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ORIGINAL ARTICLE

Isotopic estimates of sugar intake are related to chronic disease risk factors but not obesity in an Alaska native (Yup'ik) study population

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BACKGROUND/OBJECTIVES: Sugar intake may be causally associated with chronic disease risk, either directly or by contributing to obesity. However, evidence from observational studies is mixed, in part due to the error and bias inherent in self-reported measures of sugar intake. Objective biomarkers may clarify the relationship between sugar intake and chronic disease risk. We have recently validated a biomarker of sugar intake in an Alaska Native (Yup'ik) study population that incorporates red blood cell carbon and nitrogen isotope ratios in a predictive model. This study tested associations of isotopic estimates of sugar intake with body mass index (BMI), waist circumference (WC) and a broad array of other physiological and biochemical measures of chronic disease risk in Yup'ik people.

SUBJECTS/METHODS: In a cross-sectional sample of 1076 Yup'ik people, multiple linear regression was used to examine associations of sugar intake with BMI, WC and other chronic disease risk factors.

RESULTS: Isotopic estimates of sugar intake were not associated with BMI ($P=0.50$) or WC ($P=0.85$). They were positively associated with blood pressure, triglycerides (TG) and leptin, and are inversely associated with total-, high-density lipoprotein- and low-density lipoprotein-cholesterol and adiponectin.

CONCLUSIONS: Isotopic estimates of sugar intake were not associated with obesity, but were adversely associated with other chronic disease risk factors in this Yup'ik study population. This first use of stable isotope markers of sugar intake may influence recommendations for sugar intake by Yup'ik people; however, longitudinal studies are required to understand associations with chronic disease incidence.

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Keywords: carbon; nitrogen; stable isotope ratio; chronic disease; risk factors; caloric sweeteners

INTRODUCTION

There has been considerable controversy over whether sugar intake is causally associated with chronic disease risk,¹ either directly or by contributing to obesity. Intake of a high sugar diet is associated with elevated plasma triglycerides (TG),^{2,3} and consumption of high sugar snacks has been experimentally linked with elevated glucose and insulin levels.⁴ Furthermore, many studies have found that sugars (particularly fructose),⁵ consumed in beverage form (sugar-sweetened beverages; SSB) are associated with type 2 diabetes and coronary heart disease risk,^{6–8} as well as chronic disease risk factors including increased body mass index (BMI) or body weight,^{9,10} visceral adiposity,⁹ dyslipidemia,^{8–10} elevated blood pressure,¹¹ insulin resistance¹² and markers of inflammation.^{8,13} However, other studies have shown weak or no associations between either sugar or SSB intake and chronic disease risk factors.^{14,15} All observational studies of the association between sugar or SSB intake and chronic disease risk factors have relied on self-reported measures of food intake, which are subject to substantial error and bias.¹⁶ Therefore, associations are likely attenuated, which may, in part, explain the inconsistency of these findings.

A biomarker of sugar or SSB intake would strengthen inferences from observational studies and help to resolve the role of sugar intake in the development of chronic disease. Measures of 24-h urinary sugars have recently been validated as biomarkers of total and added sugars intake.¹⁷ However, these measurements require multiple urine collections to reliably estimate intake; therefore, they may be impractical to collect for large study samples. Alternatively, we and others have shown that naturally occurring variations in stable isotope ratios can be used as objective measures of diet.^{18,19} Specifically, the carbon stable isotope ratio ($\delta^{13}\text{C}$) is elevated in corn- and cane sugar-based sweeteners,²⁰ and has been proposed as a low-burden, economical and easy to measure indicator of usual sugar intake in several US populations.^{18,21,22} We have recently validated an improved isotopic model of sugar intake in an Alaska native (Yup'ik) study population,¹⁹ which incorporates both the carbon and nitrogen ($\delta^{15}\text{N}$) isotope ratios. A model based on both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ is improved because $\delta^{15}\text{N}$ accounts for confounding dietary effects on $\delta^{13}\text{C}$. In this Yup'ik study population, the dual isotope model explained 48% of the variability in reported total sugar intake.

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The overall objective of this study was to use this dual isotope model to investigate associations of sugar intake with measures of obesity and chronic disease risk in Yup'ik people. In this study, we were interested in sugar intake from both food and beverages; therefore, we examined associations with isotopic estimates of total sugar intake. Our study sample was a community-based, cross-sectional sample of 1076 Yup'ik people. We were interested in whether sugar intake was linked to obesity and other chronic disease risk factors in this study population because their intake of high sugar foods has increased substantially over the last several decades,²³ and the impact of this increase on Yup'ik people's health is unknown. Our aims were twofold. First, we investigated associations of isotopic estimates of total sugar intake with two measures of obesity: BMI and waist circumference (WC). Our hypothesis was that sugar intake would be positively associated with both BMI and WC. Second, we investigated whether isotopic estimates of total sugar intake were associated with biomarkers of chronic disease risk, independently of BMI. On the basis of previous studies in other study populations, we hypothesized that these measures of sugar intake would be positively associated with blood pressure, fasting TG, total cholesterol, C-reactive protein, fasting glucose and insulin resistance. Determining whether sugar intake is associated with obesity and other chronic disease risk factors may influence dietary recommendations for Yup'ik people, and will provide additional evidence toward our understanding of the role of sugar intake in the etiology of obesity and related chronic diseases.

MATERIALS AND METHODS

Participant recruitment and procedures

Data are from the Center for Alaska Native Health Research (CANHR) study, a cross-sectional, community-based participatory research study of the nutritional, genetic and psychosocial factors affecting obesity and related disease risk in the Yup'ik population. This study was approved by the University of Alaska Fairbanks Institutional Review Board, the National and Area Indian Health Service Institutional Review Boards and the Yukon-Kuskokwim Health Corporation Human Studies Committee.

Between 2003 and 2012, a community-based sample of 1510 participants aged 14–94 was recruited from 10 communities in rural Southwest Alaska, as described elsewhere.²⁴ At entry into the study, participants completed questionnaires to provide information on demographics, medical history and smoking status (current: yes or no). Biological samples and anthropometric measurements were also collected.

Study sample

For comparison of isotope-based estimates of total sugar intake with measures of obesity and chronic disease risk factors, we excluded 341 participants aged <19 years, 87 participants with missing stable isotope measurements and 6 participants with missing BMI measurements. This left a study sample of $n = 1076$. However, because data were missing for individual risk factors, the sample size for each analysis varied from 783 to 1039, with the exception of interleukin-6 (IL-6) and insulin-like growth factor 1 (IGF-1), which were available only on a subset of the first seven communities enrolled in the study ($n = 360$ and 363 , respectively). From within these communities, samples were balanced across age and sex, as described in detail elsewhere.²⁵

Anthropometric and biochemical measurements

Anthropometric measurements, including height, weight and blood pressure, were measured by trained staff using protocols from the NHANES III Anthropometric Procedures Manual,²⁶ as described by Boyer et al.²⁴ Blood samples were collected into ethylenediaminetetraacetic acid (EDTA) tubes from participants after a minimum 8-h fast, and processed locally: serum, lymphocyte and red blood cell (RBC) fractions were separated using a portable centrifuge and stored at -15°C . Within 6 days, samples were shipped to the University of Alaska Fairbanks and stored at -80°C . Biomarkers of chronic disease risk, including TG, total cholesterol, high-density lipoprotein (HDL) cholesterol (HDL), low-density lipoprotein (LDL) cholesterol (LDL), adiponectin, blood glucose, HbA1c, insulin, leptin,

ghrelin, C-reactive protein (CRP), insulin-like growth factor I and IL-6, were assayed in serum as previously described.^{24,25} Insulin resistance was assessed using the homeostasis model of insulin resistance (HOMA-IR) index: (fasting insulin (mU/ml) \times fasting glucose (mg/dl))/405.²⁷

Stable isotope analysis

RBCs were pipetted into tin capsules, autoclaved and prepared for isotopic analysis as previously described.²⁸ Neither autoclaving nor the use of ethylenediaminetetraacetic acid tubes affects RBC carbon or nitrogen isotope ratios.²⁹ Samples were analyzed at the Alaska Stable Isotope Facility by continuous-flow isotope ratio mass spectrometry, using a Costech ECS4010 Elemental Analyzer (Costech Scientific Inc., Valencia, CA, USA) interfaced to a Finnigan Delta Plus XP isotope ratio mass spectrometer via the ConFlo III interface (Thermo-Finnigan Inc., Bremen, Germany). The conventional means of expressing natural abundance isotope ratios is as delta values in permil (‰) relative to international standards as $\delta X = (R_{\text{sample}} - R_{\text{standard}}) / (R_{\text{standard}}) \times 1000\%$. Here, R is the ratio of heavy to light isotope ($^{15}\text{N}/^{14}\text{N}$ or $^{13}\text{C}/^{12}\text{C}$). The standards are Vienna PeeDee Belemnite for carbon and atmospheric nitrogen for nitrogen. To assess analytical precision, an internal standard was analyzed for every 10 samples (peptide: $\delta^{15}\text{N} = 7.0$, $\delta^{13}\text{C} = -15.8$). Precision was measured in two ways: as the s.d. and the coefficient of variation of these analyses. Accuracy was within 0.1%, and precision was within 0.2% for both isotopes, and the coefficient of variation for these analyses was 3.3% for $\delta^{15}\text{N}$ and 0.6% for $\delta^{13}\text{C}$. Because biological samples from this study have a lower $^{13}\text{C}/^{12}\text{C}$ than Vienna PeeDee Belemnite, $\delta^{13}\text{C}$ values are negative. The term ' $\delta^{13}\text{C}$ values' is hereafter abbreviated as $\delta^{13}\text{C}$, and the term ' $\delta^{15}\text{N}$ values' is abbreviated as $\delta^{15}\text{N}$.

Estimating sugar intake using stable isotope ratios

Total sugar intake was estimated using a dual isotope model, which was calibrated in a sample of 68 Yup'ik participants based on self-reported total sugar intake from 4, weekly 24-h recalls:¹⁹

$$\ln(\text{total sugar intake}) = 13.07 + 0.33(\delta^{13}\text{C}) - 0.23(\delta^{15}\text{N})$$

This predictive equation explained 48% of the variation in self-reported total sugar intake in the calibration data set. Although the calibration population was drawn from two of the 10 Yup'ik communities participating in the present study, it is possible that the calibration equation might differ slightly for the larger population studied here. Furthermore, because our isotopic model of total sugar intake was calibrated against self-reported data, it may have incorporated reporting bias. For these reasons, we tested whether our isotopic model of sugar intake was associated with a second, unbiased marker of total sugar intake recently validated for Yup'ik people: the carbon isotope ratio of RBC alanine ($\delta^{13}\text{C}_{\text{ALA}}$).³⁰ We tested this association by measuring $\delta^{13}\text{C}_{\text{ALA}}$ in a random sample of 50 research participants from the present study. Our estimates of total sugar intake calibrated from self-report were significantly correlated with $\delta^{13}\text{C}_{\text{ALA}}$ (Pearson's $r = 0.46$), which gives us further confidence that our estimate of sugar intake is objective and valid for use in this study population. Hereafter, total sugar intake estimated using this dual isotope model is referred to as 'sugar intake'.

Statistical analyses

We examined the associations of sugar intake with the following measures: systolic blood pressure (SBP) diastolic blood pressure (DBP), TG, total cholesterol, LDL, HDL, ghrelin, leptin, adiponectin, HbA1c, glucose, insulin, HOMA-IR, insulin-like growth factor I, IL-6 and CRP. TG, leptin, insulin, HOMA-IR, IL-6 and CRP were log-transformed for analysis. Outlying values of chronic disease risk biomarkers (>4 s.d. above the mean) were excluded because they were judged to be physiologically unreasonable. We excluded the following values: SBP ($n = 2$), TG ($n = 7$), total cholesterol ($n = 1$), glucose ($n = 4$), HbA1c ($n = 3$), leptin ($n = 1$), insulin ($n = 5$), HOMA-IR ($n = 5$), adiponectin ($n = 1$), CRP ($n = 14$) and IL-6 ($n = 5$). For IL-6, values below the limit of detection ($n = 95$) were replaced by the limit of detection divided by the square root of 2.³¹ Finally, participants taking blood pressure ($n = 142$), cholesterol-lowering ($n = 43$) or diabetes ($n = 12$) medications were excluded for analyses of associations with blood pressure, lipids and glucose/HbA1c/insulin/HOMA-IR, respectively.

We assessed whether sugar intake differed by demographic and health characteristics using one-way analysis of variance models. To determine whether sugar intake and chronic disease risk factors were associated with BMI or WC, we used age- and sex-adjusted multiple regression models.

To determine whether sugar intake was associated with biomarkers of chronic disease risk, we used BMI-adjusted multiple linear regression models. In addition to BMI adjustment, models were also adjusted for age (continuous), sex, current smoking status (yes or no) and year of data collection. Control for chewing tobacco (*iqmiq*) and pulse did not affect the results; therefore, these variables were not included in the models presented. A smaller number of study participants had physical activity (actiheart counts per day; $n = 246$)³² and self-reported dietary intake information ($n = 230$).²⁸ In these subsets, sugar intake was not associated with reported total energy intake (β (95% confidence interval, CI) = 0.92 (− 1.09, 2.95), $P = 0.37$) but was positively associated with physical activity (β (95% CI) = 190.3 (104.7, 275.8), $P < 0.0001$). In this smaller subset, estimated sugar intake was associated with DBP, TG, total cholesterol, HDL and LDL only; however, the associations with these risk factors were not affected by the adjustment for physical activity (data not shown).

Both linear and quadratic associations were assessed. We used a conservative criterion ($P < 0.01$) for reporting quadratic associations owing to the likelihood that multiple contrasts would lead to chance associations. We calculated the unadjusted P -value using a significance level of 0.05, and also indicated which tests remained statistically significant after adjustment using the Bonferroni–Holm method to account for multiple testing.³³ All statistical analyses were performed using JMP version 8 (SAS Institute, Cary, NC, USA) or STATA I/C version 12 (StataCorp. 2011, College Station, TX, USA).

RESULTS

Table 1 gives associations of demographic and health-related characteristics with sugar intake. The total study population ranged in age from 19 to 94 years (mean = 42 years \pm 15); 55% were women and 68% were overweight or obese. Mean sugar intake was 93 g/day, which is lower than has been reported for other US populations using data from the National Health and Nutrition Examination Survey.^{34,35} Sugar intake ranged from 24 to 217 g/day and was 7% higher in men, 22% higher in current smokers and 95% higher in participants aged 19–40 years compared with those aged > 60 years.

After control for age and sex, neither BMI nor WC was associated with sugar intake (Table 2). Table 2 also gives associations of sugar intake with BMI and WC stratified by age and sex. The only significant association between sugar intake and either measure of obesity was an inverse association with BMI in participants over the age of 60 years.

Table 3 gives the linear associations of total sugar intake with chronic disease risk factors. Independent of BMI, sugar intake was positively associated with SBP, DBP, TG, leptin, and inversely associated with HDL, LDL, total cholesterol and adiponectin. There were marginally non-significant positive associations with insulin

and HOMA-IR. The largest differences in chronic disease risk factors were seen with leptin, adiponectin and HOMA-IR, which were 13.8%, 13.0% and 6.7% higher in quartile 4 than quartile 1 of estimated sugar intake, respectively. LDL, HDL and total cholesterol were 11.3%, 9.6% and 7.6% lower in quartile 4 than quartile 1 of sugar intake, respectively. There were no associations of sugar intake with ghrelin, glucose, HbA1c, insulin-like growth factor I, CRP or IL-6. After the Bonferroni–Holm correction, the associations with DBP, TG, total cholesterol, HDL, LDL and leptin remained statistically significant.

DISCUSSION

There were strong associations of isotopic estimates of sugar intake with chronic disease risk factors in this cross-sectional sample of Yup'ik people. Contrary to our original hypothesis, there was no association of isotopic estimates of sugar intake with BMI or WC. However, these estimates of sugar intake were associated with increased blood pressure, TG, leptin and decreased HDL, LDL, total cholesterol and adiponectin. These results suggest that, although sugar intake is not directly associated with obesity in this Yup'ik study population, it may be independently associated with higher risk of developing chronic disease risk factors, including hypertension and dyslipidemia.

The finding that isotopic estimates of sugar intake were not associated with measures of obesity (BMI or WC) is consistent with some observational studies;^{14,36} however, other studies have demonstrated significant and positive effects of either sugar or SSB intake on BMI, body weight or risk of obesity.⁷ This association is likely mediated, at least in part, by a higher energy intake.³⁷ We were unable to assess the association between sugar and total energy intake in the complete study sample; however, in a substantially smaller subset of participants with self-reported dietary data ($n = 230$), we found no association between our measure of sugar intake and reported total energy intake. This suggests that our findings were likely not confounded by total energy intake, although this smaller sample size will have reduced our power to detect an association with our marker of sugar. However, this finding is consistent with a previous study by our group that found no association between dietary patterns and total energy intake.³⁸ Furthermore, traditional food intake as measured using $\delta^{15}\text{N}$ ²⁸ showed a marginal but positive association with BMI (β (95% CI) = 0.35 (0.04, 0.66), $P = 0.027$); therefore, the higher fat content of the marine-based traditional Yup'ik diet³⁸ coupled with the negative relationship between intake of sugar and traditional foods³⁰ may have obscured any potential positive relationship between sugar intake and BMI or WC.

Sugar intake showed a marginally non-significant positive association with insulin and HOMA-IR, and was not associated with glucose or HbA1c. The results of other observational studies that examine associations of sugar or SSB intake with indicators of glucose tolerance or insulin resistance have been inconsistent.^{8,12,39–41} Determining whether sugar intake is associated with glucose, HbA1c, insulin and HOMA-IR is of particular relevance to the health of Yup'ik people, because, although diabetes prevalence is low (2010: 27/1000), it increased 38% between 1990 and 2004,⁴² and recent studies have suggested a positive relationship between sugar intake and type 2 diabetes.^{6,43} Larger, longitudinal studies are needed to clarify the association of sugar intake with insulin and HOMA-IR in Yup'ik people, and to address whether sugar intake will lead to the development of hyperglycemia or insulin resistance over time.

Sugar intake was positively associated with both SBP and DBP independently of BMI and current smoking status, a finding that is consistent with several other observational studies.^{11,39,40} Proposed mechanisms for this relationship include changes to the uric acid pathway induced by fructose consumption⁴⁴ or

Table 1. Associations of demographic and health-related characteristics with sugar intake

	<i>n</i> (%)	Sugar intake ^{a,b} g/day	<i>P</i>
Total study population	1076 (100)	95.3 (93.1, 97.5)	
Sex			0.0017
M	499 (46)	99.1 (95.8, 102.4)	
F	577 (54)	92.0 (89.1, 94.9)	
Age			< 0.0001
19 to < 40 years	549 (51)	112.5 (109.7, 115.2)	
40 to < 60 years	375 (35)	85.3 (82.0, 88.7)	
> 60 years	152 (14)	57.9 (54.3, 61.6)	
Smokers			< 0.0001
Current	333 (31)	108.9 (105.1, 112.8)	
Non smoker	722 (67)	88.6 (86.0, 91.2)	
BMI			0.0004
< 25 kg/m ²	384 (36)	100.9 (97.4, 104.5)	
25 to < 30 kg/m ²	354 (33)	94.0 (90.1, 97.9)	
> 30 kg/m ²	338 (31)	90.3 (86.2, 94.3)	

Abbreviations: BMI, body mass index; CI, confidence interval. ^aMean (95% CI). ^bSugar intake was estimated using the formula: $\ln(\text{sugar intake}) = 13.07 + 0.33(\delta^{13}\text{C}) - 0.23(\delta^{15}\text{N})$.¹⁹

Table 2. Associations of sugar intake with BMI and waist circumference, stratified by age category and sex^{a,b}

	n	BMI, kg/m ²				Waist circumference, cm		
		Mean ± s.e.	BMI >30, %	β ^c (95% CI)	P	Mean ± s.e.	β ^c (95% CI)	P
Total	1076	28.1 ± 0.2	31	-0.09 (-0.37, 0.18)	0.50	92.0 ± 0.4	0.07 (-0.63, 0.77)	0.85
Age								
19 to <40 years	549	27.7 ± 0.3	28	-0.30 (-0.67, 0.07)	0.12	89.5 ± 0.6	-0.67 (-1.60, 0.26)	0.16
40 to <60 years	375	28.3 ± 0.3	34	0.19 (-0.23, 0.60)	0.37	93.2 ± 0.7	0.44 (-0.64, 1.51)	0.43
>60 years	152	29.0 ± 0.5	38	-1.38 (-2.44, -0.32)	0.011	98.0 ± 1.3	-2.42 (-5.34, 0.49)	0.10
Sex								
M	499	26.5 ± 0.2	19	-0.00 (-0.30, 0.31)	0.96	91.5 ± 0.6	0.24 (-0.71, 1.10)	0.67
F	577	29.5 ± 0.3	42	-0.20 (-0.65, 0.27)	0.39	92.5 ± 0.6	0.23 (-1.14, 1.00)	0.90

Abbreviations: BMI, body mass index; CI, confidence interval; F, female; M, male; WC, waist circumference. ^aAssociations in the complete study sample are age and sex adjusted. Associations within age and sex strata are sex and age adjusted, respectively. ^bSugar intake was estimated using the formula: $\ln(\text{sugar intake}) = 13.07 + 0.33(\delta^{13}\text{C}) - 0.23(\delta^{15}\text{N})$. ^cSlopes are interpreted as change in obesity measure (BMI or WC) for each 25 g increase in total sugar intake.

Table 3. Linear associations of sugar intake with chronic disease risk factors, and means of those risk factors stratified by quartile of sugar intake (n = 380–1039)^a

	n	Sugar intake ^{b,c} (range, g/day)				β ^d (95% CI)	P
		Quartile 1 (23 to <66)	Quartile 2 (66 to <93)	Quartile 3 (93 to <121)	Quartile 4 (121 to <217)		
SBP, mm Hg	879	116.7 ± 1.0	118.4 ± 0.8	117.7 ± 0.8	120.2 ± 0.8	0.78 (0.14, 1.43)	0.018
DBP, mm Hg	881	68.6 ± 0.8	70.7 ± 0.6	70.8 ± 0.6	71.5 ± 0.7	0.88 (0.39, 1.37)	<0.0001 ^e
Triglycerides, mg/dl ^f	911	66.0 (62.4, 69.8)	74.8 (71.3, 78.5)	81.0 (77.1, 85.0)	81.9 (77.6, 86.4)	6.09 (3.98, 8.20)	<0.0001 ^e
Cholesterol, mg/dl	921	226.3 ± 3.3	229.8 ± 2.6	215.6 ± 2.6	208.6 ± 2.9	-5.09 (-7.21, -2.97)	<0.0001 ^e
HDL, mg/dl	919	65.8 ± 1.1	64.9 ± 1.0	61.0 ± 1.1	59.7 ± 1.1	-1.62 (-2.42, -0.82)	<0.0001 ^e
LDL, mg/dl	921	148.5 ± 2.6	148.4 ± 2.2	138.7 ± 2.3	130.6 ± 2.5	-5.08 (-7.21, -2.97)	<0.0001 ^e
Leptin, ng/ml ^f	810	6.8 (6.3, 7.4)	6.9 (6.4, 7.4)	7.0 (6.5, 7.5)	7.4 (6.8, 8.0)	4.44 (1.43, 7.53)	0.004 ^e
Adiponectin, μg/ml	959	10.4 ± 0.4	9.7 ± 0.3	9.6 ± 0.3	9.5 ± 0.3	-0.32 (-0.57, -0.06)	0.015
Ghrelin, pg/ml	809	424.1 ± 11.1	424.2 ± 10.0	411.6 ± 10.8	404.5 ± 11.8	-0.65 (-9.11, 7.81)	0.88
HbA1c, %	960	5.6 ± 0.02	5.5 ± 0.02	5.5 ± 0.02	5.5 ± 0.02	-0.02 (-0.03, 0.00)	0.09
Glucose, mg/dl	1038	94.2 ± 0.7	93.0 ± 0.6	92.3 ± 0.6	92.9 ± 0.6	-0.03 (-0.51, 0.44)	0.90
Insulin, μU/dl ^f	787	12.2 (11.4, 13.0)	12.0 (11.4, 12.8)	12.6 (11.8, 13.4)	12.6 (11.8, 13.5)	2.29 (-0.14, 4.78)	0.069
HOMA-IR ^f	782	2.8 (2.6, 3.0)	2.7 (2.5, 2.9)	2.9 (2.7, 3.1)	2.9 (2.7, 3.1)	2.42 (-0.18, 5.11)	0.064
IGF-I, ng/ml	386	272.8 ± 9.5	246.3 ± 8.7	250.9 ± 8.5	248.0 ± 9.2	-3.53 (-10.34, 3.28)	0.31
CRP, mg/dl ^f	782	0.08 (0.07, 0.10)	0.11 (0.09, 0.13)	0.10 (0.08, 0.12)	0.13 (0.10, 0.15)	5.91 (-0.97, 13.3)	0.094
IL-6, μg/l ^f	379	0.08 (0.06, 0.13)	0.10 (0.07, 0.12)	0.09 (0.07, 0.12)	0.09 (0.07, 0.12)	3.94 (-6.03, 15.0)	0.45

Abbreviations: BMI, body mass index; CI, confidence interval; CRP, C-reactive protein; DBP, diastolic blood pressure; HOMA-IR, homeostasis model of insulin resistance; IGF-I, insulin-like growth factor I; IL-6, interleukin-6; SBP, systolic blood pressure. ^aSample size varies because of outliers and missing data. Multiple linear regression models were adjusted for age (continuous), sex, BMI (continuous), smoking status (yes or no) and year of data collection. ^bSugar intake was estimated using the formula: $\ln(\text{sugar intake}) = 13.07 + 0.33(\delta^{13}\text{C}) - 0.23(\delta^{15}\text{N})$. ^cMeans of chronic disease risk biomarkers by quartile of sugar intake are least squares means (± s.e.), adjusted for age (continuous), sex, BMI (continuous) and smoking status (yes or no). Geometric means (95% CI) are given for log-transformed variables. ^dSlopes are interpreted as change in chronic disease risk factor for each 25 g increase in total sugar intake. ^eAssociations remained statistically significant after the Bonferroni-Holm correction. ^fLog-transformed values were used for regression analyses; slopes have been back transformed for ease of interpretation and are interpreted as percentage change in the chronic disease risk factor for each 25 g increase in sugar intake.

increased sodium retention.⁴⁵ Although our findings are not informative regarding the mechanism by which this association may occur, they are suggestive that decreased sugar intake may be beneficial for the blood pressure of Yup'ik people.

Moreover in agreement with findings from several prospective studies^{8,39,40} was the strong positive association of sugar intake with TG. This association may be due to high intake of fructose (either directly, or as high fructose corn syrup or sucrose),⁹ which is known to increase TG levels through increased hepatic *de novo* lipogenesis^{2,3,9} and decreased rate of peripheral TG clearance.² Alternatively, sugar intake is positively associated with intake of carbohydrates in this population,⁴⁵ which may also lead to increased triglyceride levels.⁴⁶ We also found a strong inverse association of sugar intake with HDL, LDL and total cholesterol,

which was in agreement with previous findings for HDL,^{8,39} but counter to our expectations for LDL and total cholesterol.¹⁰ Here, because of the high intakes of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) from the Yup'ik traditional diet,³⁸ HDL is high and positively associated with total cholesterol (β (95% CI) = 1.12 (0.98, 1.27), *P* < 0.0001). HDL is also associated with LDL in this population (β (95% CI) = 0.31 (0.18, 0.44), *P* < 0.0001), possibly due to other high-fat components of the traditional diet.⁴⁷ Thus, this phenomenon may be due to the unique dietary patterns of this Yup'ik people and these findings may not be applicable to other study populations.

We found that sugar intake was not associated with C-reactive protein in this Yup'ik study population, which contrasts with the positive associations reported by the few studies that examined

sugar or SSB intake and inflammation.^{8,10,13} Again, this finding may be related to the overall high intakes of EPA and DHA in the Yup'ik population.³⁸ EPA and DHA promote an anti-inflammatory state,⁴⁸ and were inversely associated with CRP in a subset of this study population.²⁵ Therefore, the lack of association may be due to the unique dietary patterns of this study population and may not be relevant to other US populations.

Our findings that high sugar intake was associated with higher levels of circulating leptin and lower levels of adiponectin are also in contrast to those from other observational studies, which have demonstrated an inverse⁸ or no^{10,49} association of reported sugar intake with leptin and no association with adiponectin.^{8,49} However, one experimental study has demonstrated decreased adiponectin following 10 weeks of sugar consumption.⁵⁰ Higher leptin and lower adiponectin levels may lead to increased risk of cardiovascular disease, renal disease⁵¹ and type 2 diabetes;⁴⁷ therefore, our results suggest that high sugar intake may adversely affect chronic disease risk in Yup'ik people. These results remained statistically significant when adjusted for either BMI or WC, measures of adiposity that are known to affect these adipokines.

Finally, we note that some of the associations presented here, particularly those with blood lipids, are the inverse of those found with biomarkers of EPA and DHA intake in a subset of this study population.³⁸ Thus, these relationships could partially reflect the strong inverse association between intakes of sugar and traditional foods,³⁰ which are high in EPA and DHA. We tested whether adjustment for RBC EPA and DHA affected associations between sugar intake and chronic disease risk factors in a subset of this study population ($n = 279\text{--}361$).²⁵ In this substantially smaller study sample, associations of sugar intake with SBP, DBP, adiponectin, insulin and HOMA-IR were statistically insignificant. Associations of sugar intake with total cholesterol, LDL and leptin were weakened, but remained statistically significant after an adjustment for RBC EPA or DHA. However, associations with TG and HDL became non-significant after EPA or DHA adjustment. These findings suggest that sugar intake does affect blood lipids and leptin, independent of EPA and DHA intake, in this study population. We suggest that longitudinal studies are warranted to disentangle the effects of sugar and traditional food intake on chronic disease risk in Yup'ik people.

The primary strength of this study is that it uses biomarker-based estimates of sugar intake to examine associations with chronic disease risk in a large sample of Yup'ik people. Although stable isotope ratios have shown potential as biomarkers of sugar intake in several other non-Native US populations,^{18,21,22} this is the first study to use these markers to evaluate associations with chronic disease risk. These biomarker-based estimates were likely less biased than self-reported measures, and were available on a much large number of study participants than would have been available had we assessed diet using self-report. This study is also one of very few that examine the association of non-traditional food intake and chronic disease risk in Yup'ik people.⁵² The primary limitation of this study is that it is based on a cross-sectional study sample; therefore, we cannot exclude the possibility of reverse causality, or residual confounding from intake of other dietary components or lifestyle factors. Furthermore, a limitation of isotopic biomarkers of sugar intake, more generally, is that they cannot indicate intake of sugars that are not ¹³C enriched, such as beet sugar and honey. Consumption of these sugars was likely low in our Yup'ik study population, due to restricted access to commercial foods in rural Yup'ik communities;³⁰ therefore, we expect the impact of this limitation on this study to be minimal.

This study is the first examination of the effects of sugar intake on Yup'ik health, and used an objective biomarker of sugar intake that was developed specifically for use with Yup'ik people. We found that isotopic estimates of sugar intake was not associated with BMI or WC in this Yup'ik study population, but

that it was positively associated with blood pressure, TG, insulin, insulin resistance and leptin, and inversely associated with HDL, LDL, total cholesterol and adiponectin. These findings suggest that, although sugar intake is not associated with obesity in the Yup'ik population, high intakes of sugar may have adverse effects on chronic disease risk factors often related to obesity. Longitudinal studies are warranted to confirm the findings of this study and better understand associations of sugar intake with disease risk in the Yup'ik population.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

DMO and BBB designed research; SEH, AB and BBB conducted research; KLS, PJH, SHN and KC performed laboratory analyses; SHN analyzed data; SHN, DMO and ARK wrote the manuscript. SHN had primary responsibility for final content. All authors contributed to the final version of the manuscript.

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