Sex, Adiposity, and Hypertension Status Modify the Inverse Effect of Marine Food Intake on Blood Pressure in Alaska Native (Yup'ik) People^{1–3}

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Abstract

Background: Alaska Native people currently have a higher prevalence of hypertension than do nonnative Alaskans, although in the 1950s hypertension was rare among Alaska Native people. A novel biomarker of marine foods, the nitrogen isotope ratio ($\delta^{15}\text{N}$) in RBCs was shown to be negatively associated with systolic and diastolic blood pressure. Few studies have examined how individual characteristics modify the association of marine food intake with blood pressure.

Objective: This exploratory analysis examined whether sex, adiposity, and hypertension modify the inverse association between marine food intake and blood pressure.

Methods: We used covariate-adjusted linear models to describe the association between $\delta^{15}\text{N}$ and blood pressure in 873 adult Alaska Native (Yup'ik) people who resided in 8 communities in southwest Alaska. We separately stratified by sex, body mass index (BMI) group, abdominal obesity, and hypertension status and assessed the interaction between $\delta^{15}\text{N}$ and participant characteristics on blood pressure via likelihood ratio tests.

Results: The association between $\delta^{15}\text{N}$ and systolic blood pressure was modified by sex, BMI status, and abdominal obesity, with the inverse association observed only in the male ($\beta = -1.5$; 95% CI: $-2.4, -0.6$), nonobese BMI ($\beta = -1.7$; 95% CI: $-2.5, -1.0$), and non-abdominally obese ($\beta = -1.6$; 95% CI: $-2.4, -0.9$) strata (all P -interaction < 0.0001). A reduction in diastolic blood pressure associated with $\delta^{15}\text{N}$ was observed in the nonobese BMI ($\beta = -1.1$; 95% CI: $-1.7, -0.5$) and non-abdominally obese ($\beta = -1.1$; 95% CI: $-1.7, -0.5$) strata, although only the interaction between BMI group and $\delta^{15}\text{N}$ with diastolic blood pressure was significant. The inverse association between $\delta^{15}\text{N}$ and both systolic and diastolic blood pressure was observed in nonhypertensive individuals, although the comparison had limited power. The results were consistent with those identified by using combined RBC concentrations of eicosapentaenoic acid and docosahexaenoic acid as the biomarker of marine food intake, although the associations identified by using $\delta^{15}\text{N}$ were larger.

Conclusions: Obesity status modified the inverse association between marine food intake and both systolic and diastolic blood pressure in adult Alaska Native (Yup'ik) people. The inverse association between $\delta^{15}\text{N}$ and systolic blood pressure was also modified by sex. *J Nutr* 2015;145:931–8.

Keywords: biomarker, effect modification, hypertension, indigenous people, marine food intake

Introduction

Alaska Native people currently have a higher prevalence of hypertension than do nonnative Alaskans (1), although in the 1950s hypertension was rare among Alaska Native people (2, 3).

Increased access to market foods has substantially affected the nutritional intake of Alaska Native people. For example, in the Yup'ik people, one of the most numerous of the Alaska Native groups, macronutrient intake has shifted from a traditional dietary pattern of low carbohydrate, high protein, and high fat to a high-carbohydrate pattern that is similar to that observed in

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³ Supplemental Tables 1 and 2 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at <http://jn.nutrition.org>.

a US national sample (1, 4, 5). The Center for Alaska Native Health Research (CANHR), established in 2001, has been investigating protective factors and correlates of chronic metabolic disease risk in the Yup'ik people of southwest Alaska (6).

CANHR's efforts have been focused primarily in the Yukon-Kuskokwim Delta region of Alaska, where there are ~23,000 Yup'ik individuals inhabiting 58 rural villages, none of which are accessible by roads (4). The Yup'ik diet includes a combination of traditional and Western foods (7), with traditional foods contributing 22% of total calories (5). Marine food sources, primarily fish and seal oil, contributed 69% of energy intake to the traditional component of the Yup'ik diet (5).

CANHR has been developing objective biomarkers of traditional intake based on stable isotope ratios in order to reduce the bias and error associated self-reported measures (8, 9). The nitrogen stable isotope ratio of ^{15}N to ^{14}N ($\delta^{15}\text{N}$; as defined in Methods) in RBCs is a specific marker of marine protein and has been shown to be a reliable marker of overall marine food intake. RBC $\delta^{15}\text{N}$ is significantly correlated with self-reported measures of marine food intake as well as with RBC concentrations of EPA (20:5n-3) and DHA (22:6n-3) ($r = 0.83$ and 0.75 , respectively) (4, 6). The use of $\delta^{15}\text{N}$ instead of EPA and DHA as a biomarker of marine intake has the advantage of having higher throughput and lower cost.

Our group previously evaluated the association between 3 biomarkers of marine food intake, specifically RBC EPA, DHA, and $\delta^{15}\text{N}$ and systolic and diastolic blood pressure in a cohort of Yup'ik people (10). $\delta^{15}\text{N}$ was shown to be inversely associated with systolic ($\beta = -0.97$, $P = 0.02$) and diastolic ($\beta = -0.92$, $P \leq 0.01$) blood pressure; EPA exhibited similar associations. This study did not, however, examine whether the association differed by individual characteristics such as sex, obesity, and clinical hypertensive status.

Multifactorial diseases occur in a complex biological context, and there is an increasing awareness of the importance of considering underlying biology when examining specific exposure-disease relations. Indeed, several recent studies highlighted effect modification in the relation between various predictors and disease outcomes (11–15). It is well known that the risk of hypertension varies with sex and obesity (15–20), and it is plausible that the observed benefit of marine food intake may differ by these characteristics. For example, the effects of EPA and DHA (10) may noticeably affect blood pressure in nonobese individuals, but may be overwhelmed by the milieu of metabolic dysfunction that accompanies obesity and therefore not have an impact in this context. Studies also suggest that the benefits of marine intake may vary by hypertension status (21, 22). Thus, the objective of the current analysis was to examine whether the inverse association between marine food intake and blood pressure is modified by sex, adiposity, and clinical hypertension status.

Methods

Participant recruitment and procedures. Data are from the CANHR study, a cross-sectional, community-based participatory research study aimed at understanding the interaction of biological, genetic, nutritional, and psychosocial risk and protective factors for obesity and related disease in the Yup'ik people (23). The CANHR study protocol was approved by the University of Alaska Institutional Review Board, the National and Alaska Area Indian Health Service Institutional Review Board, and the Yukon-Kuskokwim Health Corporation Human Studies Committee.

The study protocol was described previously (4, 24). In brief, between 2003 and 2008, a convenience sample of men and women were recruited from 8 communities in the Yukon-Kuskokwim River Delta region of southwest Alaska. A total of 1003 individuals were enrolled in the study. Interested residents were invited to the local community center or other community building, where they completed a general interview covering demographic characteristics, educational level, ethnicity, and medical history. Clinical examinations were conducted for each participant to obtain anthropometric measurements (25). Blood samples were collected by venipuncture after at least 8 h of fasting, and certified interviewers conducted 24-h nutritional recall interviews ($n = 547$).

Biochemical assessment and dietary biomarker measurements. Blood samples were processed locally into serum, lymphocyte, and RBC fractions by using a portable centrifuge and stored at -15°C in a portable freezer. Within 6 d, the samples were shipped to the University of Alaska Fairbanks and stored at -80°C (10). Blood samples were analyzed to obtain serum values for total cholesterol, HDL cholesterol, LDL cholesterol, glucose, and TGs, as described elsewhere (25).

RBC FAs were extracted with isopropanol and chloroform by using the procedure developed by Rose and Oklander (26), and FAMES were analyzed by GC-LC, as described previously (27), to obtain individual FA concentrations, specifically DHA and EPA. RBC FA measurements were available for 497 of 1003 participants enrolled in the study.

The measurement of RBC stable nitrogen isotope ratios was completed at the Alaska Stable Isotope Facility at the University of Alaska Fairbanks, as described elsewhere (28, 29). Stable isotope ratios at natural abundance are expressed in permil (‰) abundance of ^{15}N relative to an international standard: $\delta^{15}\text{N} = ((^{15}\text{N}/^{14}\text{N})_{\text{sample}} - (^{15}\text{N}/^{14}\text{N})_{\text{standard}}) / (^{15}\text{N}/^{14}\text{N})_{\text{standard}} \cdot 1000\text{‰}$, where the standard is atmospheric nitrogen ($^{15}\text{N}/^{14}\text{N} = 0.003677$) (28, 30). Stable isotope ($\delta^{15}\text{N}$) measurements were available for all 1003 participants who completed the CANHR 1 study (28).

Anthropometric measurements. Trained staff completed anthropometric measurements with the use of protocols established by the NHANES III Anthropometric Procedures Manual, as described by Boyer et al. (24). Weight was measured to the nearest 0.1 kg. Standing height measurements were obtained to the nearest 1/8 inch. BMI was calculated by dividing weight (kg) by height (m^2). Waist circumference measurements were obtained twice, immediately below the lateral portion of the rib cage, by using a Guillick II 150-cm tape measure (Rolyn Prest) with a tensioning device. If the difference between repeated measurements exceeded 2.0 cm, a third measurement was taken and recorded. Resting systolic and diastolic blood pressure values were measured 3 times after a 5-min rest period by using an Omron Hem-907 (Omron Healthcare) automated system; reported values are the mean of the 3 measurements (4, 24).

Dietary assessment. Nutritional intake was assessed by using 24-h recall interviews ($n = 547$), as described in detail elsewhere (4, 24). In brief, participants were instructed to list all food and beverages consumed in a 24-h period, and the lead nutritionist reviewed all responses and followed up as necessary. Portion sizes were estimated by using methods developed by the Fred Hutchinson Cancer Research Center (4). A demographic/medical questionnaire was also administered that included questions on tobacco use.

Nutrient analysis was conducted by using the University of Minnesota's Nutrition Data System for Research (NDS-R; software version 4.06, 2003) Food and Nutrient Database, which contains most traditional foods. If foods were missing from the databases, similar food items were substituted when appropriate or the missing item was added to the database. Appropriate calculations were made to obtain values for total fat intake (g/d), total energy intake (kJ), and total n-3 FA intake.

Inclusion criteria. Nonnative individuals ($n = 17$) and participants who reported taking antihypertensive medications ($n = 128$) were excluded from the analysis, leaving 873 participants in the analysis. Nutritional intake information and RBC FA measurements were available for 466 and 451 of the 873 participants eligible for inclusion in the study,

respectively. In addition, participants who were unable to recall ≥ 1 meals during the 24-h recall period ($n = 8$) and participants with improbable reported intake values ($n = 27$) were excluded from univariate analysis of the cohort's nutrient intake; 4 participants with a total fat intake of >325 g/d and 23 participants with a caloric intake >5000 kcal/d or <600 kcal/d were excluded.

Statistical analysis. BMI (in kg/m^2) groups were established according to WHO guidelines: <18.5 = underweight, 18.5 – 24.9 = normal weight, 25.0 – 29.9 = overweight, and ≥ 30.0 = obese (31). Abdominal obesity was defined by using the WHO recommendations: ≥ 101.6 cm for men and ≥ 88.9 cm for women (32). Participants were classified as hypertensive according to the guidelines established by the NIH, National Heart, Lung, and Blood Institute: individuals having a systolic blood pressure >140 mm Hg or a diastolic blood pressure >90 mm Hg (33). We classified a participant as a tobacco user if he or she responded “yes” to any of the following 4 questions included in the demographic/medical questionnaire:

- 1) Have you smoked at least 100 cigarettes in your entire life?
- 2) Do you smoke regularly (>1 cigarette a day for the past 3 mo)?
- 3) Do you use chewing tobacco such as Redman Garret or *iqmik* (a form of smokeless tobacco used among Alaska Native people) (34)?
- 4) Do you use snuff such as Skoal or Copenhagen?

The primary outcomes of interest were systolic and diastolic blood pressure and the primary predictor of interest was the stable nitrogen isotope ratio ($\delta^{15}\text{N}$). To identify potential confounders, we first estimated unadjusted bivariate associations between the stable isotope ratio ($\delta^{15}\text{N}$) and participant characteristics including age and sex, anthropometric measurements, educational level, variables of nutritional intake, and blood pressure.

On the basis of these results, we constructed linear regression models with systolic or diastolic blood pressure as the outcomes, $\delta^{15}\text{N}$ as the primary predictor, and age (continuous), sex, BMI (continuous), and educational level (continuous) as covariates. We conducted sensitivity analyses to evaluate the impact of tobacco use, total fat intake, total caloric intake, and RBC total cholesterol by individually adding these into the adjusted models. We also examined the potential nonlinear association of $\delta^{15}\text{N}$ with systolic and diastolic blood pressure by modeling $\delta^{15}\text{N}$ quartile groups with the first quartile (0–25%) as the reference group. We subsequently conducted a test for trend by modeling the $\delta^{15}\text{N}$ quartiles as a single ordinal predictor and conducting a Wald test on the coefficient.

We conducted additional analysis to evaluate the interactions of participant characteristics and the stable isotope ratio on both systolic and diastolic blood pressure. We separately stratified the study cohort by sex, BMI group (excluding underweight), abdominal obesity, and clinical hypertension status. We then constructed adjusted linear regressions to evaluate the association between $\delta^{15}\text{N}$ and blood pressure by stratum. We performed a likelihood ratio test to evaluate the interaction of $\delta^{15}\text{N}$ and each stratified variable (e.g., sex). In sensitivity analyses, each model was subsequently adjusted for cholesterol intake, total fat intake, tobacco use, and combined RBC concentration of EPA and DHA.

Last, we examined whether the results we observed using $\delta^{15}\text{N}$ were consistent with those found using combined RBC concentration of EPA and DHA as the predictor of interest. We repeated the adjusted linear regressions as well as the likelihood ratio tests described in the preceding paragraph, replacing $\delta^{15}\text{N}$ with the combined RBC concentration of EPA and DHA.

All statistical tests were evaluated with a $P = 0.05$ significance level. All statistical analysis was performed by using Statistical Analysis Software (version 9.3i; SAS Institute).

Results

Participant characteristics

Of the 873 participants included in our analysis, 56% were overweight or obese, 47.5% were male, and 7% were classified

as clinically hypertensive on the basis of their measured systolic or diastolic blood pressure. Participant characteristics stratified by BMI group, sex, and hypertensive status are shown in Table 1 and Supplemental Tables 1 and 2, respectively. As shown in Table 1, self-reported energy and fat intake did not differ by weight status. The mean biomarkers of marine food intake ($\delta^{15}\text{N}$, RBC DHA, and RBC EPA) were significantly higher in overweight and obese participants than in those of normal weight. The mean systolic and diastolic blood pressures were also higher in overweight and obese participants.

Men had a lower mean BMI than women (25.7 vs. 28.3) (Supplemental Table 1). On average, men had lower measured $\delta^{15}\text{N}$ and RBC DHA and higher self-reported total energy and fat intake. Mean systolic blood pressure was higher among men than among women, whereas mean diastolic blood pressure was similar in both sexes.

Hypertensive participants had a mean BMI that was 2.4 units higher than nonhypertensive participants. They also had a mean waist circumference that was 7.1 cm larger. Although there was no difference in self-reported energy and fat intake by hypertensive status, hypertensive participants had higher mean $\delta^{15}\text{N}$ and RBC DHA and EPA concentrations than did nonhypertensive participants.

Association of $\delta^{15}\text{N}$ and blood pressure: complete cohort

After adjustments for sex, age (continuous), BMI (continuous), and education (continuous), both $\delta^{15}\text{N}$ and combined RBC EPA and DHA were negatively associated with diastolic and systolic blood pressure (Table 2). The association between $\delta^{15}\text{N}$ and blood pressure was not significantly altered after adjustment for tobacco use, self-reported total fat intake and total energy intake, and RBC total cholesterol (data not shown).

Quartiles of $\delta^{15}\text{N}$ were linearly associated with systolic blood pressure (P -trend < 0.01); participants in the fourth quartile of $\delta^{15}\text{N}$ had a systolic blood pressure that was 3.8 mm Hg lower than those in the first quartile (95% CI: -6.5 , -1.2 mm Hg). The linearity of the association between $\delta^{15}\text{N}$ and diastolic blood pressure was less prominent (P -trend < 0.05).

Effect modification

$\delta^{15}\text{N}$ and systolic blood pressure. The association between $\delta^{15}\text{N}$ and systolic blood pressure was modified by BMI and abdominal obesity status, sex, and hypertensive status (Table 3). Although each permil increase in $\delta^{15}\text{N}$ was associated with a -1.5 to -1.7 mm Hg lower systolic blood pressure in normal-weight and overweight participants, there was no association in obese participants (P -interaction < 0.0001). Similarly, in non-abdominally obese participants, each permil increase in $\delta^{15}\text{N}$ was associated with a -1.6 mm Hg (95% CI: -2.4 , -0.9 mm Hg) difference in mean systolic blood pressure, whereas there was no observed association in abdominally obese individuals (P -interaction < 0.0001). The association of $\delta^{15}\text{N}$ with systolic blood pressure also varied substantially by sex: each permil increase in $\delta^{15}\text{N}$ was associated with a 1.5-mm Hg decrease (95% CI: -2.4 , -0.6 mm Hg) in mean systolic blood pressure in men, although no association was observed in women (P -interaction < 0.0001). In analyses stratified by participant hypertension status, $\delta^{15}\text{N}$ showed an inverse association in nonhypertensive individuals only (P -interaction < 0.05); each permil increase in $\delta^{15}\text{N}$ was associated with a -0.6 mm Hg (95% CI: -1.2 , 0.0 mm Hg) difference in mean systolic blood pressure in this stratum.

TABLE 1 Participant characteristics of adult Alaska Native (Yup'ik) people¹

	Overall (n = 873)	Normal weight (n = 356)	Overweight (n = 271)	Obese (n = 221)
Demographic characteristics				
Age, y	34.4 ± 15.7	29.4 ± 15.3	37.1 ± 15.1***	38.5 ± 13.6***
Tobacco users, ² n (%)	742 (85.0)	293 (82.3)	238 (87.8)	191 (86.4)
Education: last grade completed, y	10.5 ± 2.6	10.3 ± 2.3	10.7 ± 2.7	10.8 ± 2.5*
Anthropometric measurements				
Weight, kg	70.7 ± 15.8	59.8 ± 7.7	71.2 ± 7.8***	89.1 ± 15.5***
Height, m	1.62 ± 0.08	1.63 ± 0.08	1.62 ± 0.08**	1.60 ± 0.09***
Waist circumference, cm	88.6 ± 14.2	77.5 ± 5.7	89.6 ± 6.8***	106.7 ± 11.5***
BMI, ³ kg/m	27.1 ± 5.8	22.4 ± 1.6	27.2 ± 1.4***	34.9 ± 4.8***
Nutritional intake ³				
Energy, kJ/d	8310 ± 3600	8330 ± 3450	8160 ± 3940	8130 ± 3350
Total fat, g/d	81.6 ± 50.4	76.6 ± 46.0	85.3 ± 55.2	85.8 ± 50
n-3 FA, g/d	5.0 ± 7.8	3.8 ± 5.0	6.5 ± 10.8**	5.0 ± 6.1
RBC analytes				
Cholesterol, ⁴ mg/dL	212 ± 46.2	200 ± 45.2	222 ± 47.1***	221 ± 41.6***
δ ¹⁵ N, ⁴ ‰	9.0 ± 1.5	8.6 ± 1.3	9.2 ± 1.5***	9.3 ± 1.7***
DHA, ⁵ % total FAs	6.2 ± 1.8	5.8 ± 1.7	6.4 ± 1.8**	6.5 ± 1.9**
EPA, ⁵ % total FAs	2.3 ± 1.8	1.9 ± 1.4	2.6 ± 1.9***	2.6 ± 1.9***
Blood pressure, mm Hg				
Diastolic blood pressure	69.9 ± 9.8	66.2 ± 8.7	70.2 ± 9.2***	76.0 ± 8.9***
Systolic blood pressure	118 ± 13.3	116 ± 12.7	119 ± 12.3*	121 ± 14.1***

¹ Values are means ± SDs unless otherwise indicated. Two-sample, 2-tailed *t* tests were performed to assess the significance of differences in participant characteristics of both overweight and obese strata relative to normal weight. *****P* < 0.001, ****P* < 0.01, ***P* < 0.05. δ¹⁵N, nitrogen stable isotope ratio.

² We classified a participant as a tobacco user if they responded "yes" to any of the 4 questions concerning tobacco use that were included in the demographic/medical questionnaire.

³ Values based on 431 participants with 24-h recall data (35 participants excluded for unreliable or implausible values).

⁴ Values based on 862 participants with RBC stable isotope measurements.

⁵ Values based on 451 participants with individual RBC FA data.

δ¹⁵N and diastolic blood pressure. The association between δ¹⁵N and diastolic blood pressure was modified by obesity status (Table 3). Each permil increase in δ¹⁵N was associated with a −1.1-mm Hg (95% CI: −1.9, −0.3 mm Hg) and a −1.0-mm Hg (95% CI: −1.9, −0.1 mm Hg) difference in normal-weight and overweight participants, respectively, whereas no association was observed in obese participants (*P*-interaction < 0.05). In addition, an inverse association between δ¹⁵N and diastolic blood pressure was observed only in the non-abdominally obese stratum, although the interaction did not reach statistical significance. When stratifying by hypertension status, δ¹⁵N

showed an inverse association in nonhypertensive individuals only, although the interaction was not statistically significant. No interaction was observed when examining participants stratified by sex.

Adjustment for RBC concentrations of EPA and DHA attenuated the associations in the stratified subgroups. With the exception of diastolic blood pressure in non-abdominally obese participants, the association between δ¹⁵N and blood pressure was not significant in all subgroups, although the directions of the associations were consistent (data not shown).

TABLE 2 Association between δ¹⁵N percentiles and blood pressure in adult Alaska Native (Yup'ik) people¹

Predictor	Systolic blood pressure		Diastolic blood pressure	
	Change (mm Hg) per unit of predictor	<i>P</i> -trend	Change (mm Hg) per unit of predictor	<i>P</i> -trend
RBC EPA and DHA ² (%)	−0.4 (−0.9, 0.1)		−0.3 (−0.6, 0.1)	
δ ¹⁵ N (‰)	−0.9 (−1.6, −0.3)		−0.6 (−1.1, −0.2)	
δ ¹⁵ N quartiles				
1 (<7.83‰)	1.0		1.0	
2 (7.83‰ to <8.71‰)	−0.6 (−2.9, 1.6)		−0.4 (−2.0, 1.3)	
3 (8.71–9.86‰)	−2.0 (−4.4, 0.4)		−0.1 (−1.9, 1.6)	
4 (>9.86‰)	−3.8 (−6.5, −1.2)	<0.01	−2.3 (−4.2, −0.3)	<0.05

¹ Values are β-coefficients (95% CIs) from linear regression models with systolic or diastolic blood pressure as the outcome and predictors that included δ¹⁵N or δ¹⁵N quartiles, age (continuous), sex, BMI (continuous), and educational level (continuous); *n* = 873. *P* values were from a test for linear trend where δ¹⁵N quartiles were fit as a single ordinal predictor. δ¹⁵N, nitrogen stable isotope ratio.

² Values based on 451 participants with individual RBC FA data.

TABLE 3 Associations of $\delta^{15}\text{N}$, a marker of marine food intake, and blood pressure in adult Alaska Native (Yup'ik) people¹

Stratified analysis	Systolic blood pressure	Diastolic blood pressure
Sex		
Men (<i>n</i> = 408)	−1.5 (−2.4, −0.6)	−0.7 (−1.4, 0.0)
Women (<i>n</i> = 449)	−0.6 (−1.5, 0.3)	−0.6 (−1.2, 0.0)
<i>P</i> -interaction ²	<0.0001	0.4
BMI group ³		
Normal (<i>n</i> = 356)	−1.5 (−2.6, −0.5)	−1.1 (−1.9, −0.3)
Overweight (<i>n</i> = 269)	−1.7 (−2.8, −0.5)	−1.0 (−1.9, −0.1)
Obese (<i>n</i> = 221)	0.3 (−0.9, 1.5)	0.1 (−0.7, 1.0)
<i>P</i> -interaction	<0.0001	< 0.05
Abdominally obese ⁴		
Not obese (<i>n</i> = 585)	−1.6 (−2.4, −0.9)	−1.1 (−1.7, −0.5)
Obese (<i>n</i> = 272)	0.0 (−1.2, 1.2)	0.0 (−0.8, 0.9)
<i>P</i> -interaction	<0.0001	0.4
Hypertensive ⁵		
No (<i>n</i> = 796)	−0.6 (−1.2, 0.0)	−0.7 (−1.1, −0.2)
Yes (<i>n</i> = 61)	0.1 (−2.0, 2.1)	0.7 (−0.9, 2.3)
<i>P</i> -interaction	<0.05	0.2

¹ Values are β -coefficients (95% CIs) from linear regression models, stratified by participant characteristics (as listed) with systolic blood pressure as the outcome and predictors that included $\delta^{15}\text{N}$, age (continuous), education (continuous), sex (where applicable), and BMI [(continuous); where applicable]; *n* = 873. $\delta^{15}\text{N}$, nitrogen stable isotope ratio.

² *P*-interaction values were derived from a likelihood ratio test of interaction.

³ BMI (in kg/m²) groups were defined according to WHO guidelines: <18.5 = underweight, 18.5–24.9 = normal weight, 25.0–29.9 = overweight, and ≥ 30.0 = obese; underweight participants were excluded from the analysis.

⁴ Abdominal obesity was defined by using the WHO recommendations: ≥ 101.6 cm for men and ≥ 88.9 cm for women.

⁵ Participants having a systolic blood pressure >140 mm Hg or a diastolic blood pressure >90 mm Hg were classified as hypertensive.

Associations between combined RBC concentration of EPA and DHA and blood pressure

The results observed by using $\delta^{15}\text{N}$ were consistent with those obtained by using the combined RBC concentration of EPA and DHA as our predictor of interest (Table 4). An inverse association between combined RBC concentration of EPA and DHA and systolic blood pressure was observed in the male (*P*-interaction < 0.001) and non-abdominally obese (*P*-interaction < 0.01) strata. No interaction was observed when examining participants stratified by BMI group, although the direction of the association is consistent with that observed with $\delta^{15}\text{N}$. No significant effect modification was observed when examining the association between combined RBC concentration of EPA and DHA and diastolic blood pressure, although the direction of the associations was consistent with those obtained by using $\delta^{15}\text{N}$.

Discussion

The nitrogen stable isotope ratio is a marker of marine protein intake, which is correlated with intakes of EPA, DHA, and marine foods. Given its broad association with marine food intake, $\delta^{15}\text{N}$ could capture aspects of marine intake beyond marine protein, such as other macro- and micronutrients. We observed an inverse association between marine food intake and blood pressure, which was modified by participant characteristics. Specifically, the inverse association of marine food intake with systolic blood pressure was observed only in male, nonobese, and nonhypertensive participants, and the inverse

effect on diastolic blood pressure was observed only in nonobese individuals.

Clinical trials in general US and European populations (21, 22, 35) as well as with the observational findings from the Genetic of Coronary Artery Disease in Alaskan Natives study (36) found a protective association between marine food intake and blood pressure, although few studies explored whether this association is modified by participant characteristics.

In this Yup'ik study population, the inverse association between marine food intake and diastolic blood pressure was observed in both men and women. However, marine food intake was associated only with systolic blood pressure in men. Surprisingly, modification of the association between marine food intake and blood pressure by sex has been largely unexplored in the literature, and many relevant clinical trials focus only on men (21, 22). In a meta-regression analysis by Geleijnse et al. (37) of 36 trials, the association of fish oil with systolic and diastolic blood pressure was not statistically different in trials that included men and women compared with those that included only men; however, the authors stated that they could not effectively evaluate effect modification by sex because of the lack of randomized trials in women and the lack of sex-stratified results in trial reports. A study in an adult Inuit population (*n* = 1861) from Greenland did look at marine food intake and blood pressure stratified by sex (38). In this study population, whole-blood mercury concentration (another biomarker of marine food intake) was associated with a decrease in diastolic blood pressure in men, whereas no significant association

TABLE 4 Associations of combined RBC concentrations of EPA and DHA and blood pressure in adult Alaska Native (Yup'ik) people¹

Stratified analysis	Systolic blood pressure	Diastolic blood pressure
Sex		
Men (<i>n</i> = 408)	−0.7 (−1.4, 0.0)	−0.5 (−1.0, 0.0)
Women (<i>n</i> = 449)	−0.2 (−0.8, 0.5)	−0.1 (−0.5, 0.4)
<i>P</i> -interaction ²	<0.001	0.9
BMI group ³		
Normal (<i>n</i> = 356)	−0.7 (−1.5, 0.1)	−0.5 (−1.2, 0.1)
Overweight (<i>n</i> = 269)	−0.5 (−1.3, 0.3)	−0.4 (−1.0, 0.2)
Obese (<i>n</i> = 221)	0.3 (−0.7, 1.2)	0.1 (−0.5, 0.7)
<i>P</i> -interaction	0.2	0.2
Abdominally obese ⁴		
Not obese (<i>n</i> = 585)	−0.7 (−1.2, −0.1)	−0.5 (−0.9, −0.1)
Obese (<i>n</i> = 272)	0.0 (−0.9, 0.9)	0.0 (−0.6, 0.6)
<i>P</i> -interaction	<0.01	0.8
Hypertensive ⁵		
No (<i>n</i> = 796)	−0.2 (−0.6, 0.1)	−0.3 (−0.6, 0.1)
Yes (<i>n</i> = 61)	−0.1 (−2.0, 1.8)	0.0 (−1.1, 1.1)
<i>P</i> -interaction	<0.05	0.1

¹ Values are β -coefficients (95% CIs) from linear regression models based on 451 participants with individual RBC FA data, stratified by participant characteristics (as listed) with systolic blood pressure as the outcome and predictors that included RBC EPA and DHA intake, age (continuous), education (continuous), sex (where applicable), and BMI [(continuous); where applicable]; total *n* = 873.

² *P*-interaction values were derived from a likelihood ratio test of interaction.

³ BMI (in kg/m²) groups were defined according to WHO guidelines: <18.5 = underweight, 18.5–24.9 = normal weight, 25.0–29.9 = overweight, and ≥ 30.0 = obese; underweight participants were excluded from the analysis.

⁴ Abdominal obesity was defined by using the WHO recommendations: ≥ 101.6 cm for men and ≥ 88.9 cm for women.

⁵ Participants having a systolic blood pressure >140 mm Hg or a diastolic blood pressure >90 mm Hg were classified as hypertensive.

was observed among women; the authors did not conduct a statistical test for interaction.

The biological mechanism by which marine food intake could differentially affect men and women is unknown. It is possible that EPA- and DHA-mediated release of the vasodilator NO may vary by sex, as was observed in previous studies in rabbits (39) and rats (40, 41). Studies by McCulloch and Randall (42), Villar et al. (43), Scotland et al. (44), and Pak et al. (45) suggest that NO is the predominant mediator of vasodilation in men, whereas vasodilation in women seems to be primarily regulated by endothelium-derived hyperpolarizing factor (EDRF). If marine food intake differentially affects NO and EDRF release, then it is possible that marine intake may differentially affect blood pressure in males and females.

In this study population, the inverse association between marine food intake and systolic blood pressure was observed only in individuals with nonobese BMIs and waist circumferences compared with those with obese levels of overall and central adiposity. Although most studies on marine food intake and blood pressure controlled for adiposity, we are not aware of any other studies that tested for effect modification by obesity status. The Geleijnse et al. (37) meta-regression analysis on fish oil and blood pressure attempted to explore this question by comparing 20 trials in participants with a BMI >26.8 to 7 trials in participants with a BMI ≤26.8; they did not observe a significant difference in effect size between these 2 groups of trials. Although provocative, this meta-regression analysis is limited by its inability to examine associations on an individual level. Effect modification by obesity status is plausible given that adipose tissue produces adipokines and inflammatory cytokines, including IL-6 (46) and adiponectin (47), which affect vasoconstriction (48). It is possible that these adipose-derived products modify the association between marine food intake and blood pressure. In addition, obesity is a strong independent risk factor for hypertension (15, 19, 20) and the harmful effects of obesity on blood pressure may overwhelm the inverse effects of increased marine food intake.

In this study, we observed an inverse association between marine food intake and blood pressure in nonhypertensive individuals. An association in nonhypertensive individuals has been identified in a number of randomized controlled trials, although several studies observed a larger effect of n-3 supplementation in hypertensive individuals (21, 22, 37). Although we only observed the inverse association of marine food intake with blood pressure in nonhypertensive individuals, we cannot rule out an inverse association in hypertensive individuals given the limited number of individuals with clinical hypertension ($n = 61$) included in our analysis.

This study has several key strengths and limitations. The unique study population had 50-fold variability in PUFA intake and the mean intake of EPA and DHA was 20 times higher than that of the general US population (49), allowing for the identification of novel findings that could broaden our understanding of the incidence of hypertension in general. Moreover, we used an objective biomarker of marine food intake to explore effect modification by individual characteristics. Our study was limited by its observational design, and thus we could not determine causality or exclude the possibility of confounding from other dietary components that are associated with a traditional lifestyle. Moreover, the small number of uncontrolled hypertensive individuals in our study cohort limited our power to examine the association between marine food intake and blood pressure in this group. In addition, although $\delta^{15}\text{N}$ is highly correlated with overall marine food

intake, our study could not specifically explore which nutritional components of marine food intake are protective. In addition, future work should assess the impact of other components of the Yup'ik diet (e.g., berry consumption, sodium intake) and lifestyle factors (e.g., level of physical activity) that may confer additional health benefits and/or potentially confound our observed associations. Given the high incidence and lack of variability in tobacco use in our study cohort, we were also unable to appropriately evaluate the effect of tobacco use on the associations we observed. Our findings need to be replicated in other cohorts of Alaska Native people, and clinical prospective studies could be conducted within this population. Future studies should also examine whether $\delta^{15}\text{N}$ interacts with obesity and hypertension to determine other outcomes, including blood TGs, cholesterol, insulin, and glucose.

Our findings have important implications for both our general understanding of dietary effects on hypertension and Alaska Native health. Our results suggest that the large amount of marine foods in the traditional Yup'ik diet has beneficial effects on blood pressure, and this inverse association may be most pronounced in nonobese individuals. Importantly, we also observed an inverse association between marine food intake and blood pressure in nonhypertensive individuals, a group that incurs the majority of cardiovascular events (50). The modifications we observed underline the importance of considering effect modification in future studies in the field.

In conclusion, we found that the inverse association of marine food intake with systolic and diastolic blood pressure was greater in nonobese individuals. We also found a larger inverse association between marine food intake and systolic blood pressure in men than in women.

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