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Urinary Excretion of Sodium, Nitrogen, and Sugar Amounts Are Valid Biomarkers of Dietary Sodium, Protein, and High Sugar Intake in Nonobese Adolescents

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Abstract

Background: Objective indicators of dietary intake (e.g., biomarkers) are needed to overcome the limitations of self-reported dietary intake assessment methods in adolescents. To our knowledge, no controlled feeding studies to date have evaluated the validity of urinary sodium, nitrogen, or sugar excretion as dietary biomarkers in adolescents.

Objective: This investigation aimed to evaluate the validity of urinary sodium, nitrogen, and total sugars (TS) excretion as biomarkers for sodium, protein, and added sugars (AS) intake in nonobese adolescents.

Methods: In a crossover controlled feeding study design, 33 adolescents [12–18 y of age, 47 ± 25th percentile (mean ± SD) of body mass index (BMI; in kg/m^2) for age] consumed 5% AS [low added sugars (LAS)] and 25% AS [high added sugars (HAS)] isocaloric, macronutrient-matched (55% carbohydrate, 30% fat, and 15% protein) diets for 7 d each, in a randomly assigned order, with a 4-wk washout period between diets. On the final 2 d of each diet period, 24-h urine samples were collected. Thirty-two adolescents completed all measurements (97% retention).

Results: Urinary sodium was not different from the expected 90% recovery (mean ± SD: 88% ± 18%, $P = 0.50$). Urinary nitrogen was correlated with protein intake ($r = 0.69$, $P < 0.001$), although it was below the 80% expected recovery (62% ± 7%, $P < 0.001$). Urinary TS values were correlated with AS intake during the HAS diet ($r = 0.77$, $P < 0.001$) and had a higher R^2 value of 0.28 than did AS intake ($R^2 = 0.36$). TS excretion differed between LAS (0.226 ± 0.09 mg/d) and HAS (0.365 ± 0.16 mg/d) feeding periods ($P < 0.001$).

Conclusions: Urinary sodium appears to be a valid biomarker for sodium intake in nonobese adolescents. Urinary nitrogen is associated with protein intake, but nitrogen excretion rates were less than previously reported for adults, possibly owing to adolescent growth rates. TS excretion reflects AS at 25% AS intake and was responsive to the change in AS intake. Thus, urinary biomarkers are promising objective indicators of dietary intake in adolescents, although larger-scale feeding trials are needed to confirm these findings. This trial was registered at clinicaltrials.gov as NCT02455388. *J Nutr* 2017;147:2364–73.

Keywords: dietary biomarkers, urinary sodium, urinary nitrogen, urinary sugars, adolescent dietary intake assessment

Introduction

Valid methods for assessing dietary intake in adolescents are necessary to improve current understanding of the relation between dietary components and the development of adverse health outcomes. Self-reported dietary intake data have known limitations, particularly in overweight or obese adolescents, who are more likely to underreport energy intake (EI) (1). Low energy reporters are also more likely to misreport soft drinks and sweet or savory snacks (2), which contain nutrients of public health concern (3). Biomarkers can eliminate the biases associated with

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Abbreviations used: AS, added sugars; EI, energy intake; HAS, high added sugars; ICC, intraclass correlation; LAS, low added sugars; MS/MS, tandem mass spectrometry; PAQ-A, Physical Activity Questionnaire for Adolescents; TS, total sugars; UPLC, ultra-performance liquid chromatography.

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self-reported data by serving as objective indicators of dietary intake (4). As such, biomarkers that reflect the consumption of food categories emphasized by the Dietary Guidelines for Americans are needed (5). In adults, urinary biomarkers can provide information about some dietary components, such as sodium, protein, and added sugars (AS) (6–8), using minimally invasive sample collection procedures (9).

Urinary sodium is excreted at a known value of ~90% of dietary intake in adults (6). Sodium excretion has been associated with diet quality in 6-y-olds (10) and body weight or body fat in 3- to 18-y-olds (11); however, to our knowledge, no controlled feeding studies to date have evaluated the validity of urinary sodium excretion as a biomarker of sodium intake in adolescents. Physical activity, environmental temperature, and puberty onset could affect the amount of sodium excreted through sweat (6, 12), potentially impacting urinary excretion.

Urinary nitrogen is excreted at a known rate of ~80% of dietary nitrogen intake for adults in nitrogen balance (7, 13). Weighed food records have been used to estimate protein intake in younger populations, but these estimations were validated using one 24-h urine collection, nitrogen excretion was assumed to reflect 80% of intake, and validity was lowest among adolescents (14). To our knowledge, no controlled feeding studies to date have evaluated the validity of urinary nitrogen excretion as a biomarker of protein intake in adolescents. Because of a lack of data, current RDAs for protein intake in adolescents are estimated from data in younger children and adults, with "an additional amount" added to promote growth (15).

Urinary excretion of sucrose and fructose is minimal relative to dietary intake (16); however, sucrose is excreted when it passes through the gastrointestinal mucosa without being hydrolyzed into glucose and fructose, and fructose is excreted when it is absorbed in the small intestine but not taken up by other tissues (17). In adults, urinary sugar excretion is more strongly correlated with extrinsic (added) sugars than intrinsic (natural) sugars when AS comprise a larger portion of total sugar intake (18), and urinary sugars are capable of reflecting changes in sugar consumption regardless of BMI (in kg/m^2) (19). To our knowledge, no controlled feeding studies to date have evaluated the validity of urinary sucrose, fructose, or total sugars (TS) excretion as biomarkers of AS intake in adolescents. The current study utilized a randomized controlled crossover feeding design to evaluate the validity of urinary sodium, nitrogen, sucrose, fructose, and TS as objective indicators of dietary intake in adolescents.

Methods

Study sample and design. The Virginia Tech Institutional Review Board approved all study procedures before the onset of study recruitment. Adolescents were recruited from a local campus community in Southwest Virginia between June 2015 and July 2016 via flyers and word of mouth for participation in a randomized controlled crossover design feeding study. Interested adolescents were included if they met the criteria for age (12–18 y) and BMI (<95th BMI percentile) (20), had no special dietary needs or restrictions, obtained parental permission (age 12–17 y) or provided informed consent (age 18 y), and agreed to comply with all study procedures. Individuals were excluded if they did not meet the inclusion criteria or declined to participate. The investigation is part of a trial designed to evaluate biomarkers of AS intake, which is registered at clinicaltrials.gov (NCT02455388).

Procedures. The schedule of study visits is depicted in **Figure 1**. Baseline measurements were obtained through methods including a questionnaire to assess health history and demographic characteristics, the use of a wall-mounted stadiometer (model 216; Seca) to measure height, the use of a digital scale (Scale-Tronix 5002; Welch Allyn) to assess weight, and evaluation of BMI percentile (20). Female participants completed a menstrual calendar to account for the timing of each participant's menstrual cycle during scheduling, to the extent possible. Four 24-h dietary recalls (3 weekdays, 1 weekend day) were completed to determine habitual intake, with parents providing additional information if necessary. Data on dietary intake during baseline (four 24-h

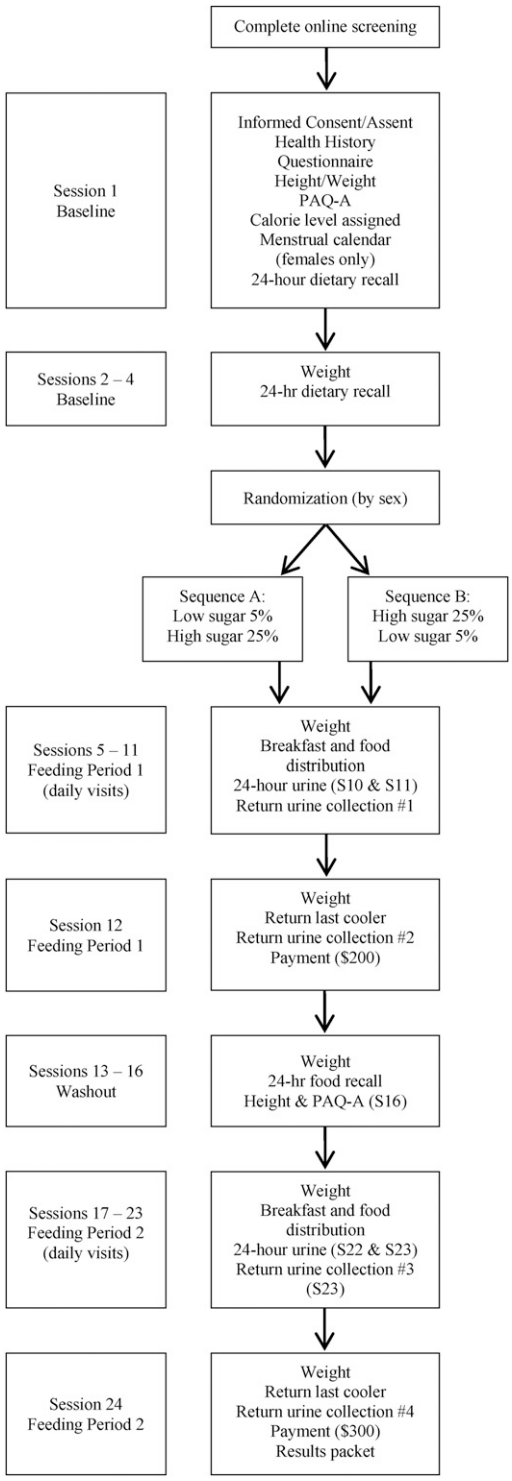

FIGURE 1 Study protocol for nonobese adolescents consuming low- and high-sugar diets. PAQ-A, Physical Activity Questionnaire for Adolescents; S, session.

dietary recalls), the washout period (four 24-h dietary recalls), and each controlled feeding period (menus for controlled diets) were collected and analyzed using the Nutrition Data System for Research (version 2013). The Institute of Medicine equations for boys and girls 9–18 y were used to assess resting energy expenditure (21) and were multiplied by an activity factor derived from the Physical Activity Questionnaire for Adolescents (PAQ-A) (22) to estimate total energy needs. Participants were randomly assigned to a diet sequence to eliminate the possibility of an order effect. Each participant completed either 1 wk of the low added sugars (LAS) diet (5% AS) or 1 wk of the high added sugars (HAS) diet (25% AS) and 4 wk of washout, followed by the second feeding period. A washout period was included to allow participants to resume their habitual dietary intake, thus eliminating the possibility of a carryover effect. Energy needs were re-assessed before the start of the second feeding period using height, weight, and PAQ-A data collected during the washout phase.

Controlled feeding. Diets (**Table 1**) were matched for macronutrient content (55% carbohydrate, 15% protein, and 30% fat) and the form of AS (67% solid, 33% liquid), which is in accordance with typical US intake (24). Sample menus for the LAS and HAS diets are provided in **Supplemental Tables 1** and **2**. Animal protein and nonsweetener corn intake amounts were also maintained across diets to account for the confounding effects of nonsweetener corn intake on other biomarkers being investigated that were not included in this analysis (25). Diets were isocaloric, as evidenced by weight stability; body weight was assessed each morning during the controlled diet periods. Two optional snack modules (150 kcal each) were offered daily to promote weight stability; these matched the nutrient composition of the controlled diet. Calorie amounts were adjusted accordingly for participants whose weight fluctuated above or below their weight stability range [±1 lb (0.45 kg) from their first weigh-in that week] for 3 consecutive days.

All food was prepared in a metabolic kitchen using a digital benchtop scale (Practum 5101-1S; Sartorius). Provided amounts were ±0.8 g of menu amounts. Caloric intake was calculated using standardized values for the metabolizable energy of macronutrients (26), and menu sodium content was adjusted based on package labels to reflect the specific brands of food items provided. Breakfast was consumed in the dining laboratory and supervised daily by research staff; all other meals, snacks, and beverages were provided. Participants were instructed to consume all provided food and drink, with the option to consume the following: 2 snack modules, 3 plain water bottles (to encourage adequate hydration), and 3 sucralose (Splenda) packets (to increase palatability, if desired). Menus were provided with meal and snack suggestions and microwave instructions for heating frozen or refrigerated items. Participants were instructed to return food and beverage containers unwashed so they could be weighed to record actual consumption; they were also asked to report any food or drink consumption that was inconsistent with the controlled diet.

TABLE 1 Nutrient composition of the 7-d low- and high-sugar controlled feeding period diets provided to nonobese adolescents[1]

Nutrient	5% AS	25% AS
Energy, kcal/d	2000–4500	2000–4500
Carbohydrates, % kcal	55	55
AS,[2] % kcal	5	25
Liquid	33	33
Solid	67	67
Total sugars,[3] % kcal	23	31
Sucrose, % kcal	6.1	16.5
Fructose, % kcal	6.6	5.5
Fat, % kcal	30	30
Protein, % kcal	15	15
Animal protein	42	42
Dairy protein	20	20
Vegetable or other	38	38
Nitrogen,[4] g/d	17 ± 4 (12–28)	17 ± 4 (11–27)
Sodium, mg/d	3550 ± 750 (2710–5960)	3790 ± 750 (2960–6170)

[1] Values are ranges, percentages, or means ± SDs (ranges) unless otherwise indicated. AS, added sugars.
[2] The Nutrition Data System for Research variable ''added sugars by total sugars'' was used (23).
[3] Total sugars is the sum of glucose, fructose, galactose, sucrose, lactose, and maltose (23).
[4] Nitrogen was calculated from protein content and food-specific nitrogen conversion factors or a general conversion factor of 6.25 (23).

24-h urine collections. Participants completed 24-h urine collections on 2 consecutive days at the end of each controlled feeding period (day 6 and day 7). Subjects were provided plastic 3-L urine collection containers, each containing 6.75 mL of thymol to prevent bacterial growth and preserve urinary sugars. Participants were provided verbal and written instructions for flushing their first morning urine sample, recording that as their start time on a provided worksheet, collecting their urine for a 24-h period, and recording their stop time. Participants were asked to report missed collections and spills on the same worksheet, which was reviewed upon return of each urine sample. Then participants were provided with new containers and instructions to begin collecting their urine for a second consecutive 24-h period.

Urine processing and analysis. Total urine volume was measured for each 24-h sample upon delivery and was reported as the total volume of urine minus the volume of thymol added to the container(s) before collection, with the exception of the urinary sugars assays, which accounted for the dilution with thymol. Samples were refrigerated for 1–2 d or kept on ice packs and sent directly to Solstas Lab Partners, a Clinical Laboratory Improvement Amendments- and College of American Pathologists-certified laboratory, for processing. Trained Solstas technicians ran all assays on the Roche Cobas 8000 modular analyzer. The 24-h sodium content was assessed by the indirect ion selective electrode assay, a method with <3% variation (6). The 24-h nitrogen content was assessed by a kinetic test for urease and glutamate dehydrogenase (27). Finally, 24-h creatinine was assessed using a kinetic calorimetry assay based on the Jaffe method (28).

Urinary sucrose and fructose assays were completed using MS. The urine samples were preserved in 2-mL cryotubes, frozen at −81°C during initial processing, and thawed once before completing the assays. Urinary sugars were measured as described by Camilleri et al. (29) with the following modifications: 200 μL of urine was combined with 800 μL of the internal standard [0.25 g/L $^{13}C_6$-glucose in acetonitrile:water (94:6)], vortexed, and centrifuged at room temperature. After centrifugation (5 min; 17,000 × *g*), 500 μL of the supernatant was analyzed by ultra-performance liquid chromatography (UPLC) tandem mass spectrometry (MS/MS) (5 μL injected). UPLC separation was performed on a Waters Acquity H-Class system equipped with an Acquity UPLC BEH amide column (2.1 × 50 mm, 1.7-μm particle size). Elution was performed at 0.7 mL/min using the following binary mobile phase system: phase A comprised acetonitrile:water (65:35) with 0.2% (vol:vol) triethylamine, and phase B comprised 0.1% (vol:vol) formic acid in acetonitrile. The linear elution gradient was 1% A (starting), 99% A (3 min), 1% A (3.05 min), and 1% A (5.5 min). Column and sample temperatures were ambient (25°C and 10°C, respectively). Detection by MS/MS was performed on a Waters Acquity triple quadrupole detector. Negative-mode electrospray ionization was performed with a capillary voltage of −4 kV and source and desolvation temperatures of 150°C and 450°C, respectively. Desolvation and cone gasses were at flow rates of 900 and 100 L/h, respectively. For MS/MS, the collision gas was argon. The cone voltages, collision energy, and multiple reaction monitoring transitions for each compound were as follows: *1*) fructose: $[M–H]^-$, 179 *m/z*; daughter ion, 89 *m/z*; cone voltage, 18 V; and collision energy, 8 eV; *2*) sucrose: $[M–H]^-$, 341 *m/z*; daughter ion, 179 *m/z*; cone voltage, 34 V; and collision energy, 12 eV; and *3*) internal standard $^{13}C_6$-glucose: $[M–H]^-$, 185 *m/z*; daughter ion, 92 *m/z*; cone voltage, 16 V; and collision energy, 8 eV. Peak widths were ~5 s, and AutoDwell was employed with required points-per-peak set at 12. The interscan delay time was 0.02 s. Data acquisition,

processing, and quantification were performed using Waters MassLynx software (version 4.1). Urine sugar concentrations were calculated from standard curves of sucrose and fructose and then multiplied by 24-h urine volume to represent daily excretion.

Urine collection completeness criteria. Urine collections were deemed incomplete if any one of the following criteria applied: *1*) creatinine excretion <0.1 mmol · kg body weight^{-1} · d^{-1} (30), *2*) reported collection time outside the time frame of 20–28 h (31), *3*) reported as missing ≥2 collections (19, 32), and *4*) urine volume <500 mL (19, 32). If data were missing, urine specimens were evaluated for completeness based on the remaining criteria. Samples considered complete by these criteria were time-adjusted to 24 h (31). Samples missing duration times were included and assumed to be 24 h unless deemed incomplete by one of the other criteria.

Statistical analysis. Data were analyzed using IBM SPSS Statistics statistical analysis software (version 24). All statistical tests were set with an a priori significance of $\alpha = 0.05$. Descriptive statistics were used to characterize participant demographics, estimated energy needs, and habitual dietary intake. Self-reported dietary intake (baseline and washout) was averaged and compared with mean caloric intake during both controlled feeding periods using paired *t* tests. Weight stability was analyzed using paired *t* tests to compare each subject's weight from the beginning (day 1) to the end (day 8) of each feeding period. Dietary compliance was assessed from metabolic kitchen records, and paired *t* tests were used to evaluate whether dietary compliance differed between feeding periods. Correlations were used to evaluate urinary creatinine excretion as an indicator of body weight and height. Intraclass correlations (ICCs) were used to evaluate the reliability of urinary creatinine excretion across all four 24-h collections, which can indicate urine collection compliance when body mass remains constant. For all urinary data, either 24- or 48-h collections were compared to corresponding 24- or 48-h dietary intake, respectively, based on the amount of complete collections available per participant per feeding period. For the LAS feeding period, 25 participants had 2 complete collections, 5 participants had 1 complete collection, and 2 participants had no complete collections. For the HAS feeding period, 27 participants had 2 complete collections, 4 participants had 1 complete collection, and 1 participant had no complete collections. Data were analyzed to include all participants, those with 2 complete collections, and those with ≥1 complete collection. One outlier was excluded from data analysis involving the variable of urinary nitrogen recovery and 3 outliers were excluded from data analysis involving the variable of urinary sucrose excretion as extreme values (IQR >3) (33).

Evaluating validity. Descriptive statistics were used to evaluate the percentage of sodium intake recovered by urinary sodium excretion. A 1-sample *t* test was used to evaluate the validity of urinary sodium as a biomarker of dietary sodium by comparing actual excretion to the excretion expected (90%) (6) based on corresponding sodium intake. Correlations were used to determine the strength of the relation between urinary nitrogen excretion and protein intake. A 1-sample *t* test was used to evaluate the validity of urinary nitrogen as a biomarker of dietary protein by comparing actual excretion to the excretion expected (80%) (7, 13) based on corresponding nitrogen intake. To address the assumption that our adolescent population was in nitrogen balance, Pearson's correlations were conducted between protein intake (per kilogram of body weight) and urinary nitrogen recovery for the full sample and subsets by sex. To evaluate the impact of dietary fiber intake on the urinary nitrogen biomarker (13, 34, 35), paired *t* tests were used to assess for differences between diet periods. Correlations were used to evaluate the validity of urinary fructose, sucrose, and TS as objective indicators of dietary AS and TS. Urinary excretion of each sugar was compared with dietary intake of the corresponding sugar and AS intake during each feeding period, and the amount of dietary sugar intake recovered through urinary excretion was calculated. Relations between TS excretion and AS/TS intake were plotted. A paired *t* test was used to evaluate changes in urinary sugars in response to changes in AS intake (i.e., sensitivity).

Evaluating reliability. ICCs were used to evaluate the reliability of the percentage of urinary sodium recovered from the 4 diet days corresponding to 24-h urine collections. The percentage of recovery was used to account for differences in daily sodium intake. ICCs were used to evaluate the reliability of urinary fructose, sucrose, and TS when AS intake was held constant. Fructose and sucrose excretion could only be assessed for participants with 2 complete 24-h urine collections during the same feeding period. ICC estimates and their 95% CIs were calculated based on an average-measures, absolute-agreement, 2-way mixed-effects model.

Results

Eligibility, participant enrollment, and completion numbers are shown in **Figure 2**. One participant was removed from the study because of poor compliance with the controlled diet. Another participant repeated the LAS feeding period (after a second washout period) after reporting noncompliance on 1 d of the controlled diet. Participant demographic characteristics, estimated energy needs, and baseline self-reported dietary intake are provided in **Table 2**. The population was representative of both sexes and all ages within the 12- to 18-y-old range; participants were primarily non-Hispanic white, nonobese, and low-active per PAQ-A mean score. Self-reported dietary intake was not significantly different between baseline (Table 2) and the washout period, with respect to energy intake (2480 ± 600 kcal, $P = 0.66$), macronutrient distribution (carbohydrate: 50% ± 5%, $P = 0.64$; protein: 16% ± 3%, $P = 0.06$; and fat: 34% ± 4%, $P = 0.47$), AS intake as a percentage of EI (12% ± 4%, $P = 0.83$), and sodium intake (3760 ± 1310 mg, $P = 0.71$). However, self-reported EI differed from EI during the controlled feeding periods (2800 ± 620 kcal, $P = 0.003$). Participants' weight did not change significantly from the beginning to the end of either feeding period (mean weight changes: LAS: −0.063 ± 0.69 kg, $P = 0.61$; HAS: −0.016 ± 0.58 kg, $P = 0.88$), and weight change did not differ between feeding periods ($P = 0.88$). Recorded intake data indicate that participants were compliant with both diets, consuming 99% (LAS) and 98% (HAS) of the food and beverage items provided to them, with no difference in dietary compliance between feeding periods ($P = 0.11$).

Urinary creatinine excretion, the indicator of urine collection compliance, was strongly correlated with measures of body weight ($r = 0.87$, $n = 29$, $P < 0.001$) and height ($r = 0.76$, $n = 29$, $P < 0.001$) for the full sample during both feeding periods (creatinine data were missing for 3 participants). ICCs for urinary creatinine excretion ($n = 29$, 4 items) indicated good-to-excellent (36) test-retest reliability (ICC: 0.89; 95% CI: 0.802, 0.942; $P < 0.001$).

Biomarker validity. Sodium intake was recovered through urinary sodium excretion at rates of 86–90% for both feeding periods, including the full sample and the sample restricted to participants with complete 24-h urine collections. Mean dietary sodium recovered through urinary excretion did not differ significantly from the expected recovery of 90% for the full sample (88% ± 18%, $P = 0.50$) or within any subset of the data (urine collection completeness, PAQ-A physical activity status, age) with the exception of sex. Female participants recovered more sodium than expected (96% ± 11%, $P = 0.045$), and male participants recovered less urinary sodium than females (79% ± 21%, $P = 0.012$). Sodium intake differed between feeding periods (LAS: 3550 ± 690 mg/d; HAS: 3660 ± 720 mg/d; $P < 0.001$), but urinary sodium excretion did not differ significantly when the sample was restricted to participants

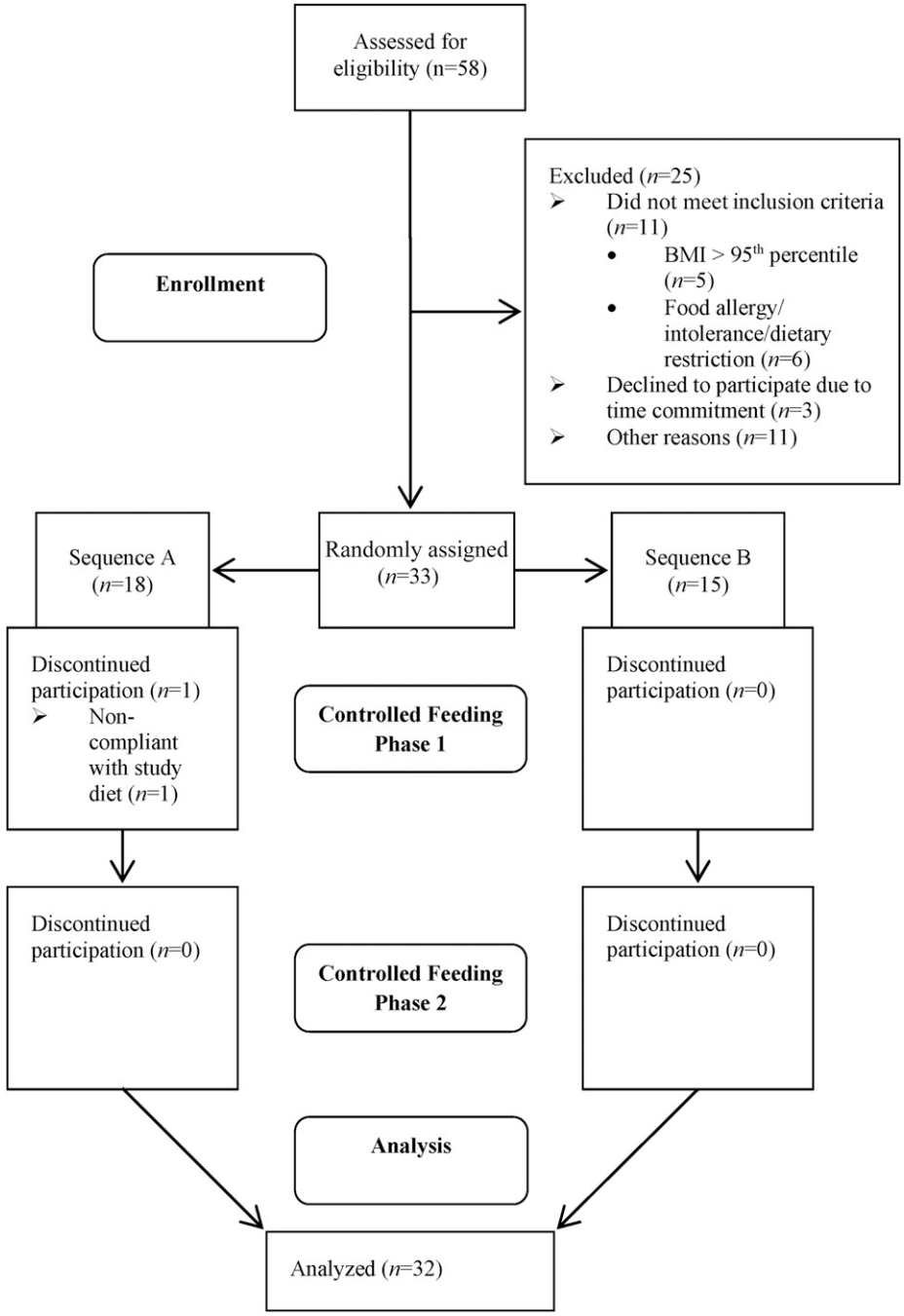

FIGURE 2 Consolidated Standards of Reporting Trials diagram for non-obese adolescents consuming low- and high-sugar diets. Sequence A comprised a 5% AS diet in controlled feeding phase 1 and a 25% AS diet in phase 2. Sequence B comprised a 25% AS diet in controlled feeding phase 1 and a 5% AS diet in phase 2. AS, added sugars.

with 4 complete 24-h urine samples (LAS: 3080 ± 730 mg/d; HAS: 3460 ± 1060 mg/d; $P = 0.06$).

We found that 24-h urinary nitrogen excretion (10.4 ± 2.3 g/24 h) was correlated with 24-h dietary protein intake (105 ± 23 g/d) for the full sample ($n = 29$, $r = 0.69$, $P < 0.001$) and within subsets grouped by feeding period and urine collection completeness, yielding moderate to strong r values ($r = 0.53$–0.83). However, mean dietary nitrogen recovered through urinary nitrogen excretion was significantly different from the expected excretion of 80% for the full sample (62% ± 7%, $P < 0.001$) and within subsets, regardless of grouping by urine collection completeness, physical activity status, sex, or age. The correlation between dietary protein intake (expressed in g/kg body weight) and urinary nitrogen recovery (expressed in %) was significant and negative for the full sample ($n = 28$, $r = -0.47$, $P = 0.012$), and this correlation was stronger for males ($n = 12$, $r = -0.56$, $P = 0.06$) than females ($n = 16$, $r = -0.16$, $P = 0.55$). To determine whether the negative correlation was attributable to growth, the height change between baseline and the washout period was evaluated. A significant negative correlation was found between height change (expressed in cm) and urinary nitrogen recovery (expressed in %) for the full sample ($n = 28$, $r = -0.47$, $P = 0.012$). This correlation was also significant for males ($n = 12$, $r = -0.67$, $P = 0.018$), with a mean height increase of 0.53 ± 0.53 cm, but it was not significant for females ($n = 16$, $r = -0.23$, $P = 0.39$), with a mean height

TABLE 2 Participant characteristics of nonobese adolescents consuming low- and high-sugar diets[1]

Participant characteristic	Value
Sex	
Male	15 (46.9)
Female	17 (53.1)
Age,[2] y	15.3 ± 1.6 (12–18)
12	2 (6.3)
13	2 (6.3)
14	4 (12.5)
15	11 (34.4)
16	6 (18.8)
17	3 (9.4)
18	4 (12.5)
Race/ethnicity	
White	32 (100)
BMI-for-age percentile,[3] kg/m^2	47 ± 25 (2–93)
Obese	0 (0.0)
Overweight	1 (3.1)
Healthy weight	28 (87.5)
Underweight	3 (9.4)
PAQ-A[4]	2.08 ± 0.52 (1.09–3.19)
Estimated energy needs,[5] kcal/d	2920 ± 700 (1940–4770)
Baseline self-reported dietary intake	
Energy, kcal/d	2520 ± 560 (1680–3770)
Added sugar, % kcal	12 ± 3 (6–18)
Sodium, mg/d	3820 ± 1120 (1920–7080)
Carbohydrates, % kcal	51 ± 4 (43–58)
Protein, % kcal	15 ± 3 (9–19)
Fat, % kcal	34 ± 3 (27–40)

[1] Values are *n* (%) or means ± SDs (ranges) unless otherwise indicated (*n* = 32 participants). PAQ-A, Physical Activity Questionnaire for Adolescents.
[2] The number of participants by age and sex was as follows: 12 y, 2 males and 0 females; 13 y, 0 males and 2 females; 14 y, 1 male and 3 females; 15 y, 6 males and 5 females; 16 y, 4 males and 2 females; 17 y, 1 male and 2 females; and 18 y, 1 male and 3 females.
[3] CDC weight status categories were as follows (19): obese (≥95th percentile), overweight (85th to <95th percentile), healthy weight (5th to <85th percentile), and underweight (<5th percentile).
[4] The PAQ-A was scored on a scale of 1 (low physical activity) to 5 (high physical activity) (21).
[5] Energy needs were estimated based on Institute of Medicine equations (20) and PAQ-A scores (21).

increase of 0.23 ± 0.60 cm. Although dietary nitrogen intake did not differ between feeding periods (LAS: 17.1 ± 3.6 g; HAS: 16.9 ± 3.7; $P = 0.32$), urinary nitrogen excretion (LAS: 9.9 ± 2.1 g/d; HAS: 11.1 ± 2.5 g/d; $P < 0.001$) and the percentage of dietary nitrogen recovered as urinary nitrogen (LAS: 58% ± 7%; HAS: 66% ± 8%; $P < 0.001$) were significantly lower during the LAS feeding period. Fiber intake, which has been reported to affect nitrogen excretion (13, 34, 35), was significantly different between feeding periods (LAS: 36 ± 8 g/d; HAS: 22 ± 5 g/d; $P < 0.001$) and between males (LAS: 42 ± 6 g/d; HAS: 25 ± 4 g/d) and females (LAS: 31 ± 4 g/d; HAS: 19 ± 2 g/d) for both controlled feeding periods ($P < 0.001$).

Table 3 presents urinary sugar excretion (sucrose, fructose, TS) and the percentage of dietary sugar intake recovered through urinary excretion. During the LAS feeding period, urinary sucrose, fructose, and TS excretion were not correlated with dietary intake of the corresponding sugar or with AS intake. During the HAS feeding period, all urinary sugar measures yielded significant correlations with dietary intake of the corresponding sugar and AS intake (Table 3). **Figure 3** depicts the relations between urinary TS excretion and AS/TS intake. Both slopes differ from zero ($P < 0.001$) and R^2 values for the comparisons between urinary TS excretion and AS intake (Figure 3B) show a better fit with the linear regression equation. All urinary sugars were responsive to the change in dietary intake, including sucrose (LAS: 0.016 ± 0.01 mg/d; HAS: 0.034 ± 0.03 mg/d; $P = 0.001$), fructose (LAS: 0.209 ± 0.09 mg/d; HAS: 0.331 ± 0.15 mg/d; $P < 0.001$), and TS (LAS: 0.226 ± 0.37 mg/d; HAS: 0.365 ± 0.16 mg/d; $P < 0.001$) for the full sample.

Biomarker reliability. ICCs for urinary sodium recovery of the full sample ($n = 32$, 4 items) indicated moderate (36) test-retest reliability (ICC: 0.52; 95% CI: 0.22, 0.74; $P = 0.001$). Sodium recovery for the full sample was more reliable during the HAS diet (ICC: 0.49; 95% CI: −0.02 to 0.75; $P = 0.010$) than the LAS diet (ICC: 0.20; 95% CI: −0.33 to 0.56; $P = 0.20$). ICCs for LAS sucrose excretion indicated poor (36) test-retest reliability ($n = 24$; ICC: 0.01; 95% CI: −1.09 to 0.55; $P = 0.49$). ICCs for LAS fructose excretion ($n = 25$; ICC: 0.50; 95% CI: −0.04 to 0.77; $P = 0.03$) and TS excretion ($n = 24$; ICC: 0.52; 95% CI: −0.03 to 0.79; $P = 0.03$) indicated moderate (36) test-retest reliability. HAS sucrose excretion ($n = 25$; ICC: 0.67; 95% CI: 0.23, 0.85; $P = 0.005$) indicated moderate (36) test-retest reliability, whereas HAS fructose excretion ($n = 27$; ICC: 0.80; 95% CI: 0.41, 0.92; $P < 0.001$) and HAS TS excretion ($n = 25$; ICC: 0.84; 95% CI: 0.52, 0.94; $P < 0.001$) indicated good (36) test-retest reliability.

Discussion

To our knowledge, this investigation represents the first controlled feeding study to evaluate the validity of urinary sodium, nitrogen, sucrose, fructose, and TS as objective indicators of dietary sodium, protein, and AS intake in nonobese adolescents. Our findings indicate that urinary sodium is a valid indicator of dietary sodium intake for adolescents, because excretion was not significantly different from the expected 90% excretion rate (6). Urinary nitrogen excretion was significantly different (i.e., lower) from the expected excretion of 80% of dietary nitrogen intake (7, 13) but was correlated with protein intake. Urinary sucrose, fructose, and TS appear to be valid indicators of dietary AS at higher amounts of AS intake. Finally, urinary sugars excretion is reliable at high amounts of AS intake and responsive to changes in AS intake.

Previously reported sodium intake (self-reported) and excretion values from 10- to 18-y-olds (11) are comparable to our findings. Male adolescents may have recovered less sodium as a result of poorer compliance with urine collections or increased sodium excretion via sweat attributable to physical activity (not captured by the PAQ-A), differences in body surface area, or seasonality factors (i.e., more male participants completed urine collections during the spring or summer months). Urinary sodium recovery was only moderately reliable when all 4 samples for each participant were included, further justifying the need for multiple 24-h collections.

Previous studies in adolescents revealed similar but slightly lower values for 24-h urinary nitrogen excretion (after conversion of reported amounts to grams per day), along with substantially lower estimates of protein intake (14, 37). However, these studies relied on 3-d weighed food records; if protein intake is underreported and urinary nitrogen excretion is valid

TABLE 3 Correlations between urinary sugars excretion, dietary intake of the corresponding sugar, and dietary AS intake in nonobese adolescents consuming low- and high-sugar diets[1]

Feeding period[2]	Sugar	Urinary sugar excretion,[3] mg/24 h		Dietary sugar intake, g/24 h	r	P value	Dietary sugar intake recovered through urinary excretion, %
		Value	Participants, n				
5% AS	AS	—	25	34 ± 8	0.16[4]	0.42	—
		—	25	34 ± 8	0.14[5]	0.51	—
		—	25	34 ± 8	0.15[6]	0.49	—
	Sucrose	0.015 ± 0.01	24	44 ± 10	0.17	0.42	0.00003
	Fructose	0.199 ± 0.07	25	46 ± 13	0.10	0.62	0.00046
	TS	0.213 ± 0.07	24	165 ± 42	0.16	0.47	0.00014
25% AS	AS	—	27	172 ± 40	0.66[4]	<0.001*	—
		—	27	172 ± 40	0.75[5]	<0.001*	—
		—	27	172 ± 40	0.77[6]	<0.001*	—
	Sucrose	0.028 ± 0.01	25	121 ± 28	0.69	<0.001*	0.00002
	Fructose	0.348 ± 0.15	27	36 ± 10	0.74	<0.001*	0.00096
	TS	0.369 ± 0.16	25	213 ± 52	0.77	<0.001*	0.00017

[1] Values are means ± SDs unless otherwise indicated. *Significant at $\alpha = 0.05$. AS, added sugars; TS, total sugars.
[2] Sample was restricted to participants with 2 complete 24-h urine collections.
[3] For 5% AS, one outlier was excluded as a result of SPSS identification as an extreme value for sucrose excretion. The second outlier identified was already excluded via incomplete sample. For 25% AS, 2 outliers were excluded as a result of SPSS identification as extreme values for sucrose excretion; a third outlier identified was already excluded via incomplete sample.
[4] Correlations between AS intake and sucrose excretion.
[5] Correlations between AS intake and fructose excretion.
[6] Correlations between AS intake and TS excretion.

and reliable, then the amount of dietary nitrogen recovered as urinary nitrogen would be falsely inflated, making adolescent data appear more comparable to adult amounts of nitrogen recovery (13, 38), as opposed to our results, which indicate lower amounts of recovery.

Lower nitrogen recovery can be attributed to the positive nitrogen balance in growing children and adolescents (14). Participants in the current study consumed $1.85 \pm 0.25\ g \cdot kg^{-1} \cdot d^{-1}$ on average, which is adequate to achieve positive nitrogen balance regardless of growth or increases in fat-free mass, according to a study in adolescent athletes (39). The association between higher amounts of protein intake (expressed in g/kg body weight) and lower amounts of urinary nitrogen recovery (expressed in %) further suggests that the extent of positive nitrogen balance was greater for some adolescents. Participants with the highest protein-calorie requirements were likely experiencing the most rapid growth, so although they were consuming more nitrogen, they were excreting less, relative to dietary intake. Peak growth typically occurs between 11 and 14 y for females and 15 and 18 y for males (40), so the age range recruited (12–18 y) overlapped peak growth rates, particularly for our male participants, who experienced more vertical growth during the study. This accumulation of lean mass may explain why males recovered less nitrogen.

The difference in urinary nitrogen excretion and recovery between feeding periods could be attributed to the dietary fiber content of each diet. Fiber intake can increase the amount of nitrogen excreted in the feces (13, 34, 35). A controlled feeding study in a small sample of adults showed an increase in fecal nitrogen excretion from 1.4 to 2.49 g/d with the addition of 30 g dietary fiber (34). Since fermentable carbohydrates provide energy to the gut microflora, dietary intake stimulates the transfer of urea from the blood to the large intestine for use in microbial protein synthesis before excretion in the feces (41, 42), resulting in less urinary nitrogen excretion. As a result, consumption of 36 g fiber (LAS) resulted in significantly less urinary nitrogen excretion than did consumption of 22 g fiber (HAS). Furthermore, because males consumed more dietary fiber than their female counterparts on both controlled diets

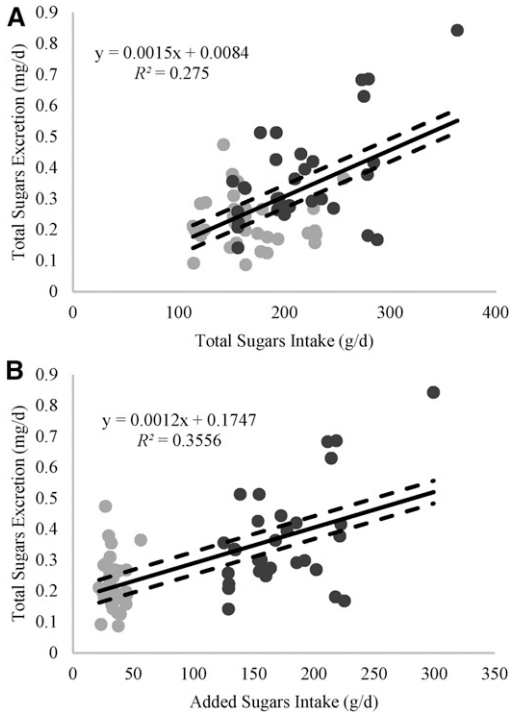

FIGURE 3 Association between urinary total sugar excretion and total sugars intake (A) and AS intake (B) in nonobese adolescents consuming low- and high-sugar diets. Each participant is denoted by a solid gray circle during the 5% AS diet and by a solid black circle during the 25% AS diet. The linear regression trendline is denoted by a solid black line, with dotted black lines indicating upper and lower 95% CIs. AS, added sugars.

(as a result of higher estimated energy needs), they could have excreted even more nitrogen in the feces relative to the urine.

Strong correlations between excretion of urinary sugars and 25% AS intake demonstrate that urinary sugar biomarkers are likely a valid measure of AS intake for adolescents, who consume >16% of their energy from AS (3). Urinary sugars also appear to be a better biomarker for dietary AS than dietary TS because of the slightly higher R^2 values indicating a better fit with the linear regression equation, although this cannot be confirmed with the current study design. Graphical representations between TS excretion and dietary AS and TS intake indicate some degree of sensitivity; however, the regression equations for predicting dietary AS and TS intake from TS excretion suggest low predictive value, only accounting for 36% (AS) and 28% (TS) of variation around mean values. Urinary sugars biomarkers may not be valid indicators of AS intake at lower amounts of intake (5% AS) because sucrose and fructose are already excreted in such small quantities (16). Urinary sugar excretion values for the current study were much lower than prior research suggests (8, 18, 19) relative to our controlled dietary intake amounts of 5% and 25% AS (23% and 31% TS). To our knowledge, the only study known to assess fructose excretion in prepubertal males and females was not a controlled feeding study, fructose intake was not estimated, and urinary sugars were analyzed using enzymatic analysis; however, sugar intake was estimated at 15% AS and 23% TS, yielding urinary fructose excretion of 19.8 mg/d in males and 20.7 mg/d in females (43). A previous study validated the detection and quantification of urinary sucrose via LC/MS analysis of known concentrations (44). The highest concentrations of urinary sucrose (70 and 450 μM) were found to be within 1% and 2%, respectively, of their corresponding theoretical results, whereas the lowest concentrations (2 and 20 μM) were within 15% and 7% of their respective accurate values (44). Lower values in our adolescent population could also be a result of reduced intestinal permeability relative to adults, in light of the deteriorating intestinal barrier function observed in aged monkeys (45). The responsiveness of urinary sucrose, fructose, and TS to a dietary change demonstrates the potential for these biomarkers to serve as objective indicators of dietary AS; however additional research is needed to determine the sensitivity of urinary sugars between 5% and 25% of AS intake and also to evaluate the effect of different sources of AS in the diet. Less reliability at the lower amount of AS intake may be a result of the already low quantities of sugar excretion (16), in combination with the many factors being discovered that affect intestinal permeability [e.g., inflammation, gut microbiota (46, 47), medication use, running (48)]. However, good reliability at a high amount of AS consumption is promising, given the current dietary intake of adolescents (3).

This study has strengths and limitations. The validation of urinary sodium, urinary nitrogen, and urinary sugars as objective indicators of dietary intake in adolescents is a novel topic. A limited amount of research has focused on this population even though adolescent obesity rates continue to increase (49), adolescents are the highest consumers of AS (3), and targeting overweight or obese adolescents appears to protect their cardiometabolic health (50, 51). Additional strengths include the use of a randomized controlled crossover feeding approach with a high completion rate, subjective and objective compliance measures, and collection of two 24-h urine samples per feeding period, considering that two to three 24-h urine collections yield a reliability index of 0.8 for most biomarkers (52). Urinary biomarkers are minimally invasive, urinary sugar measures reflect all sources of dietary sugar (53), and multiple biomarkers can be assessed from the same urine sample. Finally, participants arrived fasted each morning (for fingerstick blood samples related to other study objectives), which made urine collections more likely to reflect all nutrients consumed during each 24-h collection, because sodium intake for a given day is thought to be excreted within the next 18–31 h (6), although this is still a limitation.

Overall, the sample size was small and lacked diversity; additional research is needed to determine whether these findings apply to larger, more racially diverse nonobese and obese adolescents. Diet calculations may be limited by the potential inaccuracy of values representing the metabolizable energy of macronutrients (26) and the need to calculate sodium intake from package labels. Urinary measurement error is possible, including the subtraction of the thymol preservative from the total urine volume for measures of urinary sodium, nitrogen, and creatinine, and feces were not collected to confirm that dietary fiber intake shifts nitrogen excretion from the urine to the feces. Finally, the need for multiple 24-h urine collections is burdensome to participants, which could have impacted compliance; however, spot urine samples are being evaluated for validity (6, 9, 17, 31), which would reduce participant burden in future studies.

Urinary sodium appears to be a valid indicator of dietary sodium intake for adolescents across the age range and physical activity levels of the study population, although older adolescents (15–18 y) and females may be more capable of collecting 24-h urine samples, and multiple collections are likely needed as a result of moderate reliability. Strong correlations between urinary nitrogen excretion and dietary protein intake suggest that urinary nitrogen may be a valid biomarker for protein intake in some populations, but the varying growth rates of adolescents put them in positive nitrogen balance, decreasing the percentage of dietary nitrogen recovered through urinary excretion. The extent of positive nitrogen balance remains unknown, but it appears to vary by sex in accordance with the timing of peak growth and the amount of lean body mass accrued. Furthermore, a 14-g difference in fiber intake significantly impacted urinary nitrogen excretion. Therefore, determining adolescent protein intake from urinary nitrogen excretion would require prediction equations that are specific to sex, growth rate, and dietary fiber intake. Urinary fructose, sucrose, and TS biomarkers appear to be valid and reliable at 25% AS intake, and TS excretion may be a better indicator of dietary AS intake than TS intake. All urinary sugars measured were responsive to the 20% change in dietary AS, but the sensitivity of each urinary sugar biomarker to smaller dietary changes has not yet been established and predictive value appears low. Further research is warranted to *1*) examine the effects of adolescent growth rate, dietary fiber intake, and sex-based differences on urinary nitrogen excretion; *2*) validate urinary sucrose, fructose, and TS biomarkers at lower amounts of AS intake (<25% AS) as well as in diets varying in TS intake and in sources of AS; and *3*) determine the sensitivity of urinary sugar biomarkers to smaller dietary changes, all with larger, more diverse samples.

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statistical analysis and had primary responsibility for the final content; and all authors: conducted the research, wrote and revised the paper, and read and approved the final manuscript.

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