

Stable Isotope Models of Sugar Intake Using Hair, Red Blood Cells, and Plasma, but Not Fasting Plasma Glucose, Predict Sugar Intake in a Yup'ik Study Population^{1,2}

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Abstract

Objectively measured biomarkers will help to resolve the controversial role of sugar intake in the etiology of obesity and related chronic diseases. We recently validated a dual-isotope model based on RBC carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotope ratios that explained a large percentage of the variation in self-reported sugar intake in a Yup'ik study population. Stable isotope ratios can easily be measured from many tissues, including RBCs, plasma, and hair; however, it is not known how isotopic models of sugar intake compare among these tissues. Here, we compared self-reported sugar intake with models based on RBCs, plasma, and hair $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in Yup'ik people. We also evaluated associations of sugar intake with fasting plasma glucose $\delta^{13}\text{C}$. Finally, we evaluated relations between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in hair, plasma, RBCs, and fasting plasma glucose to allow comparison of isotope ratios across tissue types. Models using RBCs, plasma, or hair isotope ratios explained similar amounts of variance in total sugar, added sugar, and sugar-sweetened beverage intake (~53%, 48%, and 34%, respectively); however, the association with $\delta^{13}\text{C}$ was strongest for models based on RBCs and hair. There were no associations with fasting plasma glucose $\delta^{13}\text{C}$ ($R^2 = 0.03$). The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of RBCs, plasma, and hair showed strong, positive correlations; the slopes of these relations did not differ from 1. This study demonstrates that RBC, plasma, and hair isotope ratios predict sugar intake and provides data that will allow comparison of studies using different sample types. *J. Nutr.* 144: 75–80, 2014.

Introduction

Biomarkers of sugar intake will strengthen inferences from observational studies and will help to resolve the controversial role of sugar intake in the development of obesity and related chronic diseases (1). The carbon stable isotope ratio ($\delta^{13}\text{C}$) has been proposed as a low burden, economical measure of sugar intake, because this isotope ratio is elevated in corn-based sweeteners and cane sugar (2–5). We recently validated an improved isotopic model of sugar intake based on RBC carbon and nitrogen ($\delta^{15}\text{N}$) isotope ratios (2) that explains nearly 50% of the variation in self-reported intake in an Alaska Native (Yup'ik) population (6) and is improved because $\delta^{15}\text{N}$ accounts for confounding dietary effects on $\delta^{13}\text{C}$. Furthermore, we and others have shown that $\delta^{13}\text{C}$ and

$\delta^{15}\text{N}$ are correlated among different tissue types, including RBCs, clot, serum, and hair (3, 4), suggesting that this measure of sugar intake could also be applied to stored serum or plasma samples or used to noninvasively assay sugar intake by using hair. However, whether our model performs similarly in different tissue types is not known.

An alternative approach to isotopic measures of sweetener intake has been proposed by Cook et al. (5), who found that plasma glucose $\delta^{13}\text{C}$ was strongly associated with postprandial corn and cane-based sweetener intake. However, the effect was not observed for fasted samples, limiting the utility of plasma glucose $\delta^{13}\text{C}$ as a measure of longer-term or “usual” intake. Because the Cook study was based on a 7-d controlled feeding study, it is possible that the duration of dietary treatment was too short to sufficiently affect endogenous sources of fasting glucose. However, whether longer term differences in sugar intake, such as the differences in usual intake among individuals, could affect fasting plasma glucose $\delta^{13}\text{C}$ has not been tested.

This study tested whether models of sugar intake based on stable isotope measurements from RBCs, plasma, and hair had similar validity in a Yup'ik study population. We also examined whether usual sugar intake was associated with fasting plasma glucose $\delta^{13}\text{C}$. Finally, we determined the associations between the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of RBCs, plasma, fasting plasma glucose,

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and hair. Participants in this study were from 2 Yup'ik communities in rural southwest Alaska. This study will provide information to determine which sample types can be used to provide isotopic predictions of sugar intake in future studies and will enable comparison among existing stable isotope studies based on RBCs, plasma, and hair.

Methods

Participant recruitment and procedures. Data are from the Center for Alaska Native Health Research *Neqem Nallunaikuta* ("Foods' Marker") study. This study was approved by University of Alaska Fairbanks Institutional Review Board and the Yukon-Kuskokwim Health Corporation Human Studies Committee.

Between 2008 and 2009, a community-based sample of 68 Yup'ik participants aged 14–79 y was recruited from 2 coastal communities in southwest Alaska. At entry into the study, participants completed a demographic questionnaire and the first of four 24-h recall dietary interviews (24HR). Three more dietary interviews were conducted during the next 4 wk, as described in detail elsewhere (2).

Of the 68 participants enrolled in the *Neqem Nallunaikuta* study, 65 had isotopic measurements for RBCs, plasma, and fasting plasma glucose and 54 had isotopic measurements for hair. Our comparisons of the dual isotopic models of sugar intake were based on 52 participants that had a full suite of isotopic measurements. Our evaluation of associations between fasting plasma glucose $\delta^{13}\text{C}$ and sugar intake was based on the sample of 65 participants with fasting plasma glucose stable isotope measurements. Finally, associations between the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of RBCs, plasma, hair, and fasting plasma glucose values were based on the 52 participants who had a full suite of isotopic measurements.

Assessment of dietary intake. 24HRs were collected from each participant by certified interviewers using algorithm-driven, computer-assisted software [Nutrition Data System for Research (NDSR) software 2008; University of Minnesota]. Participants were asked to recall all food and beverages consumed the day prior to the interview using a multiple pass approach. All participants were given portion estimation tools (measuring cups, rulers, and food models or portion estimation guides; Fred Hutchinson Cancer Research Center). Although most participants were bilingual, a native Yup'ik speaker conducted interviews for participants who did not speak English. No recalls were excluded due to unreasonable intake (6).

The NDSR food and nutrient database (7) was used to calculate sugar intake. In this study, sugar intake is measured in 3 ways: as total sugars, added sugars, and SSBs. Total sugar intake (g/d) is defined as the total sum of all mono- and di-saccharides consumed and includes primarily fructose, glucose, and sucrose. Added sugar intake (g/d) was defined as the sum of sugars and syrups added to foods during food preparation or commercial food processing. SSB intake was calculated from the NDSR food codes as the sum of servings of sweetened soft drinks and sweetened fruit drinks [servings/d, 237 mL (8 fl oz)/serving].

Biological sample collection. Fasting blood samples were collected into EDTA tubes and processed in rural communities using a portable centrifuge. RBCs and plasma were aliquotted and stored at -15°C in a portable freezer. Within 6 d, samples were shipped to the University of Alaska Fairbanks and stored at -80°C . Hair was collected by cutting ~50 hairs from the back of the head as close to the scalp as possible. Samples were taped with the cut end labeled and stored in plastic bags.

Biological samples were collected at least 2 wk after the completion of dietary interviews, so that their average age would approximately match the period during which interviews were conducted. RBCs have a lifespan of 90–120 d (8–10) and a mean age of 50 d (9). Hair grows at a rate of 1 cm/mo (11–13); we sampled the 2 cm of hair closest to the scalp to reflect the last 2 mo of intake (4). Plasma components turn over at differing rates, but the turnover rate of albumin, which comprises the bulk of plasma proteins, is 10%/d (14). Therefore, we assume that plasma isotope ratios represent approximately the last 2–3 wk of intake.

Stable isotope analysis. RBCs and plasma aliquots were autoclaved and prepared for stable isotope analysis as described elsewhere (15). Neither autoclaving nor the use of EDTA-treated tubes affects RBC carbon or nitrogen isotope ratios (16). Hair samples were cleaned and prepared for stable isotope analysis as described elsewhere (4). RBC, whole plasma, and hair samples were analyzed at the Alaska Stable Isotope Facility by continuous-flow isotope ratio MS by using a Costech ECS4010 Elemental Analyzer (Costech Scientific) interfaced to a Finnigan Delta Plus XP isotope ratio mass spectrometer via the ConFlo III interface (Thermo-Finnigan). The conventional means of expressing natural abundance isotope ratios is as δ values in permil (‰). This reflects the ratio of the abundance of the heavy and light isotopes relative to internationally recognized standards: $\delta X = (R_{\text{sample}} - R_{\text{standard}}) / (R_{\text{standard}}) \cdot 1000\text{‰}$ (17). Here, R is the ratio of heavy:light isotope ($^{15}\text{N}:^{14}\text{N}$, $^{13}\text{C}:^{12}\text{C}$) and 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; precision was measured as the CV of these analyses (3.3% for $\delta^{15}\text{N}$ and 0.6% for $\delta^{13}\text{C}$). The purity of the samples was assessed through the molar C:N ratios, which were 3.3 ± 0.1 for RBCs, 3.0 ± 0.1 for hair, and 3.7 ± 0.1 for plasma. The C:N ratios for hair and RBCs were consistent with those previously published (4,18,19). Expected C:N ratios for plasma have not been previously published; however, our values were consistent with that expected for a protein-rich blood fraction that also contains lipid.

We measured fasting plasma glucose $\delta^{13}\text{C}$ values following the methods of Rembacz et al. (20), with modifications as described by Cook et al. (5). Briefly, 250 μL of plasma was added to a 10-mL Exetainer (Labco Limited). Plasma was then treated with 20 μL 5% acetic acid to remove circulating bicarbonate, vortexed, and dried under a steady stream of nitrogen. Samples were resuspended with 500 μL DI water, flushed with helium gas to provide anaerobic conditions, and capped with screw-top caps containing a rubber septum. Commercially available baker's yeast (Red Star Yeast) was used as a 20- μL yeast/water suspension (5 g/L) and injected through the rubber septum. Exetainers were incubated at 30°C for 16 h and the ^{13}C abundance of the resulting CO_2 was analyzed at the Alaska Stable Isotope Facility using a Finnigan Delta Plus XP isotope ratio mass spectrometer equipped with a Gas Bench inlet system. Samples were analyzed in triplicate and the mean SD was 0.8‰.

Because biological samples from this study have a lower $^{13}\text{C}:^{12}\text{C}$ than Vienna PeeDee Belemnite, all $\delta^{13}\text{C}$ 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 $\delta^{15}\text{N}$.

Statistical analyses. SSBs (servings/d + 1) and total and added sugars (g/d + 1) were log transformed for analyses. The ability of dual isotopic models based on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ measured in RBCs, plasma, and hair to predict reported sugar intake was assessed using multiple linear regression models in which isotope ratios were the independent variables. How well fasting plasma glucose $\delta^{13}\text{C}$ predicted sugar intake was assessed using simple linear regression, because plasma glucose does not contain any nitrogen. The β -coefficients for these models were back transformed and are interpreted as percentage change in the dietary variable for every 1‰ change in isotope ratio. To test relations among isotope ratios of RBCs, plasma, fasting plasma glucose, and hair, we used simple linear regression models. Agreement between measures was evaluated in 2 ways: first, whether the slope of their relation was equal to 1, and second, as the mean and SD of their differences, according to Bland and Altman (21). Agreement was considered good if the SD of the mean difference was within 0.5‰. All 2-sided tests of significance were set at an α of 0.05. All statistical analyses were performed using JMP version 8 (SAS Institute) and STATA/IC version 12 (StataCorp).

Results

The demographic and dietary intake characteristics for the 68 participants in the *Neqem Nallunaikuta* study and the subset of participants who had a full suite of RBC, plasma, and hair isotopic measurements are given in Table 1 ($n = 52$). The mean

TABLE 1 Participant characteristics and reported sugar intake for all participants in the Center for Alaska Native Health Research *Neqem Nallunaikuta* study and in the set of participants with a full suite of RBC, plasma, and hair isotopic measurements¹

	<i>Neqem Nallunaikuta</i> study (n = 68)	RBC, plasma, and hair isotope subset (n = 52)
Age, y	40 ± 18	40 ± 17
14–20	11 (16)	6 (12)
21–40	22 (32)	19 (37)
41–60	27 (40)	22 (42)
>60	8 (12)	5 (10)
Sex		
Male	35 (51)	22 (42)
Female	33 (49)	30 (58)
BMI, kg/m ²	27 ± 6	28 ± 6
<18.5	1 (1)	—
18.5–<25	30 (44)	21 (40)
25–<30	16 (24)	14 (27)
≥30	21 (31)	15 (29)
Sugar intake		
Total sugar, g/d	89 (77, 103)	85 (72, 100)
Added sugar, g/d	74 (62, 88)	72 (61, 85)
SSB, servings/d	1.09 (1.07, 1.10)	1.40 (1.09, 1.76)

¹ Values are means ± SDs or n (%); geometric means (95% CIs) are given for log-transformed variables (sugar intake). SSB, sugar-sweetened beverage.

reported total sugar intake was 89 g/d in the complete study population and 85 g/d in the isotope subset (n = 52), which is lower than has been reported for U.S. whites (22, 23) and other native circumpolar populations (24).

The associations of reported sugar intake with isotopic models of sugar intake for those participants who had RBC, plasma, and hair isotope measurements (n = 52) are given in **Table 2**. The strongest associations with sugar intake were observed for models based on isotope measurements in RBCs and hair, which both explained ~52%, 47%, and 34% of the variance in total sugars, added sugars, and SSBs, respectively. Models of sugar intake based on plasma isotope ratios were slightly less strong and the associations of plasma $\delta^{13}\text{C}$ with sugar intake were only significant for SSBs, despite plasma $\delta^{15}\text{N}$ showing strong inverse associations in all models. In the larger sample of 65 participants, however, plasma $\delta^{13}\text{C}$ was associated with total sugars (β : 22.1;

95% CI: 4.1, 43.3; $P = 0.0148$) and SSBs (β : 25.4; 95% CI: 7.2, 46.6; $P = 0.0054$), and the association with added sugars was only marginally nonsignificant (β : 22.1; 95% CI: -0.69, 50.1; $P = 0.06$). **Figure 1** shows the relation between reported total sugar intake and the predicted values using our dual isotope model for RBCs, plasma, and hair.

There were no significant associations of sugar intake with fasting plasma glucose $\delta^{13}\text{C}$ (n = 65). Fasting plasma glucose $\delta^{13}\text{C}$ was not associated with intake of total sugars (β : 5.3; 95% CI: -1.7, 12.8; $R^2 = 0.03$; $P = 0.14$), added sugars (β : 5.6; 95% CI: -2.4, 14.3; $R^2 = 0.03$; $P = 0.17$), or SSBs (β : 5.4; 95% CI: -0.75, 12.0; $R^2 = 0.05$; $P = 0.09$).

Table 3 gives means and distributions of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for all sample types (n = 52). In agreement with previous results from this population (4), hair $\delta^{13}\text{C}$ values were consistently elevated over RBC $\delta^{13}\text{C}$ values by $1.9 \pm 0.3\text{‰}$. Plasma and RBC $\delta^{13}\text{C}$ values did not differ from one another but were elevated relative to fasting plasma glucose $\delta^{13}\text{C}$ by $4.2 \pm 2.1\text{‰}$ and $4.0 \pm 2.1\text{‰}$, respectively. Hair $\delta^{15}\text{N}$ was elevated $1.5 \pm 0.5\text{‰}$ relative to RBC $\delta^{15}\text{N}$ (4) but did not differ from plasma $\delta^{15}\text{N}$. Plasma $\delta^{15}\text{N}$ was elevated over RBC $\delta^{15}\text{N}$ by $1.4 \pm 0.4\text{‰}$.

Figure 2 shows the associations between the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of RBCs, plasma, and hair (n = 52). The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of RBCs, hair, and plasma were strongly associated (all $P < 0.0001$) and the slopes of these relations did not differ from 1, with the exception of the association between hair and plasma $\delta^{15}\text{N}$ (β : 0.87; 95% CI: 0.81, 0.93). Agreement among RBC, plasma, and hair isotope ratios was mostly good once the differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were accounted for. For $\delta^{13}\text{C}$, the SD of the mean difference was 0.39‰ for RBCs compared with plasma, 0.35‰ for RBCs compared with hair, and 0.39‰ for plasma compared with hair. For $\delta^{15}\text{N}$, the SD of the mean difference was 0.41‰ for RBCs compared with plasma, 0.53‰ for RBCs compared with hair, and 0.47‰ for plasma compared with hair. There were weak but significant associations of fasting plasma glucose $\delta^{13}\text{C}$ with RBC $\delta^{13}\text{C}$ (β : 0.08; 95% CI: 0.01, 0.16; $r = 0.31$; $P = 0.03$), plasma $\delta^{13}\text{C}$ (β : 0.11; 95% CI: 0.02, 0.20; $r = 0.33$; $P = 0.022$), and hair $\delta^{13}\text{C}$ (β : 0.14; 95% CI: 0.05, 0.22; $r = 0.44$; $P = 0.002$).

Discussion

This study tested whether isotope measurements from RBCs, plasma, and hair had similar validity when used to model sugar

TABLE 2 Associations of reported sugar intake with RBC, plasma, and hair isotope ratios for all participants in the *Neqem Nallunaikuta* study with a full suite of RBC, plasma, and hair isotopic measurements (n = 52)

Isotope ratio	Total sugars (g/d)			Added sugars (g/d)			SSB ¹ (servings/d)		
	β^2	95% CI	R^2	β^2	95% CI	R^2	β^2	95% CI	R^2
RBC			0.53			0.48			0.34
$\delta^{13}\text{C}$	37.7**	13.1, 67.6		31.4*	6.8, 61.6		33.8**	10.1, 62.7	
$\delta^{15}\text{N}$	-21.0***	-26.1, -15.5		-20.4***	-25.8, -14.5		-13.5***	-19.1, -7.5	
Plasma			0.48			0.43			0.28
$\delta^{13}\text{C}$	11.5	-4.9, 30.8		10.3	-6.6, 30.3		20.5*	1.6, 42.8	
$\delta^{15}\text{N}$	-17.8***	-23.1, -12.2		-17.1***	-22.7, -11.1		-10.5**	-16.7, -3.9	
Hair			0.52			0.47			0.34
$\delta^{13}\text{C}$	22.5*	2.5, 46.3		21.1*	0.49, 45.8		27.5**	7.1, 51.7	
$\delta^{15}\text{N}$	-17.0***	-22.0, -11.7		-16.4***	-21.6, -10.8		-9.6**	-14.9, -4.0	

¹ SSB, sugar-sweetened beverage.

² Indicates change in the dependent variable (sugar intake) for every 1‰ change in isotope ratio. All dietary variables were transformed for analysis; therefore, estimates of β are interpreted as percentage change in the dietary variable for every 1‰ change in isotope ratio. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.0001$.

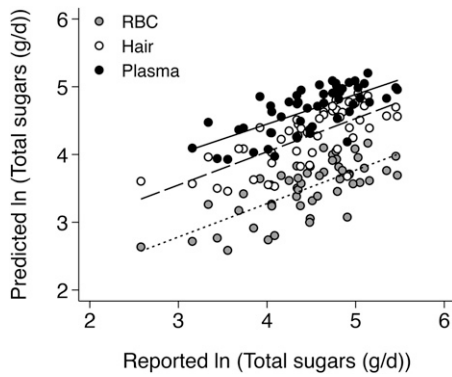


FIGURE 1 Associations of reported total sugar intake with predicted total sugar intake for all participants in the *Neqem Nallunaikuta* study with a full suite of RBC, plasma, and hair isotopic measurements ($n = 52$). Total sugar intake was predicted using the formulae that were generated from the models of total sugar intake using RBC (dotted line), hair (dashed line), and plasma (solid line) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ as predictors (Table 2).

intake in a Yup'ik population. We found that that a dual isotopic model of sugar intake-based $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ explained nearly identical amounts of variance in total sugars, added sugars, and SSBs when isotope ratios were measured in hair or RBCs. These models slightly outperformed models based on plasma isotope ratios in which plasma $\delta^{13}\text{C}$ was associated with SSBs but not total or added sugars. These dual isotope models can be used to generate predictive equations for estimating dietary sugar intake (2), which will have utility for assessing associations of sugar intake with chronic disease risk in this Alaska Native population. We also showed that fasting plasma glucose $\delta^{13}\text{C}$ was not associated with differences in self-reported usual sugar intake, consistent with the findings of a recent 7-d controlled feeding study (5). Finally, we showed strong, positive associations and good agreement between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ measured in RBCs, plasma, and hair. These results support the use of isotopes measured in RBCs, hair, and plasma to indicate sugar intake, although they suggest that measurements from RBCs and hair are preferable to those from plasma. Importantly, this study also provides a basis for comparing studies using these different sample types.

RBC and hair isotope ratios provided a better measure of reported sugar intake than did plasma isotope ratios. This difference in performance could be related to the difference in turnover time between these biological fractions; the timing of our dietary interviews, as well as our sampling strategy for the hair, was timed to reflect the average age of RBCs rather than plasma. However, despite the lack of association between total or added sugars and plasma $\delta^{13}\text{C}$, the variance explained by the dual isotope model using plasma isotope ratios was high for all sugar variables (28–48%). This is because of the strong inverse association between reported sugar intake and plasma

TABLE 3 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for participants in the *Neqem Nallunaikuta* study with a full suite of RBC, plasma, and hair isotopic measurements¹

	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
	‰	‰
RBC	-19.7 ± 0.6	9.3 ± 1.8
Plasma	-19.9 ± 0.7	10.7 ± 1.8
Hair	-17.8 ± 0.7	10.8 ± 2.0
Fasting plasma glucose	-15.5 ± 2.3	—

¹ Values are means \pm SDs, $n = 52$.

$\delta^{15}\text{N}$ (25), a measure of traditional food intake in Yup'ik people (15).

The finding that hair isotope ratios were similar to RBCs in their ability to predict sugar intake is important, because hair can be collected noninvasively and without the need for specialized personnel or storage (4). Such a noninvasive biomarker will be useful for studies involving children as well as in studies that do not otherwise require a blood draw. Furthermore, in rural study populations, trained community aides can easily collect hair samples, reducing the cost and difficulty of assessing dietary intake using this biomarker. Finally, hair has an additional advantage over blood and plasma: because hair grows continuously and does not remodel after growth, it provides a continuous record of the biomarker back through time. This may be useful to assess the impact of any intervention designed to reduce sugar consumption over time. The ability of hair isotope ratios to predict sugar intake in other, non-Native populations has not been assessed; however, we expect associations with hair to be similar to those previously reported for whole blood and serum (26, 27) based on our findings in this study.

Our finding that fasting plasma glucose $\delta^{13}\text{C}$ was not associated with total sugars, added sugars, or SSBs is consistent with that from a recent controlled feeding study reported by Cook et al. (5), which demonstrated no effect of C_4 sugar intake on

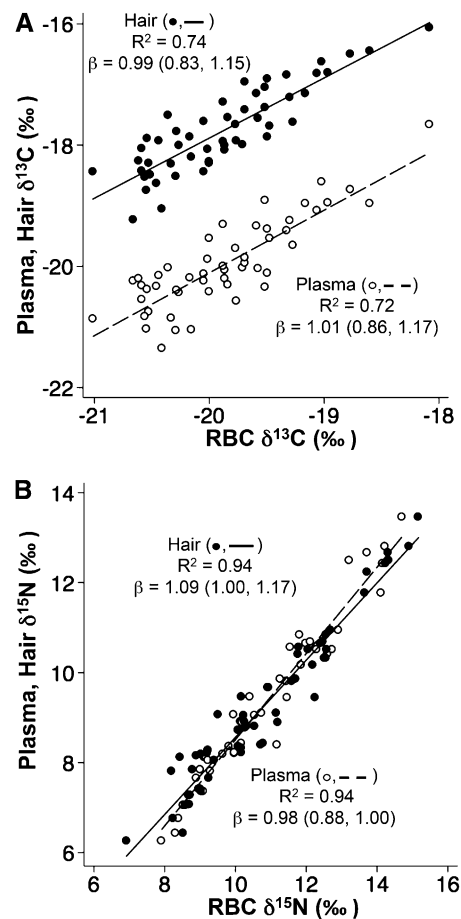


FIGURE 2 Relations [R^2 ; β (95% CI)] of RBC $\delta^{13}\text{C}$ (A) and RBC $\delta^{15}\text{N}$ (B) isotope ratios with hair and plasma isotope ratio intake for all participants in the *Neqem Nallunaikuta* study with a full suite of RBC, plasma, and hair isotopic measurements ($n = 52$). The associations between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of hair and plasma were also strong and positive [$R^2 = 0.72$; β : 0.90; 95% CI: 0.74, 1.06 ($\delta^{13}\text{C}$)] and [$R^2 = 0.94$; β : 0.87; 95% CI: 0.81, 0.93 ($\delta^{15}\text{N}$)]. All $P < 0.0001$.

fasting plasma glucose $\delta^{13}\text{C}$. This study built upon their work by testing whether fasting plasma glucose $\delta^{13}\text{C}$ could be associated with dietary differences that are of a longer duration than a 7-d intervention. However, our results confirm that fasting plasma glucose $\delta^{13}\text{C}$ does not reflect sugar intake even in the context of differences in usual consumption. This is likely due to plasma glucose carbon not solely deriving from dietary sugars; rather, we suggest that fasting plasma glucose incorporates carbon from other dietary sources through gluconeogenesis.

The differences between carbon and nitrogen isotope ratios in RBCs, plasma, and hair presented here were consistent with previous reports for RBCs and hair in Yup'ik people (4) and plasma protein and hair for a small sample ($n = 10$) of Chicago residents (28). To our knowledge, comparisons of RBCs and plasma isotope ratios have not been previously reported for humans; however, a recent study of Baltimore residents found very similar results for blood clot and serum (3). Associations among RBCs, hair, and plasma isotope ratios were strong and followed the expected 1:1 relations, which is also in agreement with previous work (3, 4). These findings will be useful in comparing isotopic studies conducted in hair, RBCs, and plasma in Yup'ik people and will provide a standard for comparison in similar studies in other, non-Native populations.

The primary limitation of this study is that we tested our proposed biomarkers against self-reported intakes, which are subject to random and systematic errors. Very little is known about factors that bias dietary self-report in Yup'ik people; however, sex and obesity have been identified as factors that may influence reporting bias in other populations (29). Neither sex nor BMI was associated with $\delta^{13}\text{C}$ in this study population; therefore, these potential biases could not explain the associations we found with sugar intake. In this study, the assay for fasting plasma glucose $\delta^{13}\text{C}$ was less precise than that of $\delta^{13}\text{C}$ measured in bulk plasma, RBCs, and hair; however, intra-sample variation was significantly smaller than inter-sample variation, suggesting that the null result with sugar intake was not simply due to insufficient analytical precision.

Several limitations of these and other isotope biomarkers of sugar intake warrant further discussion. First, our models underestimated reported sugars at high levels of intake for plasma and hair and at all levels of intake for RBCs (Fig. 1). This underestimation was not observed in our initial validation of the RBC model in a larger sample (2); therefore, it may be an artifact of the smaller sample size presented here. Second, the $\delta^{13}\text{C}$ biomarker has the potential to be affected by other sources of enriched ^{13}C in the Yup'ik diet; our model controls for most, but not all, of these effects (2). Finally, one limitation of the $\delta^{13}\text{C}$ biomarker of sugar intake more generally is that it is not associated with intake of sugars that are not ^{13}C enriched, such as beet sugar, honey, and intrinsic sugars found in fruit and dairy products (30). Consumption of these sugars by Yup'ik people is low (31); therefore, we do not expect our results to be affected by their consumption. However, we anticipate associations of $\delta^{13}\text{C}$ with total sugar intake will be attenuated in populations that consume sugars that are not ^{13}C enriched (27).

In summary, this study demonstrated that the stable isotope ratios of RBCs, plasma, and hair, but not fasting plasma glucose, can be used to assess sugar intake in Yup'ik people and provided data that will allow comparison of isotope ratios in these different sample types. In particular, the finding that hair isotope ratios are as predictive of sugar intake as RBC isotope ratios is important, because it suggests that sugar intake can be measured noninvasively and without the need for specialized sampling equipment. Further studies are warranted to determine the utility

of this dual isotope model of sugar intake in other, non-Native populations.

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