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Correlation of Vitamin E, Uric Acid and Diet Composition with Histologic Features of Pediatric Nonalcoholic Fatty Liver Disease

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Abstract

Objectives—Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in children in the United States. Although changes in diet are often recommended to improve NAFLD, little is known regarding diet influence on histologic features of the disease.

Methods—This was a prospective, cross-sectional registry based study. Children (n=149) enrolled in the multi-center NASH Clinical Research Network had demographic, anthropometric, clinical, laboratory and histology data obtained, including the Block Brief Food Questionnaire. Subjects were grouped by presence or absence of steatohepatitis and grades of histologic features according to NASH-CRN criteria.

Results—No significant differences were found between children with steatosis compared to steatohepatitis for fraction of calories from fat, carbohydrates, and protein. Sugar sweetened beverage consumption was low and did not correlate with histologic features, although uric acid, a surrogate marker for fructose intake, was significantly increased in those with definite NASH (p=.008). For all groups, vitamin E consumption was insufficient compared to the recommended daily allowance. Median consumption of vitamin E was lower in children with higher grade of steatosis

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Abbreviations: Alanine aminotransferase (ALT), aspartate aminotransferase (AST), body mass index (BMI), gamma glutamyl transferase (GGT), nonalcoholic fatty liver disease (NAFLD), nonalcoholic steatohepatitis (NASH), NAFLD activity score (NAS)

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(8.4 vs 6.1 vs 6.9 for grade I, II and III respectively, $p = .05$). Those consuming less vitamin C had increased ballooning degeneration ($p = 0.05$).

Conclusions—Children with NAFLD have a diet that is insufficient in vitamin E and this may contribute to the pathophysiology of NAFLD. In children with NAFLD, reported sugar sweetened beverage consumption is low; however uric acid, which may reflect total fructose consumption, was significantly associated with NASH and should be further evaluated.

Keywords

NASH; Nutrition; Vitamin C; Sugar Sweetened Beverages

Introduction

Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in children in the United States(1). The term NAFLD refers to a spectrum of disease, ranging from isolated steatosis to non-alcoholic steatohepatitis (NASH) to cirrhosis(2) and children can present in any stage along this continuum. Typically found in overweight individuals, NAFLD is associated with features of the metabolic syndrome including dyslipidemia, visceral adiposity, hypertension and insulin resistance(3).

Similar to other constituents composing the metabolic syndrome, it is poorly understood why some obese children have normal livers while others develop steatosis alone or florid steatohepatitis. Although NAFLD is much more common in overweight children, a body mass index (BMI) above a certain threshold is a poor predictor of NAFLD severity(4).

Diet composition is an environmental factor that might influence NAFLD severity. Previous attempts have been made at correlating diet and NAFLD in children; however, precedent studies did not utilize liver biopsy or reliable imaging techniques for case ascertainment(5, 6). The importance of specific micro- or macro- nutrients may lie more in their influence on cell injury, fibrosis, inflammation or degree of steatosis rather than categorical change in liver fat or alterations in surrogate markers. These features can only be assessed with a biopsy-validated study design.

The National Institute of Diabetes and Digestive and Kidney Diseases organized and funded a Nonalcoholic Steatohepatitis Clinical Research Network (NASH CRN) beginning in 2002. Clinical, laboratory, anthropometric and demographic and nutritional data collected as part of the NASH CRN provide a unique resource because liver histology underwent a systematic, masked central review by an expert panel of pathologists and clinical factors were assessed near the time of liver biopsy. The objective of this study is to examine dietary data from a large cohort of NAFLD patients and correlate diet variables with specific histopathologic features. We hypothesized that children with decreased antioxidant vitamin consumption and increased sugar sweetened beverage consumption would have worse pathologic features of NAFLD.

Materials and Methods

The NAFLD Database is a prospective, observational registry of subjects with known or suspected NAFLD, which includes children 2-17 years. The NAFLD Database studies have Institutional Review Board approval at each of the Clinical Centers participating in the NASH CRN (appendix). Written consent was obtained from a parent or guardian and written assent obtained from all children 8 years and older prior to participation. Participants were eligible for inclusion in this diet study if they had baseline clinical data collected within 6 months of liver biopsy (most were assessed within 2 months) and their liver biopsy

specimens had undergone masked central review and scoring by the Pathology Committee of the NASH CRN by November 2007 (Table 1). Because liver biopsy was an inclusion criteria into the study, baseline clinical data including diet information was collected after the liver biopsy and diagnosis of NAFLD.

Demographic data were obtained via structured interview and questionnaires. Height, weight, waist and hip measurements were taken in duplicate while standing and wearing light clothing. Height and weight were measured without shoes to the nearest 0.1cm and 0.1kg, respectively. BMI was calculated as weight (kg) divided by height (m) squared. BMI percentile was determined according to age and gender based on data from the Centers for Disease Control and Prevention and converted to a z-score for comparison.

Diet information was collected using the complete Block Brief questionnaire. This questionnaire includes 77 food items and was developed from the NHANES 1999-2002 dietary recall data. Individual portion size is asked, and pictures are provided improving quantification of nutrient intake and allowing detailed comparisons of diet and histopathology. Sugar sweetened beverage per week information was compiled by totaling the “glasses or cans” per year reported for fruit juices, Kool Aid® and similar drinks, fruit punches, and soda and dividing by 52 weeks. We chose to include 100% fruit juice because juices contain large amounts of fructose, a sugar known to induce fatty liver in animal models(7). Fasting whole blood samples were obtained by venipuncture following overnight fast of more than 12 hours and processed for plasma and serum within 2 hours. Each clinical center performed reported laboratory assays on site.

Biopsies were evaluated for NAFLD features according to the validated histological scoring system for the NASH CRN(8): Steatosis [grade 0 (<5% macrovesicular fat), grade 1 (5-33%), grade 2 (34-66%), grade 3(>66%)], portal inflammation (grade 0-2), lobular inflammation (grade none, <2, 2-4 and >4) and ballooning degeneration (none, few and many). A NAFLD activity score (NAS) was tabulated by summing scores for steatosis, lobular inflammation, and ballooning degeneration (score range 1-7). For analyses, cases with low NAS (range 1-3) were compared to those with high scores (range 4-8). A diagnostic categorization was also determined for each case: “Not NASH”, “Borderline zone 3”, “Borderline zone 1”, or “Definite NASH”(8). “Definite NASH” unequivocally fulfills previously defined criteria for steatohepatitis, while the category of “Not NASH” encompasses cases of NAFLD in which the changes are so mild or non-specific that more specific classification cannot be made. The borderline zone 1 designation resembles a pattern of steatosis with predominant portal inflammation and portal fibrosis, without significant lobular inflammation or perisinusoidal fibrosis. This pattern is similar to, but not identical to that described previously in children as “type 2 NASH”(9).

Analyses included children 6-17 years of age at enrollment. Unadjusted comparisons of characteristics by steatohepatitis status, NAS, steatosis grade, lobular inflammation and ballooning were conducted using either Wilcoxon rank sum tests and Kruskal-Wallis tests (categorical variables), or Fisher's exact tests (measured variables). The medians of selected variables are presented with the first and third quartile to demonstrate distribution. Additional comparisons of steatohepatitis status limited to the “Not NASH” and “Definite NASH” groups were also conducted.

Results

A total of 149 children were eligible and included for analyses. Of those subjects, 110 (73%) were boys and 79 (53%) were Hispanic, primarily Mexican-American. The mean age was 13 ± 2.6 years and the mean BMI z-score 2.3 ± 0.4. The subjects were fairly evenly distributed

over the 4 categories of NAS disease severity (Table 1). Demographic variables were similar between children when compared by NASH status, except age, which was younger in both borderline zone 3 and borderline zone 1 groups. There were significant differences in baseline laboratory values as displayed in Table 1. Triglycerides, glucose, GGT and AST/ALT tended to be higher with greater NASH severity, as reported previously(4). Uric acid was highest in the definite NASH group and had a significant variation across all 4 groups ($p=.008$), with the lowest level seen in the borderline Z1 group. Uric acid for “Definite NASH” was higher compared to those with steatosis alone (not present) although this did not reach significance ($p=.07$). In general, as shown in Table 2, total calorie consumption was similar between groups and was lower than reported in nationally representative survey data for these age groups(10). For all children, most calories came from carbohydrates (median percentage of calories from carbohydrates was 50.4% (range 27 – 74%)). Most consumed a moderate fat diet (median % kcal from fat was 35% (range 18-54%)). Fifty percent or less of the children in all 4 groups consumed > 6 sugar-sweetened beverages per week and this was not different between groups. When the amount of specific daily nutrients was compared between the 4 groups, no significant differences were found (Table 2). All groups consumed greater than the recommended daily allowance (RDA) for Vitamin C, Vitamin A and Vitamin D(11). All groups consumed less than the RDA for folate (RDA=400 mcg/d for boys and girls) and less than half of the RDA for Vitamin E (RDA=22.5IU/day)(11).

Comparison between those without NASH (Not present) and those with definite NASH (Definite) also showed no significant differences. We also compared the subjects by high and low NAS score and there was again no difference for total calories, % kcal from fat, carbohydrates or protein (data not shown). Reported consumption of saturated fat, unsaturated fat and dietary cholesterol was similar when compared both by NASH status and NAS.

Diet variables were also compared for 3 histologic features: steatosis, lobular inflammation and ballooning. Median consumption of Vitamin E was lower in children with higher grade of steatosis (Grade I: 8.4 (5.9, 11.5), Grade II: (6.1, (4.4, 9.6), Grade III: 6.9, (5.7, 10.3), $p = .05$) (Table 3). Total calories, percent protein, fat and carbohydrates and well as vitamin C, A and D were not significantly different by steatosis grade. When diet variables were examined by amount of lobular inflammation, only dietary fiber differed significantly and was increased in the 8 subjects with a score of 3 (highest level) compared to the 73 and 70 subjects with low and moderate inflammation ($p = 0.03$) (data not shown). Increased ballooning was seen in those with reduced Vitamin C consumption: None: 106.9, (65.8, 160.6), Few: 139.9, (82.1, 202.3), Many: 72.6, (59.6, 120.5) ($p = 0.05$) (Table 4). As shown, carbohydrate consumption in grams varied significantly for ballooning, although percentage of calories from carbohydrates was not significantly different between groups.

Discussion

Despite substantial scientific interest in dietary influences on NAFLD, little information is available about diet in children with NAFLD compared to the histologic features of the disease. In this registry based study, we were able to examine self reported diet and compare this to rigorously measured histology. We found that diet (after the diagnosis of NAFLD) did not differentiate simple steatosis and NASH. We also found several interesting associations that suggest areas for further investigation, including very low consumption of vitamin E, weak associations between vitamin E and C and increased histologic severity and finally, strong associations between uric acid (a surrogate marker for fructose consumption) and histologic severity.

We did not find any significant differences in diet when we compared children with “NASH” to “not NASH.” Each group reported similar consumption of fat, sugar -sweetened beverages, antioxidants and other micronutrients. Diet comparisons by NAS score, representing a composite of these findings, also did not differ significantly. These findings suggest that diet is not the primary cause of whether a child with NAFLD has NASH or not.

In our cohort of children with NAFLD, the median reported consumption of vitamin E in all groups was less than half of the US recommended daily allowance of Vitamin E (for adolescents 22.5 IU/day)(11). Because of the registry design, our study did not include obese children without NAFLD so we do not have a disease-free control group for comparisons. However, in general, obese children have not been identified as having lower reported intake of vitamin E and C. Data from the National Health and Nutrition Examination Survey (1988-1994) demonstrated that children who were obese had similar reported intake of these vitamins as well as fruits and vegetables compared to non-obese children.(12)

In our study, we found weak associations with vitamin E and C consumption compared to steatosis and ballooning respectively. Both, vitamin C and vitamin E function as scavengers of hydroxyl, peroxy and superoxide radicals and protect against plasma lipid and low-density lipoprotein peroxidation(13) and other oxidative stress and thus could be important in preventing the progression of NAFLD. Vitamin E has been tested as a treatment for NAFLD(14-17) in part because antioxidant deficiency may lead to increased lipid peroxidation and cell death due to mitochondrial compromise (evident as ballooning on liver biopsy). In the recently published PIVENS randomized, controlled -trial comparing pioglitazone, vitamin E and placebo in adults with NASH, vitamin E therapy was associated with improved ALT, AST, steatosis, lobular inflammation and ballooning(18), suggesting a protective role in hepatocytes. In the TONIC treatment trial of children and adolescents with NAFLD, 58% of those with Vitamin E had histologic resolution of NASH, with significant improvement over the 28% with NASH receiving placebo (19).

There are several previous non-pathology based published studies that also examine the diet of children with and without presumptive NAFLD based on surrogate markers. Quiros-Tejeira et al found a small increase in consumption of dietary cholesterol in the suspected NAFLD children compared to than normal ALT subjects(20). De Piano et al studied 43 adolescents, including 13 with NAFLD (based on ultrasound evaluation) and found no significant differences in total energy, % protein, % carbohydrates, % fat or cholesterol consumption compared to obese adolescents without echogenic livers(6). Panpodreou et al compared adolescents with and without NAFLD (using ultrasound) and total energy, % protein and % fat were similar. However, they found an increase in carbohydrates and sugar intake in children with NAFLD(21).

We were also interested in examining sugar consumption in our subjects because added sugars are known to be associated with dyslipidemia(22) and fructose can be used to induce fatty liver in animal models(23). Adolescents are the highest consumers of both added sugars and fructose, making them a high risk group for potential effects(24). Because the Block Brief questionnaire lacks a detailed breakdown of sugar, we utilized sugar sweetened beverages (the largest source(24)) as a surrogate marker of fructose intake. Interestingly, we did not find any difference in reported sugar sweetened beverage consumption between groups. Previous studies of adult NAFLD patients, including a registry study using a similar design to ours(25), have found increased reported intake of sugar sweetened beverages, elevated uric acid levels (26-29) and associations between uric acid and fibrosis severity(25). Uric acid levels increase with fructose intake(30) and intake of fructose correlates with uric acid levels in the general population(31). Because of this it has been

used as a surrogate marker of fructose intake. Despite the lack of difference in reported sugar sweetened beverage consumption, our groups differed significantly in uric acid level with the highest levels found in the definite NASH group ($p=.008$). When definite NASH was compared to steatosis alone (not NASH), there was a trend towards a higher uric acid level in those with definite NASH ($p=.07$). Because sugar sweetened beverages only account for an average of ~40% fructose in the diet(24), our subjects may have substantial fructose intake from other sources (such as processed foods) that we were unable to measure given the limitations of our dietary instrument. In addition, it is possible that uric acid has an independent effect in NAFLD, unrelated to fructose intake. Further studies with both histology and more detailed diet information will be needed to understand the relationship of fructose to NAFLD and more specifically to NASH in children.

A possible limitation of our study may be a reporting or recall bias because most participants reported a relatively healthy diet. For example, median sugar sweetened beverage intake was reported as 1 glass or can or less per week. In the United States, average intake of sugar sweetened beverages for children age 12 to 19 years represented 356 calories per day(32), which would translate to 2.5 cans of a typical 12 oz can of soda or more than three 8 oz glasses of fruit-like drink per day. Thus, our subjects report a much lower than average consumption of sugar sweetened beverages. In addition, less than one-fourth of our subjects reported >40% fat intake, the definition of a high fat diet. There are several factors that may account for this finding. All of our participants had already undergone a substantial medical procedure (liver biopsy) and had been given a diagnosis of NAFLD. This event may have been an effective trigger for lifestyle improvements, including alterations in diet. At the time of diagnosis, many patients are instructed to decrease sugar sweetened beverages, increase fruits and vegetables and reduce fat intake as part of standard therapy. Most of our subjects were evaluated at 1-2 months after the liver biopsy, possibly a peak time for implementing an improved diet. The Block Brief Questionnaire asks participants to reflect on their diet over the past year, however the recall may be more influenced by their current diets or they may wish to appear in conformance with recently provided nutritional advice for a healthy diet. Overall, these effects may be less important because it would likely affect all participants, regardless of histologic variation.

Several of our findings had p values of .05. Use of multiple comparisons could lead to p values identified as significant that are actually random. However, there is a pathophysiological basis for these findings, and the results support work by Strauss et. al. that obese children are low in vitamin E (12) and the PIVENS trial. In our study, the trends for uric acid, vitamin E and vitamin C were not consistent across the 3 groups. This is likely as result of the difficulties of assigning scores and cutoffs to continuously variable pathology findings. An alternative hypothesis is that vitamins may have threshold levels and are associated with worsened pathology only when they fall below certain levels.

The Block Brief questionnaire has inherent limitations. Cullen et al compared a similar Block survey (Block Kids) in children to two 24 hour dietary recalls and found that the Block overestimated the percent energy from carbohydrates and found significant differences in the means for most food groups and nutrients between the two methods(33). They found that the Block was more accurate for children > 12 years of age and for nutrients (compared to food groups), both bolstering the findings in this study since our mean age was > 13.0 years and we examined specific nutrients, not food group servings. Mexican American diets are not well-represented in the Block Brief Questionnaire, and some of our subjects are Mexican Americans.

In summary, macronutrients did not differentiate between simple steatosis and NASH in the children in our study. We found that children with NAFLD consumed less than the

recommended amounts of vitamin E and that there was a weak association between lower consumption of both vitamin E and C and pathologic severity of NAFLD. Uric acid, a surrogate marker of dietary fructose, was significantly increased in those children with definite NASH compared to milder forms of NAFLD. Prospective studies are needed to evaluate diet in potential subjects prior to diagnosis and nutritional counseling (and ideally before NAFLD onset) in order to better confirm dietary contributors to this disease.

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Role of Authors

- Miriam Vos and Joel Lavine – conception of study design, initiation of ancillary study application, review of data, writing and revision of paper
- Colvin, Ryan – statistical analysis
- Patricia Belt, Daphne Bryan and James Tonascia – study design, data analysis design
- Jean Molleston, Karen Murray, Philip Rosenthal, Jeffery Schwimmer, and Aynur Unalp – recruitment of subjects, review and writing of the article.

Table 1

Demographics and laboratory values by NASH status

	NASH				P*	P†
	Not present (n=39)	Borderline Z3 (n=27)	Borderline Z1 (n=36)	Definite (n=47)		
	Median					
Demographics						
Age, years	14	12	11	14	<0.0001	0.94
Race, %					0.08	0.33
White, non-Hispanic	43.6	30.8	25.0	53.2		
Hispanic	51.3	57.7	69.4	36.2		
Other	5.1	11.5	5.6	10.6		
Male gender, %	66.7	66.7	86.1	70.2	0.18	0.82
Time between biopsy and registration, days	51	27	39.5	30	0.38	0.61
Anthropomorphic variables						
BMI, kg/m2	32.1	32.9	31.5	34.2	0.31	0.20
BMI z-score	2.19	2.34	2.44	2.38	0.33	0.10
Laboratory values						
HOMA-IR	5.9	11.1	22.8	30.9	0.09	0.01
Triglycerides, mg/dL	121.5	109	114	146	0.11	0.03
HDL Cholesterol, mg/dL	39	37	38	35	0.10	0.03
Uric acid > 5.5 mg/dL, %	61.5	61.5	38.2	76.1	0.008	0.07
AST, U/L	41	47	52.5	60	0.0002	<0.0001
ALT, U/L	70	84	83.5	93.5	0.01	0.0009
GGT, U/L	29.5	37	40.5	49	0.001	<0.0001

^{*} P values for 4 groups calculated using Kruskal-Wallis test for continuous variables and Fisher's exact test for categorical variables.

[†] P values for Not present vs. Definite only.

Table 2

Dietary indicators by NASH status

	NASH				P*	P†
	Not present (n=39)	Borderline Z3 (n=27)	Borderline Z1 (n=36)	Definite (n=47)		
	Median					
Daily nutrients from food						
Calories, Kcal	1526	1502	1844	1653	0.44	0.55
Protein, g	62.4	67.9	75.2	62.2	0.41	0.65
Total fat, g	58.3	63.7	72.5	61.9	0.45	0.34
Carbohydrate, g	193.8	206.7	227.1	194.3	0.42	0.76
Total dietary fiber, g	13.4	14.6	15.7	12.3	0.49	0.63
Saturated fat, g	19.7	20.5	24.2	21.5	0.37	0.31
Monounsaturated fat, g	24.8	23.6	26.3	23.7	0.43	0.42
Polyunsaturated fat, g	10.9	11.4	11.8	14.1	0.53	0.23
Dietary cholesterol, mg	205.2	191.4	200.6	204.6	0.90	0.90
Vitamin C, mg	99.5	109.0	147.1	82.1	0.15	0.17
Folate, mcg	274.5	328.6	390.7	299.2	0.34	0.69
Vitamin A, RE	953.6	906.8	1100.0	1004.4	0.50	0.66
Vitamin E, α-TE, IU	6.6	6.7	7.7	7.0	0.49	0.95
Vitamin D, IU	121.6	119.0	225.1	121.4	0.38	0.64
Percent of calories from food sources						
Carbohydrates, % of Kcal	50.8	48.9	51.9	47.9	0.67	0.28
Fat, % of Kcal	34.9	34.8	36.2	35.8	0.66	0.33
Protein, % of Kcal	15.9	16.5	16.3	16.2	0.29	0.90
Sweets and desserts, % of Kcal	7.4	5.2	7.4	7.0	0.53	0.82
Sweetened beverage‡ consumption						
Sweetened beverage consumption per week	4.6	4.2	6.8	5.5	0.50	0.80
> 6 sweetened beverages per week, %	47.4	44.4	50.0	40.4	0.85	0.66

* P values for 4 groups calculated using Kruskal-Wallis test for continuous variables and Fisher's exact test for categorical variables.

† P values for Not present vs. Definite only.

✦ Including fruit juices, Kool Aid, fruit punches and soda.

Table 3

Dietary indicators by steatosis grade

	Steatosis grade			P [*]
	0-33% (n=51)	34-66% (n=45)	>66% (n=55)	
	Median	Median	Median	
Daily nutrients from food				
Calories, Kcal	1742	1513	1616	0.64
Protein, g	75.2	68.6	65.0	0.29
Total fat, g	69.4	58.1	64.5	0.28
Carbohydrate, g	203.1	220.0	197.7	0.94
Total dietary fiber, g	16.2	12.1	12.8	0.37
Saturated fat, g	23.2	19.7	21.2	0.43
Monounsaturated fat, g	26.0	21.5	24.7	0.46
Polyunsaturated fat, g	14.6	9.6	11.5	0.08
Dietary cholesterol, mg	234.8	184.6	190.8	0.11
Vitamin C, mg	100.1	103.1	109.0	0.97
Folate, mcg	363.0	307.2	290.8	0.56
Vitamin A, RE	1147.9	949.2	918.6	0.44
Vitamin E, α -TE, IU	8.4	6.1	6.9	0.05
Vitamin D, IU	150.0	136.2	131.2	0.97
Percent of calories from food sources				
Carbohydrates, % of Kcal	49.1	50.9	51.2	0.43
Fat, % of Kcal	37.1	34.3	35.0	0.49
Protein, % of Kcal	17.3	16.4	15.9	0.33
Sweets and desserts, % of Kcal	5.2	8.1	6.3	0.84
Sweetened beverage[†] consumption				
Sweetened beverage consumption per week	5.5	6.0	5.9	0.74
> 6 sweetened beverages per week, %	48.0	44.4	36.7	0.94

* P values for 3 groups calculated using Kruskal-Wallis test for continuous variables and Fisher's exact test for categorical variables.

[†] Including fruit juices, Kool Aid, fruit punches and soda.

Table 4

Dietary indicators by amount of ballooning

	Amount of ballooning			p [*]
	None (n=74)	Few (n=47)	Many (n=30)	
	Median	Median	Median	
Daily nutrients from food				
Calories, Kcal	1522	1800	1573	0.09
Protein, g	64.9	74.8	60.4	0.19
Total fat, g	58.9	66.4	63.4	0.16
Carbohydrate, g	188.4	236.9	176.0	0.05
Total dietary fiber, g	14.2	16.2	11.7	0.28
Saturated fat, g	20.3	23.2	20.1	0.27
Monounsaturated fat, g	23.7	28.3	23.7	0.19
Polyunsaturated fat, g	10.8	14.3	12.8	0.06
Dietary cholesterol, mg	190.0	216.2	203.0	0.71
Vitamin C, mg	106.9	139.9	72.6	0.05
Folate, mcg	286.8	390.7	278.9	0.09
Vitamin A, RE	955.6	1031.4	1014.0	0.87
Vitamin E, α -TE, IU	6.6	7.9	7.1	0.30
Vitamin D, IU	137.1	165.4	92.9	0.14
Percent of calories from food sources				
Carbohydrates, % of Kcal	50.6	51.2	46.5	0.23
Fat, % of Kcal	34.9	34.7	37.4	0.13
Protein, % of Kcal	16.3	16.0	16.1	0.41
Sweets and desserts, % of Kcal	6.2	9.1	6.7	0.54
Sweetened beverage[†] consumption				
Sweetened beverage consumption per week	4.7	7.4	3.3	0.30
> 6 sweetened beverages per week, %	45.2	55.3	33.3	0.17

* P values for 3 groups calculated using Kruskal-Wallis test for continuous variables and Fisher's exact test for categorical variables.

[†] Including fruit juices, Kool Aid, fruit punches and soda.