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Plasma marine n-3 polyunsaturated fatty acids and cardiovascular risk factors – data from the ACE 1950 Study

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Disclosures

The authors declare no disclosures or conflict of interest.

The results presented in this paper have not been published previously and is not under consideration for publication anywhere else.

Author Contributions:

A.C, I.A.E and M.S designed the present study.

H.R, M.N.L, T.B, T.O and A.T designed and organized the ACE 1950 Study including baseline examinations and data collection.

T.V, H.I.-H, E.B.O and O.M.R performed carotid ultrasound and baseline examinations.

E.B.S was responsible for the fatty acid analyses.

A.C, I.A.E and M.N.L analysed the data.

A.C, I.A.E, E.B.S, T.O and M.S edited the manuscript, H.R, T.V, H.I.-H, E.B.O, O.M.R, M.N.L, T.B and A.T co-edited the manuscript.

All the authors approved the final version of the manuscript.

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Eicosapentaenoic acid

Docosahexaenoic acid

Abbreviations:

ACE	Akershus Cardiac Examination
ACEi	Angiotensin converting enzyme inhibitor
ARB	Angiotensin receptor blocker
BMI	Body mass index
cIMT	Carotid intima-media thickness
CKD	Chronic kidney disease
CI	Confidence interval
CRP	C-reactive protein
CV	Cardiovascular
CVD	Cardiovascular disease
DHA	Docosahexaenoic acid
eGFR	Estimated glomerular filtration rate
EPA	Eicosapentaenoic acid
FFQ	Food frequency questionnaires
HbA1c	Glycated hemoglobin
HDL	High-density lipoprotein
IQR	Interquartile range
LDL	Low-density lipoprotein

PUFA	Polyunsaturated fatty acid
Std. β-coeff.	Standardized regression coefficient
Unstd. β-coeff.	Unstandardized regression coefficient
wt%	Weight percentage

Abstract

Purpose: A high intake of marine n-3 polyunsaturated fatty acids (PUFAs) might improve cardiovascular (CV) health. We conducted a cross-sectional study to investigate associations between plasma phospholipid levels of marine n-3 PUFAs and CV risk factors, educational level, physical activity and smoking habits.

Methods: A total of 3,706 individuals from a general population, all born in 1950 and residing in Akershus County, Norway, were included in this study. The main statistical approach was multivariable adjusted linear regression.

Results: Plasma marine n-3 PUFA levels ranged from 2.7 to 20.3 wt%, with a median level of 7.7 wt% (interquartile range 4.3 to 11.1 wt%). High levels of plasma marine n-3 PUFAs were associated with lower serum triglycerides (Standardized regression coefficient [Std. β-coeff.] -0.14, $p<0.001$), body mass index (Std. β-coeff. -0.08, $p<0.001$), serum creatinine (Std. β-coeff. -0.03, $p=0.05$), C-reactive protein levels (Std. β-coeff. -0.03, $p=0.04$), higher levels of serum high-density lipoprotein cholesterol (Std. β-coeff. 0.08, $p<0.001$) and low-density lipoprotein cholesterol (Std. β-coeff. 0.04, $p=0.003$). High levels of plasma marine n-3 PUFAs were also associated with lower glycated hemoglobin (Std. β-coeff. -0.04, $p=0.01$), however, only in individuals without diabetes. We found no associations between plasma marine n-3 PUFA levels and fasting plasma glucose or carotid intima-media thickness. High levels of plasma marine n-3 PUFAs were associated with higher educational level, more physical activity and lower prevalence of smoking.

Conclusion: In this cross-sectional study of Norwegian individuals born in 1950, high levels of plasma marine n-3 PUFAs were favourably associated with several CV risk factors, suggesting that fish consumption might improve CV health.

Introduction

The major marine n-3 polyunsaturated fatty acids (PUFAs), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are essential fatty acids provided by consumption of fatty fish and other seafoods [1]. A high intake of marine n-3 PUFAs has been associated with reduced risk of cardiovascular (CV) mortality in epidemiological studies [2-4]. Although recent clinical trials have shown mixed results on CV outcomes [5-8], marine n-3 PUFAs are generally considered cardioprotective based on epidemiological and mechanistic studies [9]. Over the last decades, fish consumption in Norway has decreased following a shift towards a more Western type of diet, characterized by high intake of processed food, red meat and refined sugars [10]. Type 2 diabetes, obesity and cardiovascular disease (CVD) are some of the conditions associated with Western diet [11]. As Norwegian dietary habits are changing with a continuous decrease in fish consumption, the beneficial effects of marine n-3 PUFAs on CV health could be attenuated [12].

A high intake of fatty fish has been linked to higher education and healthier lifestyle [13,14]. In a recent study, higher plasma EPA and DHA levels were associated with an increased likelihood of healthy aging [15]. EPA and DHA improve CV health by both shared and separate molecular pathways [16]. Mechanistic studies and clinical trials report that EPA and DHA influence CV risk factors such as blood lipids and inflammation differently [16].

The Akershus Cardiac Examination (ACE) 1950 Study is a large population-based study with extensive characterization of CV risk factors in a Norwegian general population [17]. To our knowledge, no previous large observational study in a Norwegian population, focusing on CV health, have measured plasma marine n-3 PUFA level as a marker of fatty fish consumption. The study had three objectives: 1) To study associations between plasma marine n-3 PUFA levels and multiple CV risk factors, with additional separate analyses for plasma EPA and

DHA levels. 2) To study associations between plasma marine n-3 PUFA levels and educational level, physical activity and smoking habits. 3) To validate a fatty fish consumption frequency questionnaire using plasma marine n-3 PUFA levels as reference.

Materials and methods

Study design and participants

The ACE 1950 Study aimed to examine the cardio- and cerebrovascular health of individuals born in 1950 and resident in Akershus County, Norway.

The study is a collaborative project between the Cardiothoracic Research Group, Akershus University Hospital and the Department of Medical Research, Bærum Hospital, Vestre Viken Hospital Trust.

From a total of 5,827 eligible individuals, invited for study participation by letters and subsequent phone calls, 3,706 (64%) individuals were enrolled in the study at Akershus University Hospital and Bærum Hospital from September 2012 through May 2015 (Figure 1). The remaining 2,121 (36%) invited individuals did not respond or declined participation without further explanation. Written consent was obtained before final enrollment. The study design has previously been presented [17]. The study was approved by the Norwegian Regional Ethics Committee (September 7th 2011. Ref. number 2011/1475) and performed in accordance with the Declaration of Helsinki. It was registered at clinicaltrials.gov with registration number NCT01555411.

Data collection and procedures

Study procedures and questionnaires have previously been described in detail [18].

History of CV and cerebrovascular disease was obtained and cross-checked with medical records. Individuals completed a study-specific food frequency questionnaire (FFQ) and

questionnaires regarding educational level, physical activity and smoking habits. Higher education was defined as > 12 years of formal education. High physical activity was defined as > 2 sessions of exercise per week. Smoking habits were recorded as either current smoker or non-smoker.

Individuals were asked to indicate the frequency of fatty fish consumption in the FFQ where they could select one of the following categories: zero to three times per month, one to three times per week, four to six times per week or daily intake. Data on consumption of lean fish was not included in this study.

Overnight fasting blood samples were obtained and stored at -80 °C. Ultrasound examination of the right and left carotid arteries was performed for the assessment of carotid intima-media thickness (cIMT), as previously described [19]. The mean cIMT was obtained from the average of right and left cIMT measurements. Hypertension was defined as current use of anti-hypertensive medication, or a mean systolic blood pressure ≥ 140 mmHg or a mean diastolic blood pressure ≥ 90 mmHg obtained at inclusion from three measurements. Hypercholesterolemia was defined as current use of lipid-lowering agents, total serum cholesterol ≥ 6.2 mmol/L or low-density lipoprotein (LDL) cholesterol ≥ 4.1 mmol/L [20]. Diabetes mellitus was defined as self-reported diabetes, current use of glucose-lowering medication or glycated hemoglobin (HbA1c) ≥ 6.5%. World Health Organizations definition was used to define obesity (body mass index [BMI] [kg/m^2] ≥ 30) [21]. Estimated glomerular filtration rate (eGFR) was calculated using The Chronic Kidney Disease Epidemiology Collaboration equation [22], and chronic kidney disease (CKD) stages 3-5, defined as eGFR <60 ml/min/1.73m^2 was recorded.

From stored blood samples, aliquots of plasma were sent to The Lipid Research Center, Aalborg University Hospital for analysis of fatty acid composition. In brief, total lipids were extracted from serum using a modified Folch method [23]. The phospholipid fraction was

isolated from other lipids using the Burdge method [24]. Fatty acids were derived from transesterification of phospholipid fractions that were transferred to gas chromatographic tubes. By using a Varian 3900 gas chromatograph (Varian, Middleburg, The Netherlands) with 60 m x 0.25 mm capillary columns, individual fatty acids were identified, and quantified as weight percentage (wt%) of total plasma phospholipid fatty acids. Plasma marine n-3 PUFA levels were defined as the sum of plasma EPA and DHA. Plasma marine n-3 PUFAs were not adequately analyzed for six individuals and for 17 individuals there was not enough plasma for fatty acid analysis. (Figure 1).

Statistical analysis

We used tertiles of plasma marine n-3 PUFA levels for presentation of demographic and clinical data. Results are presented as percentage for categorical data and mean values (standard deviation) for continuous data. Differences between groups were evaluated using Chi square for dichotomous data, Kruskal-Wallis test for non-normally distributed variables like triglycerides, fasting plasma glucose, HbA1c and C-reactive protein (CRP), and ANOVA for other continuous data.

The main statistical approach was multivariable linear regression for assessment of cross-sectional associations between plasma marine n-3 PUFA, EPA and DHA levels and CV risk factors, educational level, physical activity and smoking habits. Predefined covariates were included in the multivariable models ($p<0.10$ for inclusion) by stepwise forward procedure. For some dependent variables, plasma marine n-3 PUFA, EPA and DHA levels were eliminated from the fully adjusted regression model by the stepwise forward procedure, in which case they were forced into the final models. Unstandardized regression coefficients (Unstd. β-coeff.) with corresponding 95% confidence intervals (CI), standardized regression coefficients (Std. β-coeff.), p-values and explained variance (R^2) are given for the fully

adjusted final model. Since serum triglycerides, fasting plasma glucose, HbA1c, and serum creatinine levels were non-normally distributed, they were truncated to obtain a normal distribution, before they were entered into the regression models. Because of extreme skewness, CRP was logarithmically transformed before entered as a variable in the regression analyses. Hence, the presented Unstd. β-coeff. and corresponding 95% CI represent the anti-logarithm of obtained results for CRP.

Pearson correlation coefficient was used for assessing correlation between fatty fish consumption frequency data and plasma marine n-3 PUFAs levels. Associations between categories of self-reported fatty fish consumption and plasma marine n-3 PUFA level were assessed by ANOVA. Statistical analyses were performed using SPSS® version 25.0 (IBM, NY, US).

Results

Demographic and clinical characteristics are presented in Table 1. Plasma marine n-3 PUFA levels ranged from 2.7 to 20.3 wt%, with a median level of 7.7 wt% (interquartile range [IQR] 4.3 to 11.1 wt%). A gender difference was identified with a higher proportion of women in the upper tertile of plasma marine n-3 PUFA levels. Individuals with high levels of plasma marine n-3 PUFAs had a lower prevalence of diabetes mellitus, obesity and CKD. Higher education, more physical activity and a lower prevalence of smoking were seen in individuals with high compared with low levels of plasma marine n-3 PUFAs.

Unadjusted and multivariable adjusted associations between plasma marine n-3 PUFA levels and CV risk factors are presented in Table 2. High levels of plasma marine n-3 PUFAs were associated with higher serum high-density-lipoprotein (HDL) cholesterol levels, low-density-lipoprotein (LDL) cholesterol levels, lower serum triglycerides levels, HbA1c, BMI, serum

creatinine and CRP levels in crude and multivariable adjusted analyses (Table 2). No associations were found between plasma marine n-3 PUFA levels and fasting plasma glucose or cIMT in the fully adjusted multivariable models (Table 2).

We performed gender-stratified analysis, where plasma marine n-3 PUFA levels were associated with serum LDL cholesterol levels in males (n=1863, Unstd. β-coeff. 0.02, Std. β-coeff. 0.06, p=0.005), but not in females (n=1764, Unstd. β-coeff. 0.01, Std. β-coeff. 0.03, p=0.13). However, after further adjustment for prevalent hypercholesterolemia, plasma marine n-3 PUFA levels and serum LDL cholesterol levels were no longer significantly associated in males (n=923, Unstd. β-coeff. 0.01, Std. β-coeff. 0.03, p=0.35). No gender differences were identified for the other dependent variables.

We assessed associations with fasting plasma glucose and HbA1c for plasma marine n-3 PUFA levels for individuals with and without diabetes separately. Fasting plasma glucose was not associated with plasma marine n-3 PUFA levels in individuals diagnosed with diabetes (n=310, Unstd. β-coeff 0.01, Std. β-coeff. 0.012, p=0.83) nor in individuals without diabetes (n=3336, Unstd. β-coeff 0.001, Std. β-coeff. 0.001, p=0.99). On the other hand, HbA1c was associated with plasma marine n-3 PUFA levels in individuals without diabetes (n=3331, Unstd. β-coeff -0.006, Std. β-coeff. -0.046, p=0.008), but not in individuals with diabetes (n=309, Unstd. β-coeff -0.016, Std. β-coeff. -0.042, p=0.45).

Associations between plasma EPA and DHA levels and CV risk factors were examined in separate multivariable linear regression analyses. Higher levels of both plasma EPA and DHA were associated with lower serum triglycerides and BMI (Table 3). We found significant associations with serum HDL cholesterol levels and renal function for plasma EPA levels, while plasma DHA levels were significantly associated with serum LDL cholesterol levels, HbA1c and CRP levels (Table 3).

Self-reported fatty fish consumption frequency was moderately correlated with plasma marine n-3 PUFA levels, with the highest plasma levels seen among individuals with daily fish consumption (Pearson correlation coefficient 0.30, $p<0.001$, Figure 2). Plasma marine n-3 PUFA levels were higher across categories of self-reported fatty fish consumption ($p<0.001$); zero to three times per month: median 6.3 wt% (IQR 3.7 - 11.1 wt%), one to three times per week: median 7.6 wt% (IQR 4.3 - 10.9 wt%), four to six times per week: median 8.9 wt% (IQR 5.5 - 14.4 wt%) and daily: median 9.5 wt% (IQR 5.5 - 13.5 wt%).

Discussion

In this large cross-sectional study of elderly Norwegian residents, high levels of plasma marine n-3 PUFAs were associated with lower serum triglycerides, HbA1c, BMI, serum creatinine and CRP levels as well as higher levels of serum HDL and LDL cholesterol. In addition, individuals with high levels of plasma marine n-3 PUFAs were generally more physically active and had a lower prevalence of smoking, suggesting a healthier lifestyle.

Marine n-3 PUFAs and CV risk factors

Data from most large epidemiological studies report a positive association between intake of marine n-3 PUFAs and CV mortality [4,25]. However, for some CV risk factors, such as lipoproteins and markers of glucose homeostasis, reports on associations with marine n-3 PUFA consumption are inconsistent [1]. In populations with low consumption of fish, levels of marine n-3 PUFAs in target organs might not exceed thresholds for effects on specific CV risk factors, while for populations with high intake of fatty fish there might be ceiling effects [12]. With a current decline in fish consumption in Norway during the last few decades, effects of marine n-3 PUFA intake on a population level today would likely differ from data

obtained in the previous era, where the Norwegian population had a very high fish consumption [10]. We conducted this study in an attempt to better understand how the current intake of marine n-3 PUFAs in the Norwegian population influence CV risk profile.

The triglyceride lowering effect of marine n-3 PUFA is well documented in clinical trials, where both EPA and DHA supplementation have similar triglyceride lowering effects [26]. This has primarily been shown in studies with marine n-3 PUFA supplementation exceeding 2 g/day [27], and the effect is also related to triglyceride levels at baseline, with a greater reduction achieved in individuals with higher baseline triglyceride levels [28]. In our study, plasma levels of both EPA and DHA were negatively associated with serum triglycerides. Interestingly, the mean daily intake of EPA and DHA is about 0.7 g in Norway [29], which is considerably lower than the previously proposed triglyceride-lowering dose. Rather than threshold values for marine n-3 PUFA effects, our findings suggest a linear relationship between marine n-3 PUFA intakes and triglycerides. Thus, some effect on triglycerides might also be achieved by increased fatty fish consumption and not solely with supplements.

EPA, but not DHA, was associated with higher levels of HDL cholesterol, similar to data from a previous Norwegian observational study [29]. Data from clinical trials indicate that DHA supplements are more efficient in increasing serum HDL cholesterol levels than EPA supplements [26]. However, these effects are seen at much higher doses of EPA and DHA, which are not possible to achieve in a regular diet and definitely not comparable to an epidemiological setting. In a recent meta-analysis, it was concluded that supplementation with marine n-3 PUFAs only have a little effect on HDL [30].

High levels of plasma marine n-3 PUFAs were associated with higher serum LDL cholesterol levels, but only in individuals with hypercholesterolemia. Patients with hypercholesterolemia might have received advice to increase their intake of marine n-3 PUFAs, which could possibly explain a positive association between plasma marine n-3 PUFA levels and serum LDL cholesterol in our study. In clinical trials, the effect of marine n-3 PUFA consumption on serum LDL-cholesterol has been controversial. Whereas several interventional studies have shown an increase in serum LDL cholesterol after marine n-3 PUFA supplementation [31], a recent meta-analysis showed neutral effect [30].

Epidemiological studies on fish consumption and the risk for developing type 2 diabetes have shown diverging results, with reports of both positive, neutral and negative associations [32]. In the present study, we found a lower prevalence of diabetes mellitus among individuals in the upper tertile of plasma marine n-3 PUFA levels, but no association between plasma marine n-3 PUFA levels and fasting plasma glucose. Furthermore, high levels of plasma marine n-3 PUFAs were associated with low HbA1c only in individuals without diabetes, representing majority of the study population, and not in individuals with diabetes. We speculate that plasma marine n-3 PUFA levels in individuals without diabetes in the present study might be due to a confounder effect, explained by healthier lifestyle and not related to any direct effect on glucose metabolism.

A modest weight loss of 5-10% body weight can improve dyslipidemia and insulin resistance [33], improving the CV risk profile. We found a lower prevalence of obesity in the upper tertile of plasma marine n-3 PUFA levels, and higher plasma marine n-3 PUFA levels were associated with lower BMI. We cannot, however, exclude the possibility that this inverse association was confounded by a healthier lifestyle in these individuals.

The anti-inflammatory properties of marine n-3 PUFAs are well documented. EPA competitively inhibits arachidonic acid as substrate for prostaglandin synthesis and both EPA and DHA serve as precursors of anti-inflammatory and pro-resolving protectins, maresins and resolvins [34]. In the present study, high levels of plasma marine n-3 PUFAs were associated with slightly lower CRP levels. When analyzed separately, plasma levels of DHA, but not EPA, was associated with lower CRP levels. However, CRP levels were generally low as expected in a population study, and furthermore, we did not measure high-sensitive CRP, which is a better marker of low-grade inflammation. Therefore, these findings should be interpreted with caution.

A high intake of marine n-3 PUFAs is associated with lower prevalence of CKD [35], similar to what we found in the present study, and is suggested to prevent age-associated renal function decline in adults [36]. In clinical trials, marine n-3 PUFA supplementation in patients with CKD reduced the risk of progression to end-stage renal disease [37], and prevented decline in kidney function in patients with history of myocardial infarction [38].
EPA and DHA are proposed as potential renoprotective agents due to their anti-inflammatory and anti-fibrotic properties [39]. In the present study, plasma EPA, but not DHA, levels were associated with lower serum creatinine. As previously mentioned, EPA compete with arachidonic acid in eicosanoid metabolism and therefore possess more direct anti-inflammatory properties than DHA [40], which could be a possible explanation for our findings.

In contrast to previous epidemiological studies, showing an inverse association between marine n-3 PUFAs and cIMT [41,42], we found no associations between plasma marine n-3

PUFA levels and cIMT in the present study. The discrepant results might be related to the amount of fish consumed in various populations. In a Chinese study, only participants with a low intake of marine n-3 PUFAs had an inverse association with cIMT [43]. Although fish consumption is decreasing in Norway, it still remains one of the countries with the highest fish intake per capita worldwide [44]. The high overall intake of marine n-3 PUFAs in our study could make it difficult to show a difference between the individuals with regard to cIMT. Age and hypertension are considered strong predictors of cIMT progression [45] while HDL cholesterol was inversely associated with cIMT progression in a large meta-analysis of over 21.000 individuals [46]. We only found a weak association between plasma n-3 PUFA levels and HDL cholesterol and furthermore plasma marine n-3 PUFA levels were not associated with blood pressure, which might explain the lack of associations with cIMT in our study.

Fish consumption as a marker of a healthy lifestyle

Fish consumption has been associated with a healthy lifestyle, high educational level and high socioeconomical status in general populations of other countries [13,14]. Persons with high fish consumption tend to smoke less, are more physically active and eat less processed meat than persons with low fish consumption [13,47].

In the present study, plasma levels of marine n-3 PUFAs were moderately correlated with self-reported fatty fish consumption frequency. High plasma marine n-3 PUFA levels were associated higher educational level, in line with previous epidemiological studies [13,14]. High plasma marine n-3 PUFA levels were also associated with lower prevalence of smoking and more physical activity, indicating an overall healthier lifestyle among individuals with higher fatty fish intake frequency. We cannot rule out that an overall healthier lifestyle might create a confounder effect leading to an overestimation of the benefits of marine n-3 PUFAs

on CV risk factors. Thus, adjustment for lifestyle related variables seems reasonable when assessing associations between plasma marine n-3 PUFA levels and CV health.

Strengths and limitations

This study has major strengths, including a large and well-described study population with little missing data and several CV risk factors included in multivariable regression models. Previous studies report differences in fatty fish intake across age groups [48]. All the participants in our study were born in 1950, thus removing age as the otherwise most influential confounding factor.

Plasma phospholipid fatty acids levels were measured by gas chromatography, providing a valid and reliable measure of marine n-3 PUFA consumption. In contrast, dietary questionnaires will be subject to recall bias [48]

In addition to the cross-sectional design, this study also has several limitations. Fatty acid levels in plasma phospholipids do not reflect the long-term intake of fatty acids as good as erythrocyte or adipose tissue levels [48]. However, since weekly intake of fatty fish usually is relatively stable, we assume that plasma fatty acid composition in the present study represents the long-term average fatty acid profiles for the majority of individuals [49].

Adjustments were made for smoking habits, physical activity and educational level in the multivariable regression analyses. However, we cannot rule out residual confounding influencing associations between plasma marine n-3 PUFA levels and CV risk factors.

Self-reported fatty fish consumption did not include quantities of fish consumed.

Finally, due to the relatively high intake of fish in a Norwegian population, our findings might not apply to other regions with lower intake.

Conclusion

In this cross-sectional study of a Norwegian general population, high levels of plasma marine n-3 PUFAs were associated with lower serum triglycerides, HbA1c, BMI, serum creatinine, CRP levels and higher levels of serum HDL and LDL cholesterol. In addition, high plasma marine n-3 PUFA levels were associated with higher educational level, more physical activity and lower prevalence of smoking, signalling a generally healthier lifestyle. Although this might act as a confounding factor, that cannot be completely adjusted for in statistical analyses, the findings in our study suggest a favourable association between plasma marine n-3 PUFA levels and CV risk factors.

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Figure Captions

Fig. 1

Flowchart of the inclusion of the study participants

Fig. 2

Relationship between plasma marine n-3 PUFA levels and self-reported fatty fish consumption frequency

Table 1. Characteristics of study participants according to tertiles of plasma n-3 polyunsaturated fatty acid levels

	All patients	Low	Medium	High	p
n-3 PUFA level (wt%)	**2.7-20.3**	**≤ 6.62**	**6.63 – 8.86**	**≥ 8.87**	
Number of participants	3683	1221	1236	1226	
EPA	2.6 (1.4)	1.4 (0.4)	2.3 (0.5)	4.0 (1.3)	<0.001
DHA	5.5 (1.4)	4.0 (0.7)	5.4 (0.6)	7.0 (1.0)	<0.001
Age, *years*	63.9 (0.6)	63.9 (0.6)	63.9 (0.6)	63.6 (0.7)	0.92
Gender (Male), *%*	51.3	55.8	50.6	47.6	<0.001
Fatty fish intake frequency, *%*					
0-3 servings/month	13.0	22.6	10.3	6.1	<0.001
1-3 servings/week	69.3	69.2	73.3	65.5	<0.001
4-6 servings/week	12.8	6.5	12.2	19.8	<0.001
Daily	4.9	1.7	4.3	8.7	<0.001
Daily fruit intake, *%*	51.2	50.3	51.6	51.7	0.58
Daily vegetable intake, *%*	58.9	56.4	59.7	60.7	0.44
Current smoker, *%*	14.5	20.3	14.0	9.2	<0.001
Physical activity (≥ 2 times weekly), *%*	61.7	54.5	62.2	68.6	<0.001
Higher education, *%*	46.5	39.7	48.3	51.6	<0.001
Systolic blood pressure, *mmHg*	138 (19)	138 (18)	138 (19)	137 (19)	0.45
Diastolic blood pressure, *mmHg*	77 (10)	77 (10)	77 (10)	77 (10)	0.18
Total cholesterol, *mmol/L*	5.4 (1)	5.4 (1)	5.4 (1)	5.5 (1)	0.01
HDL cholesterol, *mmol/L*	1.5 (0.5)	1.5 (0.5)	1.5 (0.5)	1.6 (0.5)	<0.001
LDL cholesterol, *mmol/L*	3.3 (1)	3.2 (1)	3.3 (1)	3.3 (1)	0.04
Triglycerides, *mmol/L*	1.4 (0.7)	1.5 (0.7)	1.4 (0.7)	1.2 (0.6)	<0.001
Fasting plasma glucose, *mmol/L*	5.5 (1.0)	5.6 (1.0)	5.5 (1.0)	5.4 (0.9)	<0.001
HbA1c, *%*	5.8 (0.6)	5.8 (0.6)	5.8 (0.6)	5.7 (0.5)	<0.001
Body mass index, kg/m^2	27.1 (4.4)	27.6 (4.7)	27.4 (4.4)	26.5 (4.1)	<0.001
eGFR, *ml/min x 1.73m2*	83 (12)	83 (12)	83 (12)	84 (11)	0.14
Creatinine, *µmol/L*	75.9 (14.4)	76.7 (14.6)	76.2 (14.3)	74.8 (14.3)	0.003

cIMT, *mm*	0.73 (0.1)	0.73 (0.1)	0.73 (0.1)	0.72 (0.1)	0.04
CRP, *mg/L*	2.0 (1.9)	2.1 (2.0)	2.0 (1.9)	1.9 (1.9)	<0.001
Hypertension, *%*	62.0	63.4	61.5	61.1	0.45
Hypercholesterolemia, *%*	52.6	47.6	54.2	55.8	0.001
Cerebrovascular disease, *%*	3.7	4.6	3.6	3.1	0.14
Coronary artery disease, *%*	7.0	7.8	6.9	6.4	0.42
Diabetes mellitus, *%*	8.5	10.7	9.1	5.7	<0.001
Obesity (BMI ≥ 30), *%*	22.6	26.7	23.2	17.8	<0.001
CKD stages 3-5 (eGFR <60 ml/min x 1.73m^2), *%*	3.9	5.2	3.9	2.5	0.003
Medication, *%*					
Diuretics	3.1	3.1	3.0	3.1	0.98
Beta blockers	13.4	14.2	13.3	12.6	0.51
Calcium channel blockers	8.1	7.1	10.4	6.9	0.002
ACEi or ARB	26.9	27.6	26.4	26.8	0.79
Lipid lowering drugs	26.1	25.3	26.1	27.1	0.61

Results are presented as percentage for categorical data and mean value (standard deviation) for continuous data. Differences between groups were evaluated using Chi square for dichotomous data, the Kruskal-Wallis test for triglycerides, fasting plasma glucose, HbA1c and CRP, and ANOVA for other continuous data.
Abbreviations: EPA: Eicosapentaenoic acid. DHA: Docosahexaenoic acid. HDL: High density lipoprotein. LDL: Low density lipoproteins HbA1c: Hemoglobin A1c. eGFR: Estimated glomerular filtration rate (CKD-EPI formula). cIMT: Carotid intima-media thickness. CRP: C-reactive protein. BMI: Body mass index. CKD: Chronic kidney disease. ACEi: Angiotensin converting enzyme inhibitor. ARB: Angiotensin receptor blocker.

Table 2. Associations between plasma n-3 polyunsaturated fatty acid levels and cardiovascular risk factors

Univariable linear regression analysis					
Cardiovascular risk factors	**n**	**Unstd. β-coeff. (95% CI)**	**Std. β-coeff.**	**p**	**R^2**
HDL cholesterol, *mmol/L*	3680	0.03 (0.02, 0.03)	0.14	<0.001	0.02
LDL cholesterol, *mmol/L*	3657	0.01 (0.001, 0.02)	0.03	0.04	0.001
Triglycerides, *mmol/L*	3680	-0.05 (-0.06, -0.04)	-0.20	<0.001	0.04
Fasting glucose, *mmol/L*	3675	-0.03 (-0.04, -0.02)	-0.07	<0.001	0.01
HbA1c, *%*	3669	-0.02 (-0.03, -0.002)	-0.10	<0.001	0.01
BMI, *kg/m²*	3683	-0.18 (-0.24, -0.13)	-0.11	<0.001	0.01
Creatinine, *μmol/L*	3663	-0.25 (-0.43, -0.07)	-0.05	0.006	0.002
cIMT, *mm*	3661	-0.002 (-0.003, 0.00)	-0.04	0.02	0.001
CRP, *mg/L*	3669	-1.02 (-1.03, -1.01)	-0.07	<0.001	0.004
Multivariable linear regression analysis					
Cardiovascular risk factors	**n**	**Unstd. β-coeff. (95% CI)**	**Std. β-coeff.**	**p**	**R^2**
HDL cholesterol, *mmol/L* [a]	3650	0.015 (0.01, 0.02)	0.08	<0.001	0.27
LDL cholesterol, *mmol/L* [b]	3627	0.016 (0.01, 0.03)	0.04	0.003	0.27
Triglycerides, *mmol/L* [c]	3650	-0.04 (-0.05, -0.03)	-0.14	<0.001	0.18
Fasting glucose, *mmol/L* [d]	3646	-0.001 (-0.01, 0.01)	-0.002	0.89	0.38
HbA1c, *%* [e]	3640	-0.01 (-0.014, -0.002)	-0.04	0.006	0.41
BMI, *kg/m* [f]	3617	-0.14 (-0.19, -0.08)	-0.08	<0.001	0.10
Creatinine, *μmol/L* [g]	3633	-0.16 (-0.31, -0.002)	-0.03	0.05	0.28
cIMT, *mm* [h]	3634	-0.001 (-0.002, -0.001)	-0.01	0.42	0.05
CRP, *mg/L* [i]	3639	-1.01 (-1.02, -1.00)	-0.03	0.04	0.05

[a] Gender, smoking, diabetes mellitus, BMI, lipid lowering drugs
[b] Gender, smoking, diabetes mellitus, BMI, lipid lowering drugs
[c] Gender, smoking, diabetes mellitus, BMI, lipid lowering drugs
[d] Gender, smoking, BMI, diabetes medication
[e] Gender, smoking, BMI, diabetes medication
[f] Gender, smoking, diabetes mellitus, physical activity, higher education
[g] Gender, smoking, diabetes mellitus, BMI, hypertension
[h] Gender, smoking, diabetes mellitus, BMI, lipid lowering drugs, hypertension
[i] Gender, smoking, diabetes mellitus, obesity

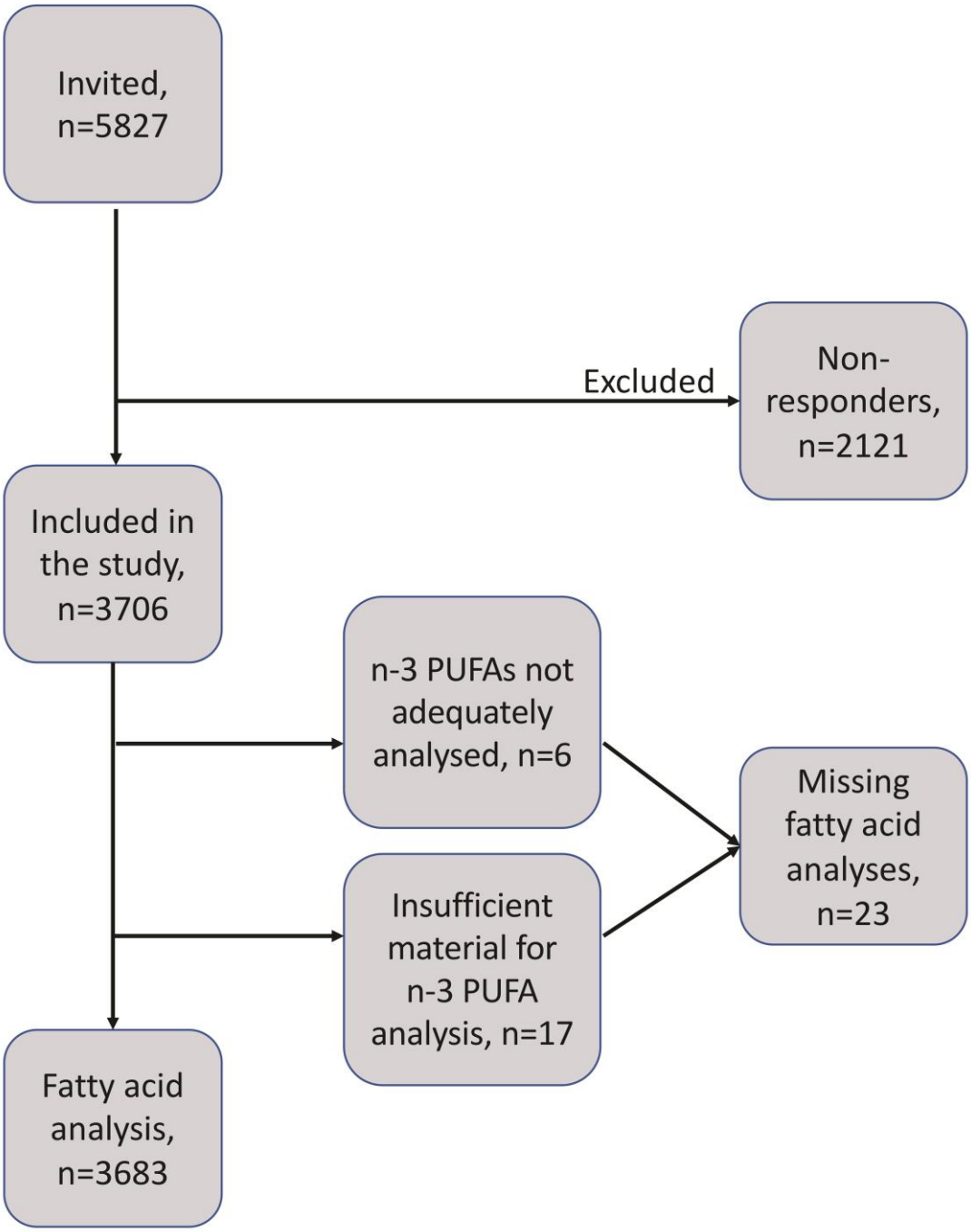
Unstandardized β coefficients (Unstd. β-coeff.) with corresponding 95% confidence intervals (CI), standardized β coefficients (Std. β-coeff.), p-values and explained variance (R^2) are given for the fully adjusted final model. The listed covariates were included in fully adjusted multivariable models (p<0.10 for inclusion).
Abbreviations: HDL: High-density lipoprotein. LDL: Low-density lipoprotein. HbA1c: Hemoglobin A1c. BMI: Body mass index. cIMT: Carotid intima-media thickness. CRP: C-reactive protein.

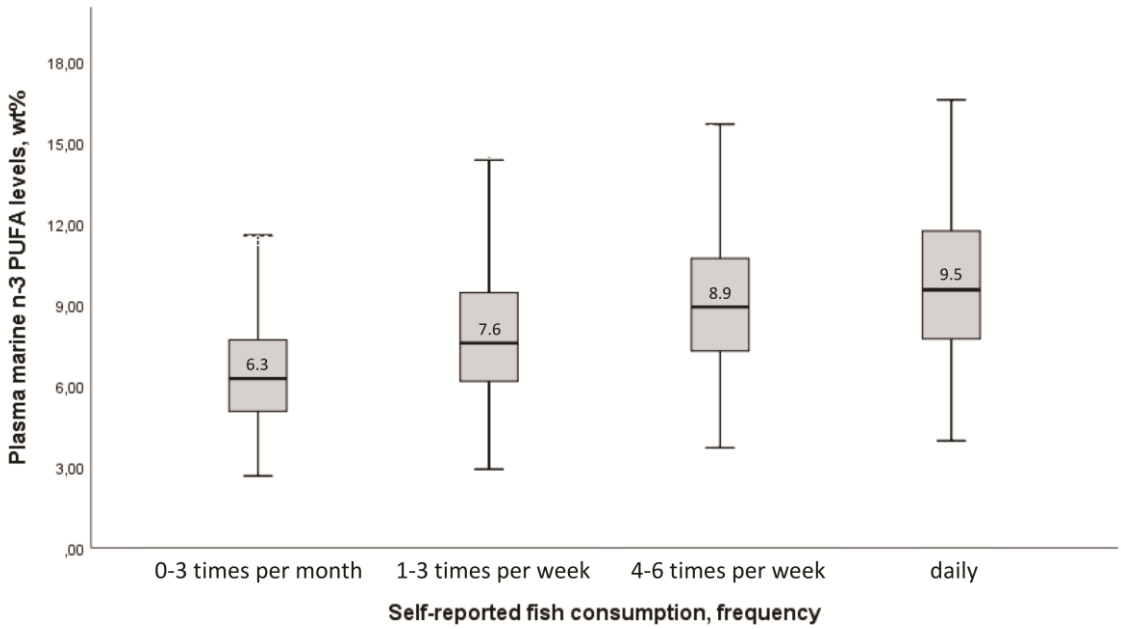
Table 3. Multivariable adjusted associations between plasma eicosapentaenoic acid and docosahexaenoic acid levels and cardiovascular risk factors

	Eicosapentaenoic acid			
Cardiovascular risk factors	**Unstd. β-coeff. (95% CI)**	**Std. β-coeff.**	**p**	**R^2**
HDL cholesterol, *mmol/L* [a]	0.05 (0.04, 0.06)	0.13	<0.001	0.29
LDL cholesterol, *mmol/L* [b]	0.01 (-0.01, 0.03)	0.01	0.36	0.27
Triglycerides, *mmol/L* [c]	-0.09 (-0.10, -0.07)	-0.17	<0.001	0.19
Fasting glucose, *mmol/L* [d]	0.02 (-0.003, 0.03)	0.02	0.10	0.38
HbA1c, *%* [e]	-0.01 (-0.02, 0.001)	-0.02	0.09	0.41
BMI, *kg/m²* [f]	-0.20 (-0.30, -0.10)	-0.06	<0.001	0.10
Creatinine, *μmol/L* [g]	-0.38 (-0.67, -0.08)	-0.04	0.01	0.28
cIMT, *mm* [h]	-0.002 (-0.004, 0.001)	-0.02	0.19	0.05
CRP, *mg/L* [i]	-1.01 (-1.03, -1.00)	-0.03	0.18	0.05
	Docosahexaenoic acid			
Cardiovascular risk factors	**Unstd. β-coeff. (95% CI)**	**Std. β-coeff.**	**p**	**R^2**
HDL cholesterol, *mmol/L* [a]	0.01 (-0.004, 0.02)	0.02	0.25	0.27
LDL cholesterol, *mmol/L* [b]	0.04 (0.03, 0.06)	0.07	<0.001	0.28
Triglycerides, *mmol/L* [c]	-0.04 (-0.06, -0.03)	-0.09	<0.001	0.17
Fasting glucose, *mmol/L* [d]	-0.02 (-0.03, 0.001)	-0.02	0.07	0.38
HbA1c, *%* [e]	-0.02 (-0.03, -0.01)	-0.04	0.001	0.41
BMI, *kg/m²* [f]	-0.27 (-0.36, -0.17)	-0.09	<0.001	0.10
Creatinine, *μmol/L* [g]	-0.18 (-0.46, 0.11)	-0.02	0.23	0.28
cIMT, *mm* [h]	0.001 (-0.003, 0.002)	-0.003	0.84	0.05
CRP, *mg/L* [i]	-1.02 (-1.03, -1.00)	-0.04	0.02	0.05

[a] Gender, smoking, diabetes mellitus, BMI, lipid lowering drugs
[b] Gender, smoking, diabetes mellitus, BMI, lipid lowering drugs
[c] Gender, smoking, diabetes mellitus, BMI, lipid lowering drugs
[d] Gender, smoking, BMI, diabetes medication
[e] Gender, smoking, BMI, diabetes medication
[f] Gender, smoking, diabetes mellitus, physical activity, higher education
[g] Gender, smoking, diabetes mellitus, BMI, hypertension
[h] Gender, smoking, diabetes mellitus, BMI, lipid lowering drugs, hypertension
[i] Gender, smoking, diabetes mellitus, obesity

Unstandardized regression coefficients (Unstd. β-coeff.) with corresponding 95% confidence intervals (CI), standardized regression coefficients (Std. β-coeff.), p-values and explained variance (R^2) are given for the fully adjusted final model. The listed covariates were included in fully adjusted multivariable models (p<0.10 for inclusion).
Abbreviations: HDL: High-density lipoprotein. LDL: Low-density lipoprotein. HbA1c: Hemoglobin A1c. BMI: Body mass index. cIMT: Carotid intima-media thickness. CRP: C-reactive protein.


Invited, n=5827
Excluded
Non-responders, n=2121
Included in the study, n=3706
n-3 PUFAs not adequately analysed, n=6
Missing fatty acid analyses, n=23
Insufficient material for n-3 PUFA analysis, n=17
Fatty acid analysis, n=3683


Plasma marine n-3 PUFA levels, wt%
18,00
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8.9
9.5
0-3 times per month
1-3 times per week
4-6 times per week
daily
Self-reported fish consumption, frequency