southwestern Virginia, between January 2014 and September 2015. Three-hundred twenty-six children and adolescents aged 6–18 y were screened online from a local university community in southwestern Virginia, between January 2014 and September 2015. Three-hundred sixty-four children (aged 6–11 y) and adolescents (aged 12–18 y) were screened online from a local university community in southwestern Virginia, between January 2014 and September 2015. Three-hundred twenty-six children and adolescents—a population that consumes even greater amounts of AS than adults (28).

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the multiple-pass method; visual aids, including food diagrams and food models, were used. The four 24-HRs were obtained on nonconsecutive days, including 1 weekend day, in a manner consistent with NHANES methodology (24, 25, 39). However, all participants were encouraged to have an adult present to aid in completing the 24-HR. The 24-HRs were analyzed with the use of NDS-R (Nutrition Data System for Research 2013; University of Minnesota, Minneapolis, MN) to quantify dietary intake variables using a 4-d average, including usual AS and SSB intake.

Non-fasting fingerstick blood samples were obtained at 2 of the 4 visits (visits 1 and 3; Figure 1) via routine fingersticks (One Touch Fine Point Lancet; Johnson & Johnson Company); Blood samples were blotted onto sterilized, binder-free glass microfiber filters (Whatman type GF/D, 2.5-cm diameter; Whatman, Inc.) and air-dried. Blood samples were analyzed in triplicate for δ13C value via natural abundance stable isotope mass spectrometry (Isoprime; Micromass UK Ltd), as described previously (29). δ15N values, measuring the relative abundance of 15N to 14N, were also determined and evaluated as possible covariates to control for the potential confounders of meat (i.e., livestock consuming corn feed) consumption on δ13C values (32, 40). Total variability across the 3 measurements was within 0.04‰.

Statistical methods. Statistical analyses were performed using statistical analysis software (Medcalc: 17.8, STATA 14, SPSS 22). Descriptive statistics (mean ± SD) are reported for participant demographic characteristics and dietary intake variables [total kilocalories, total dietary AS (grams, kilocalories), and total SSBs (milliliters, kilocalories)]. Paired-sample t tests, independent-sample t tests, chi-square tests and one-factor ANOVA were used to evaluate differences within and between assessment methods and group differences. Test-retest reliability (i.e., δ13C values obtained at the 2 laboratory visits) was assessed using the intraclass correlation coefficient (ICC), and a Pearson correlation coefficient between δ13C values and reported dietary AS intake evaluated comparative validity. Lastly, binary logistic regression analyses and non-parametric receiver operator curves (ROCs) were used to evaluate the diagnostic value of δ13C biomarker using the fingerstick blood. Participant AS and SSB intakes based on 24-HR recalls were dichotomized (≥50th percentile and <50th percentile) to reflect a measure for the diagnostic accuracy of the δ13C biomarker, with values noted in children (i.e., farther from zero, reflecting a lower AS and SSB intake), which is consistent with self-reported AS and SSB intake. In the full sample, significant differences in δ13C value were noted between normal weight and obese participants (mean difference = −0.51‰, P < 0.001), but not between normal weight and overweight participants (mean difference = −0.19‰, P = 0.11) or by gender (mean difference = 0.11‰, P = 0.14).

Significant correlations were noted between δ13C values and self-reported dietary variables including: total AS grams per day and kilocalories per day (r = 0.23, P < 0.001), SSB milliliters per day (r = 0.35, P < 0.001) and kilocalories per day (r = 0.35, P < 0.001). Scatterplots (Figure 2) show that δ13C was significantly higher in the high AS and high SSB consumers. δ13C value was correlated (P < 0.01) with BMI (r = 0.28), δ15N (r = 0.63), and age (r = 0.17), and only marginally with gender (P = 0.07). After controlling for BMI, δ15N, and age, all differences in δ13C values between high and low AS and SSB consumers were maintained.

δ13C discriminated low SSB consumers from high consumers with a sensitivity of 52% and a specificity of 77% (at a cut-off of −19.31‰; AUC = 0.70, SE = 0.03). Binary logistic regression with block entry using BMI, δ15N and gender provided a model [Model 1: 26.91 + (1.20 × δ13C) + (0.09 × BMI) – (0.65 × δ15N) – (0.74 × gender)] that significantly improved the ability to distinguish low from high SSB consumers with a specificity of 84% and a sensitivity of 60% (at a cut-off of >−19.51‰; AUC = 0.75, SE = 0.03; ROC AUC comparison: P < 0.01; see Table 2). Adding age to the above model did not improve the discrimination between high and low SSB consumers (AUC = 0.75). δ13C discriminated low AS consumers from high AS consumers with a sensitivity of 83% and a specificity of 38% (at a cut-off of >−19.85‰; AUC = 0.62, SE = 0.03). Adding BMI, δ15N, gender, and age did not improve the discrimination between high and low AS consumers (AUC = 0.61). The discrimination capability of δ13C was not significantly different between children and adolescents, or between males and females.

Results

Participant demographic characteristics, dietary intake, and biomarker values are presented in Table 1. The sample (n = 326) was balanced with regard to sex (49% males, 51% females), while 93% of participants considered themselves white (2% were Asian, 1% were African American, and 1% were other) and non-Hispanic (93%). Age ranged from 6 to 18 y (mean age: 12 ± 3 y). The sample comprised 126 participants who were children (aged 6–11 y; 44% girls) and 200 participants who were adolescents (aged 12–18 y; 56% female). Mean BMI was in the normal range with most participants categorized between the 5th and 85th percentile. Age group differences were noted between the percentage of children and adolescents classified as underweight and obese (P < 0.05). As expected, adolescents had greater RFF and reported energy intake than did children. Approximately 12% of the sample were classified as underreporters; however, reliability (r = 0.992, P < 0.001) and validity (AS g/d, kcal/d: r = 0.23, P < 0.001; SSB mL/d: r = 0.35, P < 0.001 and SSB kcal/d: r = 0.35, P < 0.001) results were not different after excluding potential underreporters.

Among children and adolescents in this sample, reported total daily AS intake was similar to the mean intake of AS by persons >2 y (83.9 g, 336 kcal/d) in the United States (8, 42). AS and SSB intakes were significantly different between children and adolescents (P < 0.001), with children reporting a lower consumption than adolescents.

Mean δ13C values at visits 1 and 3, respectively, were −19.66 ± 0.68‰ (range −22.26‰ to −17.79‰) and −19.64 ± 0.68‰ (range −22.39‰ to −17.84‰). Biomarker measurements were strongly correlated across the 2 visits (ICC = 0.99, P < 0.001) and for subsequent analyses the δ13C value at visit 1 was used. (Note: the following results do not differ when using the visit 2 δ13C value, or the average of the 2 measures.) The δ13C values differed between children and adolescents with lower values noted in children (i.e., farther from zero, reflecting a lower AS and SSB intake), which is consistent with self-reported AS and SSB intake. In the full sample, significant differences in δ13C value were noted between normal weight and obese participants (mean difference = −0.51‰, P < 0.001), but not between normal weight and overweight participants (mean difference = −0.19‰, P = 0.11) or by gender (mean difference = 0.11‰, P = 0.14).

Discussion

Our findings represent the first investigation to evaluate the validity, reliability, and sensitivity of the δ13C AS biomarker using fingerstick blood samples in children and adolescents. Only

Added-sugar biomarker in children and adolescents

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TABLE 1 Participant characteristics of the full sample and among children and adolescent subgroups in a study evaluating the validity and reliability of the $\delta^{13}$C value of fingerstick blood as a biomarker of added sugar intake.

<table>
<thead>
<tr>
<th>Participant characteristics</th>
<th>Total sample</th>
<th>Children (6–11 y)</th>
<th>Adolescents (12–18 y)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of participants, n</td>
<td>326</td>
<td>126</td>
<td>200</td>
</tr>
<tr>
<td>Male, n(%)</td>
<td>159 (49)</td>
<td>71 (56)</td>
<td>88 (44)*</td>
</tr>
<tr>
<td>Female, n(%)</td>
<td>167 (51)</td>
<td>55 (44)</td>
<td>112 (56)*</td>
</tr>
<tr>
<td>Age, y</td>
<td>12 ± 3</td>
<td>9 ± 2</td>
<td>15 ± 2</td>
</tr>
<tr>
<td>BMI status, n(%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Underweight (&lt;5th percentile)</td>
<td>12 (4)</td>
<td>9 (7)</td>
<td>3 (2)*</td>
</tr>
<tr>
<td>Normal weight (5th to &lt;85th percentile)</td>
<td>254 (78)</td>
<td>101 (80)</td>
<td>153 (77)</td>
</tr>
<tr>
<td>Overweight (85th to &lt;95th percentile)</td>
<td>35 (11)</td>
<td>11 (9)</td>
<td>24 (12)</td>
</tr>
<tr>
<td>Obese (≥95th percentile)</td>
<td>25 (8)</td>
<td>5 (4)</td>
<td>20 (10)*</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>20.0 ± 4.5</td>
<td>17.0 ± 2.8</td>
<td>21.8 ± 4.4*</td>
</tr>
<tr>
<td>BMI-for-age, percentiles</td>
<td>56.1 ± 27.9</td>
<td>49.6 ± 28.4</td>
<td>60.1 ± 26.8*</td>
</tr>
<tr>
<td>Resting energy expenditure, kcal/d</td>
<td>1891 ± 472</td>
<td>1736 ± 412</td>
<td>1992 ± 485*</td>
</tr>
<tr>
<td>Reported energy intake, kcal/d</td>
<td>2063 ± 552</td>
<td>1882 ± 418</td>
<td>2190 ± 588*</td>
</tr>
<tr>
<td>$\delta^{13}$C,‰</td>
<td>−15.65 ± 0.69</td>
<td>−19.89 ± 0.67</td>
<td>−19.56 ± 0.67*</td>
</tr>
<tr>
<td>Added sugar</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>g/d</td>
<td>82.2 ± 35.8</td>
<td>73.2 ± 28.1</td>
<td>87.8 ± 38.9*</td>
</tr>
<tr>
<td>kcal/d</td>
<td>329 ± 143</td>
<td>293 ± 112</td>
<td>351 ± 159*</td>
</tr>
<tr>
<td>Sugar-sweetened beverage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ml/d</td>
<td>222 ± 243</td>
<td>177 ± 210</td>
<td>251 ± 254*</td>
</tr>
<tr>
<td>kcal/d</td>
<td>98 ± 103</td>
<td>79 ± 88</td>
<td>110 ± 110*</td>
</tr>
</tbody>
</table>

1Values are means ± SDs unless otherwise indicated. Chi-square tests were used to compare proportions across groups; independent-sample t tests were used to compare age group means; BMI-for-age percentiles were calculated according to CDC standards (37); resting energy expenditure was calculated based upon data from the Institute of Medicine (38); reported energy intake was the average energy intake from the four 24-h dietary recalls. *Different from children, $P < 0.05$. This is likely explained by SSBs being comprised of 99% corn and cane sweeteners (36), while AS could be derived from a wider variety of sugar sources (i.e., C4 or C3 plants) (35). This may also explain the ROC results, which indicated that the biomarker was able to better discriminate between high and low SSB consumers than high and low AS consumers. While the $\delta^{13}$C biomarker can be useful for identifying high and low AS and SSB consumers, the AUC for these variables was within the acceptable range (0.7–0.9) (45) for SSB but not for AS. In part, this could be attributed to the use of self-reported dietary data, which may underestimate biomarker validity due to both dietary underreporting and the variability in self-reported intake (46). Therefore, this technique merits further investigation.

FIGURE 2 $\delta^{13}$C values of fingerstick blood in children and adolescents with low and high SSB (A) and AS (B) intakes. Values are significantly higher in the high-SSB (high, $n = 98$; low, $n = 228$) and high-AS (high, $n = 82$; low, $n = 244$) consumers. **Means differ, $P < 0.01$. After controlling for age, BMI, and $\delta^{15}$N (‰), these differences were maintained, $P < 0.01$. AS, added sugar; SSB, sugar-sweetened beverage.
using controlled feeding study designs in children and adolescents, given its potential to contribute to the investigations of the health consequences of AS and SSB intake (6, 8, 9, 13–16, 43, 47, 48).

The current investigation reported mean intakes of SSB intake for children (79 kcal/d) and adolescents (110 kcal/d) that were below the current estimates of 129 and 350 kcal/d from SSBs alone for children and adolescents, respectively, determined using a single 24-HR, in the United States (2). Johnson et al. (8) found that the mean AS intake, using two 24-HRs, for children aged 4–8 y was ∼336 kcal/d while adolescent males and females reported much higher intakes (467 and 371 kcal/d, respectively); our results are consistent with the current literature, indicating that our sample is generalizable with respect to AS and SSB intake in children. These estimates of AS and SSB intake far exceed recommendations made by the Dietary Guidelines for Americans (9), the American Heart Association (8, 41), and the WHO (6).

Strengths of this investigation include a large sample size (n = 326) with a wide age range (6–18 y). The approach used also allowed us to evaluate differences in dietary intake and biomarker values between children and adolescents. Utilizing fingerstick blood samples minimized the degree of invasiveness, which is a commonly cited limitation of biochemical data collection, particularly among children, and no adverse effects were reported.

We acknowledge several limitations in our study. Despite a large sample size, there was a lack of racial and ethnic diversity in our sample; minority populations are more likely to consume SSBs than their white counterparts (2). Pubertal status was not assessed; however, this was addressed by dichotomizing the sample into child and adolescent subgroups, which did not present any significant differences in AUC values. The δ¹³C biomarker is limited to assessing intake of sugar from C4 plants, as proposed elsewhere (1). Tested as a covariate to control for meat intake using methods determined using a single 24-HR, in the United States (49). Johnson et al. (8) also observed a significant difference in AS intake between male and female participants; our study did not observe this difference.

In conclusion, the δ¹³C biomarker holds promise as a minimally invasive, objective indicator of AS and SSB intake in children and adolescents. This biomarker has the potential to overcome limitations posed by self-reported dietary data that may aid in discovering consistent and causal relations between AS and SSB intake and health (26, 29, 35, 51). Further research is warranted using a controlled feeding study design, where AS intake and/or SSB intake is manipulated. This approach could also determine the dose-response relations between δ¹³C values and AS and SSB intake (52), which could potentially be translated into clinically relevant thresholds to objectively characterize the AS and SSB consumption of children and adolescents. As MS technology advances to include portable, lower-cost mass spectrometers (53), a valid stable isotope biomarker of AS and SSB intake could have substantial public health impact both for research and clinical practice.

Acknowledgments
The authors’ responsibilities were as follows—BMD, AHJ, JS, VEH, HAB, MIF, and JCD: designed the research; CRM, CEH, and SKR: conducted the research; BMD and AHJ: provided essential materials; CRM, BMD, and JS: analyzed the data; and all authors: wrote the paper and read and approved the final manuscript.

References

TABLE 2 AUC for the associations between AS and SSB intake with δ¹³C in children and adolescents

<table>
<thead>
<tr>
<th>Full sample</th>
<th>SSB (AUC)</th>
<th>AS (AUC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male (n = 159)</td>
<td>0.59 ± 0.05</td>
<td>0.76 ± 0.04</td>
</tr>
<tr>
<td>Female (n = 167)</td>
<td>0.64 ± 0.05</td>
<td>0.68 ± 0.05</td>
</tr>
<tr>
<td>Age group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Children (n = 126)</td>
<td>0.70 ± 0.06</td>
<td>0.78 ± 0.05</td>
</tr>
<tr>
<td>Adolescents (n = 200)</td>
<td>0.60 ± 0.05</td>
<td>0.74 ± 0.04</td>
</tr>
</tbody>
</table>

1 All values are means ± SEs. Analyses for the full sample and age groups adjusted for δ¹⁵N, BMI, and gender; analyses by gender adjusted for δ¹⁵N, BMI, and age. AS, added sugar; SSB, sugar-sweetened beverage.


