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Short-term changes in added sugar consumption by adolescents reflected in the carbon isotope ratio of fingerstick blood

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Abstract

Background: Consumption of added sugars (AS) and sugar-sweetened beverages (SSB) may adversely affect adolescents' weight and cardiovascular disease risk. Reliance on self-reported dietary assessment methods is a common research limitation, which could be overcome by dietary intake biomarkers. **Aim:** The investigation was a proof-of-concept study to evaluate the proposed carbon isotope ratio ($\delta^{13}C$) biomarker of AS intake in adolescents, using a controlled feeding design. **Methods:** Participants ($n = 33$, age 15.3 years, 53% female) underwent two seven-day controlled feeding periods in a randomly assigned order. Diets were matched in composition except for AS content (5% or 25% of total energy). Fasting fingerstick blood samples were collected daily during each diet period. **Results:** Fingerstick $\delta^{13}C$ values changed from day 1 to 8 by –0.05 $\pm$ 0.071‰ on 5% AS, and +0.03 $\pm$ 0.083‰ on 25% AS ($p \leq 0.001$). Reliability was demonstrated between day 7 and 8 $\delta^{13}C$ values on the 5% (ICC = 0.996, $p \leq 0.001$) and 25% (ICC = 0.997, $p \leq 0.001$) AS diets. **Conclusions:** Larger scale investigations are warranted to determine if this technique could be applied to population-level research in order to help assess the effectiveness of interventions aimed at reducing the consumption of AS or SSB intake.

Keywords

Added sugar, adolescents, biomarker dietary assessment, validation

Introduction

Added sugars (AS) are defined as: "... sugars and syrups added to foods during processing or preparation (Johnson et al., 2009; US Department of Agriculture, 2015)." Adolescents consume approximately 16% of daily calories as (Ervin et al., 2012; Welsh et al., 2011). Of this, sugar-sweetened beverages (SSB) comprise about 33–60% of AS intake (Ervin et al., 2012; Guthrie and Morton, 2000; Watowicz et al., 2015; Welsh et al., 2011); adolescent consumer preference for sugary beverages has been shown to be heavily influenced by unique environmental influences (Smith et al., 2017). Consumption of AS/SSB is linked to undesirable changes in weight status (Bermudez and Gao, 2010; Lim et al., 2009; Malik et al., 2013; Wang et al., 2015) and cardiovascular disease risk factors in youth (Ambrosini et al., 2013; Gyllenhammer et al., 2014). This has prompted major health organizations to establish limits for AS intake (US Department of Agriculture, 2015; Vos et al., 2016; World Health Organization, 2015). Yet, there is debate about the health effects of AS/SSB intake, partly due to the limitations of self-reported dietary data (Davy and Jahren, 2016). Because pediatric populations tend to misreport dietary intake (Bel-Serrat et al., 2016; Lioret et al., 2011; Murakami and Livingstone, 2016; Rangan et al., 2014; Ventura et al., 2006), especially for foods containing AS (Lioret et al., 2011; Rangan et al., 2014; Ventura et al., 2006), there is a need for objective dietary intake biomarkers (Kuhnle, 2012). The $\delta^{13}C$ value

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of fingerstick blood has been proposed as a biomarker for AS intake (Davy and Jahren, 2016). If validated, this technique could be applied to population-level research in order to help assess the effectiveness of interventions aimed at reducing the consumption of AS or SSB intake.

The proposed $\delta^{13}C$ AS biomarker is based upon the differential accumulation of ^{13}C to ^{12}C isotopes in plant tissues (Jahren et al., 2014). Plants that perform C4 photosynthesis undergo additional chemical reactions than the C3 pathway, which leads to elevated ^{13}C content in C4 plants (Jahren et al., 2014). The primary crops for sugar production in the United States—corn, sugarcane, and sorghum—are C4 plants (Jahren et al., 2014). Thus, the $\delta^{13}C$ of human tissue, which is influenced by diet, can reflect AS intake. Positive correlations between AS intake and the $\delta^{13}C$ value of plasma glucose (Cook et al., 2010), red blood cell (RBC) alanine (Choy et al., 2013), RBCs (Nash et al., 2014), and whole blood (Davy et al., 2011; Fakhouri et al., 2014; Yeung et al., 2010) have been reported. However, investigations have been limited to those which utilized self-reported dietary intake data (Chi et al., 2015; Choy et al., 2013; Davy et al., 2011; Fakhouri et al., 2014; MacDougall et al., 2018; Nash et al., 2013; Nash et al., 2014; Yeung et al., 2010) and one short-term controlled feeding study in adults (Cook et al., 2010). Of the two published investigations of the $\delta^{13}C$ biomarker in children (Chi et al., 2015; MacDougall et al., 2018), only one assessed dietary intake (MacDougall et al., 2018). Our recent comparison of biomarker values to self-reported SSB intake in 326 children and adolescents determined that the biomarker was able discriminate between high and low SSB consumers (MacDougall et al., 2018). Controlled feeding studies are now needed to evaluate the validity of this technique for objectively assessing AS and SSB intake.

Tissue metabolic rate and $\delta^{13}C$ turnover are positively correlated in animals (Fry and Arnold, 1982; MacAvoy et al., 2006; Tieszen et al., 1982). Since adolescents are undergoing growth and development (Rogol et al., 2000), tissue turnover times may be more rapid in adolescents than in adults, and fingerstick blood $\delta^{13}C$ may therefore change more rapidly in response to changes in AS intake. The objective of this investigation was to evaluate the sensitivity and reliability of the proposed $\delta^{13}C$ AS biomarker, assessed in fingerstick blood samples, in adolescents during a short-term controlled feeding trial using a crossover design. The investigation was a proof-of-concept study. It was hypothesized that fingerstick $\delta^{13}C$ would be a sensitive and reliable indicator of actual AS intake.

Methods

Participants

Adolescents were recruited from a local campus community in Southwest Virginia between June 2015 and July 2016 through flyers, email advertisements, and word of mouth. Eligible individuals were 12–18 years of age; with a body mass index (BMI) percentile <95% per the Centers for Disease Control and Prevention's (CDC) BMI-for-age growth charts (Centers for Disease Control and Prevention, 2015); without food allergies, intolerances, or aversions; and willing to follow a controlled diet for two separate 1-week periods. Of the 58 adolescents screened for eligibility, 33 were enrolled in the study. Based upon power analyses that assumed a minimum correlation of 0.3 within participants, plus a minimum effect size of $\eta^2 p = 0.1$, we estimated that a sample size of 30 would be sufficient to infer that the difference in $\delta^{13}C$ value of fingerstick blood samples between day 1 and day 8 of each controlled feeding period would not be zero (significance level is 0.05; 90% power). Therefore, once approximately 30 participants had enrolled, recruitment was ceased. Of those enrolled, 32 participants completed the study.

Experimental design

Figure 1 depicts the study design. Baseline measurements took place over four study sessions, which included assessment of demographic information, habitual dietary intake using four 24-h dietary recalls, habitual physical activity level, height, weight, and the collection of two fingerstick blood samples. Using a crossover design, participants next completed two seven-day controlled feeding periods in a randomly assigned order (5% "low" added sugars (LAS); 25% "high" added sugars (HAS)), with a four-week washout period between the two diet conditions. A study coordinator who was not involved with data collection or analysis was responsible for enrolling participants and assigning participants to their randomized sequence in which to complete the diets, LAS first (A) or HAS first (B), utilizing a computer-generated randomization scheme. Allocation of sequence was conducted to ensure that groups were approximately equal in distribution according to participants' gender and age. The sequence was known to researchers, but not to participants, before the controlled diets were provided. Each morning during the controlled diet periods, body weight was measured to ensure weight stability, and a fasting fingerstick blood sample was obtained. During the washout period, four 24-h dietary recalls were obtained, and physical activity level, height, and weight were reassessed. Participants were compensated US$500 for completing all study sessions, and were provided with a results packet that described their baseline self-reported dietary intake.

Experimental protocol

Controlled diet development and delivery. Age- and sex-specific equations from the Institute of Medicine (IOM) (Food and Nutrition Board and Institute of Medicine, 2005) were used to estimate energy needs. Physical activity was self-reported using the Physical Activity Questionnaire for Adolescents (PAQ-A) (Kowalski et al., 2004), which was

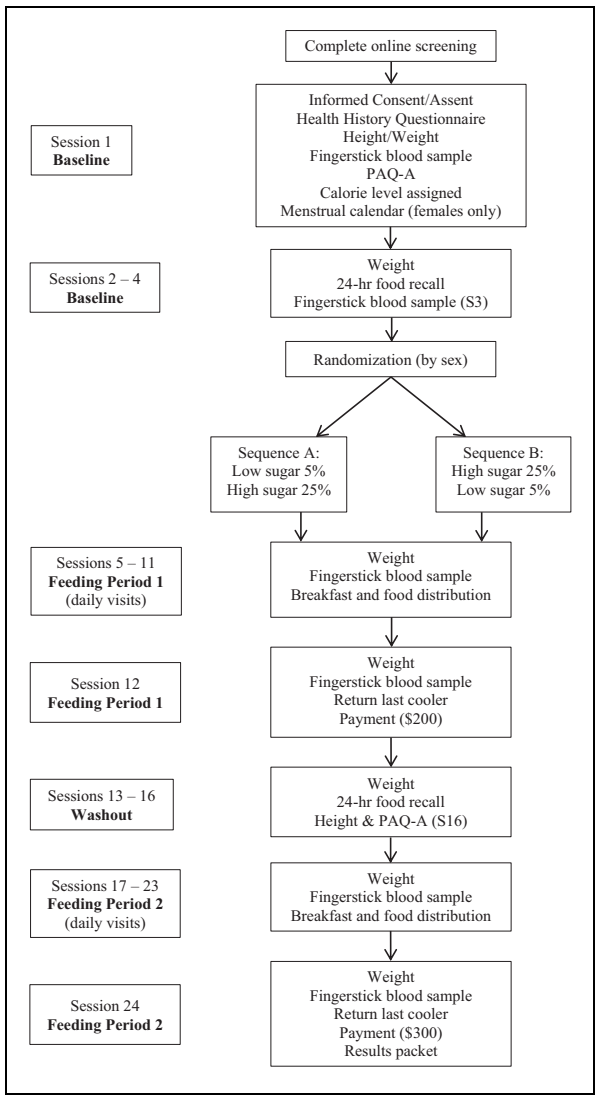

Figure 1. Study protocol. Sequence A: participants were assigned the 5%, LAS diet first, then the 25%, HAS diet. Sequence B: participants were assigned the 25%, HAS diet first, then the 5%, LAS diet. Unless otherwise indicated in parentheses, measurements/procedures listed in each panel took place at every visit indicated in the corresponding visit panel to the left.
PAQ-A: Physical Activity Questionnaire for Adolescents.

used to derive an activity factor for determining total daily energy requirements (Liu et al., 2016). The LAS and HAS diets (5% and 25% of calories from AS, respectively) were developed to meet dietary intake targets, which are listed in Table 1. Each seven-day diet consisted of a three-day rotating menu, which ranged from 1500 to 4500 kcal/day according to participants estimated energy requirements. Two optional 150 kcal snack modules were provided daily while on the controlled diet, to prevent energy deficits that could result from variation in daily activity level. Snack modules were designed to meet the same dietary targets as the overall diet.

Table 1. Dietary targets for low- and high-added sugar diets.

Nutrient	Target value
Total energy, kcal	Varies: 1500–3500
Carbohydrate, % of kcal	55%
Total AS, % of kcal	5% or 25%
Liquid AS, % of AS	33%
Solid AS, % of AS	67%
Fat, % of kcal	30%
Protein, % of kcal	15%
Animal protein, % of protein	42%
Dairy protein, % of protein	20%
Vegetable/other protein, % of protein	38%

A list of foods popular among children was used for menu planning (American Dietetic Association, 1999) to provide foods acceptable and palatable to this population. Food items were entered in nutrition analysis software (Nutrition Data System for Research (NDS-R) 2013, University of Minnesota, Minneapolis, MN). The specific foods provided on both diets were matched closely to minimize a difference in compliance between the two feeding periods (sample menus are included as Supplemental Material).

The macronutrient composition of both diets fell within the acceptable macronutrient distribution ranges prescribed for children of 4–18 years of age (Food and Nutrition Board and Institute of Medicine, 2006). The LAS diet matched the World Health Organization's conditional recommendation that AS should be limited to 5% of daily caloric intake (World Health Organization, 2015). The amount of AS on the HAS diet corresponded to the level of AS which has been associated with an inadequate intake of essential nutrients (Food and Nutrition Board and Institute of Medicine, 2006). The percentage of AS from solid versus liquid sources corresponded with that consumed by American adults (Ervin and Ogden, 2013), according to information available at the time of diet planning. Protein sources were consistent across diets, since meat sourced from corn-fed animals could influence $\delta^{13}C$ biomarker values (Jahren et al., 2014); percentages matched protein intake patterns consumed by American adults (Smit et al., 1999).

On the first day of each controlled feeding period, diet instructions were reviewed with participants and their parents. Breakfast was supervised and consumed in the dining laboratory, following the measurement of weight and the collection of a fingerstick blood sample. Remaining meals and snacks for the day were provided in a portable cooler and consumed outside of the laboratory. In addition to the optional snack modules, participants were provided with three sucralose packets and three bottles of water each day, which were optional. Any uneaten food was returned the next morning, which was weighed to determine actual food consumption and to assess compliance. Participants were also asked to report consumption of non-study provided foods. If participants consumed $\geq$90% of food

provided in the study (Hall and Most, 2005), then they were considered compliant with consuming the controlled diets.

Dietary assessment. During the baseline and washout periods (visits 1–4 and 13–16, respectively), participants completed a total of eight, non-consecutive 24-h recalls with trained research personnel. Recalls encompassed both weekday and weekend days, in accordance with recommended dietary assessment practices (Thompson and Subar, 2013). Although participants self-reported intake, most parents accompanied their children to assist when needed (e.g. reporting details of a recipe). Researchers employed the US Department of Agriculture's Multi-Pass Method (Raper et al., 2004) to standardize prompting. Additionally, participants used 2D and 3D food models to increase accuracy of reporting. Food recalls were analyzed using NDS-R to determine habitual macronutrient composition and AS intake.

Fingerstick blood sampling for $\delta^{13}C$. The primary outcome was the $\delta^{13}C$ values of fingerstick blood samples collected during the controlled feeding periods. Two non-fasting fingerstick samples were collected at baseline (MedLance Plus Universal Lancet, HTL-Strefa, Ozorków, Poland), to establish participant's usual values for $\delta^{13}C$. Each morning of both controlled feeding periods, after a 12-h fast, a fingerstick blood sample was collected to monitor change in $\delta^{13}C$ values in response to the high and low AS diet. Fingerstick blood samples were collected using sterilized binder-free glass microfiber filters (Whatman, type GF/D, 2.5 cm diameter, Whatman, Inc., Piscataway, NJ), and air-dried for 15–30 min. Next, the blood samples were analyzed for $\delta^{13}C$ via natural abundance stable isotope mass spectrometry (NA-SIMS), as previously described (Davy et al., 2011). Each sample was tested in triplicate; the mean of these results was reported. There was an analytical uncertainty of $\pm 0.046‰$ with each sample measurement for the entire sample ($n = 32$).

Statistical methods

Data were analyzed using statistical analysis software (SPSS v. 24.0 for Windows, SPSS Inc., Chicago, IL). Descriptive statistics (mean $\pm$ SD; frequencies) were reported for participant demographics (sex, age, race/ethnicity, height, weight, BMI, and BMI percentile), self-reported dietary intake and controlled dietary intake variables (total calorie intake (kcal); carbohydrate, protein, and fat intake (g and % of kcal); AS intake (g and % of kcal); sodium (mg/day)), dietary compliance (returned food weigh-back data ((consumed/provided) $\times$ 100, expressed as %), and $\delta^{13}C$ fingerstick values.

Sensitivity was evaluated using a repeated measures analysis of variance (RM-ANOVA) between day 1 and day 8 fingerstick $\delta^{13}C$ values within each diet condition. A post-hoc, paired sample t-test was used if a significant diet by condition effect was found. Reliability was assessed using day 7 and day 8 fingerstick $\delta^{13}C$ values, within each diet condition, using intra-class correlation (ICC). Paired sample *t*-tests were used to determine whether baseline fingerstick $\delta^{13}C$ values were different from day 1 fingerstick $\delta^{13}C$ value on the LAS and HAS diets. Paired sample *t*-tests were also used to determine if demographic and dietary intake data differed between the baseline and washout periods, and whether dietary variables differed between self-reported intake and controlled diets. All statistical tests were set with an a priori significance of $\alpha = 0.05$. One participant was determined to be 88% compliant (based upon returned food) during the LAS controlled diet, and analyses were conducted with and without this participant. No differences were found; therefore, biomarker analyses with the full sample are presented.

Results

As depicted in Figure 2, 58 participants were screened for eligibility. Of these, 25 failed to meet eligibility criteria ($n = 11$), declined to participate ($n = 3$), or were excluded for other reasons, e.g. failing to keep scheduled appointments ($n = 11$). Additionally, one participant discontinued the study after non-compliance to the controlled diet. Thus, 32 adolescents completed the controlled feeding study (97% completion) (see Figure 2). Participant demographic characteristics, anthropometric measurements, activity level and estimated energy requirements are provided in Table 2. The sample was primarily non-Hispanic White (97%) and female (53%); mean age was 15.3 years. BMI percentile was in the normal weight range (2), and physical activity level was reported as "low active" (37). Estimated daily energy needs were 2903 kcal/day, which ranged from 1938 kcal/day to 4745 kcal/day.

Anthropometric measurements, activity level and energy requirements were re-assessed during the washout period. Weight, PAQ-A score, and estimated energy needs did not significantly differ compared with baseline values (reported in Table 2, $p > 0.05$ for all). However, height increased by 0.4 ± 0.7 cm, from baseline to the washout period ($p \leq 0.001$). Self-reported dietary intake at baseline and washout is provided in Table 3. There were no significant differences in total energy, macronutrient (as g/day and % of kcal/day), total sugars, AS (as g/day and % of kcal/day), or sodium intake between baseline and washout periods (all $p > 0.05$).

Controlled feeding periods and dietary compliance

Estimated energy requirements were $\sim$383 kcal/day higher than baseline self-reported energy intake ($p \leq 0.001$). Weight stability and returned food were used to assess compliance to the controlled feeding periods. Weight did not significantly differ from day 1 to day 8 on either the LAS ($p = 0.613$) or HAS ($p = 0.879$) diet. Based on returned food, compliance was $98.5 \pm 0.01\%$ to the LAS diet and $97.9 \pm 0.02\%$ to the HAS diet. Three participants

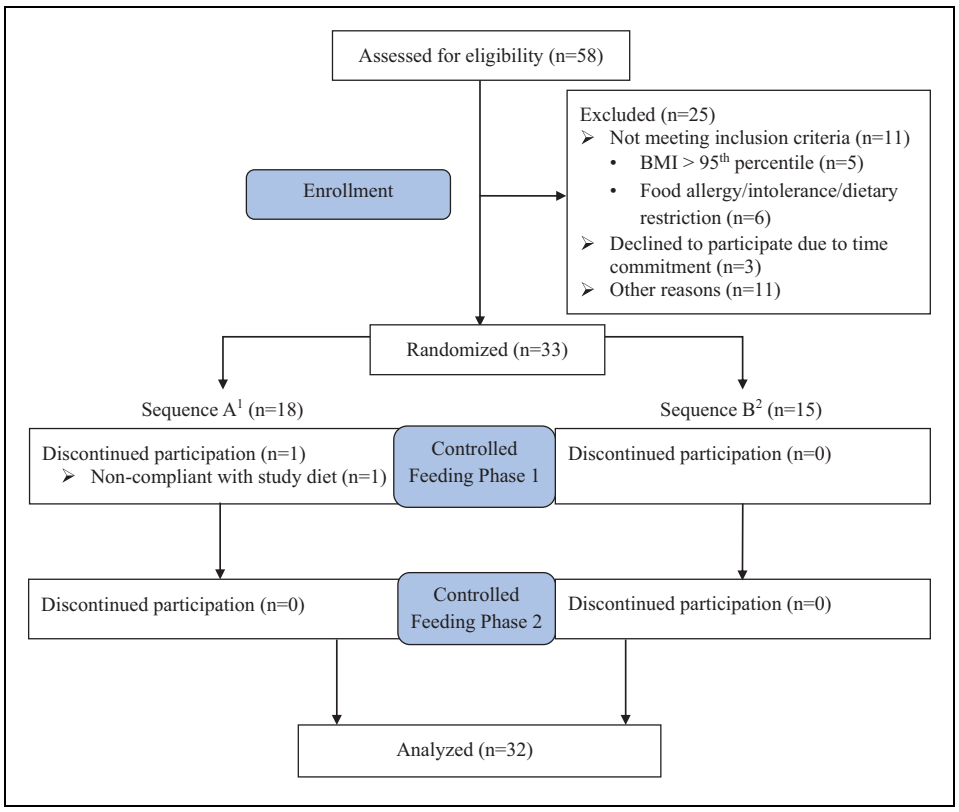

Figure 2. Consolidated Standards of Reporting Trials (CONSORT) diagram. [1]Sequence A participants were assigned the 5%, LAS diet first, then the 25%, HAS diet. [2]Sequence B participants were assigned the 25%, HAS diet first, then the 5%, LAS diet.

each reported consuming non-study foods on one day of the two seven-day controlled diet periods. Of these, one occurred during the LAS diet (one slice of cake and a sweetened sports drink), which resulted in the participant having to repeat the seven-day LAS diet period. Two occurred during the HAS diet (three breath mints (10 kcal, 0 g AS]; one s'more (126 kcal, 4 g AS)) which did not warrant repeating the seven-day controlled diet due to their minimal impact on that day's overall AS intake.

$\delta^{13}C$ biomarker: sensitivity and reliability

Fingerstick $\delta^{13}C$ values from baseline and during the controlled feeding periods are reported in Table 4. There was no significant difference between group mean fingerstick $\delta^{13}C$ values from baseline and day 1 fingerstick $\delta^{13}C$ values on the LAS diet ($p = 0.110$) or the HAS diet ($p = 0.330$), nor was there a difference between the group mean day 1 fingerstick $\delta^{13}C$ values of the LAS and HAS diets ($p = 0.052$). Additionally, no difference in results occurred according to order, or sequence, in which participants completed the controlled feeding periods.

A significant diet × time effect was noted between day 1 and day 8 $\delta^{13}C$ values ($p \leq 0.001$). Average fingerstick $\delta^{13}C$ values decreased on the LAS diet and increased on the HAS diet, which were significantly different on days 1 and 8 of both feeding periods. There was a significant mean decrease between day 1 and day 8 group mean $\delta^{13}C$ values of 0.05 ± 0.071‰ on the LAS diet ($p \leq 0.001$). On the HAS diet, group mean day 8 $\delta^{13}C$ value was 0.03 ± 0.083‰ higher compared with day 1 $\delta^{13}C$ value ($p = 0.038$). Effect size was calculated as $\eta^2 p = 0.39$.

A high degree of reliability was found between day 7 and day 8 $\delta^{13}C$ values on the LAS diet, with an ICC of 0.996 (95% confidence interval, 0.993 to 0.998, $p \leq 0.001$). Similarly, a high degree of reliability was also found between day 7 and day 8 $\delta^{13}C$ values on the HAS diet, with an ICC of 0.997 (95% confidence interval, 0.993 to 0.998, $p \leq 0.001$).

Discussion

This investigation represents the first evaluation of the proposed $\delta^{13}C$ added sugar biomarker in adolescents using a controlled feeding design. These results indicate that the biomarker changed in response to short-term changes in added sugar intake, using low and high added sugar intake levels. Importantly, the sugar intake levels investigated represent the recommended AS intake and the AS intake level that has been associated with nutrient inadequacy (Food and Nutrition Board and Institute of Medicine, 2006; World Health Organization, 2015). However, the group

Table 2. Baseline demographic characteristics of the study sample ($n = 32$).

	Mean ± SD	n[a] (%)[b]
Sex		
Female, *n*	–	17 (53)
Male, *n*	–	15 (47)
Race/ethnicity		
Non-Hispanic White, *n*	–	31 (97)
Non-Hispanic Black, *n*	–	–
Hispanic, *n*	–	–
Other/unknown, *n*	–	1 (3)
Age, years	15.3 ± 1.6	–
Education		
Middle school (grades 6 – 8)	–	10 (31)
High school (grades 9 – 12)	–	22 (69)
Height, cm	167.5 ± 9.6	–
Weight, kg	57.2 ± 10.2	–
BMI, kg/m^2	20.2 ± 2.1	–
BMI percentile, %	47.0 ± 25.1	–
PAQ-A[3] score	2.1 ± 0.6	–
EEN[3], kcal/day	2903 ± 715	–

SD: standard deviation; BMI: dody mass index percentile (Centers for Disease Control and Prevention, 2015); PAQ-A: Physical Activity Questionnaire for Adolescents(Kowalski et al., 2004), where scores range from 1 to 5 with 5 indicating "high physical activity"; EEN: Estimated energy needs, calculated using IOM age- and sex-specific equations(Food and Nutrition Board and Institute of Medicine, 2005) plus PAQ-A to determine physical activity level.

[a]The number of participants, as an absolute value, in the sample that have the specified demographic characteristic.

[b]The number of participants, as a percentage, in the sample that have the specified demographic characteristic.

mean change in fingerstick $\delta^{13}C$ exceeded measurement error (± 0.046‰) only for the LAS diet in the full sample ($n = 32$). With a longer controlled feeding period, $\delta^{13}C$ values may continue to change until they reach a steady-state value, estimated to occur at approximately 3–4 weeks (Davy and Jahren, 2016; Jahren et al., 2014). The current study's results differed from that obtained in the controlled feeding study conducted by Cook et al. (2010), which reported no change in fasting plasma glucose $\delta^{13}C$ values after a seven-day controlled feeding period in adults. Consistent with previously reported findings by Nash et al. (2013), test–retest reliability was demonstrated.

A nutritional biomarker should demonstrate validity, reliability, and sensitivity to dietary intake (Hedrick et al., 2012). Previous studies suggest that blood $\delta^{13}C$ values are a valid and reliable biomarker of AS intake when compared with self-reported intake in adults (Davy et al., 2011; Fakhouri et al., 2014; Yeung et al., 2010). Fakhouri et al. (2014) reported that for every 12 fl oz/day decrease in SSB consumption, serum $\delta^{13}C$ values also decreased by 0.12‰ within a 18-month period, although this was based on self-reported changes in AS intake. Cook et al. (2010) reported that the $\delta^{13}C$ value of plasma glucose was sensitive to AS intake in a previous meal but did not change in response to overall AS content of diet within a seven-day feeding period. However, these previous studies (except for Cook et al., 2010) may have underestimated the strength of associations of AS (or SSB) intake with the proposed $\delta^{13}C$ biomarker, due to the dietary under-reporting that occurs in both adults and youth (Kuhnle, 2012; Lioret et al., 2011; MacDougall et al., 2018; Rangan et al., 2014; Thompson et al., 2010; Ventura et al., 2006).

Another proposed biomarker of sugar intake is the 24-h urinary sucrose/fructose excretion biomarker. Although urinary sugars excretion has been validated as a biomarker for total sugars intake against both self-reported dietary intake and in controlled feeding studies (Tasevska, 2015), its lack of specificity for AS is a limitation. In pre-pubertal children, urinary sugars had a higher association with natural sugar intake than with AS intake (Johner et al., 2010). The reliability of fingerstick $\delta^{13}C$ values under controlled diet conditions in this study was higher than that of the 24-h urinary sucrose/fructose biomarker, which had an ICC of 0.67 in a controlled feeding study that included adults (Tasevska et al., 2005). Finally, although 24-h urine collections are a minimally invasive sampling method, like fingerstick blood sampling, the urinary sugars biomarker's sensitivity to dietary changes requires multiple 24-h urine collections, which introduces challenges with participant burden and compliance (Davy and Jahren, 2016).

This investigation had several strengths. This investigation was the first controlled feeding study to evaluate the fingerstick $\delta^{13}C$ biomarker. The minimally invasive fingerstick blood sampling method (Davy and Jahren, 2016; Jahren et al., 2014) could feasibly be utilized in field research settings, and in large epidemiological trials. The target study population is significant, in that adolescents are the highest consumers of AS and among the highest consumers of SSB (Guthrie and Morton, 2000). Finally, compliance to the controlled diet (~98%) and completion rate were high (32 of 33 participants).

We acknowledge several limitations. The sample size was small, plus lacked racial and ethnic diversity. The feeding periods were limited to two seven-day periods, and biomarker values may not have yet reached a steady state in response to the different AS intake levels. Some foods contained sweeteners that may have been derived from C3 plants (e.g. beet sugar, honey, maple syrup) that were included in AS calculations. Since the $\delta^{13}C$ biomarker can only assess AS intake from C4 plants, AS intake from C3 sources would not have been detected (Jahren et al., 2014). However, only 8 of 71 food items used for the study diets contained C3 sources of sugar. Of those, three food items contained $\leq$2% of a C3 sweetener, according to ingredient lists. Also, variation of $\delta^{13}C$ within plant matter may be as high as 5‰ (DeNiro and Epstein, 1978); it is possible that participants could have received diets with different $\delta^{13}C$ content based on the time of the year in which they were enrolled. However, future studies could assess $\delta^{13}C$ content of foods provided in a controlled diet. Despite these limitations, these findings may be used to justify a larger-scale feeding trial, with longer controlled diet periods.

Table 3. Dietary characteristics of self-reported diet at baseline and washout, and on the LAS and HAS controlled diets ($n = 32$).

	Baseline[a]	Washout[a]	LAS diet[b]	HAS diet[b]
Calories, kcal/day	2519 ± 577	2475 ± 605	2763 ± 624	2755 ± 617
Total fat, g/day	98.5 ± 26.9	94.1 ± 23.9	92.4 ± 20.9	92.4 ± 20.9
Fat, %	34.4 ± 3.3	33.8 ± 4.4	30.3 ± 0.2	29.9 ± 0.2
Total carbohydrate, g/day	317.3 ± 69.3	312.8 ± 91.5	377.6 ± 85.8*	378.6 ± 85.5*
Carbohydrate, %	50.8 ± 3.6	50.3 ± 4.9	54.0 ± 0.2	54.9 ± 0.1
Total protein, g/day	91.1 ± 26.0	94.4 ± 28.4	105.3 ± 23.3*	102.1 ± 22.0*
Protein, %	14.9 ± 2.6	15.8 ± 3.3	15.7 ± 0.1	15.2 ± 0.2
Total sugars, g/day	127.3 ± 33.1	133.5 ± 48.1	158.2 ± 38.0*	214.0 ± 50.4*
Added sugars, g/day	76.0 ± 25.6	77.2 ± 35.7	34.5 ± 7.9*	171.9 ± 38.7*
Added sugars, %	12.2 ± 3.3	12.4 ± 4.4	5.0 ± 0.0*	25.0 ± 0.1*
Sodium, mg/day	3820 ± 1123	3757 ± 1310	3551 ± 691	± 724

HAS: high added sugar controlled diet; LAS: low added sugar controlled diet.
[a]Self-reported derived from four 24-h recalls, mean ± SD.
[b]Provided to participants during the controlled diet period, mean ± SD.
*Significant difference from baseline, $p \leq 0.05$.

Table 4. Fingerstick $\delta^{13}C$ values at baseline and on the LAS and HAS controlled diets ($n = 32$).

Baseline 1, ‰	−19.72 ± 0.50[a]
Baseline 2, ‰	−19.72 ± 0.50
Average baseline[b], ‰	−19.72 ± 0.50

	LAS feeding period	HAS feeding period
Day 1, ‰	−19.70 ± 0.49	−19.74 ± 0.51
Day 2, ‰	−19.70 ± 0.49	−19.66 ± 0.56
Day 3, ‰	−19.71 ± 0.50	−19.73 ± 0.50
Day 4, ‰	−19.71 ± 0.49	−19.71 ± 0.50
Day 5, ‰	−19.73 ± 0.48	−19.74 ± 0.50
Day 6, ‰	−19.72 ± 0.47	−19.71 ± 0.50
Day 7, ‰	−19.75 ± 0.46	−19.71 ± 0.50
Day 8, ‰	−19.75 ± 0.46*	−19.71 ± 0.49*

HAS: high added sugar controlled diet; LAS: low added sugar controlled diet.
[a]Values reported as mean ± standard deviation.
[b]Mean of baseline 1 and baseline 2 fingerstick δ13C values.
*Significant difference from day 1 of the respective controlled diet, $p \leq 0.05$.

In conclusion, group mean changes in fingerstick $\delta^{13}C$ values changed significantly in response to known dietary AS intake within seven days, in adolescents. As with other dietary biomarkers (Brown et al., 2013), this technique may be most appropriately applied to population-level research investigating dietary intake and health outcomes. Once portable mass spectrometer technology developments are refined (Zare et al., 2009), the fingerstick $\delta^{13}C$ biomarker could be a rapid, minimally invasive objective measure of AS intake in large-scale nutrition epidemiological studies.

Author note

TMH is currently a postdoctoral research fellow in the Division of Endocrinology, Metabolism, and Diabetes, School of Medicine at the University of Colorado's Anschutz Medical Campus.

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Author contributions

BMD, AHJ, JS, and VE designed research. All authors conducted research. SVL, JS, and BMD analyzed data/performed statistical analysis. All authors wrote and revised the paper. SVL, AHJ, and BMD have primary responsibility for final content. All authors read and approved the final manuscript.

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Consent for publication

All co-authors consent to submit the article for publication and each has signed the Contributor Agreement.

Ethical approval

All study procedures were approved by the Virginia Tech Institutional Review Board in accordance with the Declaration of Helsinki. Participants and their parents provided written informed assent and consent before beginning the study. This study was registered at clinicaltrials.gov as NCT02455388 on 05/27/2015.

Declaration of conflicting interests

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Supplemental material

Supplemental material for this article is available online.

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