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Evaluation of childhood nutrition by dietary survey and stable isotope analyses of hair and breath

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Abstract

Objectives: The natural abundances of carbon, nitrogen, and sulfur stable isotopes in hair, and of carbon isotopes in breath serve as quantitative biomarkers of protein and carbohydrate sources, but applicability of isotopes for evaluating children's diet has not been demonstrated. In this study, we sought to describe the stable isotope patterns observed in the hair and breath of children and to assess dietary variations in relation to age and ethnicity, hypothesizing that these would reflect dietary differences across age and ethnic groups and would correlate with intake variables derived from a Food Frequency Questionnaire.

Methods: Data were obtained from a cross-sectional study of non-Hispanic white ($N = 115$) and Hispanic ($N = 97$) children, aged 9–16 years, in Salt Lake City, Utah. Sampling included a hair sample, breath samples (AM and PM), and a youth/adolescent food questionnaire (YAQ). Hair was analyzed for carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$), and sulfur ($\delta^{34}S$) isotopes, and breath samples for $\delta^{13}C_{AM/PM}$ of respired CO_2.

Results: Non-Hispanic whites had lower $\delta^{13}C$, $\delta^{15}N$, $\delta^{13}C_{AM}$, and $\delta^{13}C_{PM}$ values than Hispanics. Hair $\delta^{13}C$ and $\delta^{15}N$ values were correlated with protein sources, particularly for non-Hispanics. Breath $\delta^{13}C$ values were correlated with carbohydrate sources, particularly for Hispanic students. Non-Hispanic white students reported greater intake of total protein, animal protein, dairy, and grain than Hispanic students. Hispanic students reported higher intake of carbohydrates, particularly sweetened beverages.

Conclusion: While YAQ and stable isotope data reflected strong cultural influences in diet, no significant gender-based nor age-based differences were detected. Significant covariation between YAQ and isotopes existed and demonstrate the potential of stable isotopes for characterizing children's diet.

1 | INTRODUCTION

With growing concerns over the effects of childhood nutrition on growth and development and long-term adult health, quantitative tools for assessing childhood diets can play an important role in dietary studies. Two prominent dietary patterns are the excess consumption of both animal protein and added sweeteners. Over-consumption of animal protein and added sweeteners, particularly those containing corn-based sweeteners, have been associated with childhood obesity and disease development (Günther, Karaolis-Danckert, Kroke, Remer, & Buyken, 2010; Günther, Remer, Kroke, & Buyken, 2007; Hu & Malik, 2010; Ludwig, Peterson, & Gortmaker, 2001; Malik, Schulze, & Hu, 2006; Wang, Bleich, & Gortmaker, 2008). Noninvasive and cost-effective methods for dietary assessment in children are critical for

accurate evaluation, long-term trend analyses, and independent assessment of the impacts of nutritional factors on health and development (Potischman, Cohen, & Picciano, 2006; Wasantwisut & Neufeld, 2012).

Food Frequency Questionnaires (FFQ) are a typical approach for assessing diets among elementary school children (Baranowski et al., 1997; Domel et al., 1994; Rockett et al., 1997; Wang et al., 2008) and adolescents (Rockett et al., 1997; Rockett, Wolf, & Colditz, 1995). In some cases FFQs were found to be imprecise measures of nutrient intake (Wang et al., 2008). Subsequent studies have found that FFQs had a low validity rate with African American (Cullen & Zakeri, 2004; Field et al., 1999), Hispanic (Cullen & Zakeri, 2004), and low-income inner-city children (Borradaile et al., 2008). Administration of FFQ may be infeasible in some populations, and repeat administration of FFQ may become a burden on study participants. However, FFQs remain the primary method to assess dietary intake in children, and largely have been accepted as valid instruments, particularly when using relative ranks (Cullen & Zakeri, 2004).

Here, we describe a class of dietary biomarkers that are noninvasive, inexpensive, specific, and free from reporting bias associated with age, social groupings, and ethnicity. This study focused on evaluating how useful stable isotope analyses could be in accurately assessing the diets of children and adolescents (McKeown et al., 2001). To date, such biomarkers have been rarely considered in the evaluation of childhood nutrition (McKeown et al., 2001; Potischman et al., 2006).

Stable isotopes have been used extensively as biomarkers of diet in anthropological and ecological research for more than three decades. Carbon isotopes are often used to distinguish diets composed of C_3 and C_4 photosynthetic pathway plants (Farquhar, Ehleringer, & Hubick, 1989). Stable carbon, nitrogen, and sulfur isotope ratios in their natural abundance have been used as biomarkers of protein intake in humans (Bol & Pflieger, 2002; Hedges, Rush, & Aalbersberg, 2009; Kuhnle, Joosen, Kneale, & O'Connell, 2012; Nash et al., 2009, 2012; O'Brien, 2015; Petzke, Boeing, Klaus, & Metges, 2005; Petzke, Boeing, & Metges, 2005; Petzke, Fuller, & Metges, 2010), and more recently, carbon isotopes have been proposed as a potential biomarker for the consumption of dietary sweets (Cook, Alvig, Liu, & Schoeller, 2010; Jahren et al., 2006; O'Brien, 2015; Yeung et al., 2010). Several natural patterns make stable isotope ratios good potential biomarkers of protein intake: (1) animal derived proteins tend to have higher stable nitrogen isotope ($\delta^{15}N$) values than plant-derived proteins (DeNiro & Epstein, 1978, 1981; Kelly, 2000; Peterson & Fry, 1987), (2) marine derived proteins (e.g., shellfish, marine fish) tend to have higher stable sulfur isotope ($\delta^{34}S$) values than terrestrial foods (Arneson & MacAvoy, 2005; Fry, 2006; Richards, Fuller, & Hedges, 2001; Valenzuela, Chesson, O'Grady, Cerling, & Ehleringer, 2011), and (3) C_4–based protein (e.g., corn, feedlot-raised beef) have higher stable carbon isotope ($\delta^{13}C$) values than C_3–based protein (e.g., most grains, seeds, vegetables, and grass-fed beef) (Chesson, Thompson, Podlesak, Cerling, & Ehleringer, 2008; Jahren & Kraft, 2008; Martinelli et al., 2011).

Previous studies have shown that the stable isotopes of hair can be used to distinguish among Europeans with omnivorous, ovo-lacto-vegetarian, or vegan diets (Bol & Pflieger, 2002; Petzke, Boeing, Klaus, et al., 2005; Petzke, Boeing, & Metges, 2005; Petzke et al., 2010). However, these biomarkers have rarely been applied in large populations (Bender et al., 2015; Gragnani, Garavello, Silva, Nardoto, & Martinelli, 2014; Kusaka et al., 2016; Thompson et al., 2010; Valenzuela, Chesson, Bowen, Cerling, & Ehleringer, 2012; Valenzuela et al., 2011) or in the context of nutritional epidemiology (Bender et al., 2015; O'Brien, 2015; Petzke et al., 2010). While isotopic analysis of hair provides a time-integrated dietary signal, isotopic analysis of breath CO_2 provides a measure of recent carbohydrate metabolism (O'Brien, 2015). Dietary sources of refined carbohydrates (i.e., candies, cookies, soda) contain high amounts of cane sugar and/or high fructose corn syrup (HFCS), both of which are derived from C_4 plants. Distinct differences in the $\delta^{13}C$ values of these C_4–derived sweeteners make them easily identifiable from common C_3 carbohydrate sources such as wheat, oats, soy, rice, fruits, and potatoes (Jahren et al., 2006). In a recent study evaluating serum $\delta^{13}C$ values, adults with high sweetened-beverage intake had significantly higher $\delta^{13}C$ values than for those with low intakes, consistent with consumption of corn/cane sugar sources (Yeung et al., 2010).

The goals of this study were twofold. Our first objective was to describe the stable isotope patterns observed in hair and breath of children in a U.S. city and to assess dietary variations with age and ethnicity. Our second objective was to assess the relationship between the stable isotope ratio data and self-reported diets using the Youth/Adolescent Questionnaire. We hypothesized that stable isotope analyses of hair would reflect differences in protein sources and of carbohydrate intake across age and ethnic groupings (Caine-Bish & Scheule, 2009; Granner et al., 2004; Lytle, Seifert, Greenstein, & McGovern, 2000; Park, Blanck, Sherry, Brener, & O'Toole, 2012).

2 | MATERIALS AND METHODS

Data were collected from children in public schools in Salt Lake City, Utah, USA. A hair sample, two breath samples, a self-administered FFQ, and a collection of biometrics were obtained from a cross-sectional study of non-Hispanic white and Hispanic children and adolescents, with participants ranging from 9 to 16 years of age. The study was conducted

in three different schools in Salt Lake City: a public elementary school (Title 1, grades 5 and 6), a charter school (grades 6, 7, 8, and 9), and a public high school (grade 10). The students themselves reported ethnicities by self-ascription. The study was approved by the University of Utah's Institutional Review Board (IRB protocol number 00032797). Children and their parents were free to choose which sample and information they wanted to provide. Hence, the sample sizes of different variables varied.

2.1 | Hair samples and isotope analyses

A hair sample was collected from each participating child by cutting a lock of hair as close to the scalp as possible and placing the hair samples in a paper envelope. Some students opted for providing the hair sample themselves, thus we did not have control over how close to the scalp they were cut and could not standardize the length and proximity of the sample to the scalp to be analyzed. Therefore, for all samples, the entire hair length was used. Sample processing and stable isotope analyses were conducted at the Stable Isotope Ratio Facility for Environmental Research (SIRFER) at the University of Utah following the procedure described in Valenzuela et al. (2012). Briefly, hair samples were washed in a 2:1 Chloroform:Methanol mix to remove lipids and other contaminants, later air-dried and ground to a fine powder using a ball mill (Retsch, Haan, Germany) in preparation for analyses. Samples, alongside a set of laboratory reference materials, were analyzed using an isotope ratio mass spectrometer operated in continuous flow mode as described in Valenzuela et al. (2012).

The carbon and nitrogen isotope ratios of the laboratory reference materials, two glutamic acids (UU-CN-1 and UU-CN-2) and two ground keratins (DS and ORX), were +24.0‰ and +49.6‰ for UU-CN-1, −28.2‰ and −4.6‰ for UU-CN-2, −23.1‰ and +1.4‰ for DS, and −11.4‰ and +10.6‰ for ORX, respectively. These values were assigned after direct calibration against the international standards USGS40 (–26.4‰ for $\delta^{13}C$, −4.5‰ for $\delta^{15}N$) and USGS41 (+37.6‰ for $\delta^{13}C$, +47.6‰ for $\delta^{15}N$). The carbon and nitrogen isotope ratio values of a powdered keratin Quality Control material (POW) were −24.0 and +5.9‰, respectively, when calibrated against USGS40 and USGS41. POW was analyzed alongside unknown hair samples and corrected as an unknown to determine analytical precisions (1σ) in this study, which were 0.1‰ for C and 0.2‰ for N. We note that POW had been previously calibrated against international standards and used to correct measured carbon and nitrogen isotope data in earlier publications. However, its defined isotope ratios were slightly different when recalibrated using USGS40 and USGS41. Therefore, to compare the hair isotope values presented in this article with datasets previously published by our research group (for hair: Bowen et al., 2009; Thompson et al., 2010; Valenzuela et al., 2011; for food: Chesson, Ehleringer, & Cerling, 2011; Chesson et al., 2008) an offset of −0.3‰ for $\delta^{13}C$ and +0.2‰ for $\delta^{15}N$ values should be applied to previous publications. The sulfur isotope ratios of our laboratory reference materials, silver sulfide, and zinc sulfide were +17.9‰ and −31.9‰, respectively, when calibrated against the international standards IAEA-S-1, 2 and 3 (–0.30, +22.7 and −32.3‰, respectively). Similarly, our Quality Control material (powdered feathers) value was +16.9‰. The analytical precision based on long-term measurements of the Quality Control material was 0.4‰. Results for $\delta^{13}C$ values are presented on the Vienna Pee Dee Belemnite (VPDB) scale, those for $\delta^{15}N$ on the AIR scale, and for $\delta^{34}S$ on the Vienna Canyon Diablo Triolite (VCDT) scale. Stable isotope ratios are reported using the standard δ-notation relative to an international standard in units per mil (‰) as follows:

$$\delta_A = (R_A/R_{STD} - 1) * 1000 \quad (1)$$

where R_A and R_{STD} are the molar ratios of the heavy to light isotopes (e.g., $^{13}C/^{12}C$) of the sample and standard, respectively.

2.2 | Breath samples

Exhaled breath samples were collected in foil balloons (Bowling, Pataki, & Ehleringer, 2003). Participants were instructed to inhale atmospheric air and exhaled air into the balloon until the balloon volume was full. Two breath samples were requested from each participant: a baseline sample (AM) and a postlunch sample (PM). After training, participants were given a balloon to take home and asked to collect the baseline sample in the morning upon waking and prior to any food or drink consumption. Postlunch samples were collected in the one to two-hour window following lunch under teachers' supervision. AM and PM breath samples were collected and analyzed on the same day.

Exhaled breath samples were removed from balloons using a gas tight syringe with a pressure-locking valve (Pressure-Lok, Baton Rouge, Louisiana). Six hundred microliters of the sample gas was injected onto a gas chromatography column coupled to a Finnigan Delta Plus mass spectrometer operating in continuous flow mode. Stable carbon isotope ratios of AM ($\delta^{13}C_{AM}$) and PM ($\delta^{13}C_{PM}$) breath samples were analyzed alongside two in-house CO_2 reference gases with isotope values of +15.5 and −10.0‰ after Dual Inlet-IRMS calibration against a certified carbon dioxide reference gas (–3.64‰; OzTech Trading Corp. Dallas, Texas). The analytical precision (1σ), based on repeated measurements of these references, was 0.15‰.

2.3 | Dietary information

The Youth/Adolescent Questionnaire (YAQ) was used to assess the previous year's diet. The YAQ is a 152-item FFQ

designed to assess the dietary intake in 9 to 19 year old children (Rockett et al., 1997, 1995). English or Spanish YAQ were self-administered at the school during class hours in the presence of the teachers and research personnel (LOV and SPO) who gave instructions and answered any questions the children had. The YAQ were analyzed by the Channing Laboratory, Brigham & Woman's Hospital, and reported as daily nutrient intakes. Servings per week (spw) of specific food categories were also calculated from the YAQ dataset by our research group; the food categories were, Dairy, Meat, Fish, Sweetened Beverages, Sweets, All Sweets, Grain, Fruits and Vegetables, Corn, Beans, and Western dietary items; these variables are defined in Supporting Information Table S1. Dietary variables are presented as intake in total grams or servings per week and as intake relative to each individual's caloric intake (i.e., g/kcal or spw/kcal).

2.4 | Biometrics

Height and weight were measured at the time of hair collection. Age-specific BMI percentiles were calculated using the CDC's Children's BMI Tool for Schools (Centers for Disease Control and Prevention, n.d.).

2.5 | Statistics

Data were summarized as mean [median] ± standard deviation. Intakes less than 500 kcal or greater than 5000 kcal per day were considered implausible and were not included in the analyses (Rockett et al., 1997). Normality of distributions was tested using Shapiro-Wilk tests. Trends in dietary intake and isotope ratios by age were described by Spearman Rank correlations, as well as by Kruskal–Wallis analysis of variance by ranks where grade level was used as a factor. Differences between ethnic groups were assessed using the Wilcoxon-rank sum test that compares two independent samples. Correlations between isotope ratios and intake variables were also assessed using partial Spearman Rank correlations. Statistical analyses were conducted using R (R Development Core Team, 2008).

3 | RESULTS

Two hundred and twelve children from fifth through tenth grade classes participated in this study; 97 were Hispanic and 115 were non-Hispanic white (Supporting Information Table S2 describes the breakdown of sample sizes by age, gender, and ethnicity). Stable isotope ratios of nitrogen, carbon, and sulfur were analyzed for 174, 172, and 84 hair samples, respectively. Carbon isotope ratios from AM breath samples and PM breath samples were analyzed for 106 and 121 participants, respectively. YAQ-derived intake variables were calculated for 197 participants (93% respondents). Neither YAQ-derived dietary variables (Shapiro-Wilk test, $P < .05$ for all variables) nor hair isotope values were normally distributed ($\delta^{13}C$ $W = 0.96$, $P < .001$; $\delta^{15}N$ $W = 0.98$, $P = .010$; $\delta^{34}S$ $W = 0.96$, $P = .018$). However, breath isotopes were normally distributed ($\delta^{13}C_{AM}$ $W = 0.98$, $P = .180$; $\delta^{13}C_{PM}$ $W = 0.98$, $P = .100$).

3.1 | Age and gender differences

Stable isotope ratios of hair did not differ across age ($P > .05$ for all Spearman rank correlations), nor between genders (All Wilcoxon rank-sum tests $P > .05$; Table 1). The average carbon, nitrogen and sulfur isotope values of hair were $-17.0 \pm 0.7‰$, $8.8 \pm 0.4‰$, and $3.6 \pm 0.7‰$, respectively (Table 1; Supporting Information Figure S1). The isotope ratios of AM breath did not vary with age ($P > .05$), but PM breath carbon isotope ratios increased with age (Table 1; Supporting Information Figure S2; $\rho = 0.19$, $P = .043$). The isotopes of breath AM and PM did not differ between girls and boys (Wilcoxon rank-sum tests $P > .05$; Table 1) The overall average breath carbon isotope value for the morning samples was $-21.1 \pm 1.4‰$ and for the afternoon samples was $-21.0 \pm 1.4‰$.

Based on YAQ self-reports, daily energy intake was similar across age (Table 1, Supporting Information Figure S3). The average and median energy intakes for the entire dataset were 1881 and 1701 kcal, respectively (SD = 748 kcal). The consumption of macronutrients did not vary with age (Rank correlation coefficient for all tests: $-0.016 < \rho < 0.40$, $P > .05$; Table 1, Supporting Information Figure S3). The average consumptions of carbohydrates, proteins, and fats were 0.14 ± 0.02 g/kcal, 0.039 ± 0.007 g/kcal, and 0.033 ± 0.006 g/kcal, respectively, for the total sample.

Boys reported a higher total caloric intake than girls (boys = 1997 ± 707 kcal, girls = 1776 ± 653 kcal; Wilcoxon rank-sum test $W = 5739$, $P = .023$; Table 1), while girls reported a higher intake of carbohydrates than boys (girls = 0.143 ± 0.017 g/kcal, boys = 0.135 ± 0.016 g/kcal; $W = 3519$, $P < .001$). There were no significant differences in the intake of other macronutrients between girls and boys (all Wilcoxon rank-sum tests $P > .05$; Table 1).

3.2 | Ethnic differences

Age-specific BMI percentiles were lower ($W = 2787$, $P = .02$) for non-Hispanic white students (BMI-percentile = 62.6 ± 29.0, $n = 90$) than for Hispanic students (BMI-percentile = 71.5 ± 28.6, $n = 87$). The caloric intakes of non-Hispanic white students (1869 ± 664 kcal) and Hispanic students (1897 ± 845 kcal) were not significantly different ($W = 4950$, $P = .67$; Supporting Information Figure S3). However, non-Hispanic white students reported higher

TABLE 1 Self-reported macronutrient intake and measured stable isotope ratios in hair and breath of children, by grade level and gender

		Grade level			Gender		
		5–6	7–8	9–10	Girls	Boys	Total
Calories[a]	kcal	2017 ± 820 (78)	1785 ± 623 (59)	1798 ± 747 (60)	1776 ± 653 (103)	1997 ± 707 (94)	1881 ± 748 (197)
Carbohydrate[a]	g	281 ± 123	251 ± 87	250 ± 111	257 ± 117	269 ± 101	263 ± 110
	g/kcal	0.14 ± 0.02	0.14 ± 0.02	0.14 ± 0.02	0.143 ± 0.017	0.135 ± 0.016	0.14 ± 0.02
Protein	g	76 ± 31	70 ± 30	69 ± 28.5	67 ± 29	78 ± 30	72 ± 30
	g/kcal	0.038 ± 0.007	0.039 ± 0.008	0.039 ± 0.007	0.038 ± 0.007	0.039 ± 0.007	0.039 ± 0.007
Fat	g	69 ± 30	58 ± 24	51 ± 28	56 ± 27	70 ± 27	63 ± 16.4
	g/kcal	0.034 ± 0.005	0.032 ± 0.006	0.033 ± 0.006	0.032 ± 0.006	0.035 ± 0.005	0.033 ± 0.006
$\delta^{13}C_{AM}$	‰	−21.1 ± 1.3 (51)	−21.4 ± 1.1 (19)	−20.9 ± 1.7 (36)	−21.1 ± 1.5 (61)	−21.0 ± 1.3 (45)	−21.1 ± 1.4 (106)
$\delta^{13}C_{PM}$[b]	‰	−21.2 ± 1.0 (41)	−21.2 ± 1.4 (44)	−20.6 ± 1.8 (33)	−21.0 ± 1.5 (70)	−21.0 ± 1.3 (48)	−21.0 ± 1.4 (118)
$\delta^{13}C$	‰	−16.9 ± 0.7 (65)	−16.9 ± 0.6 (47)	−17.1 ± 0.8 (51)	−17.0 ± 0.8 (96)	−16.9 ± 0.5 (67)	−17.0 ± 0.7 (163)
$\delta^{15}N$	‰	8.8 ± 0.4 (65)	8.9 ± 0.4 (47)	8.7 ± 0.4 (53)	8.8 ± 0.4 (97)	8.8 ± 0.4 (68)	8.8 ± 0.4 (165)
$\delta^{34}S$	‰	3.6 ± 0.5 (35)	3.6 ± 0.5 (20)	3.6 ± 0.8 (29)	3.5 ± 0.6 (54)	3.7 ± 0.7 (30)	3.6 ± 0.7 (84)

Self-report of diet over the last year on Youth/Adolescent Questionnaire (YAQ). Sample sizes for Carbohydrate, Protein, and Fat are equal to those of Calories.
[a]Significant differences were detected between boys and girls after Wilcoxon rank-sum tests.
[b]Significant correlation was detected between age (as ordinal variable) and $\delta^{13}C_{PM}$ values (Rank correlation coefficient $\rho = 0.19$, $P = .043$).
Data are presented as mean ± standard deviation (sample size).

intake of total protein, total fat, and lower intake of carbohydrates (Figure 1, Tables 2 and 3, Supporting Information Figure S3).

3.3 | Hair isotopes: Protein intake

Both the $\delta^{13}C$ and $\delta^{15}N$ values of non-Hispanic white students were lower than those of Hispanic students (Figure 2A, Table 2). No significant difference in sulfur isotope ratios was detected between non-Hispanic white and Hispanic students (Figure 2B, Table 2). Non-Hispanic white students reported higher intake of animal protein (g/kcal), primarily a higher number of servings of dairy products than Hispanic students (Table 2). However, as a proportion of total protein, the percentage of animal protein consumed was similar between non-Hispanic white students and Hispanic students (Table 2). No differences were detected in the consumption of meat or fish (Table 2).

3.4 | Breath isotopes: Carbohydrate intake

The carbon isotope ratios of breath samples collected from Hispanic students were statistically higher than those of non-Hispanic white students for both AM and PM samples (Figure 3, Table 3). Hispanic students showed significantly higher consumption of sweetened beverages and significantly lower consumption of grain than non-Hispanic white students (Table 3). No significant differences were detected between ethnic groups in the number of servings of sweets, fruits and vegetables, corn, beans, and Western dietary items (Table 3).

3.5 | Isotope ratios as quantitative biomarkers of reported diet

3.5.1 | Hair isotopes: Protein intake

Overall, stable carbon and nitrogen isotope ratios were correlated with each other ($N = 158$, $P < .001$, $r = 0.56$), but sulfur isotope ratios were not correlated with either carbon ($N = 74$, $P = .99$) or nitrogen isotopes ($N = 74$, $P = .44$). In non-Hispanic white students $\delta^{13}C$ and $\delta^{15}N$ ($N = 86$, $P < .001$, $r = 0.61$) and $\delta^{34}S$ and $\delta^{15}N$ ($N = 44$, $P = .024$, $r = 0.34$) were positively associated, but $\delta^{13}C$ and $\delta^{34}S$ ($N = 43$, $P = .17$) were not related. In Hispanic students only $\delta^{13}C$ and $\delta^{15}N$ values ($N = 72$, $P = .001$, $r = 0.38$) were positively associated, but there was no correlation of $\delta^{34}S$ values and $\delta^{13}C$ ($N = 31$, $P = .43$) or $\delta^{15}N$ values ($N = 32$, $P = .17$).

We detected a number of significant correlations between intake variables and the stable isotopes of hair, reflecting different sources of protein (Table 4). The majority of the significant correlations followed trends that would be expected based on consumption of meat, fish, and plant-derived protein. For example, we detected positive correlations between $\delta^{15}N$ values and proportion of animal protein, $\delta^{13}C$ values and intake

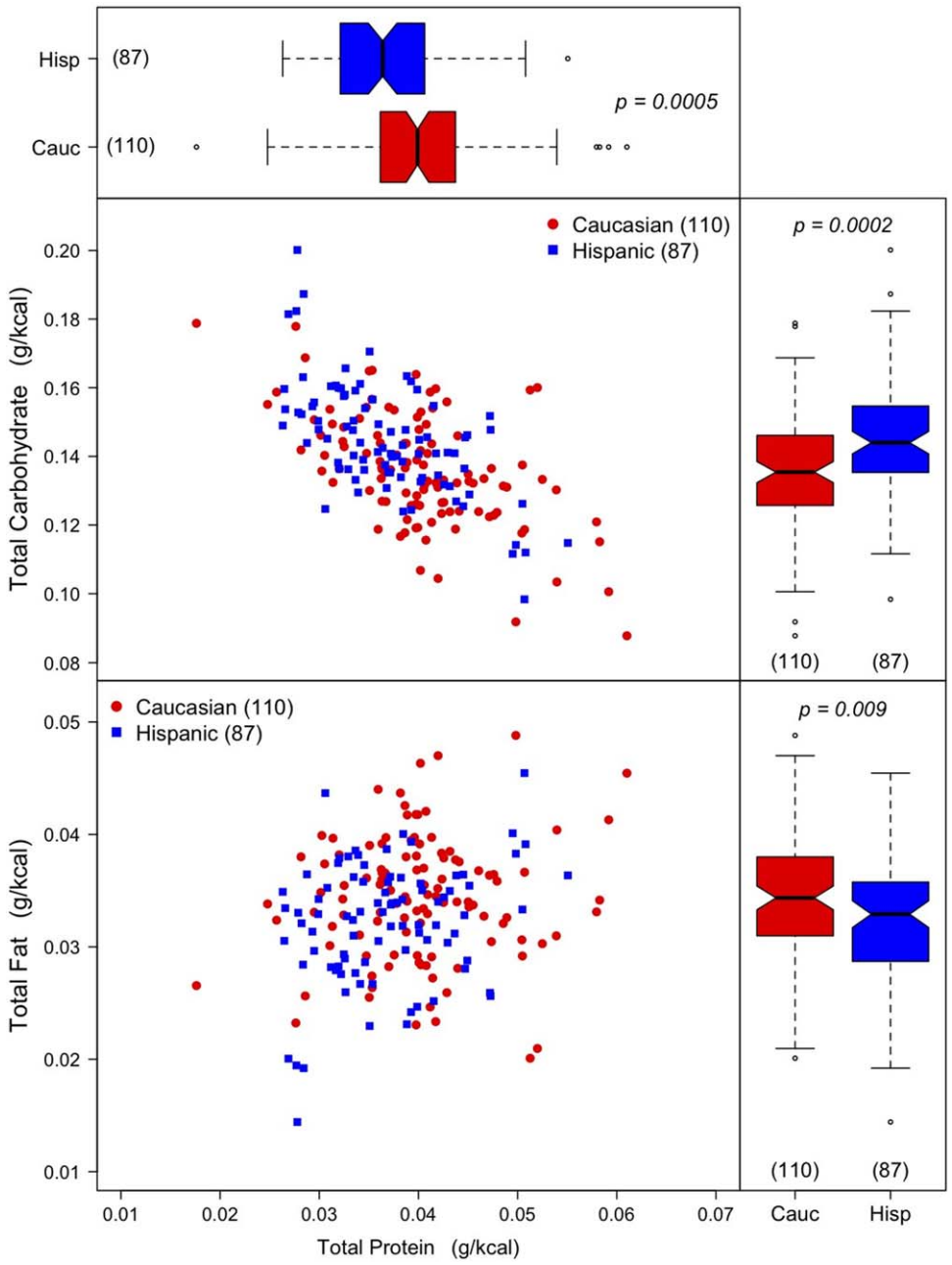

FIGURE 1 Covariation of macronutrients for non-Hispanic white and Hispanic children. Sample sizes are presented between parentheses. *P*-values after Wilcoxon rank-sum tests are presented next to the boxplots

of corn, $\delta^{34}S$ values and intake of fish (for non-Hispanic white students). We detected negative correlations for $\delta^{13}C$ and $\delta^{15}N$ values in relationship to the intake of plant-derived protein, consistent with the reported pattern that Hispanic students received less protein from vegetable sources (grains, fruits and vegetables, beans, $P < .01$) for the entire dataset and for the subset of non-Hispanic white students (Table 4).

3.5.2 | Breath isotopes: Carbohydrate intake

Carbon isotope values of AM breath samples were significantly correlated with values from PM samples, suggesting consistency in carbohydrate sources throughout the course of the day (all data $r = 0.62$, $P < .001$, $n = 86$; Figure 3). The significant correlation between AM and PM exists within each ethnic group as well; Hispanic children ($r = 0.65$, $P < .001$, $n = 44$) and non-Hispanic white children ($r = 0.56$, $P < .001$, $n = 32$). The observed isotopic differences between PM and AM values were normally distributed (Shapiro-Wilk test $W = 0.97$, $P = .08$) and not significantly different from zero (average = $0.1 \pm 1.2‰$; paired *t*-test, $t = 0.9$, $P = .34$). However, there was considerable variability among these differences suggesting that some of the students had significantly different carbohydrate sources in the AM versus the

TABLE 2 Comparisons of reported total protein, total fat, sources of animal protein in diet, and isotope values of hair for non-Hispanic white and Hispanic students

Variable		*P*-value	Non-Hispanic white	Hispanic
Total protein	g	.07	75 ± 29 (110)	69 ± 31 (87)
	g/kcal	**.0005**	0.041 ± 0.007	0.037 ± 0.007
Total fat	g	.19	64.6 ± 25.9 (110)	61.4 ± 30.3 (87)
	g/kcal	**.009**	0.034 ± 0.005	0.032 ± 0.005
Animal protein	g	.12	51 ± 24 (110)	46 ± 23 (87)
	g/kcal	.03	0.027 ± 0.008	0.025 ± 0.007
	%	.74	66 ± 12 (110)	67 ± 9.6 (87)
Dairy	spw	.02	23.6 ± 12.3 (110)	19.9 ± 12.1 (87)
	spw/kcal	**.006**	0.013 ± 0.008	0.012 ± 0.011
Meat	spw	.98	6.0 ± 7.1 (110)	6.2 ± 6.8 (87)
	spw/kcal	.51	0.003 ± 0.007	0.004 ± 0.010
Fish	spw	.14	1.1 ± 2.2 (110)	1.6 ± 3.1 (87)
	spw/kcal	.21	0.0007 ± 0.0022	0.0014 ± 0.0046
$\delta^{13}C$	‰	**.0001**	−17.3 ± 0.6 (89)	−16.6 ± 0.5 (74)
$\delta^{15}N$	‰	**.0003**	8.7 ± 0.4 (90)	9.0 ± 0.4 (75)
$\delta^{34}S$	‰	.22	3.7 ± 0.7 (48)	3.4 ± 0.5 (36)

P-values correspond to Wilcoxon rank-sum test (nonparametric *t*-test for independent samples).
Data are presented as mean ± standard deviation (sample size). Bold font highlights *P*-values < .01.

PM (range −4.1 to 3.2‰). The magnitudes of these isotopic differences were not associated with a particular ethnic group ($t = 1.3$, $P = .19$, $n_{Hisp} = 44$, $n_{non\text{-}Hisp} = 42$) or age (Pearson correlation coefficient $r = -0.01$, $P = .5$, $n = 86$).

As expected, the intake of sweetened beverages and sweets was positively correlated with $\delta^{13}C$ values from breath CO_2 (Table 4). Interestingly, these correlations were detected in both the entire dataset and in Hispanic students, but not for non-Hispanic white students (Table 4). The correlations between reported sources of carbohydrate and breath CO_2 were stronger for the AM samples than for the PM samples (Table 4).

3.6 | Correlations between hair and breath isotopes

Stable carbon isotope ratios of hair samples were significantly correlated with those of AM breath samples when the entire dataset was analyzed ($r = 0.47$, $P < .001$, $n = 96$), and when Hispanic ($r = 0.48$, $P < .001$, $n = 49$) and non-Hispanic white students ($r = 0.36$, $P = .012$, $n = 47$) were analyzed separately. Hair samples were positively associated with afternoon breath samples for the entire dataset ($r = 0.48$, $P < .001$, $n = 107$) and for non-Hispanic white students ($r = 0.45$, $P < .001$, $n = 55$); however, no correlation was detected for Hispanic students ($r = 0.33$, $P = .15$, $n = 52$). Neither nitrogen nor sulfur isotopes were correlated with breath isotopes ($P > .05$ for all comparisons).

4 | DISCUSSION

This study provides the first paired analyses of stable isotope ratios in hair and breath of children; these observations place these biomarker data in the context of reported dietary intake derived from an established FFQ, the Youth/Adolescent Questionnaire (YAQ). Our results confirm the utility of stable isotope analysis to represent broad dietary trends in children and, in some cases, to assess intake of specific food groups. The stable isotope analyses detected similar patterns as the self-reported diet data, particularly dietary differences between ethnic groups, as well as some patterns not revealed by FFQ.

The rather different temporal information provided by the YAQ and the stable isotopes of hair and breath must be considered prior to interpretation of the data. The YAQ was designed as an instrument to capture the previous year's diet (Rockett et al., 1997, 1995). The hair isotope ratios analyzed

TABLE 3 Comparisons of reported total carbohydrate intake, sources of carbohydrate, and stable carbon isotope values of breath for non-Hispanic white and Hispanic children

Variable		*P*-value	Non-Hispanic white	Hispanic
Carbohydrate	g	.41	253 ± 95 (110)	275 ± 125 (87)
	g/kcal	**.0002**	0.136 ± 0.017	0.145 ± 0.017
Sweetened Beverages	spw	.05	6.2 ± 7.0 (110)	7.2 ± 6.1 (87)
	spw/kcal	**.0036**	0.0035 ± 0.004	0.0040 ± 0.003
Sweets	spw	.73	14.7 ± 21.3 (110)	16.1 ± 22.4 (87)
	spw/kcal	.71	0.009 ± 0.017	0.012 ± 0.029
Grain	spw	**.006**	24.5 ± 15.7 (110)	21.9 ± 18.7 (87)
	spw/kcal	**.0007**	0.013 ± 0.006	0.010 ± 0.004
Fruits and vegetables	spw	.82	38.3 ± 35 (110)	41.0 ± 39.4 (87)
	spw/kcal	.89	0.022 ± 0.024	0.028 ± 0.050
Corn	spw	.38	5.3 ± 5.1 (110)	6.2 ± 6.2 (87)
	spw/kcal	.40	0.003 ± 0.004	0.004 ± 0.008
Beans	spw	.12	1.0 ± 2.1	1.0 ± 2.2 (87)
	spw/kcal	.20	0.0005 ± 0.0014	0.0009 ± 0.003
Western	spw	.08	9.1 ± 9.1 (110)	11.2 ± 11.1 (87)
	spw/kcal	.09	0.005 ± 0.008	0.008 ± 0.016
$\delta^{13}C_{AM}$	‰	.04	−21.4 ± 1.4 (52)	−20.8 ± 1.3 (54)
$\delta^{13}C_{PM}$	‰	**.0006**	−21.4 ± 1.6 (61)	−20.6 ± 1.1 (57)

P-values correspond to Wilcoxon rank-sum test (nonparametric *t*-test for independent samples).
Bold font highlights *P*-values < .01.

in bulk (entire lock), as we did in this study, reflect the average diet over the period of time integrated by the length of the hair (growth rate approximates 1 cm/month). Since we had no control over the length or how close to the scalp all hair samples were cut, our data might reflect different weeks prior to sampling. However, only a few samples were of unusual length (too short or too long); thus, we assume all hair samples represent several months of data. Breath CO_2 isotope data provide a measure of recent carbohydrate metabolism (O'Brien, 2015), but how recent might depend on the isotopic turnover and mobilization of internal pools (Ayliffe et al., 2004). The isotope signal measured in breath samples responds to what is being used for energy hours before measuring, and this could be immediate food or stored reserves. Thus, the interpretations of all comparisons, and positive or negative associations, between isotope data and YAQ variables are to be done with the assumption that there is no strong seasonal or temporal variability on the isotopic composition of the food consumed.

4.1 | Age group differences

Surprisingly, we did not detect major dietary differences among age groups assessed by either the YAQ or isotope analyses (Supporting Information Figures S1–S3, Table 1). Only the carbon isotope ratios of PM breath showed differences across age groups, which might have reflected common public school lunches. However, we have no data related to lunch composition to test this. Previous research has characterized temporal shifts in eating patterns as a decrease in the consumption of breakfast items, fruits, vegetables, and milk, and an increase in the consumption soft drinks (Caine-Bish & Scheule, 2009; Granner et al., 2004; Lytle et al., 2000). The increase in $\delta^{13}C_{PM}$ values detected across age agrees with an increase in the preference for soft drinks (mostly C_4-based carbohydrates) (Caine-Bish & Scheule, 2009; Lytle et al., 2000). With the exception of total caloric intake and carbohydrate consumption (relative to kcal), our study did not detect an influence of gender, or an interaction of age

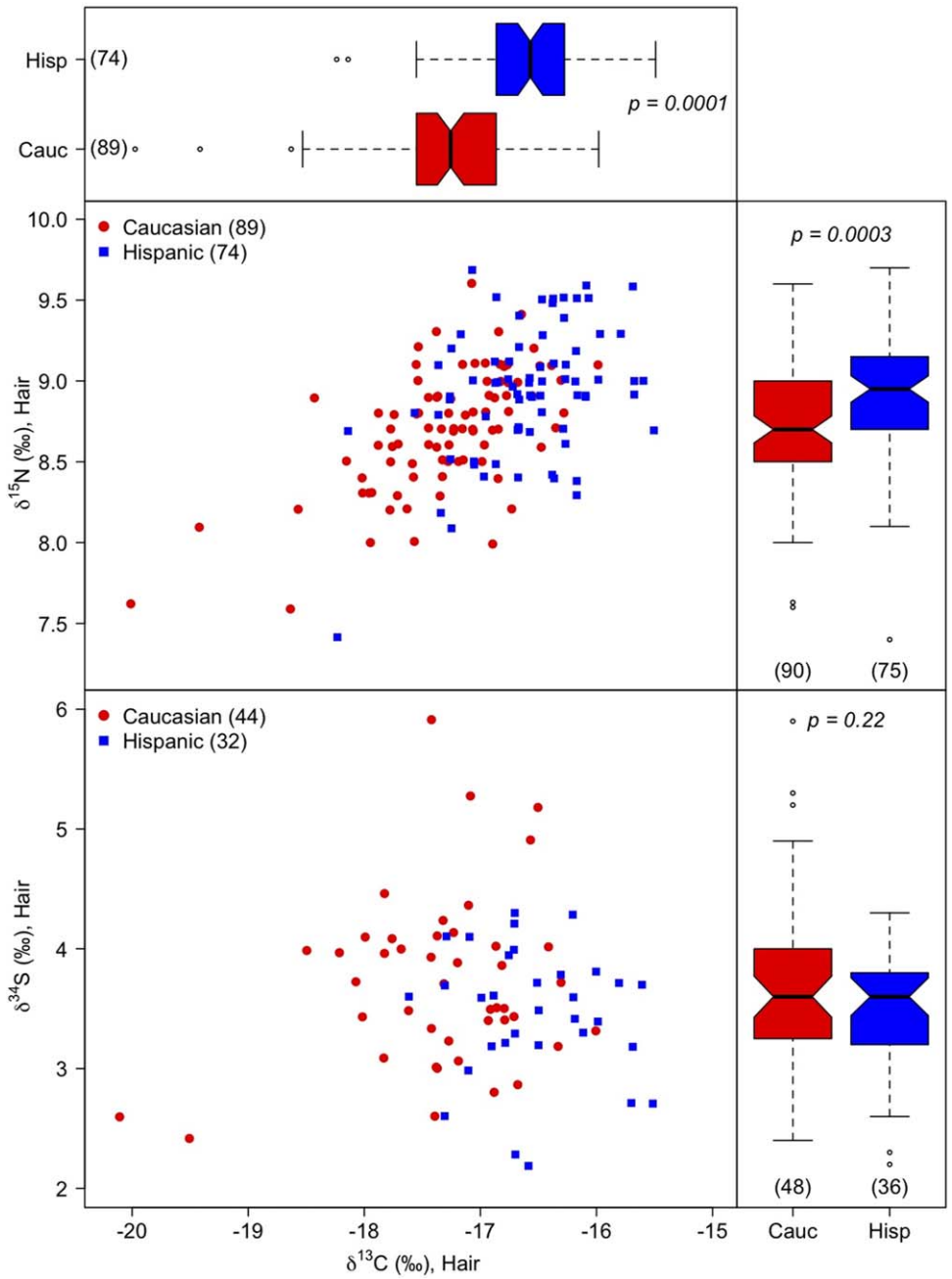

FIGURE 2 Covariation of stable isotopes of hair for non-Hispanic white and Hispanic children. Sample sizes are presented between parentheses. *P*-values after Wilcoxon rank-sum tests are presented next to the boxplots

and gender. However, the validity of the YAQ is potentially lower in the younger age groups and greater error could mask relationships (Rockett et al., 1997).

4.2 | Ethnic differences

Both the YAQ-derived variables and stable isotope ratios agreed in describing broad dietary differences between Hispanic and non-Hispanic white children. Although similar caloric intakes were reported for the two ethnic groups, the sources of calories differed between the groups.

The suggested low validity of FFQ in Hispanic populations (Cullen & Zakeri, 2004) must be considered when analyzing the data. As proposed by Cullen and Zakeri (2004), students may have difficulty remembering foods consumed over long periods of time. Also, the daily variability of intake may require students to perform arithmetic calculations for average consumption to fit into FFQ categories. In other words, children might need abstract thinking skills to complete the YAQ. For Cullen and Zakeri (2004), these could be the reasons for low validity in populations with lower academic achievement. We do not have data on academic

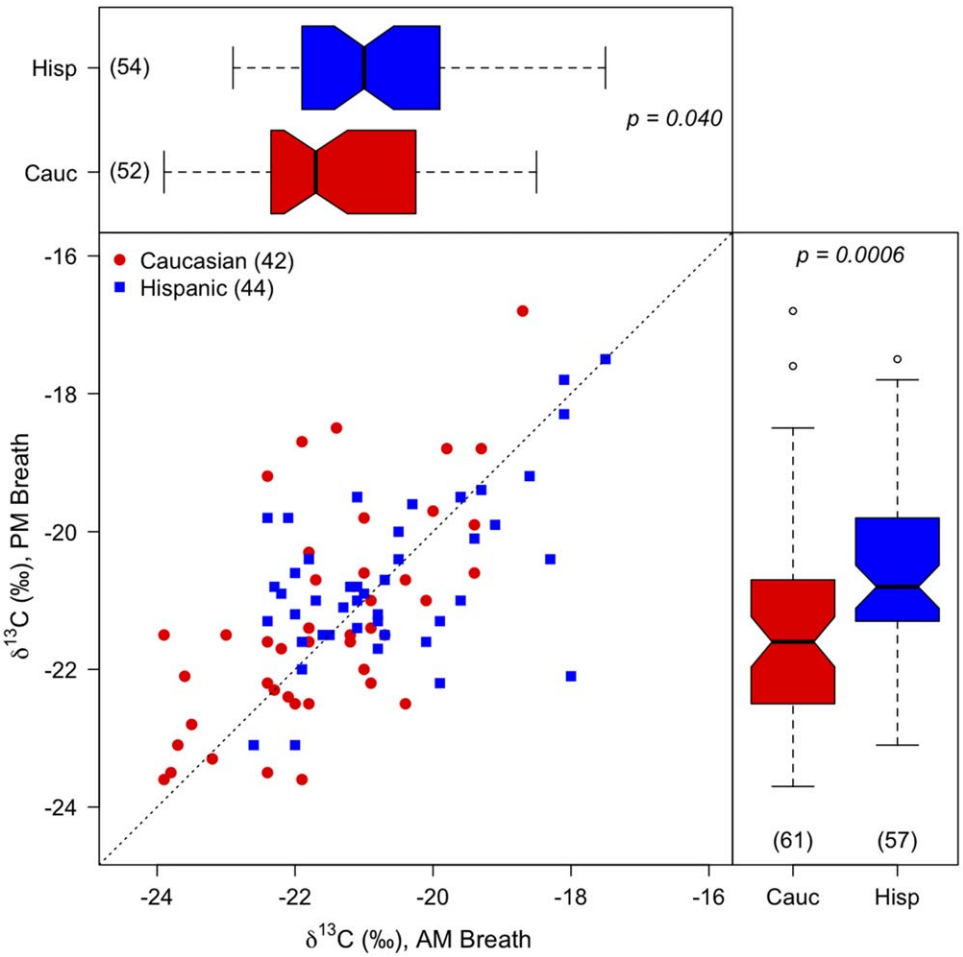

FIGURE 3 Covariation of stable isotopes of breath for non-Hispanic white and Hispanic children. The dashed line represents the 1:1 line. Sample sizes are presented between parentheses. *P*-values after Wilcoxon rank-sum tests are presented next to the boxplots

achievement for these students, but all students were in regular classes. Furthermore, when requested, YAQ forms in Spanish were used, so language should not have been a problem. Another possibility is that although the YAQ contained ethnic foods, these foods may not have represented the items usually consumed by these children.

4.2.1 | Protein intake: Hair isotopes

The significantly greater protein intake reported by non-Hispanic white students relative to Hispanic students potentially reflects a higher consumption of dairy protein and grains. However, the ability of the YAQ to provide a valid caloric-intake measure in Hispanic children has been questioned (Cullen & Zakeri, 2004). Although it is possible that the consumption of protein was underrepresented for Hispanic students in our study, our findings (more dairy and grains in non-Hispanic white relative to Hispanic students) agree with previously reported data on dietary patterns of adults across the U.S. (Smit, Nieto, Crespo, & Mitchell, 1999), and is consistent with the carbon and nitrogen isotope ratio observations of hair.

The carbon isotope ratios of modern human hair are influenced by the proportional intake of proteins from C_3 or C_4 photosynthetic origin. In a modern American diet, C_3-derived proteins are obtained by direct consumption of grains (e.g., wheat and soy), vegetables, or by consumption of animal proteins raised on C_3 pastures (i.e., grass-fed cattle). In contrast, C_4-derived proteins in the American diet are mostly obtained by direct consumption of corn or consumption of animal proteins raised on corn (i.e., feedlot cattle, hog farms, chicken farms). In the U.S., most beef, pork, and chicken are raised with corn as their main feed, and thus have high $\delta^{13}C$ values (Chesson, Ehleringer, & Cerling, 2009; Chesson et al., 2008; Jahren & Kraft, 2008). Therefore, our finding that non-Hispanic white students having hair $\delta^{13}C$ values lower (C_3-like values) than Hispanic students is likely explained by their reported consumption of more vegetables, grains, and dairy products, even though reported meat consumption by both groups was similar.

The nitrogen isotope ratios of human hair are primarily determined by the relative proportions of plant and animal protein consumed, and it is expected that the more animal protein being consumed, the higher the $\delta^{15}N$ values (20). For

TABLE 4 *P*-values of Spearman rank correlations between isotope values and dietary intake variables

Intake	Data	Hair $\delta^{13}C$, ‰	Hair $\delta^{15}N$, ‰	Hair $\delta^{34}S$, ‰	Breath $\delta^{13}C_{AM}$, ‰	Breath $\delta^{13}C_{PM}$, ‰
Total protein (g/kcal)	*Total*	**0.005 (–0.22)**	0.50	0.10	0.048 (–0.19)	0.18
	Whi	0.80	0.67	0.05	0.51	0.87
	Hisp	0.18	0.32	0.75	0.22	0.76
Animal protein (g/kcal)	*Total*	0.80	0.10	0.08	0.44	0.76
	Whi	0.50	0.12	0.13	0.75	0.55
	Hisp	0.48	0.11	0.43	0.29	0.68
Percent of animal protein	*Total*	0.08	**0.006 (+0.22)**	0.18	0.54	0.41
	Whi	**0.0001 (+0.52)**	**0.0001 (+0.40)**	0.018 (+0.34)	0.17	0.14
	Hisp	0.30	0.04 (+0.23)	0.08	0.62	0.88
Dairy (spw/kcal)	*Total*	0.20	0.55	0.40	0.29	0.57
	Whi	0.19	0.50	0.32	**0.007 (+0.36)**	0.91
	Hisp	0.10	0.38	0.88	0.66	0.77
Meat (spw/kcal)	*Total*	0.90	0.35	**0.004 (+0.32)**	0.49	0.043 (+0.19)
	Whi	0.83	0.42	0.016 (+0.35)	0.67	**0.002 (+0.39)**
	Hisp	0.36	0.20	0.19	0.48	0.79
Fish (spw/kcal)	*Total*	0.70	0.60	0.11	0.34	0.98
	Whi	0.52	0.98	0.011 (+0.37)	0.10	0.78
	Hisp	0.74	0.81	0.63	0.99	0.85
Total carbohydrate (g/kcal)	*Total*	0.34	0.42	0.053	0.60	0.30
	Whi	0.13	0.07	0.22	0.89	0.38
	Hisp	0.73	0.46	0.47	0.58	0.84
Sweetened beverages (spw/kcal)	*Total*	0.014 (+0.19)	0.11	0.28	0.020 (+0.24)	0.27
	Whi	0.31	0.10	0.07	0.21	0.99
	Hisp	0.15	0.035 (–0.24)	0.009 (+0.42)	0.16	0.17
Sweets (spw/kcal)	*Total*	0.011 (+0.20)	0.54	0.11	**0.0004 (+0.34)**	0.037 (+0.19)
	Whi	0.24	0.21	0.02 (–0.32)	0.08	0.20
	Hisp	0.021 (+0.27)	0.98	0.92	**0.0009 (+0.44)**	0.19
All sweets (spw/kcal)	*Total*	**0.0001 (+0.30)**	0.43	0.044 (–0.22)	**0.0001 (+0.37)**	0.034 (+0.19)
	Whi	0.13	0.27	0.009 (–0.36)	0.11	0.54
	Hisp	**0.0010 (+0.37)**	0.38	0.57	**0.0002 (+0.49)**	0.05
Grain (spw/kcal)	*Total*	**0.002 (–0.25)**	0.016 (–0.19)	0.015 (–0.25)	0.25	0.19
	Whi	**0.001 (–0.35)**	**0.007(–0.29)**	0.14	0.21	0.024 (–0.29)
	Hisp	0.56	0.76	0.017 (–0.40)	0.61	0.026 (+0.30)

(Continues)

TABLE 4 (Continued)

Intake	Data	Hair $\delta^{13}C$, ‰	Hair $\delta^{15}N$, ‰	Hair $\delta^{34}S$, ‰	Breath $\delta^{13}C_{AM}$, ‰	Breath $\delta^{13}C_{PM}$, ‰
Fruits and vegetables (spw/kcal)	*Total*	0.07	0.36	0.36	0.63	0.90
	Whi	**0.003 (–0.32)**	0.044 (–0.21)	0.90	0.80	0.71
	Hisp	0.53	0.040 (+0.24)	0.30	0.66	0.32
Corn (spw/kcal)	*Total*	**0.0001 (+0.31)**	0.52	0.032 (–0.23)	0.19	0.30
	Whi	0.023 (+0.24)	0.33	0.014 (–0.35)	0.50	0.63
	Hisp	0.042 (+0.24)	0.19	0.81	0.55	0.94
Beans (spw/kcal)	*Total*	0.92	0.13	0.20	0.83	0.20
	Whi	0.045 (–0.22)	**0.0005 (–0.36)**	0.94	0.30	0.66
	Hisp	0.73	0.68	0.045 (–0.34)	0.20	0.08
Western (spw/kcal)	*Total*	**0.0004 (+0.28)**	0.35	0.80	0.013 (+0.24)	**0.009 (+0.24)**
	Whi	0.013 (+0.27)	0.52	0.57	0.25	0.011 (+0.33)
	Hisp	0.07	0.79	0.98	0.043 (+0.27)	0.42
Sample sample sizes	*Total*	158	159	82	104	115
	Whi	86	87	47	51	59
	Hisp	72	72	35	53	56

Whi: non-Hispanic whites: Hispanics, *spw*: servings per week. Western: see Online Supporting Material for a complete list of items that defines the composite variable "Western", but it includes items such as pop-tart, cake, donut, cookies, milkshake, popsicle, and so forth.
Data are presented for the entire dataset (*Total*) and for both ethnic groups separately. Correlation coefficients (ρ) are presented between parentheses for those comparisons that were statistically significant ($P < .05$); bold font highlights P-values $< .01$.

example, Petzke, Boeing, and Metges (2005) reported significant differences in $\delta^{15}N$ values among vegans (6.2‰), ovo-lacto-vegetarians (7.7‰) and omnivores (9.9‰) from Germany. Accordingly, the higher consumption of animal derived protein reported by non-Hispanic white students should have been reflected in higher $\delta^{15}N$ values, but this was not the case. However, the proportion of animal protein to total protein was not different between the two ethnic groups, which might explain why the average difference in $\delta^{15}N$ values was only 0.3‰ and extensive overlap existed between the two groups. Furthermore, the nitrogen isotopes could also have been affected by the differential consumption of other sources of protein (e.g., grains or beans). Additionally, not all animal derived proteins have the same nitrogen isotope ratio. For example, chicken purchased in fast food restaurants across the USA have $\delta^{15}N$ values that were approximately 4‰ lower than beef bought in the same restaurants (Chesson et al., 2008; Jahren & Kraft, 2008). Furthermore, there have been suggestions that milk might be ^{15}N-depleted relative to the animal's average body value (Jenkins, Partridge, Stephenson, Farley, & Robbins, 2001). Thus, the observed hair $\delta^{15}N$ values could also be influenced by differential intake of several protein sources.

The lack of differences in sulfur isotope ratios among student groups is consistent with the lack of differences in reported servings of fish (including shrimp, see Supporting Information Table S1). It was expected that higher consumption of oceanic fish and other marine-derived protein would be reflected in higher $\delta^{34}S$ values of the children's hair (Arneson & MacAvoy, 2005; Fry, 2006; Richards et al., 2001). However, it is worth recalling that the composite variable "Fish" does not discriminate between freshwater (expected a wide range but mostly lower $\delta^{34}S$ values) or marine sources (expected higher $\delta^{34}S$ values; Fry, 2006).

4.2.2 | Carbohydrate intake: Breath isotopes

Stable isotope ratios of breath CO_2 were consistent with YAQ findings on the intake of carbohydrate-dense foods and drinks. Greater carbohydrate intake by Hispanic students can be attributed to significantly higher consumption of sweetened beverages (Table 3), while non-Hispanic white students

had significantly higher consumption of grain-based foods (Table 3). This difference was supported by more positive $\delta^{13}C$ values of CO_2 from breath samples for Hispanic relative to non-Hispanic white students. As mentioned above, a more positive $\delta^{13}C$ value is indicative of a higher proportion of C_4 plants in the diet. Cane sugar and HFCS, both major components of sweets and sweetened beverages, are derived from C_4 plants (Jahren et al., 2006), while the food items grouped into the "Grain" variable (e.g., bread, muffin, spaghetti, cold cereal) are mostly C_3 grain-based, with the primary ingredients being wheat, rice, soy, and oats. Hispanic cuisine (particularly Mexican and Central American) is rich in foods that are corn-based and it was surprising that the consumption of corn-based foods, as reported by the YAQ, was not significantly higher in Hispanic students as compared with non-Hispanic white students. However, as mentioned before, this conclusion might reflect the potential low validity of the YAQ for Hispanic children (Cullen & Zakeri, 2004).

4.3 | Isotope ratios as biomarkers of reported diet

Isotope ratios of hair and breath were associated in the expected dose-dependent manner with a number of nutrient variables. In the following two sections, we discuss those correlations for which there is strong statistical evidence of covariation between YAQ-derived intake variables and stable isotope values, and provide a reference for their use as quantitative biomarkers of intake and dietary patterns.

4.3.1 | Protein intake-hair isotopes

As predicted, hair $\delta^{15}N$ values increased with an increase in the proportional intake of animal protein and decreased with an increase in plant protein intake. This finding agrees well with all previous published data (see Petzke et al. (2010) for a review of the topic). Also, as predicted, hair $\delta^{13}C$ values reflected the intake of protein from different C_3 and C_4 photosynthetic origins (Bol & Pflieger, 2002; Petzke, Boeing, Klaus, et al., 2005; Petzke, Boeing, & Metges, 2005; Petzke et al., 2010; Valenzuela et al., 2012). Carbon isotope values decreased with an increase in the reported intake of C_3 plants (grains, fruits, and vegetables) and increased with an increase in the consumption of corn. The highly significant correlations of $\delta^{13}C$ values with proportion of animal protein (among non-Hispanic white students), intake of Western dietary items, and total protein are interesting and deserve further research. However, the students might respond to the pervasiveness of C_4-derived proteins in the American diet due to the consumption of animal proteins raised on corn as explained above.

The positive correlation between $\delta^{34}S$ values and fish intake for non-Hispanic white students also reflects a predicted trend. However, the highly significant correlation between meat and $\delta^{34}S$ values was unexpected, and this correlation might simply reflect that children who consume more meat also consume more marine fish. However, no broad survey data exist on the sulfur isotope ratios of food items available to American consumers to evaluate the influences of different sources of sulfur in the children's hair, including the same food from different regions across the U. S. (Valenzuela et al., 2011).

4.3.2 | Carbohydrate intake: Breath isotopes

In Hispanic children, the consumption of foods with high amounts of carbohydrates, including soft drinks and a variety of candy and baked goods, was highly correlated with the $\delta^{13}C$ values of AM breath. Interestingly, the $\delta^{13}C$ values of PM breath were not highly correlated with the consumption of any major carbohydrate source. The correlation of sweets with AM and not PM breath suggests that isotope values of breath CO_2 collected in a fasted state may be a better indicator of the average carbohydrate consumption, and, as argued below, the $\delta^{13}C_{PM}$ values might be reflecting the influence of the previously consumed school lunches.

The potential use of the stable isotope ratios of carbon as a biomarker of dietary sweetener intake was recently proposed by Jahren et al. (2006) and later validated by Cook et al. (2010) in a controlled feeding trial in which the $\delta^{13}C$ value of postprandial plasma glucose was examined relative to dietary C_4 carbohydrate content. In a more recent study, carbon isotope values of human serum were evaluated as a biomarker for consumption of sweets in adults (Yeung et al., 2010). Similar to our findings in breath, Yeung et al. (2010) found that serum $\delta^{13}C$ values for individuals with high intake of sweetened beverages were significantly higher than for those with low intake of sweetened beverages. Although the findings of our study and the Yeung et al. (2010) study are similar, we consider breath analysis to be a more direct measure of carbohydrate intake than serum, because breath carbon dioxide values reflect what is currently being metabolized by the children, while serum measurements cannot be considered specific to dietary carbohydrates because of the presence of fat and protein derivatives. A study investigating the dynamics of carbon isotope changes in horses, via diet switch, determined that nearly 70% of the isotope contribution for breath carbon isotopes came from the recent assimilation of dietary components (Ayliffe et al., 2004).

The significant covariation of breath isotopes and some YAQ-derived variables points to a temporal stability in the consumption of carbohydrate sources (as YAQ reflects yearly average and breath CO_2 a much shorter window of time). Another explanation is that children might respond with information fresh on their minds regarding food intake,

thus the YAQ-derived information might not reflect long-term but rather short-term intake.

It is worth noting the significant correlation between carbon isotopes in hair and breath (particularly AM samples) in our study; we consider this trend is a result of dietary patterns that expose children to protein and carbohydrate sources located in the same zone of the C_3-C_4 spectrum (basically those who consume more dairy, fruits, and vegetables, might consume less C_4-derived sweets). However, it is also possible that routing of carbon skeletons from carbohydrates or fat sources is used for de novo syntheses of amino acids and proteins (contributing to hair isotope values).

4.4 | Breath isotopes pre and post lunch

Although the average difference between carbon isotopes of breath samples collected in the morning and afternoon was not different from zero, considerable isotopic variability existed; range 7.5‰. This range is likely the result of strong differences in the carbohydrate sources consumed by the children before the PM samples were collected. Although no pattern related to age or ethnicity was recognized in these differences, it is possible that other variables such as the timing and type of breakfast, lunch, and snacks consumed in between sample collection play a role in introducing variability to these data. Furthermore, we did not have control over the AM breath collection, which was instructed to take place at the children's home upon waking up and before breakfast, and therefore, we rely on timing information from the children. Nonetheless, the large variability is a very interesting observation in terms of the potential use of breath isotopes to study the recent intake of carbohydrates throughout the course of the day in a noninvasive and cost-efficient way.

5 | CONCLUSIONS

Stable isotope analyses of hair and breath are a useful biomarker of food and nutrient intake in large-scale population-based dietary studies. Here, we showed that the significant associations between isotope ratios and YAQ variables provided a reference point for estimation of dietary intake with stable isotope analyses. Stable isotopes of hair and breath were associated with the proportion of animal protein and the consumption of grain and sweets regardless of age and ethnic group. Furthermore, several significant correlations between isotope ratios and YAQ-derived variables only occurred within an ethnic group with higher reported consumption of the assessed food item or macronutrient.

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AUTHORS' CONTRIBUTIONS

L.O.V., S.O.P. and J.R.E. designed the research; L.O.V., S. O.P. and L.E.E. conducted the research; L.O.V., S.O.P., M. M, C.S. and J.R.E. analyzed the data; L.O.V. and S.O.P. wrote the article; L.E.E., M.M, C.S. and J.R.E helped with comments in the writing of the article; L.O.V., S.O.P. and J. R.E. had primary responsibility for final content. All authors read and approved the final manuscript.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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