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Associations of obesity with triglycerides and C-reactive protein are attenuated in adults with high red blood cell eicosapentaenoic and docosahexaenoic acids

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Abstract

Background—N-3 fatty acids are associated with favorable, and obesity with unfavorable, concentrations of chronic disease risk biomarkers.

Objective—We examined whether high eicosapentaenoic (EPA) and docosahexaenoic (DHA) acid intakes, measured as percentages of total red blood cell (RBC) fatty acids, modify associations of obesity with chronic disease risk biomarkers.

Methods—In a cross-sectional study of 330 Yup'ik Eskimos, generalized additive models (GAM) and linear and quadratic regression models were used to examine associations of BMI with biomarkers across RBC EPA and DHA categories.

Results—Median (5^{th}–95^{th} percentile) RBC EPA and DHA were 2.6% (0.5–5.9%) and 7.3% (3.3–8.9%), respectively. In regression models, associations of BMI with triglycerides, glucose, insulin, C-reactive protein (CRP) and leptin differed significantly by RBC EPA and DHA. The GAM confirmed regression results for triglycerides and CRP: At low RBC EPA and RBC DHA, the predicted increases in triglycerides and CRP concentrations associated with a BMI increase from 25 to 35 were 99.5±45.3 mg/dl (106%) and 137.8±71.0 mg/dl (156%), respectively, for triglycerides and 1.2±0.7 mg/l (61%) and 0.8±1.0 mg/l (35%), respectively, for CRP. At high RBC

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Conflict of Interest
The authors declare no conflict of interest.

EPA and RBC DHA, these predicted increases were 13.9±8.1 mg/dl (23%) and 12.0±12.3 mg/dl (18%), respectively, for triglycerides and 0.5±0.5 mg/l (50%) and −0.5±0.6 mg/l (−34%), respectively, for CRP.

Conclusions—In this population, high RBC EPA and DHA were associated with attenuated dyslipidemia and low-grade systemic inflammation among overweight and obese persons. This may help inform recommendations for n-3 fatty acid intakes in the reduction of obesity-related disease risk.

Keywords

EPA; DHA; generalized additive models; Yup'ik Eskimos; triglycerides; C-reactive protein

Introduction

The very long chain n-3 polyunsaturated fatty acids eicosapentaenoic acid (EPA; 20:5(n-3)) and docosahexaenoic acid (DHA; 22:6(n-3)), found primarily in fish and marine mammals, are associated with reduced risks of cardiovascular disease (CVD) (Kris-Etherton *et al.*, 2002) and possibly diabetes (Nettleton and Katz, 2005). EPA and DHA have important biological functions including increased membrane fluidity, which affects nutrient transport and signal transduction (Alexander, 1998), eicosanoid metabolism (Fritsche, 2006) and gene expression and transcription (Sampath and Ntambi, 2005). In particular, high EPA and DHA intakes are associated with reduced concentrations of triglycerides (Block *et al.*, 2008; Bonaa *et al.*, 1992; Dewailly *et al.*, 2001a; Dewailly *et al.*, 2001b; Dewailly *et al.*, 2002; Ferrucci *et al.*, 2006; He *et al.*, 2008; Motoyama *et al.*, 2009; Okuda *et al.*, 2005), C-reactive protein (CRP) (Farzaneh-Far *et al.*, 2008; Klein-Platat *et al.*, 2005; Niu *et al.*, 2006) and proinflammatory cytokines, and increased concentrations of high-density lipoprotein (HDL) (Block *et al.*, 2008; Dewailly *et al.*, 2001a; Dewailly *et al.*, 2001b; Dewailly *et al.*, 2002; He *et al.*, 2008; Motoyama *et al.*, 2009; Okuda *et al.*, 2005) and anti-inflammatory cytokines (Fritsche, 2006). Most of these biomarkers are also associated, albeit in the opposite direction, with obesity (Pi-Sunyer, 2002), which raises the question of whether the associations of obesity with chronic disease risk biomarkers could be modified by high EPA and DHA intakes.

To address this question, we analyzed data from Yup'ik Eskimos living in the Yukon Kuskokwim Delta region in Southwest Alaska, a population with mean (±SD) EPA and DHA intakes >20 times the current mean intakes of the general US population (4.1±0.5 g/d versus 0.05 g/d in men and 2.8±0.3 g/d versus 0.09 g/d in women) (Bersamin *et al.*, 2008; Johnson *et al.*, 2009) and obesity prevalence similar to that of the overall US population (Mohatt *et al.*, 2007). Results of this study may provide evidence useful to developing recommendations for EPA and DHA intakes in the reduction of obesity-related disease risk.

Materials and methods

Participant recruitment and procedures

Data are from the Center for Alaska Native Health Research study, a cross-sectional community-based participatory study of obesity and disease risk in Yup'ik Eskimos. Study protocols were approved by the Institutional Review Boards of the University of Alaska, the National and Alaska Area Indian Health Service and the Yukon Kuskokwim Health Corporation Human Studies Committee.

Participant recruitment and procedures are described elsewhere (Boyer *et al.*, 2005; Mohatt *et al.*, 2007). In brief, between 2003 and 2006, 1003 participants aged ≥14 y and residing in

10 Southwest Alaskan communities were enrolled. Extensive socio-demographic and health-related data were collected through in-person interviews and self-administered questionnaires. Diet was assessed using a 24-h dietary recall and a 3-d food record. Height, weight, percent body fat (through bioelectrical impedance) and blood pressure were measured. Physical activity was measured using pedometers (counts/h). Blood samples collected in the field were separated into serum, plasma and packed RBC, frozen and stored locally at −20°C for up to 6 d and transferred to a central location and stored at −80°C.

Study sample

Blood samples in this study were from a subset of 497 participants selected from 7 out of the 10 participating communities. From 4 communities, we selected a random sample of 84 participants balanced across age strata (14–19, 20–49 and ≥50 y). The remaining 3 communities had <84 participants and thus all participants were included. We excluded 92 participants aged ≤18 y, 28 with CRP>1 mg/dl (acute inflammation), 20 with missing data on biochemical measurements, 10 with BMI<20 and 17 with BMI>40, leaving 330 for the present analyses.

Biochemical measurements

Disease risk measures examined in this study included: systolic blood pressure (SBP); diastolic blood pressure (DBP); triglycerides; low-density lipoprotein (LDL); HDL; total cholesterol; apolipoprotein A1 (apoA1); glucose; insulin; homeostasis model assessment of insulin resistance (HOMA-IR) index; insulin-like growth factor-I (IGF1); insulin-like growth factor binding protein-3 (IGFBP3); CRP; interleukin-6 (IL6); soluble tumor necrosis factor receptor-2 (sTNFR2); leptin; and adiponectin. Triglycerides, HDL, total cholesterol and apoA1 were measured with the Poly-Chem System Chemistry Analyzer (Polymedco Inc., Cortlandt Manor, NY). Intra- and inter-assay coefficients of variation (CV) were, respectively: 3.2% and 4.1% for triglycerides; 3.6% and 3.9% for HDL; 1.8% and 6.2% for total cholesterol; and 2.7% and 4.5% for apoA1. LDL was calculated as: total cholesterol −HDL−(triglycerides/5). Leptin, total adiponectin and fasting insulin were assayed using human-specific radioimmunoassay kits (Linco Research Inc., St. Charles, MO). Intra- and inter-assay CV were, respectively: 6.6% and 12.0% for leptin; 5.1% and 9.1% for adiponectin; and 5.8% and 10.2% for insulin. Fasting blood glucose was measured with a Cholestech LDX analyzer (Hayward, CA). Insulin resistance was assessed using the HOMA-IR index: (fasting insulin (μU/ml)×fasting glucose (mg/dl))/405 (Matthews *et al.*, 1985). CRP was measured with an Immulite Analyzer and high sensitivity CRP reagents (Diagnostic Products Corporation, Los Angeles, CA). Manufacturer's intra-and inter-assay CV were 2.8% and 3.3%, respectively. IGF1, IGFBP3, IL6 and sTNFR2 were assayed using ELISA kits (Biosource, Carlsbad, CA and Diagnostic Systems Laboratories Inc, Webster, TX). Intra- and inter-assay CV were, respectively: 6.5% and 5.4% for IGF1; 8.8% and 10.0% for IGFBP3; 6.4% and 7.8% for IL6; and 4.2% and 3.3% for sTNFR2.

RBC fatty acid measurements

RBC fatty acids were analyzed as previously described (O'Brien *et al.*, 2009). Briefly, fatty acids were extracted from washed RBC with 2-propanol and chloroform (Rose and Oklander, 1965). Fatty acids were converted to fatty acid methyl esters (FAME) by direct transesterification (Lepage and Roy, 1986). FAME were recovered in hexane, dried under nitrogen (40°C) and re-dissolved in hexane for gas chromatography analysis. The FAME of individual fatty acids were separated on a gas chromatograph (Model 5890B, Hewlett-Packard (HP), Agilent, Santa Clara, CA) equipped with a flame ionization detector, automatic sampler (HP 7673), electronic pressure programming (HP) and Chemstation software (HP). Quantitative precision and identification were evaluated using model mixtures of known FAME and an established control pool. The inter-assay CV was 2.7% for

EPA and 2.0% for DHA. RBC EPA and DHA composition is reported as a weight percentage of total RBC fatty acids.

Statistical analyses

Triglycerides, HOMA-IR index, CRP, IL6, sTNFR2 and leptin concentrations were log-transformed. IL6 values (n=90) below the limit of detection (0.02 pg/ml) were replaced with $0.02/\sqrt{2}$ (Hornung and Reed, 1990). Outliers for triglycerides (n=1), glucose (n=1), insulin (n=1), adiponectin (n=1), apoA1 (n=2) and sTNFR2 (n=3) were excluded because they were >4 SD above the mean and were physiologically unreasonable.

Participants' demographic and health-related characteristics are given for the entire sample and stratified by BMI (kg/m^2): <25 (normal-weight), 25–30 (overweight) and ≥30 (obese). Values are presented as means (±SD) or proportions, which were compared across BMI categories using ANOVA *F*-test and χ^2 test, respectively.

Age- and sex-adjusted means of biomarkers stratified by BMI were computed using least square means. Adjusted means are presented with SE and log-transformed means are back-transformed for ease of interpretation and given with 95% CI. Tests for significant linear trends across BMI categories were based on age- and sex-controlled linear regression models.

We used three approaches to describe the associations of BMI with disease biomarkers at different RBC EPA and DHA levels. First, we used linear regression to test whether the BMI-biomarker associations differed significantly across the following RBC EPA and DHA categories: <25th percentile, 25th–75th percentiles and >75th percentile (<1.5%, 1.5–3.9% and >3.9% for EPA; <5.8%, 5.8–8.2% and >8.2% for DHA). Because we could not assume linear associations of BMI with disease biomarkers, we examined both linear and quadratic models in which the *F*-test for the interaction of BMI with RBC EPA and DHA categories was used to determine statistical significance. We report the linear models for all biomarkers and the quadratic models only when statistically significant. Second, we used non-parametric generalized additive models (GAM) (Wood, 2006) that included a two-dimensional thin-plate regression spline over BMI and RBC EPA and DHA to graphically display the BMI-biomarker associations across RBC EPA and DHA. GAM enable the fitted associations to take their natural shapes by relaxing assumptions about the form of the functional associations. To illustrate typical BMI-biomarker associations for the three RBC EPA and DHA categories, we present the estimated two-dimensional covariate-adjusted GAM at the 10th, 50th and 90th percentiles of RBC EPA (1%, 3% and 5%) and DHA (4%, 7% and 9%). Third, based on GAM, we predicted mean concentrations (±SE) of biomarkers at the 10th and 90th percentiles of RBC EPA and DHA for BMI 25, 30 and 35 using simple case resampling over 1000 bootstrap replicates. All models were adjusted for age (continuous), sex and current smoking (yes/no). Models for IGF1 were additionally adjusted for IGFBP3. We also considered whether control for RBC n-6 fatty acids, dietary macronutrient intake and physical activity would modify interactions between BMI and RBC EPA and DHA, because these can affect disease biomarkers. However, control for these variables did not affect results and these are therefore not included in final models. Alcohol consumption is prohibited in the participating Yup'ik communities and was therefore not considered as a potential covariate.

We report results for EPA and DHA separately for two reasons. First, DHA intake is very high in this population and while RBC EPA increases linearly in response to dietary intake, RBC DHA plateaus at ≈9% of total fatty acids (O'Brien *et al.*, 2009), suggesting that RBC DHA does not reflect variability at high intake (Arterburn *et al.*, 2006; Cao *et al.*, 2006).

Second, previous studies reported differential associations of EPA and DHA with disease biomarkers (Egert *et al.*, 2009; Leigh-Firbank *et al.*, 2002; Mori *et al.*, 2000). We also present results for the n-3 index (RBC EPA+DHA) (Harris, 2009) as supplementary material.

Statistical analyses were performed using Stata/SE 11.0 (StataCorp LP, College Station, TX) and the R package mgcv version 1.4-0 (R-Project, 2009).

Results

Overall, the median age was 45.5 y, 57% were women and 71% were overweight or obese. RBC EPA and DHA ranged from 0.2% to 9.6% and 1.6% to 10.3%, respectively, with medians (5^{th}–95^{th} percentile) of 2.6% (0.5–5.9%) and 7.3% (3.3–8.9%). Women were more likely to be overweight and obese than men and smokers were less likely to be obese than non-smokers. There was a significant trend for increased BMI with increasing age. Dietary energy, total fat, carbohydrate, protein, EPA and DHA intakes, the means of RBC EPA and DHA and n-3 index and the proportions of persons in each RBC EPA and DHA and n-3 index category did not differ significantly across BMI groups (Table 1).

BMI was positively associated with higher SBP, DBP, triglycerides, glucose, insulin, HOMA-IR index, CRP and leptin, and negatively associated with HDL, apoA1 and adiponectin. Mean LDL, total cholesterol, IGF1, IL6 and sTNFR2 concentrations did not differ significantly by BMI (Table 2).

Associations of BMI with disease biomarkers across RBC EPA and DHA

Based on either the linear or quadratic models (models 1 or 2 in Table 3, respectively), the associations of BMI with triglycerides, CRP, glucose and insulin differed significantly by both RBC EPA and DHA; associations with leptin differed only by RBC DHA. To best display these associations, Figure 1 and Supplementary Figure 1 show the GAM for the BMI-biomarker associations plotted at the 10^{th}, 50^{th} and 90^{th} percentiles of RBC EPA and DHA. We also inspected the GAM for the other biomarkers and found that they were consistent with the lack of statistical significance in linear models. The linear but not quadratic models best approximated the GAM presented in Figure 1 and Supplementary Figure 1, and therefore only the linear models are described below. Similar results were obtained when using the n-3 index in the regression models (Supplementary Table) and GAMs (Supplementary Figure 2).

Triglycerides—In linear regression models, the positive associations of BMI with triglycerides were attenuated with increasing RBC EPA and DHA concentrations (Table 3). The GAM showed strong positive associations of BMI with triglycerides at low RBC EPA and DHA concentrations, which became weaker at moderate concentrations and flat at high concentrations (Figure 1a). At low RBC EPA and DHA, the predicted increases in triglyceride concentrations associated with a BMI increase from 25 to 35 were 99.5±45.3 mg/dl (106%) and 137.8±71.0 mg/dl (156%), respectively. At high RBC EPA and DHA, these predicted increases were 13.9±8.1 mg/dl (23%) and 12.0±12.3 mg/dl (18%), respectively.

C-reactive protein—In linear regression models, the positive association of BMI with CRP was modestly, but not significantly, attenuated in the moderate and high RBC EPA categories and the high RBC DHA category (Table 3). The GAM showed clear differential associations of BMI with CRP by RBC EPA and DHA at BMI>28; strong positive associations of BMI with CRP among persons with low RBC EPA and DHA were weak at moderate concentrations and flat at high concentrations (Figure 1b). At low RBC EPA and

DHA, the predicted increases in CRP concentrations associated with a BMI increase from 30 to 35 were 0.8±0.7 mg/l (32%) and 1.0±0.8 mg/l (45%), respectively. At high RBC EPA and DHA, these predicted increases were 0.1±0.6 mg/l (6%) and −0.5±0.6 mg/l (−32%), respectively. Similarly, an increase in BMI from 25 to 35 at low versus high RBC EPA and DHA was associated with similar, albeit less significant, differences in the predicted increases in CRP concentrations. These were 1.2±0.7 mg/l (61%) and 0.8±1.0 mg/l (35%) at low RBC EPA and DHA, respectively, and 0.5±0.5 mg/l (50%) and −0.5±0.6 mg/l (−34%) at high RBC EPA and DHA, respectively.

Glucose and insulin—In linear regression models, there were significant positive associations of BMI with glucose in the highest RBC EPA and DHA categories, which were significantly and similarly attenuated in the moderate and low RBC EPA and DHA categories (Table 3). For insulin, there were significant positive associations with BMI in the lowest category of RBC EPA and DHA, which were slightly, but not significantly, attenuated in the moderate and high RBC EPA and DHA categories (Table 3). The GAM for glucose and insulin (Supplementary Figure 1) were complex but overall showed no differential associations with BMI by RBC EPA and DHA.

Leptin—The positive association of BMI with leptin was significantly weaker in the high compared to the moderate and low categories of RBC DHA (Table 3). However, GAM showed no differential association by RBC DHA (Supplementary Figure 1).

Discussion

In this population-based sample of Yup'ik Eskimos, the strong positive associations of obesity with triglycerides and CRP concentrations were substantially attenuated in adults with high RBC EPA and DHA. There were suggestive findings for glucose, insulin and leptin in linear regression models, but GAM provided little support for differential associations of BMI with these biomarkers at different RBC EPA and DHA levels. We judge the GAM to better represent associations of obesity with disease biomarkers (discussed below) and therefore consider the limited findings for glucose, insulin and leptin to be uninformative. There were no differential associations of BMI with all other biomarkers by RBC EPA and DHA levels.

Most studies on the associations of EPA and DHA with chronic disease biomarkers were conducted in populations with relatively low and narrow range of EPA and DHA intakes and none of these studies examined the interactions of EPA and DHA with obesity. However, a number of published studies indirectly support our findings. First, obesity is strongly associated with elevated triglycerides (Lamon-Fava *et al.*, 1996). Second, most observational studies (Block*et al.*, 2008; Bonaa *et al.*, 1992; Ferrucci *et al.*, 2006; He *et al.*, 2008; Motoyama *et al.*, 2009), including those on populations with chronic, high EPA and DHA intakes (Dewailly *et al.*, 2001b; Dewailly *et al.*, 2002), and supplementation studies (Damsgaard *et al.*, 2008; Kelley *et al.*, 2007; Leigh-Firbank *et al.*, 2002; Maki *et al.*, 2005; Mori *et al.*, 2000; Schwellenbach *et al.*, 2006; Woodman *et al.*, 2002) reported decreased triglycerides concentrations with increasing EPA and DHA intakes.

Similarly, obesity is strongly and positively associated with CRP (Visser *et al.*, 1999). Furthermore, most observational studies reported inverse associations of EPA and DHA intakes with CRP (Fernandez-Real *et al.*, 2003; Klein-Platat *et al.*, 2005; Lopez-Garcia *et al.*, 2004; Niu*et al.*, 2006; Pischon *et al.*, 2003). The single study that addressed whether these associations differed by BMI found a stronger association of EPA with CRP among overweight versus normal-weight adolescents (Klein-Platat *et al.*, 2005), a finding consistent with ours. Most EPA and DHA supplementation studies found no effect on CRP

(Damsgaard *et al.*, 2008; Fujioka *et al.*, 2006; Madsen *et al.*, 2003; Yusof *et al.*, 2008). These studies did not examine the interaction of obesity with EPA and DHA. The effect may be specific to a BMI subgroup and therefore could not be detected in the overall sample. Also, associations of EPA and DHA with CRP may be nonlinear, as previously shown (Makhoul *et al.*, 2010), and therefore could not be detected using conventional linear regression models.

Our findings may have important clinical relevance for the prevention of some obesity-related diseases. Obesity prevalence in the US (Ogden *et al.*, 2006) and worldwide (James *et al.*, 2001) has been increasing over the past decades, with subsequent increases in rates of diabetes and other obesity-associated diseases (Pi-Sunyer, 2002). It is likely that these associations are partly mediated by the positive associations of obesity with triglycerides and CRP, two biomarkers that strongly and independently predict risks of CVD (Nordestgaard *et al.*, 2007; Pai *et al.*, 2004) and possibly diabetes (Hu *et al.*, 2009; Pradhan *et al.*, 2001; Tirosh *et al.*, 2008). Chronic, high EPA and DHA intakes, similar to those of Yup'ik Eskimos, could at least partly ameliorate the obesity-associated disease risks.

Indeed, observations in Yup'ik Eskimos suggest that EPA and DHA intakes may lower their risk for diabetes. Yup'ik Eskimos have levels of obesity similar to those observed in the general US population and yet their prevalence of type 2 diabetes is significantly lower (3.3% (Mohatt *et al.*, 2007) versus 7.7% in NHANES 2005–2006 (Cowie *et al.*, 2009)). We recognize that genetic, lifestyle and dietary factors may account for this difference; yet it is consistent with our findings that chronic, high EPA and DHA consumption in Yup'ik Eskimos may protect overweight and obese persons against obesity-associated dyslipidemia and low-grade systemic inflammation and as a result may partially explain the low prevalence of diabetes in this population.

This study has several strengths. First, our study population was uniquely suitable for examining associations among BMI, disease biomarkers and RBC EPA and DHA: Yup'ik Eskimos have a broad range of EPA and DHA intakes (Bersamin *et al.*, 2008) and also a broad BMI distribution (Mohatt *et al.*, 2007). Second, we used RBC EPA and DHA concentrations, in preference to diet assessment, because they provide unbiased dietary intake measures and they better reflect biologically relevant exposure. Third, our analytical approach of using both GAM and linear regression was unique: linear models provide straightforward inference about the dependence of BMI-biomarker associations on EPA and DHA, while GAM allow for a more data-driven quantification of these associations based on a more flexible set of model assumptions (Wood, 2006).

This study also has limitations. First, although RBC EPA and DHA were previously found to be significantly associated with HDL, LDL and total cholesterol (Makhoul *et al.*, 2010), we did not detect statistically significant differences in the associations of BMI with these biomarkers at different RBC EPA and DHA levels probably due to limited sample size in the RBC EPA and DHA categories. Second, this study was cross-sectional and associations observed may not be causal. Third, although we adjusted for several confounders, we are aware that unmeasured factors could affect RBC fatty acids composition (such as fatty acid desaturase gene polymorphisms) (Simopoulos, 2010), weight and disease biomarkers. Finally, our findings require replication in other populations.

In conclusion, high EPA and DHA intakes may attenuate the associations of obesity with dyslipidemia and low-grade systemic inflammation, two strong risk factors for CVD and diabetes. While these findings need to be replicated, they motivate experimental research on the uses of high-dose n-3 fatty acid supplementation for the attenuation of obesity-related disease risk.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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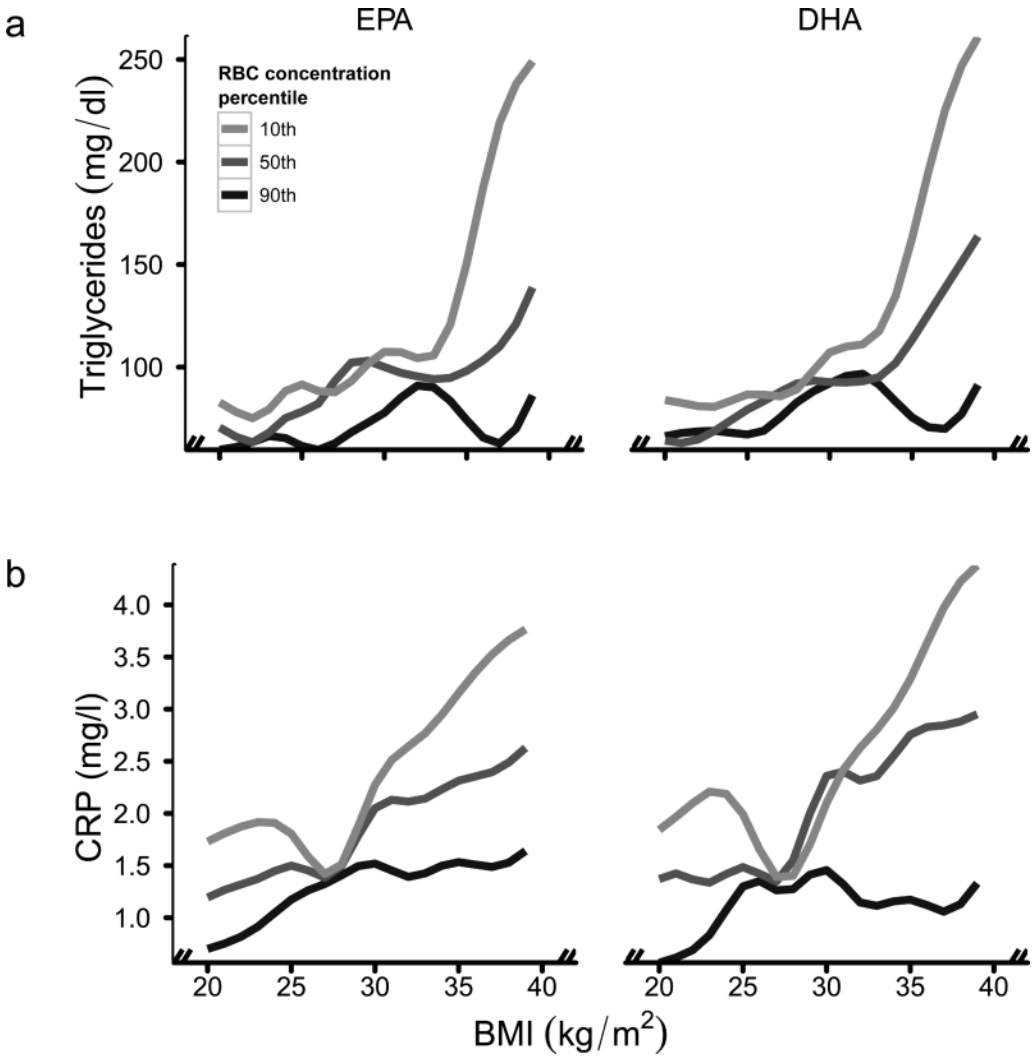

Figure 1.

Generalized Additive Models (GAM) of the associations of BMI with triglycerides (a) and CRP (b) by eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), as percentages of total fatty acids in RBC. The GAM include a bivariate smooth surface over EPA or DHA and BMI (all continuous). The estimated GAM were evaluated at mean values of age, gender and current smoking status and at the 10th (light grey line), 50th (dark grey line) and 90th (black line) percentiles of RBC EPA (1%, 3% and 5% of total fatty acids) and DHA (4%, 7% and 9% of total fatty acids). To convert triglycerides from mg/dl to mmol/l, multiply by 0.0113.

Table 1

Demographic, dietary and health-related characteristics of all study participants and stratified by BMI[a]

	All[b]	BMI[c] (kg/m²)			*P*-trend[d]
		<25	25 – 30	≥30	
n	330	96	123	111	
Sex					
Women	187 (57)	44 (23)	64 (34)	79 (42)	<0.01
Men	143 (43)	52 (36)	59 (41)	32 (22)	
Age (*years*)	44.8 ± 15.4	41.9 ± 15.4	44.8 ± 15.5	47.3 ± 15.0	0.04
18–29	65 (20)	25 (38)	26 (40)	14 (21)	0.11
30–54	169 (51)	49 (29)	58 (34)	62 (37)	
≥55	96 (29)	22 (23)	39 (41)	35 (36)	
BMI (*kg/m²*)	28.1 ± 4.8	22.7 ± 1.2	27.3 ± 1.4	33.6 ± 2.8	<0.001
Body fat (*%*)	30.9 ± 9.5	22.1 ± 6.3	29.8 ± 6.2	39.7 ± 6.7	<0.001
Current smokers	92 (28)	40 (43)	25 (27)	27 (29)	<0.01
Energy intake (*kcal/d*)	1953 ± 878	2013 ± 903	2000 ± 948	1839 ± 752	0.32
Total fat					
(g/d)	87 ± 55	89 ± 58	89 ± 61	83 ± 45	0.65
(% energy)	38 ± 11	37 ± 12	38 ± 10	39 ± 11	0.63
Carbohydrate					
(g/d)	208 ± 114	223 ± 116	207 ± 120	196 ± 103	0.27
(% energy)	44 ± 14	46 ± 13	43 ± 14	43 ± 14	0.27
Protein					
(g/d)	87 ± 47	83 ± 35	95 ± 61	82 ± 35	0.09
(% energy)	19 ± 7	18 ± 6	20 ± 8	19 ± 8	0.13
Dietary EPA (*g/d*)	1.6 ± 2.2	1.3 ± 1.9	1.9 ± 2.7	1.6 ± 2.0	0.16
Dietary DHA, (*g/d*)	1.9 ± 2.7	1.6 ± 2.2	2.2 ± 3.2	1.8 ± 2.2	0.24
RBC EPA[e] (*% of total fatty acids*)	2.8 ± 1.8	2.5 ± 1.5	2.9 ± 2.0	3.0 ± 1.8	0.09
<1.5	87 (26)	26 (30)	33 (38)	28 (32)	0.17
1.5–3.9	161 (49)	54 (33)	59 (37)	48 (30)	
>3.9	82 (25)	16 (19)	31 (38)	35 (43)	

	All[b]	BMI[c] (kg/m²)			P-trend[d]
		<25	25 – 30	≥30	
RBC DHA[e] (% *of total fatty acids*)	6.8 ± 1.8	6.6 ± 1.7	6.8 ± 1.8	7.0 ± 1.8	0.17
<5.8	84 (25)	29 (34)	30 (36)	25 (30)	0.25
5.8–8.2	168 (51)	51 (30)	64 (38)	53 (32)	
>8.2	78 (24)	16 (20)	29 (37)	33 (42)	
N-3 index[e,f] (*% of total fatty acids*)	9.6 ± 3.4	9.0 ± 3.0	9.8 ± 3.5	10.0 ± 3.4	0.11
<7.0	83 (25)	28 (34)	30 (36)	25 (30)	0.25
7.0-12.1	164 (50)	51 (31)	62 (38)	51 (31)	
>12.1	83 (25)	17 (20)	31 (37)	35 (42)	

[a] Values are means ± s.d. or *n* (%). Percentages may not sum to 100 due to rounding.

[b] Percentages are column percents.

[c] Percentages are row percents

[d] Based on tests for trend across BMI using *F* tests from ANOVA for continuous variables and χ^2 tests for categorical variables.

[e] Categorized as <25th percentile, 25th to 75th percentiles and >75th percentile.

[f] RBC EPA + DHA

Table 2

Age- and sex-adjusted means of biomarkers of chronic disease risk for all study participants and stratified by BMI[a]

	All	BMI (kg/m^2)			*P*-trend[b]
		<25	25 – 30	≥30	
n	330	96	123	111	
SBP (*mm Hg*)	124.1 ± 0.8	121.6 ± 1.5	123.4 ± 1.3	127.1 ± 1.4	0.009
DBP (*mm Hg*)	72.7 ± 0.5	68.8 ± 0.9	71.7 ± 0.8	77.0 ± 0.9	<0.001
Triglycerides[c, d] (*mg/dl*)	80.5 (76.9–84.3)	66.0 (60.9–71.6)	77.1 (71.9–82.7)	99.9 (92.7–107.7)	<0.001
Total cholesterol[e] (*mg/dl*)	223.0 ± 2.3	223.5 ± 4.3	228.5 ± 3.7	216.6 ± 4.0	0.23
LDL cholesterol[e] (*mg/dl*)	142.2 ± 2.0	138.7 ± 3.8	147.0 ± 3.3	139.9 ± 3.6	0.87
HDL cholesterol[e] (*mg/dl*)	62.7 ± 0.9	71.0 ± 1.5	64.7 ± 1.3	53.4 ± 1.4	<0.001
ApoAl (*g/l*)	1.74 ± 0.01	1.83 ± 0.02	1.77 ± 0.02	1.64 ± 0.02	<0.001
Glucose[f] (*mg/dl*)	95.1 ± 0.6	91.4 ± 1.0	94.5 ± 0.9	99.0 ± 1.0	<0.001
Insulin[g] (*μU/ml*)	15.0 ± 0.4	12.0 ± 0.6	13.9 ± 0.5	18.7 ± 0.6	<0.001
HOMA-IR[c]	3.2 (3.1–3.4)	2.5 (2.3–2.7)	3.1 (2.8–3.3)	4.2 (3.9–4.6)	<0.001
IGFl (*μg/l*)	266.6 ± 4.7	267.1 ± 8.8	265.6 ± 7.7	267.4 ± 8.2	0.98
IGFBP3 (*μg/l*)	4391 ± 51	4240 ± 93	4227 ± 81	4699 ± 86	<0.001
CRP[c] (*mg/l*)	0.9 (0.8–1.1)	0.7 (0.5–0.9)	0.9 (0.7–1.0)	1.3 (1.1–1.6)	<0.001
IL6[c] (*ng/l*)	0.08 (0.07–0.09)	0.07 (0.06–0.10)	0.07 (0.06–0.09)	0.10 (0.08–0.12)	0.17
sTNFR2[c] (*ng/l*)	1995 (1869–2129)	1963 (1734–2223)	1974 (1773–2198)	2045 (1824–2292)	0.64
Leptin[c] (*μg/l*)	8.0 (7.4–8.6)	4.1 (3.7–4.5)	8.0 (7.3–8.7)	13.9 (12.7–15.3)	<0.001
Adiponectin (*mg/l*)	8.8 ± 0.2	11.3 ± 0.4	9.0 ± 0.3	6.4 ± 0.4	<0.001

[a] Values are age- and sex-adjusted means ± SE or geometric means (95% CI) for log-transformed variables.

[b] *P* values for trend across BMI categories based on linear regression models adjusted for age and sex.

[c] Log-transformed values were used in the least square means analysis; adjusted means and 95% CIs were back-transformed.

[d] To convert triglycerides from mg/dl to mmol/l, multiply by 0.0113.

[e] To convert cholesterol (total, HDL and LDL) from mg/dl to mmol/l, multiply by 0.0259.

[f] To convert glucose from mg/dl to mmol/l, multiply by 0.0555.

[g] To convert insulin from μU/ml to pmol/l, multiply by 6.945.

Table 3

Associations of BMI with biomarkers of chronic disease risk by EPA and DHA, as percentages of total fatty acids in RBC[a]

	RBC EPA[b] (% of total fatty acids)							RBC DHA[b] (% of total fatty acids)						
	<1.5 (*n* = 87)		1.5–3.9 (*n* = 161)		>3.9 (*n* = 82)		*P*[c]	<5.8 (*n* = 84)		5.8 – 8.2 (*n* = 168)		>8.2 (*n* = 78)		*P*[c]
	β ± SE	*P*	β ± SE	*P*	β ± SE	*P*		β ± SE	*P*	β ± SE	*P*	β ± SE	*P*	
SBP (*mm Hg*)	0.3 ± 0.3	0.34	0.5 ± 0.2	0.03	0.6 ± 0.3	0.07	0.83	0.4 ± 0.4	0.29	0.6 ± 0.2	0.01	0.4 ± 0.3	0.19	0.87
DBP (*mm Hg*)	1.0 ± 0.2	<0.001	0.5 ± 0.1	<0.001	0.8 ± 0.2	<0.001	0.23	0.9 ± 0.2	<0.001	0.6 ± 0.1	<0.001	0.7 ± 0.2	0.001	0.50
Triglycerides[d, e] (*mg/dl*)														
Model 1	0.06 ± 0.01	<0.001	0.04 ± 0.01	<0.001	0.02 ± 0.01	0.02	0.01	0.06 ± 0.01	<0.001	0.04 ± 0.01	<0.001	0.02 ± 0.01	0.01	0.03
Model 2														
Linear	–0.31 ± 0.11	0.005	0.17 ± 0.07	0.01	–0.01 ± 0.09	0.93	<0.001	–0.32 ± 0.12	0.008	0.07 ± 0.07	0.30	0.12 ± 0.09	0.19	<0.001
Quadratic	0.006 ± 0.002	0.001	–0.002 ± 0.001	0.05	0.0005 ± 0.001	0.75		0.006 ± 0.002	0.002	–0.001 ± 0.001	0.62	–0.002 ± 0.001	0.28	
Total cholesterol[f] (*mg/dl*)	0.6 ± 1.0	0.50	–0.7 ± 0.7	0.32	–2.1 ± 0.9	0.02	0.12	0.5 ± 1.0	0.63	–0.7 ± 0.7	0.28	–1.8 ± 0.9	0.06	0.26
LDL cholesterol[f] (*mg/dl*)	–0.2 ± 0.9	0.82	0.1 ± 0.6	0.84	–0.6 ± 0.8	0.48	0.78	–0.2 ± 0.9	0.79	0.1 ± 0.6	0.91	–0.4 ± 0.8	0.60	0.87
HDL cholesterol[f] (*mg/dl*)	–1.2 ± 0.3	0.001	–1.4 ± 0.2	<0.001	–1.8 ± 0.3	<0.001	0.46	–1.4 ± 0.4	<0.001	–1.4 ± 0.2	<0.001	–1.7 ± 0.3	<0.001	0.78
ApoAI (*g/l*)	–0.02 ± 0.005	<0.001	–0.02 ± 0.003	<0.001	–0.02 ± 0.004	<0.001	0.99	–0.02 ± 0.005	<0.001	–0.02 ± 0.003	<0.001	–0.01 ± 0.005	0.003	0.64
Glucose[g] (*mg/dl*)	0.7 ± 0.2	0.003	0.4 ± 0.2	0.02	1.1 ± 0.2	<0.001	0.03	0.6 ± 0.2	0.01	0.5 ± 0.2	0.005	1.2 ± 0.2	<0.001	0.03
Insulin[11] (*μU/ml*)														
Model 1	0.8 ± 0.1	<0.001	0.6 ± 0.1	<0.001	0.6 ± 0.1	<0.001	0.42	0.8 ± 0.1	<0.001	0.6 ± 0.1	<0.001	0.6 ± 0.1	<0.001	0.47
Model 2														
Linear	–6.5 ± 1.7	<0.001	0.9 ± 1.1	0.41	1.5 ± 1.4	0.30	0.001	–5.5 ± 1.8	0.003	–0.2 ± 1.1	0.88	2.3 ± 1.4	0.11	0.01
Quadratic	0.12 ± 0.03	<0.001	–0.006 ± 0.019	0.76	–0.01 ± 0.02	0.53		0.11 ± 0.03	0.001	0.01 ± 0.02	0.49	–0.03 ± 0.02	0.25	
HOMA-IR[d]	0.05 ± 0.01	<0.001	0.04 ± 0.01	<0.001	0.05 ± 0.01	<0.001	0.83	0.05 ± 0.01	<0.001	0.04 ± 0.01	<0.001	0.05 ± 0.01	<0.001	0.71
IGF1[i] (*μg/l*)	–4.9 ± 1.7	0.004	–1.6 ± 1.2	0.18	–2.6 ± 1.6	0.11	0.28	–3.3 ± 1.8	0.07	–2.6 ± 1.2	0.03	–1.4 ± 1.6	0.39	0.72
IGFBP3 (*μg/l*) CRP[d] (*mg/l*)	33.9 ± 21.7	0.12	42.3 ± 15.2	0.006	57.1 ± 20.8	0.006	0.73	47.5 ± 22.8	0.04	45.8 ± 14.8	0.002	39.5 ± 20.9	0.06	0.96
Model 1	0.08 ± 0.03	0.002	0.05 ± 0.02	0.02	0.05 ± 0.03	0.05	0.49	0.06 ± 0.03	0.02	0.07 ± 0.02	<0.001	0.02 ± 0.03	0.37	0.38
Model 2														
Linear	–0.76 ± 0.33	0.02	0.04 ± 0.20	0.84	0.51 ± 0.27	0.06	0.03	–0.75 ± 0.35	0.03	0.02 ± 0.20	0.93	0.35 ± 0.27	0.20	0.07
Quadratic	0.01 ± 0.01	0.01	0.0001 ± 0.003	0.98	–0.008 ± 0.004	0.09		0.01 ± 0.01	0.02	0.001 ± 0.003	0.80	–0.005 ± 0.004	0.23	

	RBC EPA[b] (% of total fatty acids)							RBC DHA[b] (% of total fatty acids)						
	<1.5 (*n* = 87)		1.5–3.9 (*n* = 161)		>3.9 (*n* = 82)		*P*[c]	<5.8 (*n* = 84)		5.8 – 8.2 (*n* = 168)		>8.2 (*n* = 78)		*P*[c]
	β ± SE	*P*	β ± SE	*P*	β ± SE	*P*		β ± SE	*P*	β ± SE	*P*	β ± SE	*P*	
IL6[d], ng/l	0.06 ± 0.03	0.06	0.02 ± 0.02	0.31	0.01 ± 0.03	0.82	0.46	0.06 ± 0.03	0.05	0.003 ± 0.02	0.87	0.04 ± 0.03	0.13	0.23
sTNFR2[d] (ng/l)	–0.001 ± 0.015	0.97	0.004 ± 0.01	0.67	0.007 ± 0.014	0.61	0.93	0.001 ± 0.015	0.94	–0.002 ± 0.01	0.86	0.023 ± 0.014	0.10	0.33
Leptin[d] (µg/l)	0.11 ± 0.01	<0.001	0.11 ± 0.01	<0.001	0.08 ± 0.01	<0.001	0.19	0.12 ± 0.01	<0.001	0.11 ± 0.01	<0.001	0.08 ± 0.01	<0.001	0.03
Adiponectin (mg/l)	–0.36 ± 0.09	<0.001	–0.45 ± 0.06	<0.001	–0.35 ± 0.09	<0.001	0.59	–0.34 ± 0.10	0.001	–0.39 ± 0.06	<0.001	–0.45 ± 0.09	<0.001	0.71

[a] Values are β coefficients ± SE and corresponding *P* values from linear (Model 1) and quadratic (Model 2) regression models controlling for age (continuous), sex and current smoking status (yes, no). Quadratic models are reported only when statistically significant.

[b] Categories are <25th percentile, 25th to 75th percentiles and >75th percentile.

[c] *P* values for interaction are based on the likelihood ratio *F*-test test comparing linear regression models with and without interaction of BMI (continuous) with RBC EPA and DHA (categorical).

[d] Log-transformed values were used for the regression analysis.

[e] To convert triglycerides from mg/dl to mmol/l, multiply by 0.0113.

[f] To convert cholesterol (total, HDL and LDL) from mg/dl to mmol/l, multiply by 0.0259.

[g] To convert glucose from mg/dl to mmol/l, multiply by 0.0555.

[h] To convert insulin from µU/ml to pmol/l, multiply by 6.945.

[i] Regression model additionally adjusted for IGFBP3.