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A Stable Isotope Biomarker of Marine Food Intake Captures Associations between n–3 Fatty Acid Intake and Chronic Disease Risk in a Yup'ik Study Population, and Detects New Associations with Blood Pressure and Adiponectin[1,2]

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Abstract

The nitrogen isotope ratio (δ^{15}N) of RBCs has been proposed as a biomarker of marine food intake in Yup'ik people based on strong associations with RBC eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). However, EPA and DHA derive from marine fats, whereas elevated δ^{15}N derives from marine protein, and these dietary components may have different biologic effects. Whether δ^{15}N is similarly associated with chronic disease risk factors compared with RBC EPA and DHA is not known. We used covariate-adjusted linear models to describe biomarker associations with chronic disease risk factors in Yup'ik people, first in a smaller (n = 363) cross-sectional study population using RBC EPA, DHA, and δ^{15}N, and then in a larger (n = 772) cross-sectional study population using δ^{15}N only. In the smaller sample, associations of RBC EPA, DHA, and δ^{15}N with obesity and chronic disease risk factors were similar in direction and significance: δ^{15}N was positively associated with total, HDL, and LDL cholesterol, apolipoprotein A-I, and insulin-like growth factor binding protein-3 (IGFBP-3), and inversely associated with triglycerides. Based on comparisons between covariate-adjusted β-coefficients, EPA was more strongly associated with circulating lipids and lipoproteins, whereas δ^{15}N was more strongly associated with adipokines, the inflammatory marker interleukin-6, and IGFBP-3. In the larger sample there were new findings for this population: δ^{15}N was inversely associated with blood pressure and there was a significant association (with inverse linear and positive quadratic terms) with adiponectin. In conclusion, δ^{15}N is a valid measure for evaluating associations between EPA and DHA intake and chronic disease risk in Yup'ik people and may be used in larger studies. By measuring δ^{15}N, we report beneficial associations of marine food intake with blood pressure and adiponectin, which may contribute to a lower incidence of some chronic diseases in Yup'ik people. J. Nutr. 144: 706–713, 2014.

Introduction

Objective biomarkers of diet have the potential to reduce bias and error relative to self-reported measures, and thus better detect associations between specific dietary factors and health (1,2). Our group has been developing objective biomarkers of dietary intake based on stable isotope ratios for use in ongoing research to improve the health of the Yup'ik people in southwest Alaska (3–6). We have shown that RBCs and hair nitrogen isotope ratios (^{15}N/^{14}N, expressed as δ^{15}N, as defined in Participants and Methods) are strongly correlated (>0.8) with the RBC long-chain n–3 PUFAs EPA and DHA because all 3 measures are elevated in fish and marine mammals, a large component of the traditional Yup'ik diet (5–7). Intakes of EPA and DHA are high and quite variable in Yup'ik people (6); therefore, associations with health are of particular interest for further study (8–11). Because δ^{15}N is relatively simple and

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inexpensive to assay, several recent studies have used δ^{15}N as a surrogate measure of n–3 PUFA intake (10,12,13). However, EPA and DHA derive from marine fats, whereas elevated δ^{15}N derives from marine protein, and these components are sometimes rendered and eaten separately (e.g., as seal oil and seal meat). The nutrients in the fat and lean components of the marine diet may also have different biologic effects, and thus may exhibit different associations with chronic disease risk. For these reasons, the validity of using δ^{15}N to assess associations between n–3 PUFA intake and chronic disease risk needs to be tested.

This study had 2 objectives. The first was to further extend our evaluation of the use of δ^{15}N to assess EPA and DHA intake in a Yup'ik population by comparing associations with chronic disease risk factors among RBC EPA, DHA, and δ^{15}N. These risk factors include measures of blood pressure, blood lipids, blood sugar homeostasis, growth factors, inflammatory biomarkers, and adipokines, which were previously described in a Yup'ik population by our group (8,9). To these, we added 3 measures of obesity (BMI, waist circumference, and percentage of body fat) (14), glycosylated hemoglobin A1c (HbA1c)[9] (15), and grehlin (16), as is detailed in the Participants and Methods section. Previously, we showed that RBC EPA and DHA are associated with reduced triglyceride and C-reactive protein (CRP) concentrations and increased HDL cholesterol in this population (9). Replication of findings based on RBC FAs is an important criterion when considering using δ^{15}N as a biomarker of EPA and DHA intake (17). Because both percentage of RBC EPA and δ^{15}N increase linearly with marine food intake, whereas percentage of RBC DHA approaches an upper boundary at higher intakes (6,18), we expected associations with chronic disease risk factors to be strongest for EPA and δ^{15}N, and weaker for DHA. Our second objective was to use δ^{15}N to examine associations between marine food intake and both obesity and risk factors for chronic disease in a larger population sample, from which only δ^{15}N measures were available.

Participants 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 biologic, genetic, nutritional, and psychosocial risk factors for obesity and related disease in Yup'ik people. The CANHR study was approved by the University of Alaska 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 2006, 1003 men and women aged 14 and older were recruited from 10 communities in southwest Alaska, as described in detail elsewhere (19,20). At entry into the study, participants completed extensive interviewer-administered interviews covering demographic characteristics, economic status, ethnicity, and medical history. Anthropometric measurements were taken, and blood samples were collected from participants after an overnight fast. Blood was collected and processed locally into serum, lymphocyte, and RBC fractions using a portable centrifuge, 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.

Study sample. Both δ^{15}N and RBC FA measurements were available for 496 participants from the CANHR study. The selection of these participants was described in detail elsewhere (6,9). We used this sample to compare associations of obesity and chronic disease risk factors with EPA, DHA, and δ^{15}N, and excluded 105 participants aged ≤18 y, 26 participants with CRP concentrations of >1 mg/dL (indicating acute infection), and 2 participants with missing body BMI measurements, which left 363 participants. Hereafter, we will refer to this sample as the "validation sample."

Stable isotope (δ^{15}N) measurements were available for all 1003 participants in the first phase of the CANHR study (CANHR 1). We used this larger sample to investigate associations between marine food intake (using δ^{15}N), obesity, and chronic disease risk, and excluded 178 participants aged ≤18 y, 51 participants with CRP concentrations of >1 mg/dL, and 2 participants with missing BMI measurements, leaving 772 participants. Hereafter, we will refer to this sample as the "complete CANHR sample."

Anthropometric and risk factor measurements. Anthropometric measurements, including height, weight, waist circumference, percentage of body fat, and blood pressure, were measured by trained staff using protocols from the NHANES III Anthropometric Procedures Manual (21), as described by Boyer et al. (22). Biochemical risk factors for chronic disease were assayed in serum as previously described (9,22). These risk factors include blood lipids and lipoproteins (TGs, total cholesterol, HDL and LDL cholesterol, and apo A-I), measures of blood sugar homeostasis (glucose, HbA1c, insulin, and insulin resistance), hormones related to adiposity, insulin sensitivity, and appetite (leptin, adiponectin, and ghrelin), inflammatory cytokines and related markers [CRP, IL-6, and soluble tumor necrosis factor receptor type 2 (sTNFR2)], and factors influencing cellular growth and division [insulin-like growth factor-1 (IGF-1) and insulin-like growth factor binding protein-3 (IGFBP-3)]. Insulin resistance was assessed using the HOMA-IR index: [fasting insulin (μU/mL) × fasting glucose (mg/dL)]/405 (23). Measurements of IL-6, sTNFR2, IGF-1, and IGFBP-3 were only available for participants in the validation sample.

Dietary biomarker measurements. The RBC FAs EPA and DHA were analyzed at the Fred Hutchinson Cancer Research Center in Seattle, WA, as previously described (6). RBC δ^{15}N was analyzed at the Alaska Stable Isotope Facility at the University of Alaska Fairbanks, as previously described (4,6,24). Natural abundance stable isotope ratios are conventionally expressed in per mil (‰) abundance of ^{15}N relative to an international standard:

$$\delta^{15}\mathrm{N} = [(^{15}\mathrm{N}/^{14}\mathrm{N}_{\mathrm{sample}} - ^{15}\mathrm{N})/^{14}\mathrm{N}_{\mathrm{standard}}/(^{15}\mathrm{N}/^{14}\mathrm{N}_{\mathrm{standard}})] \cdot 1000‰,$$

where the standard is atmospheric nitrogen (^{15}N/^{14}N = 0.003677).

Statistical analyses. In the validation sample, we examined the associations of EPA, DHA, and δ^{15}N with each of the following measures of obesity: BMI, percentage of body fat, and waist circumference, and each of the following risk factors for chronic disease: systolic blood pressure (SBP), diastolic blood pressure (DBP), TGs, total cholesterol, HDL and LDL cholesterol, apo A-I, glucose, HbA1c, insulin, HOMA-IR, IGF-1, IGFBP-3, CRP, IL-6, sTNFR2, leptin, adiponectin, and ghrelin. TGs, insulin, HOMA-IR, CRP, IL-6, sTNFR2, and leptin were log transformed for analysis, and results were back transformed for ease of interpretation. We also examined associations of δ^{15}N with each of these variables in the complete CANHR sample, with the exception of IGF-1, IGFBP-3, IL-6, and sTNFR2, which were only available for the validation sample.

We excluded participants from certain analyses based on medication use (n = validation sample exclusions; complete CANHR sample exclusions). We excluded participants taking blood pressure medication from analyses of SBP and DBP (n = 62; 105), participants taking diabetes medication from analyses of glucose, HbA1c, insulin, and HOMA-IR (n = 7; 11), and participants taking cholesterol medication from analyses of TGs, total cholesterol, and HDL and LDL cholesterol (n = 18; 30).

Values of chronic disease risk factors that were >4 SD above the mean were judged to be physiologically unreasonable and excluded as outliers. We excluded values for the following chronic disease risk

[9] Abbreviations used: CANHR, Center for Alaska Native Health Research; CRP, C-reactive protein; DBP, diastolic blood pressure; HbA1c, glycosylated hemoglobin A1c; IGF-1, insulin-like growth factor-1; IGFBP-3, insulin-like growth factor binding protein-3; SBP, systolic blood pressure; sTNFR2, soluble tumor necrosis factor receptor type 2.

factors (n = validation sample exclusions; complete CANHR sample exclusions): waist circumference (n = 0; 1), SBP (n = 2; 4), DBP (n = 1; 1), TGs (n = 1; 4), total cholesterol (n = 0; 1), HDL (n = 1; 0), apo A-I (n = 2; 0), glucose (n = 2; 4), HbA1c (n = 3; 5), insulin (n = 0; 6), HOMA-IR (n = 0; 8), IL-6 (n = 0; 3), sTNFR2 (n = 2; 2), leptin (n = 0; 1), adiponectin (n = 1; 3), and ghrelin (n = 1; 3). For IL-6, values below the limit of detection (n = 91) were replaced by the limit of detection divided by the square root of 2 (25).

We used multiple regression models to model associations of each biomarker (EPA, DHA, and δ^{15}N) with each measure of obesity or chronic disease risk factor. Models of obesity measures (BMI, percentage of body fat, and waist circumference) were adjusted for age (continuous), sex, and regular smoking (yes/no). All other models were additionally adjusted for BMI. A smaller number of the participants in the complete CANHR sample had physical activity data [counts per day: n = 249 (26)]. In these participants, physical activity was not associated with δ^{15}N when adjusted for age, sex, BMI, and smoking (data not shown), nor was physical activity associated with any of the biochemical risk factors when adjusted for age, sex, BMI, smoking, and δ^{15}N. Physical activity was significantly inversely associated with BMI, percentage of body fat, and waist circumference when adjusted for age, sex, smoking, and δ^{15}N; however, including it as a covariate in these models did not affect associations with δ^{15}N. Finally, we tested differences in the leptin: adiponectin ratio among quartiles of δ^{15}N using ANCOVA, with Tukey's honestly significant difference test for pairwise comparisons, adjusted for sex, age, BMI, and smoking. The leptin:adiponectin ratio was log transformed for analysis.

Both linear and quadratic terms were assessed to determine nonlinearity (8,9,24). We used a conservative criterion ($P < 0.01$) for reporting quadratic associations because of the likelihood that multiple contrasts would lead to chance associations. For all tests, we give the unadjusted P value using a significance level of 0.05, and indicate in the text and tables whether P values remain significant after adjustment for multiple testing with the Bonferroni-Holm procedure (27). Because Bonferroni-Holm adjusts the significance threshold (α) in a stepwise manner, α differs among tests within a family of hypotheses based on rank order. All statistical analyses were performed using JMP version 8 (SAS Institute).

Results

The demographic characteristics of the validation sample (n = 363) and the complete CANHR sample (n = 772) are given in **Table 1**. Distributions of sex, BMI, and smoking were similar between the 2 samples; however, older participants (≥55 y) were over-represented in the validation sample (28%) relative to the complete CANHR sample (21%). Younger participants (18–29 y) were under-represented (18% vs. 25% in the validation and complete CANHR samples, respectively).

The mean values of chronic disease risk factors for each study sample are shown in **Table 2**. Means were generally similar between the 2 study samples, with the validation sample mean within 10% of the complete CANHR sample mean for most risk factors. Exceptions included insulin, HOMA-IR, and leptin, for which validation sample means exceeded those of the complete CANHR sample mean by 15%, 15%, and 17%, respectively, and CRP, for which the validation sample mean exceeded that of the complete CANHR sample by 88%.

TABLE 1 Demographic and health-related characteristics of Yup'ik participants in the validation sample and complete CANHR sample, by sex[1]

	Validation sample			Complete CANHR sample		
	Total	Men	Women	Total	Men	Women
Participants,[2] *n (%)*	363 (100)	147 (40)	216 (60)	772 (100)	347 (45)	425 (55)
Age, *y*	45 ± 15	46 ± 15	44 ± 15	42 ± 15	41 ± 15	42 ± 15
18–29	65 (18)	25 (17)	40 (19)	194 (25)	98 (28)	96 (23)
30–54	197 (54)	77 (52)	120 (56)	415 (54)	179 (52)	236 (56)
≥55	101 (28)	45 (31)	56 (26)	163 (21)	70 (20)	93 (22)
BMI, *kg/m²*	28.8 ± 6.1	26.9 ± 4.4	30.0 ± 6.8	28.4 ± 5.9	26.8 ± 4.6	29.7 ± 6.5
<25	105 (29)	53 (36)	52 (24)	248 (32)	143 (41)	105 (25)
≥25–<30	127 (35)	62 (42)	65 (30)	267 (35)	131 (38)	136 (32)
≥30–<35	74 (20)	23 (16)	51 (24)	152 (20)	55 (16)	97 (23)
≥35	57 (16)	9 (6)	48 (22)	105 (14)	18 (5)	87 (20)
Body fat, *%*	31.7 ± 10.2	23.4 ± 6.7	37.3 ± 8.1	30.4 ± 10.5	22.8 ± 7.3	36.7 ± 8.4
Current smokers, *n (%)*	95 (26)	54 (37)	41 (19)	197 (26)	138 (40)	59 (14)
RBC δ^{15}N, ‰	9.0 ± 1.4	8.8 ± 1.4	9.2 ± 1.4	9.2 ± 1.5	9.0 ± 1.5	9.4 ± 1.5
<8.0	104 (29)	52 (35)	52 (24)	192 (25)	105 (30)	87 (20)
≥8.0–<9.0	94 (26)	38 (26)	56 (26)	195 (25)	88 (25)	107 (25)
≥9.0–<10.0	82 (23)	29 (20)	55 (25)	174 (23)	74 (21)	100 (24)
≥10.0	83 (23)	28 (19)	55 (25)	211 (27)	80 (23)	131 (31)
RBC EPA, *% of total FAs*						
<1.0	71 (20)	37 (25)	34 (16)	—	—	—
≥1.0–<3.0	145 (40)	61 (41)	84 (39)	—	—	—
≥3.0–<5.0	96 (26)	29 (20)	67 (31)	—	—	—
≥5.0	51 (14)	20 (14)	31 (14)	—	—	—
RBC DHA, *% of total FAs*						
<5.0	66 (18)	37 (25)	29 (13)	—	—	—
≥5.0–<7.0	86 (24)	47 (32)	39 (18)	—	—	—
≥7–<9.0	193 (53)	58 (39)	135 (63)	—	—	—
≥9.0	18 (5)	5 (3)	13 (6)	—	—	—

[1] Values are n (%) or means ± SDs. CANHR, Center for Alaska Native Health Research.
[2] Percentages may not sum to 100 because of rounding.

TABLE 2 Risk factors for chronic disease for Yup'ik participants in the validation sample and the complete CANHR sample[1]

	Validation sample		Complete CANHR sample	
	n^2	Mean	n^2	Mean
BMI, *kg/m²*	363	29 ± 6	772	28 ± 6
Body fat, *%*	361	32 ± 10	768	30 ± 11
Waist circumference, *cm*	359	94 ± 14	765	93 ± 14
SBP,[3] *mm Hg*	298	122 ± 14	656	119 ± 14
DBP,[3] *mm Hg*	299	73 ± 10	656	72 ± 10
TGs,[4] *mg/dL*	344	82 (78, 86)	723	77 (75, 79)
Total cholesterol,[3] *mg/dL*	345	223 ± 46	731	224 ± 45
HDL cholesterol,[3] *mg/dL*	344	62 ± 17	731	63 ± 17
LDL cholesterol,[3] *mg/dL*	345	143 ± 40	730	144 ± 38
apo A-I, *mg/dL*	361	173 ± 23	760	169 ± 27
Glucose,[3] *mg/dL*	354	95 ± 11	749	93 ± 10
HbA1c,[3] *%*	353	5.5 ± 0.4	750	5.5 ± 0.4
Insulin,[3,4] *μU/mL*	355	14.2 (13.5, 14.9)	740	12.3 (11.9, 12.8)
HOMA-IR[3,4]	355	3.3 (3.1, 3.5)	735	2.8 (2.7, 2.9)
IGF-1, *μg/L*	362	259 ± 100	—	—
IGFBP-3, *μg/mL*	362	4.4 ± 1.0	—	—
CRP,[4] *mg/dL*	363	0.17 (0.15, 0.18)	754	0.09 (0.08, 0.10)
IL-6,[4] *pg/mL*	359	0.08 (0.07, 0.09)	—	—
sTNFR2,[4] *μg/L*	360	2.0 (1.9, 2.1)	—	—
Leptin,[4] *μg/L*	363	8.2 (7.4, 9.1)	761	7.0 (6.5, 7.6)
Adiponectin, *μg/mL*	362	9 ± 4	760	10 ± 5
Ghrelin, *pg/mL*	361	434 ± 174	760	415 ± 153

[1] Values are means ± SDs or geometric means (95% CIs) for log-transformed variables. CANHR, Center for Alaska Native Health Research; CRP, C-reactive protein; DBP, diastolic blood pressure; HbA1c, glycosylated hemoglobin A1c; IGF-1, insulin-like growth factor-1; IGFBP-3, insulin-like growth factor binding protein-3; SBP, systolic blood pressure; sTNFR2, soluble tumor necrosis factor receptor type 2.
[2] Sample sizes vary because of outliers, exclusions, and missing data.
[3] Participants who reported taking blood pressure, cholesterol, or diabetes medication were excluded from calculations of means of blood pressure, blood lipid, and glucose homeostasis variables, respectively.
[4] Variables were log transformed for analyses.

In the validation sample, associations of δ^{15}N with most chronic disease risk factors were similar to those with EPA and DHA (**Table 3**). Like EPA and DHA, δ^{15}N showed positive associations with total cholesterol, HDL and LDL cholesterol, apo A-I, and IGFBP-3, and an inverse association with TGs. All 3 measures had weak inverse associations with CRP, although only associations with EPA and DHA were significant (P = 0.027, 0.015, and 0.065 for EPA, DHA, and δ^{15}N, respectively). None of the measures were associated with BMI, body fat, waist circumference, SBP, DBP, blood glucose, insulin, HOMA-IR, IGF-1, and sTNFR2. The associations of δ^{15}N with total cholesterol and apo A-I became nonsignificant after adjustment for multiple testing, as did associations of EPA with HbA1c, IGFBP-3, and CRP, and associations of DHA with TGs, HDL cholesterol, IGFBP-3, and CRP. As expected, EPA exhibited more associations with chronic disease risk factors than DHA because DHA reaches an upper limit at high intakes. Therefore, we focused subsequent comparisons on EPA and δ^{15}N.

Despite these similarities, there were also differences between EPA and δ^{15}N in their associations with different types of risk factors. For example, associations with blood lipids and lipoproteins were always stronger for EPA than δ^{15}N, and EPA captured an inverse quadratic component to associations with total cholesterol and LDL cholesterol that δ^{15}N did not. However, δ^{15}N had stronger associations with hormones relating to body mass and appetite (leptin, adiponectin, and ghrelin, although associations with ghrelin and adiponectin were marginally nonsignificant: P = 0.0014, 0.070, and 0.06, respectively). δ^{15}N was associated with IL-6, unlike EPA, and the association of δ^{15}N with IGFBP-3 was 4-fold stronger than that of EPA. Finally, there was an unexpected positive association of HbA1c with EPA but not δ^{15}N; however, the association of EPA with HbA1c became nonsignificant after adjustment for multiple testing.

Associations between δ^{15}N, measures of obesity, and chronic disease risk factors in the complete CANHR sample are presented in **Table 4**. δ^{15}N had a weak positive association with BMI, which subsequent analyses revealed to be in women only [β (95% CI) = 0.61 (0.12, 1.1), P = 0.014]. Many of the relations of chronic disease risk factors with δ^{15}N in the complete CANHR sample were similar to those found in the validation sample: positive associations with total cholesterol, HDL and LDL cholesterol, and apo A-I; inverse associations with TGs and leptin; and no association with glucose, HbA1c, insulin, HOMA-IR, and CRP. However, in the larger complete CANHR sample, δ^{15}N also captured inverse quadratic components to associations with total cholesterol and HDL and LDL cholesterol, similarly to EPA in the validation sample. We also detected new associations with δ^{15}N in this larger sample; in particular, inverse associations with blood pressure (SBP and DBP) and a positive quadratic association with adiponectin, notable for a large increase in adiponectin between quartiles 3 and 4 of δ^{15}N. Because the association of δ^{15}N with leptin was inverse, the leptin:adiponectin ratio was significantly lower in quartile 4 of δ^{15}N (**Fig. 1**). The associations of δ^{15}N with BMI and SBP became nonsignificant after multiple test correction, as did the linear component of the association with adiponectin.

Discussion

This study compared associations with risk factors for chronic disease among 3 biomarkers of marine food intake measured in the RBCs of a Yup'ik population: EPA, DHA, and δ^{15}N. Among these measures, EPA exhibited the most associations with chronic disease risk, followed by δ^{15}N, and finally DHA. Generally EPA and δ^{15}N had similar associations with chronic disease risk factors; however, associations with blood lipids and lipoproteins were stronger for EPA, whereas associations with δ^{15}N were stronger for adipokines (leptin and adiponectin), IL-6, and IGFBP-3. In the complete CANHR sample, δ^{15}N detected inverse associations with blood pressure and a positive quadratic association with adiponectin that were not previously detected in this population. δ^{15}N also revealed a weak positive association between traditional marine food intake and BMI in women. This study highlights the value of δ^{15}N as a practical measure for studying the associations between traditional marine food intake and chronic disease risk in Yup'ik people, and improves our understanding of those relations.

The systematic differences in chronic disease risk factor associations between δ^{15}N and EPA are interesting given the strong association (r > 0.8) between these measures in the Yup'ik population (5,6). EPA, DHA, and high δ^{15}N values derive from traditional marine foods in this population; however, FAs (EPA, DHA) derive from the lipid portion of those foods, whereas marine protein (the source of elevated δ^{15}N) derives from the lean portion of those foods. We found that EPA was a more sensitive index related to associations of marine food intake with

TABLE 3 Regression coefficients for associations of chronic disease risk factors with 3 biomarkers of marine food intake: EPA, DHA, and δ^{15}N in Yup'ik participants in the validation sample (n = 298–363)[1]

		EPA (20:5n3)			DHA (22:6n3)			δ^{15}N		
	n	$\beta \pm$ SE	β_s[2]	P	$\beta \pm$ SE	β_s[2]	P	$\beta \pm$ SE	β_s[2]	P
BMI (kg/m^2)	363	−0.1 ± 0.2	−0.04	0.54	−0.4 ± 0.2	−0.11	0.08	0.4 ± 0.3	0.09	0.13
Body fat (%)	361	−0.1 ± 0.3	−0.01	0.82	−0.4 ± 0.3	−0.06	0.18	0.3 ± 0.3	0.04	0.35
Waist circumference (cm)	359	−0.5 ± 0.5	−0.07	0.28	−0.9 ± 0.5	−0.11	0.075	0.4 ± 0.6	0.04	0.46
SBP[3] (mm Hg)	298	−0.4 ± 0.6	−0.05	0.49	−0.1 ± 0.6	−0.01	0.89	−0.6 ± 0.7	−0.05	0.41
DBP[3] (mm Hg)	299	−0.5 ± 0.4	−0.08	0.19	0.1 ± 0.4	0.01	0.87	−0.9 ± 0.4	−0.12	0.058
TGs[3,4] (mg/dL)	344	−8.5 (−11.0, −6.0)	−0.36	<0.0001[5]	−4.0 (−6.7, −1.2)	−0.16	0.0061	−6.7 (−9.8, −3.4)	−0.23	<0.0001[5]
Total cholesterol[3] (mg/dL)	345	9.0 ± 1.7	0.35	<0.0001[5]	7.6 ± 1.5	0.29	<0.0001[5]	5.1 ± 1.9	0.16	0.0066
Quadratic		−1.7 ± 0.5	−0.18	0.0016[5]						
HDL cholesterol[3] (mg/dL)	344	2.5 ± 0.5	0.26	<0.0001[5]	1.6 ± 0.5	0.17	0.0041	1.9 ± 0.6	0.17	0.0025[5]
LDL cholesterol[3] (mg/dL)	345	8.8 ± 1.5	0.39	<0.0001[5]	7.8 ± 1.3	0.34	<0.0001[5]	5.2 ± 1.6	0.18	0.0016[5]
Quadratic		−1.6 ± 0.5	−0.19	0.0007[5]						
apo A-I (mg/dL)	361	3.1 ± 0.7	0.24	<0.0001[5]	2.3 ± 0.7	0.18	0.0022[5]	2.4 ± 0.9	0.15	0.0069
Glucose[3] (mg/dL)	354	0.4 ± 0.4	0.06	0.30	0.5 ± 0.4	0.07	0.21	0.5 ± 0.4	0.06	0.24
HbA1c[3] (%)	353	0.03 ± 0.01	0.14	0.0098	0.05 ± 0.01	0.20	0.0003[5]	0.02 ± 0.02	0.07	0.22
Insulin[3,4] (μU/mL)	355	0.4 (−2.4, 3.3)	0.01	0.80	0.9 (−2.0, 3.8)	0.03	0.54	1.7 (−1.7, 5.2)	0.05	0.33
HOMA-IR[3,4]	355	0.7 (−2.4, 3.9)	0.02	0.65	1.3 (−1.8, 4.6)	0.05	0.40	2.2 (−1.5, 6.0)	0.06	0.24
IGF-1 (μg/L)	362	−1.4 ± 3.1	−0.02	0.66	−0.2 ± 3.1	−0.003	0.95	−6.1 ± 3.6	−0.09	0.092
IGFBP-3 (μg/mL)	362	0.26 ± 0.09	0.45	0.0042	0.07 ± 0.03	0.13	0.036	1.3 ± 0.3	1.92	<0.0001[5]
Quadratic		−0.04 ± 0.01	−0.55	0.0003[5]				−0.07 ± 0.02	−2.04	<0.0001[5]
CRP[4] (mg/dL)	363	−1.3 (−2.3, −12.9)	−0.13	0.027	−1.4 (−2.5, 0.3)	−0.14	0.015	−1.2 (−2.5, 0.1)	−0.10	0.065
IL-6[4] (pg/mL)	359	2.4 (−6.6, 12.2)	0.03	0.61	2.9 (−6.1, 12.9)	0.04	0.54	21.4 (9.1, 35.0)	0.24	0.0004[5]
sTNFR2[4] (μg/L)	360	−0.002 (−0.006, 0.003)	−0.05	0.44	−0.003 (−0.007, 0.002)	−0.08	0.20	0.003 (−0.002, 0.009)	0.08	0.20
Leptin[4] (μg/L)	363	−3.0 (−5.9, 0.04)	−0.06	0.053	−1.2 (−4.3, 1.9)	−0.03	0.43	−5.7 (−9.0, −2.3)	−0.09	0.0014[5]
Adiponectin (μg/mL)	362	0.2 ± 0.1	0.07	0.23	−0.1 ± 0.1	−0.03	0.65	0.3 ± 0.2	0.10	0.070
Ghrelin (pg/mL)	361	−9.2 ± 6.1	−0.09	0.13	−2.0 ± 6.1	−0.02	0.75	−13.5 ± 7.1	−0.11	0.058

[1] Models were adjusted for age (continuous), sex, and smoking (yes/no). Models of blood pressure and biochemic risk factors were also adjusted for BMI (continuous). CRP, C-reactive protein; DBP, diastolic blood pressure; HbA1c, glycosylated hemoglobin A1c; IGF-1, insulin-like growth factor-1; IGFBP-3, insulin-like growth factor binding protein-3; SBP, systolic blood pressure; sTNFR2, soluble tumor necrosis factor receptor type 2.
[2] Standardized βs.
[3] Participants who reported taking blood pressure, cholesterol, or diabetes medication were excluded from analyses of blood pressure, blood lipid, and blood glucose/insulin variables, respectively.
[4] Variables were log transformed for analysis; estimates of β were back-transformed for ease of interpretation and were interpreted as percentage change in the chronic disease risk factor for each percentage increase in n–3 FA or ‰ increase in δ^{15}N.
[5] Associations remained statistically significant after Bonferroni-Holm correction.

blood lipid markers (e.g., HDL and LDL cholesterol, total cholesterol, TGs, and apo A-I), most likely because EPA has direct regulatory links to pathways involved in lipid metabolism (28). However, there were other associations of chronic disease risk factors with marine food intake to which δ^{15}N was more sensitive, including hormones relating to adiposity, insulin sensitivity and energy balance (leptin, adiponectin, and ghrelin), IGFBP-3, and the inflammatory cytokine IL-6. This finding raises the possibility that nutrients deriving from the lean portion of the marine diet are more directly associated with these specific chronic disease risk factors. This suggestion is supported by a recent study showing that fish intake was more strongly associated with adiponectin than equivalent long-chain n–3 PUFA intake as fish oil (29). These findings highlight the likelihood that multiple nutrients from traditional marine foods (fish and marine mammals) may be involved in chronic disease risk or protection, not simply EPA and DHA (30).

Although the results of this study indicate differences between δ^{15}N and EPA in risk factor associations, these differences do not invalidate the use of δ^{15}N to evaluate associations between marine n–3 PUFA intake and chronic disease risk in Yup'ik people. In the validation sample, δ^{15}N detected the same associations with blood lipids that EPA did, with the exception of the quadratic component of associations with total cholesterol and LDL cholesterol. However, in the larger, complete CANHR sample, δ^{15}N did detect quadratic associations with total cholesterol and HDL and LDL cholesterol, suggesting that δ^{15}N will duplicate associations between EPA and blood lipids when there is sufficient statistical power. Thus, this study provides strong support for the use of δ^{15}N as a biomarker of marine n–3 PUFA intake in studies of Yup'ik people.

The associations of marine food intake with HDL cholesterol (+) and TGs (−) have been previously described for this population (9); however, by using δ^{15}N to assess relations with chronic disease risk factors in a larger sample, we also found new associations. δ^{15}N was negatively associated with both DBP and SBP, consistent with findings from other populations based on intake of fish oils (31). δ^{15}N exhibited a strong positive quadratic association with adiponectin with an 18% increase between quartiles 3 and 4, suggesting that very high marine food intakes may be insulin-sensitizing and anti-inflammatory in this population (32–36). Because δ^{15}N was also inversely associated with leptin, there was a marked decrease in the leptin:adiponectin ratio in the highest quartile of δ^{15}N. A low leptin:adiponectin ratio is associated with improved insulin sensitivity and endothelial function (32), and is characteristic of lower risk of metabolic syndrome, independent of obesity (37,38). Thus, the association of high marine food intakes with adipokines may

TABLE 4 Associations of chronic disease risk factors with δ^{15}N, presented as least square means by quartile of δ^{15}N and regression coefficients, in Yup'ik participants in the complete CANHR sample[1]

		δ^{15}N, ‰				Linear	
	n	Quartile 1 (*n* = 193)	Quartile 2 (*n* = 195)	Quartile 3 (*n* = 193)	Quartile 4 (*n* = 192)	*β*	*P*
BMI, *kg/m*^{2}	772	28.5 ± 0.5	27.8 ± 0.4	28.0 ± 0.4	29.0 ± 0.5	0.3 ± 0.2	0.037
Body fat, *%*	768	30.1 ± 0.6	29.8 ± 0.6	30.0 ± 0.6	30.8 ± 0.6	0.4 ± 0.2	0.057
Waist circumference, *cm*	765	93.2 ± 1.1	93.2 ± 1.1	92.3 ± 1.0	94.3 ± 1.1	0.6 ± 0.4	0.12
SBP,[2] *mm Hg*	656	121.5 ± 1.1	119.8 ± 1.0	117.0 ± 1.0	117.2 ± 1.1	−1.0 ± 0.4	0.022
DBP,[2] *mm Hg*	656	73.6 ± 0.8	72.0 ± 0.8	71.3 ± 0.9	69.4 ± 0.9	−0.9 ± 0.3	0.0017[4]
TGs,[2,3] *mg/dL*	723	79.3 (74.6, 84.4)	82.5 (77.8, 87.5)	76.2 (72.1, 80.1)	68.7 (64.7, 73.0)	−5.1 (−6.8,−2.6)	<0.0001[4]
Total cholesterol,[2] *mg/dL*	731	212.3 ± 3.4	223.0 ± 3.2	235.6 ± 3.1	230.9 ± 3.2	56.1 ± 9.6	<0.0001[4]
Quadratic						−2.7 ± 0.5	<0.0001[4]
HDL cholesterol,[2] *mg/dL*	731	59.2 ± 1.2	59.5 ± 1.2	64.4 ± 1.2	65.9 ± 1.3	42.4 ± 8.4	<0.0001[4]
Quadratic						−2.0 ± 0.4	<0.0001[4]
LDL cholesterol,[2] *mg/dL*	730	133.8 ± 2.9	145.5 ± 2.8	153.7 ± 2.7	151.3 ± 2.9	13.6 ± 3.6	0.000[4]
Quadratic						−0.6 ± 0.2	0.0008[4]
apo A-I, *mg/dL*	760	163.5 ± 2.0	164.7 ± 1.9	172.7 ± 1.9	172.7 ± 2.0	19.2 ± 5.8	0.001[4]
Quadratic						−0.9 ± 0.3	0.0029[4]
Glucose,[2] *mg/dL*	749	93.1 ± 0.8	92.3 ± 0.8	94.2 ± 0.7	94.4 ± 0.7	0.3 ± 0.3	0.23
HbA1c,[2] *%*	750	5.5 ± 0.02	5.5 ± 0.03	5.5 ± 0.03	5.5 ± 0.02	0.0 ± 0.01	0.70
Insulin,[2,3] *μU/mL*	740	12.4 (11.5, 13.3)	12.2 (11.4, 13.0)	12.0 (11.3, 12.7)	11.8 (11.1, 12.7)	−1.7 (−4.2, 0.4)	0.17
HOMA-IR[2,3]	735	2.8 (2.6, 3.0)	2.7 (2.6, 2.9)	2.8 (2.6, 2.9)	2.7 (2.5, 2.9)	−1.7 (4.4, 0.6)	0.21
CRP,[3] *mg/dL*	754	0.10 (0.08, 0.12)	0.11 (0.09, 0.13)	0.09 (0.07, 0.10)	0.08 (0.07, 0.10)	−4.3 (−9.8, 1.5)	0.14
Leptin,[3] *μg/L*	761	7.2 (6.6, 7.8)	7.0 (6.5, 7.6)	6.8 (6.3, 7.4)	6.2 (5.7, 6.7)	−5.1 (−7.7, −2.4)	0.0002[4]
Adiponectin, *μg/mL*	760	9.3 ± 0.4	8.5 ± 0.4	9.4 ± 0.4	11.0 ± 0.4	−2.7 ± 1.1	0.017
Quadratic						0.2 ± 0.1	0.0027[4]
Ghrelin, *pg/mL*	760	419.2 ± 12.2	407.3 ± 11.6	408.1 ± 11.0	399.0 ± 11.8	−6.2 ± 4.1	0.13

[1] Models were adjusted for age (continuous), sex, smoking (yes/no), and BMI (continuous), with the exception of models for BMI, body fat, and waist circumference, which were not BMI-adjusted. Means of chronic disease risk biomarkers by δ^{15}N are least square means (±SEs), adjusted for age (continuous), sex, BMI (continuous), and smoking (yes/no). Geometric means (95% CIs) are given for log-transformed variables. Slope of the regression model (β) is given as β ± SE, or β (95% CI), where dependent variables were log transformed. CANHR, Center for Alaska Native Health Research; CRP, C-reactive protein; DBP, diastolic blood pressure; HbA1c, glycosylated hemoglobin A1c; SBP, systolic blood pressure.

[2] Participants who reported taking blood pressure, cholesterol, or diabetes medication were excluded from analyses of blood pressure, blood lipid, and blood glucose/insulin variables, respectively.

[3] Variables were log transformed for analysis; estimates of β were back transformed for ease of interpretation and were interpreted as percentage change in the chronic disease risk factor for each percentage increase in n–3 FA or ‰ increase in δ^{15}N.

[4] Associations remained statistically significant after Bonferroni-Holm correction.

contribute to the low prevalence of type 2 diabetes in this (20) and other native Alaskan populations (39,40). Our data suggest that δ^{15}N may be a particularly sensitive and practical marker for these associations.

A weak positive association of δ^{15}N with BMI suggests a link between traditional marine food intake and obesity in women. Traditional Yup'ik foods are high in fat and protein, and in a subset of the CANHR population (n = 531), self-reported fat intake increases from 32% to 44% of energy by quintile of traditional food intake (41). A previous study found no relation between traditional food intake and energy intake (41); however, self-reported energy intake is particularly prone to bias and misreporting (42), which could obscure a modest association. The mean BMI of women in the CANHR study is high (29.7 kg/m^2) (20), and further investigation of the dietary factors contributing to obesity in Yup'ik women is warranted.

This study has several key strengths and limitations. Intake of marine foods varies widely in the study population, creating an ideal scenario for testing associations of chronic disease risk factors with candidate biomarkers of marine food intake. However, biomarker-risk associations are likely to differ in populations with lower intakes of marine foods, and validation in a more representative population is required before applying δ^{15}N as a measure of marine food intake more broadly. The complete CANHR sample is large for a study using an objective dietary biomarker to examine diet-health relations, which gave us the power to detect new health benefits of traditional food intake. However, because this study is cross-sectional, we cannot determine causality or exclude the possibility of confounding from other dietary components or lifestyle factors (e.g., physical activity, which is known to influence HDL cholesterol) (43). Traditional marine food intake is inversely associated with intake of market foods, including sugars (44). A recent study from our group found associations of sugar intake with circulating lipids, blood pressure, adiponectin, and leptin that were the reverse of those described here (24), and it is possible that covarying sugar intake may contribute to associations with chronic disease risk. However, associations of EPA and DHA intake with blood lipids and adipokine expression have strong a priori support (45,46), and the association of marine food intake with adiponectin has a different shape and a significantly higher magnitude compared with that of sugar intake with adiponectin (24). Thus, we feel it is likely that marine food intake contributes significantly to these associations, particularly those with blood lipids and adipokines, but that further longitudinal studies will be needed to better clarify the contribution of specific nutrients to disease risk.

In summary, δ^{15}N shows similar associations with most chronic disease risk factors to RBC EPA and DHA. Associations with blood lipids and lipoproteins tended to be stronger with RBC EPA, whereas associations with blood pressure, adipokines, and

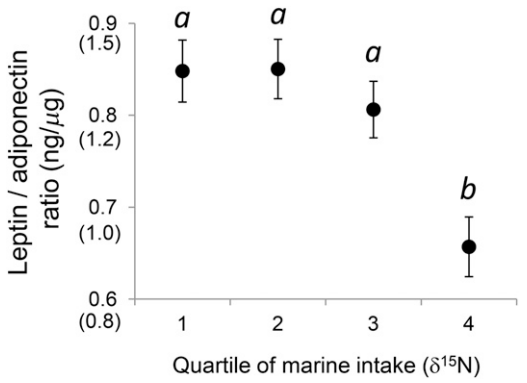

FIGURE 1 Differences in the leptin:adiponectin ratio by quartile of δ^{15}N in the complete CANHR sample of Yup'ik participants. Values are least square means ± SEs, n = 192–195, adjusted for age, sex, BMI, and smoking. Differences were tested using ANCOVA with Tukey's honestly significant difference for pairwise contrasts. Means without a common letter differ, $P < 0.05$. The lepin:adiponectin ratio was transformed as log (1 + leptin/adiponectin), and back-transformed values are given in parentheses on the y-axis. CANHR, Center for Alaska Native Health Research.

growth factors were stronger with δ^{15}N. These differences notwithstanding, the associations were similar enough for δ^{15}N to be used as a valid proxy measure of EPA and DHA intake in studies of chronic disease risk. Because measurement of δ^{15}N is relatively simple and has high throughput, it is much more feasible to apply to larger studies than measurement of RBC FAs. When we used δ^{15}N to assay marine food intake in a larger Yup'ik study population, we found new associations with blood pressure and adiponectin that were not previously detected. The finding with adiponectin is particularly significant because it may help to explain the low prevalence of type 2 diabetes in Yup'ik people despite a high prevalence of obesity.

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