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Serum individual non-esterified fatty acids and risk of heart failure in older adults

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Abstract

Background: Heart failure (HF) is highly prevalent among older adults and is associated with high costs. Although serum total non-esterified fatty acids (NEFAs) have been positively associated with HF risk, the contribution of each individual NEFA to HF risk has not been examined.

Objective: To examine the association of individual fasting NEFAs with HF risk in older adults.

Methods: In this prospective cohort study of older adults, we measured 35 individual NEFAs in 2,140 participants of the Cardiovascular Health Study using gas chromatography. HF was ascertained using review of medical records by an endpoint committee.

Results: Mean age was 77.7±4.4 years and 38.8% were men. During a median follow-up of 9.7 years (maximum 19.0), 655 new cases of HF occurred. In a multivariable Cox regression model controlling for demographic and anthropometric variables, field center, education, serum

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albumin, glomerular filtration rate, physical activity, alcohol consumption, smoking, hormone replacement therapy, unintentional weight loss, and all other measured NEFAs, we observed inverse associations [HR (95% CI) per standard deviation] of non-esterified pentadecanoic (15:0)[0.73 (0.57-0.94)], ɤ-linolenic (GLA) [0.87 (0.75-1.00)], and docosahexaenoic (DHA) [0.73 (0.61-0.88)] acids with HF and positive associations of non-esterified stearic (18:0) [1.30 (1.04-1.63)] and nervonic (24:1n-9) [1.17 (1.06-1.29)] acids with HF.

Conclusion: Our data are consistent with a higher risk of HF with non-esterified stearic and nervonic acids and a lower risk with non-esterified 15:0; GLA; and DHA in older adults. If confirmed in other studies, specific NEFAs may provide new targets for HF prevention.

Keywords

Epidemiology; heart failure; risk factors; fatty acids

Introduction

With improved life expectancy and a growing aging US population, heart failure (HF) remains a major public health issue[1]. Approximately 5 million Americans live with HF and each year nearly one million Americans are diagnosed with HF[1]. The prevalence of HF is expected to continue to rise, partly due to improved survival after myocardial infarction, a shift in demographics to an older population, and increase prevalence of type 2 diabetes (T2D) and obesity (two important risk factors for HF[2-7]). Furthermore, HF remains one of the leading causes of office visits among older adults and is associated with a high mortality[8-10]. This underscores the need for identification of novel and modifiable risk factors that can be used to improve prevention of HF. Non-esterified fatty acids (NEFAs) are byproducts of lipolysis[11] and can impair insulin signaling in skeletal muscle, promote hepatic gluconeogenesis, and reduce insulin secretion[12, 13], with subsequent development of T2D. NEFAs can also impair endothelial function[14] and might contribute to ischemic cardiomyopathy. Our group[15] has previously shown that serum total NEFAs were associated with a higher risk of T2D[16] and HF[17].Because total serum NEFAs comprise >30 individual fatty acids with various carbon lengths and degree of unsaturation, it is unclear whether the observed elevated risk of HF with serum total NEFAs results from equal contribution of each individual NEFA or from a subset of individual NEFAs. For example, myocardium preferentially uses long-chain esterified/non-esterified fatty acids to meet energy demand via beta-oxidation[18]. Animal experiments have shown that beta-oxidation of long-chain monounsaturated fatty acids can cause cardiotoxicity[19]. In humans, phospholipid source of nervonic acid (24:1n-9) has been positively associated with cardiovascular risk factors and with a higher risk of HF,[20] while very long-chain saturated fatty acids (20:0, 22:0, and 24:0) have been associated with a lower risk of HF[21]. No previous study has examined the association of non-esterified nervonic acid or other individual NEFAs with HF. Identification of specific NEFAs that play a major role in the development of HF could provide potential new targets for future dietary and/or pharmacologic interventions. Hence, we sought to comprehensively examine this gap using an agnostic approach to test the primary hypothesis that some but not all individual NEFAs are associated with HF risk in older adults. In a secondary analysis, we tested the hypothesis that the association of individual NEFAs with

HF is stronger when restricted to the first five years of follow up as previously reported between serum total NEFAs and incidence of diabetes and HF in the same cohort[15, 17].

Materials and methods

We used data collected on participants of the Cardiovascular Health Study (CHS), which is a prospective cohort consisting of 5,888 men and women aged 65 years and older who were selected for recruitment from four US communities (Forsyth County, NC; Washington County, MD; Sacramento County, CA; and Pittsburgh, PA). A description of design, methods, and procedures in the CHS has been previously published[22]. Briefly, 5,201 adults aged ≥65 years were enrolled between 1989 and 1990. In addition, a supplemental cohort of 687 predominantly African American adults was recruited between 1992 and 1993 from three of the same communities (excepting Washington County). Of the 4,413 participants who attended the 1996-1997 visit (year 9), the current study focused on 2,402 CHS participants who underwent an oral glucose tolerance test at the 1996-97 examination to characterize both individual fasting NEFAs and post OGTT NEFAs. Of the 2,402 people with OGTT, we selected 2,145 participants with unthawed fasting and post-OGTT serum samples due to sensitivity of individual NEFAs to thaw-freeze cycles. After excluding specimens that were oxidized or hemolyzed (n=5), fasting NEFA measurements were available on 2,140. In this report, we focused on fasting NEFAs and we excluded participants with prevalent HF (n =137), resulting in a final analysis sample of 2,003 participants. The institutional review board of each participating center approved the study, and all participants gave informed written consent to participate in the study. A separate exemption approval to use de-identified archived serum samples to measure individual NEFAs was obtained from the Tufts University/Tufts Medical Center Institutional Review Board.

Serum lipids were extracted using previously described techniques[23, 24] and the NEFA subfraction was separated via solid-phase chromatography using aminopropyl column[25], followed by methylation to form fatty acid methyl esters[24, 26]. We used an Autosystem XL gas chromatogram (Perkin Elmer, Boston, MA) equipped with a 100 m x 0.25 mm capillary column (HP INNOWax, Agilent Technologies, CA) to separate the fatty acid methyl esters. We identified 35 fatty acids using authenticated standards (NuCheck Prep, MN). Inter-assay coefficients of variation were 0.5% to 4.3% for fatty acids with concentrations >5 mol%; 1.8% to 7.1% for those present at concentrations between 1-5 mol%; and 2.8% to 11.1% for those present at concentrations <1 mol%. All measurements for NEFAs were completed at the Jean Mayer USDA Human Nutrition Research Center on Aging, Tufts University, Boston, MA.

HF events were adjudicated by the CHS Events Committee as previously described[27, 28]. Briefly, HF validation required symptoms (shortness of breath, fatigue, orthopnea, paroxysmal nocturnal dyspnea); clinical signs including pulmonary rales, peripheral edema, gallop rhythm, and displaced left ventricular apical impulse; pulmonary edema and increasing cardiomegaly on chest X-rays; and use of diuretics, digitalis, or vasodilators for HF treatment. Incident HF was ascertained upon review of pertinent data on hospitalization or outpatient visits such as medical history, physical examination, report of chest X-ray,

and medications. HF sub-type (reduced or preserved ejection fraction) was based on left ventricular ejection fraction (<45% and ≥ 45%, respectively).

We collected self-reported information on age, sex, ethnicity, educational attainment, smoking status, unintentional weight-loss >10 pounds, health status, hormone replacement therapy (HRT), and alcohol consumption. Leisure-time activity (kcal/week) was assessed with a modified Minnesota Leisure-Time Activities questionnaire[29]. Weight, waist circumference, and height were measured using standardized protocols. Serum albumin was measured using standard methods. Hypertension was defined as systolic blood pressure ≥ 140 mm Hg, diastolic blood pressure ≥ 90 mm Hg, or physician diagnosis of hypertension plus treatment with blood pressure lowering medications. T2D was defined if a participant was using insulin or oral hypoglycemic agents; had a fasting glucose level of ≥ 7 mmol/L (126 mg/dL) or a non-fasting glucose level of ≥ 11.1 mmol/L (200 mg/dl). Glomerular filtration rate was estimated based on cystatin C as previously described[30].

We used generalized additive models to test for departures from linearity for the association of each individual NEFA with incident HF. Only one individual NEFA (20:5n-3) showed evidence of non-linearity in a model without adjustment for the other NEFAs; however, in the model with adjustment for other individual NEFAs, there was little evidence of meaningful non-linearity. Therefore, we present only the estimates from the linear model for simplicity and clarity. We used Cox proportional hazards regression that included all 35 NEFAs to estimate hazard ratios with 95% confidence intervals for incident HF associated with a 1 standard deviation (SD) increment in each NEFA. Time at risk was calculated as the interval in days from the date of the 1996-97 visit to the earliest of: date of incident HF, date of death, date of loss to follow-up, or administrative censoring date (June 2015). Model 1 adjusted for age, sex, race, field center, and all other NEFAs. Model 2 controlled for all variables in model 1 plus education, serum albumin, body mass index, waist circumference, estimated glomerular filtration rate using cystatin, physical activity, alcohol, smoking status, HRT, and unintentional weight loss. We also examined additional control for potential intermediate factors including prevalent diabetes, coronary artery disease, atrial fibrillation, hypertension, and use of blood pressure medications using model 2. We found no departure from proportional hazards; however, because our previous analysis of total NEFAs and HF showed a stronger association within the first 5 years of follow-up[17], we conducted a secondary analysis in the present study limiting follow-up to the first 5 years. Additional secondary analyses included association of fasting serum total NEFAs measured on samples collected during 1996-97 study visit with HF risk. Finally, we conducted sensitivity analyses excluding 370 people with prevalent coronary artery disease and excluding the first two years of follow up.

Results

The mean age of the 2,003 study participants at baseline (1996-97) was 77.7±4.4 years, 38.8% were men, and 14.2% were black. Table 1 presents baseline characteristics by quartiles of non-esterified nervonic acid. Higher serum concentration of nervonic acid was associated with older age, female sex, high blood pressure, and high alcohol consumption (Table 1). Mean (SD) of the most abundant individual NEFAs (μmol/L) were 150.0 (62.9)

for 18:1n-9 (oleic); 124.0 (44.5) for 16:0 (palmitic); 78.7 (32.5) for 18:2n-6 (linoleic); and 60.3 (17.2) for 18:0 (stearic)[Supplemental Table 1]. During a median follow up of 9.4 years, 655 (32.7%) participants developed HF, including 152 cases with reduced and 250 with preserved left ventricular ejection fraction. In a multivariable-adjusted Cox regression model that included all 35 NEFAs, each SD increment of non-esterified stearic acid (18:0) was associated with a 30% higher risk of HF (95% CI: 4% to 63%) , adjusting for age, sex, race, field center, education, serum albumin, body mass index, waist circumference, glomerular filtration rate based on cystatin C, leisure time physical activity, alcohol, smoking status, HRT, unintentional weight loss, and all other NEFAs (Table 2); corresponding values for non-esterified nervonic acid (24:1n-9) was 17% (95% CI: 6% to 29%), Table 2. In contrast, non-esterified pentadecanoic acid (15:0), GLA (18:3n-6), and DHA (22:6n-3) were associated with a 27% (95% CI: 6-43%), 13% (95% CI: 0-25%), and 27% (95% CI: 12-39%) lower risk of HF, respectively, in a fully adjusted model (Table 2). Additional adjustment for potential mediators (type 2 diabetes, hypertension and blood pressure lowering medications, CHD, and atrial fibrillation) did not materially alter the results [HR (95% CI) per SD increment: 0.78 (0.60-1.10) for 15:0; 1.30 (1.04-1.63) for 18:0; 1.16 (1.05-1.28) for 24:1n-9; 0.86 (0.74-1.00) for 18:3n-6; and 0.72 (0.60-0.87) for 22:6n-3. In a secondary analysis restricted to the first five years of follow up, we observed associations of non-esterified pentadecanoic acid (15:0) [HR per SD: 0.55 (95% CI: 0.36-0.85)] and non-esterified nervonic acid [HR per SD: 1.24 (95% CI: 1.08-1.42)] with HF in a multivariable adjusted model (Supplemental Table 2). Additionally, we observed associations of individual NEFAs with HF that were not apparent with the longer follow-up period (supplemental Table 2). Exclusion of the first two years of follow up, or of 370 participants with prevalent coronary artery disease did not alter the conclusions. For example, for individual DHA (22:6n-3), [HR (95% CI) per SD increment went from 0.73 (0.61-0.88) in main analysis to 0.73 (0.60-0.89) after exclusion of the first 2 years of follow up and 0.74 (0.60-0.92) after exclusion of prevalent CAD. Corresponding values for stearic acid (18:0) were 1.30 (1.04-1.63); 1.35 (1.06-1.71); and 1.34 (1.03-1.74). In exploratory analyses, fasting serum total NEFAs (per SD increment) measured contemporaneously with individual NEFAs was not associated with HF risk (multivariable adjusted HR: 1.00 (95% CI: 0.91-1.09). Lastly, examination of HF subtypes showed similar direction of effect sizes albeit lack of precision due to limited cases of HF with preserved (n=250) and reduced (n=152) ejection fraction (Supplemental Table 3).

Discussion

In this large prospective study of US older adults, we found a higher risk of HF with two individual NEFAs (stearic and nervonic acids) and a lower risk with three NEFAs (pentadecanoic acid, GLA, and DHA) in a multivariable adjusted model. Of these five NEFAs associated with HF, only two (pentadecanoic and nervonic acids) showed associations when follow up was restricted to the first five years of the study in a secondary analysis.

To the best of our knowledge, this is the first study to comprehensively examine the association of individual NEFAs with HF risk in a large prospective cohort of older adults. These findings extend the interpretation of our previous work reporting a positive

association of serum total NEFAs with HF risk[17] in the same cohort of older adults by suggesting that evaluation of the association of serum total NEFAs with HF risk may depend on the relative composition of individual NEFAs and direction of those associations (i.e., lower HF risk with 15:0,18:3n-6, and 22:6n-3 versus higher risk with 24:1n-9 and 18:0 NEFAs). Furthermore, assessment of individual NEFAs can help identify a sub-class of NEFAs associated with higher versus lower risk of HF and inform future dietary and/or pharmacological approaches to alter serum concentrations of those specific NEFAs for HF benefit. Although we observed new individual NEFAs associated with HF when analysis was restricted to the first 5 years of follow up, these findings are only exploratory and merit confirmation in future studies with adequate data to evaluate the impact of updating NEFAs over time on HF outcome. If confirmed in future studies, these findings might provide an opportunity to develop (i) new pharmacologic interventions that can alter the concentration of nervonic acid for example, or (ii) new dietary recommendations to shift the balance between protective (i.e., DHA, pentadecanoic, gamma-linolenic acids) and deleterious (i.e. stearic and nervonic acids) NEFAs to protect against HF.

In two large prospective cohorts, phospholipid very long-chain monounsaturated fatty acids were positively associated with cardiovascular risk factors and with a higher incidence of HF[20]; comparing the highest to the lowest quintile of plasma phospholipid nervonic acid (24:1n-9), multivariable adjusted hazard ratios were 1.75 (95% CI: 1.23–2.50) in the Cardiovascular Health Study (CHS) and 1.92 (95% CI: 1.22–3.03) in the Atherosclerosis Risk in Communities (ARIC) Study[20]. It is important to acknowledge that we cannot directly compare associations of phospholipid fraction with NEFAs as former tend to reflect longer term intake while NEFAs reflect a balance between lipolytic factors (i.e., catecholamines) and inhibitors of lipolysis (i.e., insulin or adiponectin). Nonetheless, it is important to note that nervonic acid from two different compartments (phospholipid vs. non-esterified fractions) showed similar association with HF. Of note is that very long-chain fatty acids including nervonic acid (24:1n-9) are predominantly oxidized in peroxisomes, instead of mitochondria and a lack of membrane-transporting enzymes for very long-chain fatty acids in peroxisomes[31, 32] can lead to accumulation of reactive oxygen species and other metabolites from beta-oxidation with subsequent apoptosis[33, 34]. Lower risk of HF observed with non-esterified DHA is consistent with observational and clinical trial data relating red blood cell membrane, phospholipid marine omega-3 fatty acids[35], or EPA/DHA supplements with HF[36].

The current study has some limitations including the observational study design which cannot preclude unmeasured or residual confounding as source of bias. Second, we only had one measurement of individual NEFAs at baseline and were not able to account for changes in serum NEFAs over time. Third, our cohort consisted of older adults with mean age of 77 years, thereby limiting generalizability of our findings to the general population. Fourth, we had limited statistical power and precision to evaluate associations of individual NEFAs with heart failure type (preserved vs. reduced ejection fraction). Fifth, we did not adjust for multiple comparisons, and our findings should be considered hypothesis-generating. Despite these limitations, our study has numerous strengths including the novel aspect of examining association of individual NEFAs with HF risk; availability of data on major confounding factors; relatively long follow up in CHS; multi-center design with participants

from different geographic regions of the US; standardized approach to adjudicate HF by CHS Endpoint Committee; and objective and accurate measurement of individual NEFAs with excellent coefficients of variation for individual NEFAs with high concentrations.

Given the current burden of HF in the US, our data provide preliminary evidence for potential novel targets for pharmacological and/or dietary intervention to modify HF risk factors and with the ultimate goal to prevent development of HF. Thus, replication of our findings while addressing some of the shortcomings outlined above is warranted.

In conclusion, we have demonstrated in this large prospective study of older adults, that individual NEFAs are differentially associated with HF risk. Specifically, we showed that higher serum levels of non-esterified DHA, GLA, and pentadecanoic acid were associated with lower incident HF risk, while higher levels of non-esterified stearic and nervonic acids were associated with a higher risk of HF.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1.

Characteristics of CHS participants by quartiles of non-esterified nervonic acid (24:1n-9)

Characteristic	Quartiles of serum concentration of 24:1n-9 (umol/L)			
	≤0.27	>0.27-0.32	>0.32-0.39	>0.39
Age (y)	77.2 ± 4.0	77.6 ± 4.3	78.0 ± 4.6	78.2 ± 4.7
Male	208 (41.5%)	178 (35.5%)	192 (38.3%)	200 (40.0%)
Black race	71 (14.2%)	75 (15.0%)	65 (13.0%)	74 (14.8%)
Field center				
North Carolina	146 (29.1%)	128 (25.5%)	103 (20.6%)	86 (17.2%)
California	153 (30.5%)	142 (28.3%)	129 (25.7%)	155 (31.0%)
Maryland	85 (17.0%)	97 (19.4%)	108 (21.6%)	113 (22.6%)
Pennsylvania	117 (23.4%)	134 (26.7%)	161 (32.1%)	146 (29.2%)
Educational attainment				
<High school	104 (20.8%)	102 (20.4%)	111 (22.2%)	104 (20.8%)
High school	129 (25.9%)	150 (30.1%)	147 (29.4%)	148 (29.6%)
>High school	266 (53.3%)	247 (49.5%)	242 (48.4%)	248 (49.6%)
Smoking status				
Never	234 (46.7%)	222 (44.3%)	206 (41.1%)	216 (43.2%)
Former	228 (45.5%)	252 (50.3%)	256 (51.1%)	249 (49.8%)
Current	39 (7.8%)	27 (5.4%)	39 (7.8%)	35 (7.0%)
Alcoholic drinks/week				
0	301 (60.3%)	288 (57.7%)	265 (52.9%)	240 (48.1%)
1-6	142 (28.5%)	158 (31.7%)	161 (32.1%)	153 (30.7%)
7-14	37 (7.4%)	27 (5.4%)	43 (8.6%)	63 (12.6%)
>14	19 (3.8%)	26 (5.2%)	32 (6.4%)	43 (8.6%)
Physical activity, kcals/week	1487 ± 1910	1392 ± 1712	1272 ± 1389	1374 ± 1723
Body mass index (kg/m2)	27.1 ± 4.3	26.7 ± 4.0	27.0 ± 4.7	25.9 ± 4.5
Waist circumference, cm	97.5 ± 12.3	96.0 ± 11.9	96.4 ± 12.9	95.0 ± 13.4
Systolic BP (mm Hg)	136.5 ± 20.1	134.7 ± 19.6	137.3 ± 20.3	138.3 ± 21.1
Cholesterol (mg/dl)	194.4 ± 37.4	203.6 ± 37.3	206.5 ± 38.6	209.3 ± 39.6
Albumin (g/dl)	3.7 ± 0.3	3.8 ± 0.3	3.8 ± 0.3	3.8 ± 0.3
eGFR-cystatin	72.3 ± 17.1	73.0 ± 19.2	72.3 ± 17.8	72.3 ± 19.3
C-reactive protein (mg/L)	4.3 ± 7.8	4.0 ± 7.1	4.6 ± 7.6	4.7 ± 8.6
NTproBNP, pg/ml	279.3 ± 387.4	240.6 ± 299.7	316.0 ± 502.7	347.3 ± 658.4
Prevalent diabetes	28 (5.6%)	22 (4.4%)	31 (6.2%)	33 (6.6%)
Hypertension	297 (59.4%)	293 (58.6%)	290 (58.2%)	308 (61.7%)
Anti-hypertensive medication	257 (51.3%)	262 (52.3%)	245 (49.0%)	250 (50.1%)
Aspirin use > 2 days in 2 weeks	216 (44.1%)	204 (41.3%)	194 (39.2%)	173 (35.2%)
Unintentional weight loss	20 (4.3%)	14 (3.0%)	27 (5.7%)	30 (6.3%)
Self-reported health				

Characteristic	Quartiles of serum concentration of 24:1n-9 (umol/L)			
Excellent	38 (7.6%)	20 (4.0%)	38 (7.6%)	21 (4.2%)
Very good	159 (31.7%)	147 (29.3%)	131 (26.2%)	141 (28.2%)
Good	218 (43.5%)	246 (49.1%)	246 (49.2%)	246 (49.2%)
Fair	84 (16.8%)	86 (17.2%)	80 (16.0%)	84 (16.8%)
Poor	2 (0.4%)	2 (0.4%)	5 (1.0%)	8 (1.6%)

Table 2.

Hazard ratio (95% CI) of incident heart failure per SD increment of individual NEFAs

	Model 1		Model 2	
NEFA, μmol/L	**Hazard ratio (95% CI)**	**p**	**Hazard ratio (95% CI)**	**p**
12:0	0.99 (0.90-1.08)	0.76	0.97 (0.88-1.07)	0.56
14:0	1.15 (0.86-1.55)	0.35	1.08 (0.79-1.48)	0.62
15:0 Pentadecanoic acid	**0.72 (0.57-0.92)**	**0.01**	**0.73 (0.57-0.94)**	**0.01**
16:0	1.12 (0.83-1.49)	0.46	1.02 (0.74-1.41)	0.90
18:0 stearic acid	**1.18 (0.96-1.45)**	**0.13**	**1.30 (1.04-1.63)**	**0.02**
20:0	0.98 (0.84-1.14)	0.77	0.92 (0.78-1.08)	0.30
22:0	1.03 (0.94-1.13)	0.57	1.01 (0.91-1.12)	0.89
24:0	0.98 (0.89-1.07)	0.59	0.98 (0.90-1.07)	0.65
14:1n-5	0.97 (0.75-1.25)	0.83	0.92 (0.71-1.20)	0.55
16:1n-9	1.21 (0.94-1.56)	0.14	1.15 (0.88-1.51)	0.29
16:1n-7	0.98 (0.71-1.37)	0.92	1.19 (0.84-1.69)	0.32
18:1n-9 oleic acid	0.65 (0.40-1.05)	0.08	0.69 (0.42-1.14)	0.15
18:1n-7	1.27 (0.88-1.85)	0.20	1.05 (0.71-1.56)	0.82
20:1n-9	0.91 (0.71-1.16)	0.44	0.95 (0.74-1.21)	0.67
22:1n-9	1.00 (0.92-1.10)	0.94	1.00 (0.91-1.10)	0.97
24:1n-9 nervonic acid	**1.12 (1.06-1.19)**	**0.0002**	**1.17 (1.06-1.29)**	**0.0018**
18:2n-6	1.28 (0.94-1.75)	0.12	1.16 (0.85-1.60)	0.35
18:3n-6 ɣ-linolenic acid	**0.85 (0.75-0.97)**	**0.01**	**0.87 (0.75-1.00)**	**0.05**
20:2n-6	0.93 (0.80-1.06)	0.27	0.96 (0.83-1.10)	0.55
20:3n-6	1.11 (0.93-1.33)	0.26	1.09 (0.91-1.32)	0.35
20:4n-6	0.87 (0.75-1.02)	0.08	0.89 (0.75-1.04)	0.14
22:4n-6adrenic acid	1.07 (0.99-1.16)	0.07	1.08 (0.99-1.17)	0.07
22:5n-6	0.98 (0.86-1.12)	0.78	1.00 (0.87-1.14)	0.97
18:3n-3	0.88 (0.72-1.08)	0.23	0.98 (0.80-1.20)	0.84
18:4n-3	1.03 (0.93-1.13)	0.59	1.01 (0.91-1.12)	0.90
20:5n-3 EPA	1.16 (1.01-1.32)	0.04	1.15 (0.99-1.33)	0.06
22:5n-3 DPA	1.11 (0.90-1.38)	0.34	1.19 (0.95-1.50)	0.14
22:6n-3 DHA	**0.75 (0.63-0.89)**	**0.0015**	**0.73 (0.61-0.88)**	**0.0011**
16:1n-9T	0.86 (0.68-1.08)	0.20	0.83 (0.64-1.06)	0.14
16:1n-7T	1.14 (0.90-1.44)	0.28	1.13 (0.88-1.45)	0.33
18:1n10-12T	1.25 (0.96-1.62)	0.10	1.19 (0.91-1.57)	0.21
18:1n-9T	1.03 (0.81-1.30)	0.83	1.09 (0.85-1.39)	0.51
18:1n-7T	0.86 (0.68-1.08)	0.19	0.86 (0.67-1.10)	0.23
18:2T*	0.97 (0.90-1.06)	0.53	0.98 (0.90-1.07)	0.61
18:2CLA	0.98 (0.87-1.11)	0.79	1.02 (0.89-1.17)	0.74

Model 1 adjusts for age, sex, race, field center, and other NEFAs (n=2003)

Model 2 adjust for Model 1 covariates plus education, serum albumin, BMI, waist circumference, estimated glomerular filtration rate using cystatin, physical activity, alcohol, smoking status, HRT, unintentional weight loss, and other NEFAs (n=1849); EPA: eicosapentaenoic acid; DPA: docosapentaenoic acid; DHA: docosahexaenoic acid