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ORIGINAL CONTRIBUTION

Associations between intake of fish and n-3 long-chain polyunsaturated fatty acids and plasma metabolites related to the kynurenine pathway in patients with coronary artery disease

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Abstract
Purpose Enhanced tryptophan degradation via the kynurenine pathway has been related to several pathological conditions. However, little is known about the effect of diet on individual metabolites of this pathway. We investigated cross-sectional associations between reported intake of fish and omega-3 (n-3) long-chain PUFA (LC-PUFA) and plasma metabolites related to the kynurenine pathway.

Methods Participants were 2324 individuals with coronary artery disease from the Western Norway B Vitamin Intervention Trial. Fish and n-3 LC-PUFA intakes were assessed using a food frequency questionnaire. Plasma concentrations of tryptophan, kynurenine, kynurenic acid, anthranilic acid, 3-hydroxykynurenine, xanthurenic acid, 3-hydroxyanthranilic acid, neopterin, and kynurenine-to-tryptophan ratio (KTR) were analyzed. Associations were investigated using partial Spearman's rank correlations and multiple linear regressions.

Results Median age at inclusion was 62 years (80 % males), and 84 % had stable angina pectoris. Intake of fatty fish and n-3 LC-PUFA was inversely associated with plasma 3-hydroxykynurenine. Consumption of total fish, lean fish, and n-3 LC-PUFA was inversely associated with plasma neopterin. Intake of total fish, fatty fish, and n-3 LC-PUFA was inversely associated with KTR. All these correlations were weak (ρ between -0.12 and -0.06, $P < 0.01$). In 306 patients with diabetes, lean fish intake was positively associated with plasma 3-hydroxyanthranilic acid ($\rho = 0.22$, $P < 0.001$, P for interaction $= 0.01$), and total fish intake was inversely associated with KTR ($\rho = -0.17$, $P < 0.01$, P for interaction $= 0.02$).

Conclusion Fish intake was not an important determinant of individual metabolites in the kynurenine pathway. However, some correlations were stronger in patients with diabetes. The inverse associations of fish or n-3 LC-PUFA with neopterin and KTR may suggest a slightly lower IFN-γ-mediated immune activation with a higher intake.

Keywords Fish intake · Omega-3 polyunsaturated fatty acid · Neopterin · Kynurenine pathway

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Introduction

Tryptophan (Trp) is an essential amino acid important for synthesis of proteins and as a precursor for serotonin and niacin [1]. The major catabolic route of Trp is the kynurenine (Kyn) pathway, where Trp is converted into compounds collectively termed kynurenines [1]. In the first step, Trp is converted to Kyn catalyzed by the enzymes indoleamine 2,3-dioxygenase (IDO) or tryptophan 2,3-dioxygenase (TDO) [1]. Kyn is in turn metabolized to either kynurenic acid (KA), anthranilic acid (AA), or 3-hydroxykynurenine (HK), which is converted to xanthurenic acid (XA) or 3-hydroxyanthranilic acid (HAA; Fig. 1). The enzymes involved in the synthesis of KA, AA, HK, XA, and HAA depend on vitamins B_2 or B_6 as cofactors [2, 3].

The cytokine interferon-γ (IFN-γ) stimulates the conversion of Kyn from Trp by up-regulating IDO [1]. IFN-γ also stimulates the formation of neopterin in macrophages [4]. Thus, the Kyn-to-Trp ratio (KTR) is a marker of both IDO and TDO activities and IFN-γ-mediated immune activation, whereas neopterin is a marker of IFN-γ activity [4, 5]. Factors known to be associated with systemic kynurenines and neopterin are age, sex, BMI, renal function, and smoking [6].

Activation of the Kyn pathway and/or increased circulating neopterin has been linked to various pathologic conditions [7–9]. Specifically, dysregulation of the Kyn pathway might be associated with insulin resistance [10] and type 2 diabetes mellitus [11]. Recently, we reported that elevated plasma kynurenines predicted increased risk of acute myocardial infarction in patients with coronary artery disease (CAD), and the risk associations were generally stronger in patients with pre-diabetes or diabetes [12]. Diet is a modifiable lifestyle factor that has been related to both cardiovascular disease (CVD) and type 2 diabetes mellitus. Specifically, dietary intake of fish and omega-3 (n-3) long-chain polyunsaturated fatty acid (LC-PUFA) has been associated with reduced risk of CVD and mortality [13, 14]. In contrast, fish and n-3 LC-PUFA consumption has been related, depending on geographical region, to both decreased and increased risks of type 2 diabetes mellitus [15, 16]. Studies on associations between dietary intake and the Kyn pathway may provide new knowledge of mechanisms involved in nutrition-disease relationships. However, data are scarce on associations between diet and circulating kynurenines. Associations between intake of fish or n-3 LC-PUFA and kynurenines have, to the best of our knowledge, not been studied in humans. Therefore, we investigated the associations between fish and n-3 LC-PUFA intakes and circulating kynurenines, neopterin, and KTR in a large Norwegian cohort including patients with CAD. In addition, we evaluated whether any associations were modified by diabetes.

Subjects and methods

Study population

The current work was a cross-sectional study based on participants from the Western Norway B Vitamin Intervention Trial, which included 3090 men and women at baseline. This trial is registered at clinicaltrials.gov as NCT00354081 and has been described in detail elsewhere [17]. Briefly, participants underwent coronary angiography for suspected CAD at Haukeland University Hospital (Bergen, Norway) or Stavanger University Hospital (Stavanger, Norway) between 1999 and 2004. The participants answered a food frequency questionnaire (FFQ) at baseline. Participants without a completed FFQ ($n = 606$), those who left more than one blank page in the FFQ ($n = 96$), and those reporting very low (<3000 kJ/day for women and <3300 kJ/day for men) or very high (>15,000 kJ/day for women and >17,500 kJ/day for men) total energy intake ($n = 37$) were excluded. In addition, 27 individuals without

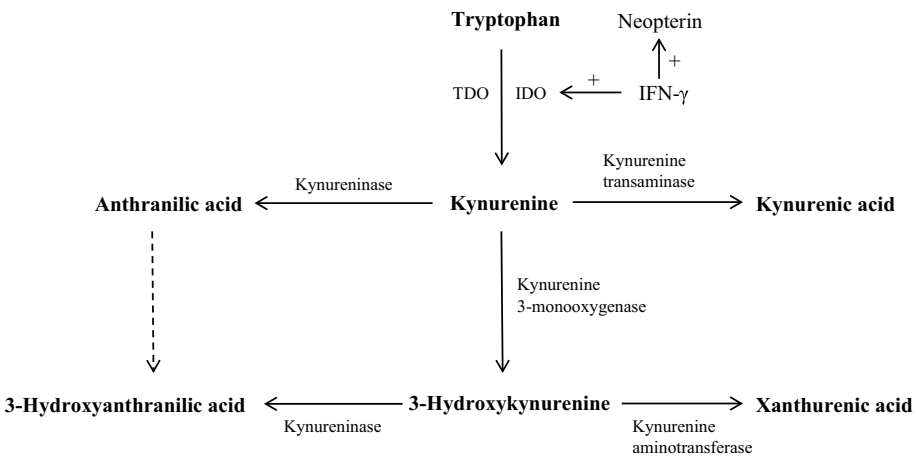

Fig. 1 Overview of the tryptophan–kynurenine pathway. *IDO* indoleamine 2,3-dioxygenase, *IFN-γ* interferon-γ, *TDO* tryptophan 2,3-dioxygenase

plasma kynurenines or neopterin measures at baseline were excluded, leaving 2324 individuals for the current analyses. The study protocol was in accordance with principles of the Declaration of Helsinki, and the study was approved by the Regional Committee for Medical Research Ethics, the Norwegian Medicines Agency, and the Data Inspectorate. All participants gave written informed consent.

Dietary assessment

Habitual dietary intake, reflecting intake during the previous year, was assessed in a 169-item semiquantitative FFQ developed at the Department of Nutrition, University of Oslo, Norway [18, 19]. The FFQ was given to participants at enrollment and was returned by mail or collected at the follow-up visit 1 month later. Questions on use of dietary supplements were included in the FFQ. The frequency alternatives ranged from once a month to several times per day. Daily food (g) and nutrient intakes (including dietary supplements) were calculated using a food database and software system developed at the Department of Nutrition, University of Oslo (Kostberegningssystem, version 3.2; University of Oslo, Norway). The food database is mainly based on the official Norwegian food composition table, with some additional foods [20].

In addition to 'total fish' (finfish and shellfish), fish intake was divided into 'fatty fish' (mackerel, herring, trout, salmon), 'lean fish' (cod, saithe, haddock), 'processed fish' (fish fingers, fish soup, fish pudding, etc.), and 'fish on sandwich' (mackerel, salmon, trout, sardines, pickled herring, sandwich caviar). Total (diet and supplements) n-3 PUFA intake (g/day) was the sum of α-linolenic acid, eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA), and n-3 LC-PUFA intake (g/day) included EPA, DPA, and DHA. Reported consumption of cod liver oil or fish oil was defined as use of supplements. The FFQ has been evaluated against weighed diet records, serum carotenoids, and fatty acid composition of plasma phospholipids [18, 21, 22]. Reported fish intake and n-3 LC-PUFA concentration in plasma phospholipids were positively correlated ($r = 0.37$) [21]. Correlation coefficients of reported intake and concentrations in plasma phospholipids were 0.51 and 0.49 for EPA and DHA, respectively. Compared with plasma phospholipid fatty acid concentration, the ability of the FFQ to classify into the same or adjacent quartile was 81 and 78 % for EPA and DHA, respectively [21].

Covariates and baseline data

Demographic and clinical data were collected at baseline by study personnel. Diabetes was defined by self-reported diagnosis of type 1 or 2 diabetes mellitus, fasting plasma glucose ≥7.0 mmol/L, or non-fasting plasma glucose ≥11.0 mmol/L. Hypertension was assumed if subjects were treated with antihypertensive drugs. Current smokers were defined as self-reported current smokers, smokers with cessation within the last month before baseline, or participants having cotinine (main metabolite of nicotine) concentrations ≥85 nmol/L [23]. Estimated glomerular filtration rate (eGFR) and extent of CAD were assessed as previously described [24].

Biochemical analyses

Fasting and non-fasting blood samples were collected at baseline, and plasma was stored at −80 °C until analyzed. Standard blood laboratory parameters were analyzed from fresh samples by the hospital laboratories. Plasma neopterin, KA, AA, HK, XA, HAA, and cotinine were measured by liquid chromatography/tandem-mass spectrometry [25], and Trp and Kyn by gas chromatography–mass spectrometry [26] at Bevital AS (www.bevital.no). To calculate KTR, the plasma concentration of Kyn (nmol/L) was divided by the plasma concentration of Trp (μmol/L).

Statistical analyses

Continuous variables are presented as medians (25th, 75th percentiles) and categorical variables as percentages. Testing for trend for baseline characteristics across quartiles of total fish intake was analyzed using quantile regression [27] for continuous variables and logistic regression for categorical variables. Differences between males and females were assessed by the Mann–Whitney *U* test.

All dietary variables related to fish or n-3 LC-PUFA intake were energy-adjusted using the residual method, while other dietary intake variables were energy-adjusted with the residual or density method [28]. All median dietary intake values not presented as percent of total energy intake are not energy-adjusted values. Because most variables were non-normally distributed, the associations between fish and n-3 LC-PUFA intakes and Trp, kynurenines, neopterin, and KTR were evaluated using partial Spearman's rank correlations. Age (continuous), sex, eGFR (continuous), BMI (continuous), smoking (dichotomous), and energy intake (continuous) were included as covariates in all partial Spearman's rank correlations, which also were performed in a subgroup with diabetes. Statistical testing of effect modification by diabetes was performed by including an interaction product term between fish or n-3 LC-PUFA intakes and diabetes into a simple linear regression. Both the dependent and independent variables in these analyses were ranked.

Multiple linear regressions were performed to assess the association of intake of fish and n-3 LC-PUFA (quartiles) with plasma Trp, kynurenines, neopterin, and KTR. Fish and n-3 LC-PUFA variables were represented in the model with indicator variables for each of the three non-reference

quartiles (quartiles 2–4), and age (continuous), sex, eGFR (continuous), BMI (continuous), smoking (dichotomous), and energy intake (continuous) were included simultaneously. In an additional model, adjustment for consumption of alcohol (0, >0–15.0, ≥15.0 g/day), fiber (quartiles), and vegetables (quartiles) was performed.

The computer statistical software SPSS for Windows, version 21 (IBM, NY, USA), and the quantreg package of R version 3.1.2 (The R Foundation for Statistical Computing, Vienna, Austria) were used for statistical analyses. Due to multiple testing, two-sided P values <0.01 were considered statistically significant.

Results

Baseline characteristics

Baseline characteristics in the total cohort and across quartiles of daily total fish intake are presented in Table 1. Participants ranged in age from 28 to 85 years, 80.1 % of the patients were male, and a majority (84.2 %) had stable angina pectoris. The use of acetylsalicylic acid, statins, and beta blockers was 76.9, 72.5, and 68.6 %, respectively (Table 1). Thirty-seven percent of the patients were fasting during blood sampling. Patients with higher intake of total fish were older, less likely to smoke, and more likely to have hypertension. Plasma Trp, Kyn, KA, and HAA increased with increasing total fish intake, and C-reactive protein (CRP) was reduced with higher fish intake (Table 1).

Median values of plasma Trp, KA, XA, and HAA were lower ($P < 0.01$), whereas median HK was higher in women compared with men ($P < 0.01$). Plasma Kyn and AA did not differ between women and men. The six plasma kynurenines were inversely associated with eGFR and positively associated with BMI and age. Neopterin and KTR were higher in women compared with men ($P < 0.01$). Neopterin was inversely associated with eGFR ($\rho = -0.48$, $P < 0.001$) and positively associated with age ($\rho = 0.36$, $P < 0.001$).

Baseline dietary intake

Baseline dietary intakes in the total cohort and across quartiles of daily total fish intake are given in Table 2. Dietary intake of protein, vegetables, and fruit and berries increased with increasing total fish intake. Furthermore, the proportion of cod liver oil consumption increased across quartiles of total fish intake. Median (25th, 75th percentiles) daily reported fish intake was 97.2 (65.2, 141) g/day (Table 2), and the consumption of lean fish was higher than that of fatty fish. Fish oil and/or cod liver oil was consumed by 40.6 % of the participants, and 89.2 % had an intake of EPA + DHA ≥ 250 mg/day. The median proportional intakes of the n-3 LC-PUFAs EPA, DPA, and DHA (g/day) were 35.9, 8.1, and 55.7 %, respectively.

Correlation between total fish intake and kynurenines, neopterin, and KTR

Adjusted correlation coefficients between total fish, types of fish, and n-3 LC-PUFA intakes and plasma concentrations of Trp, kynurenines, neopterin, and KTR are presented in Fig. 2. Total fish intake was positively correlated with Trp ($\rho = 0.09$, $P < 0.001$). There were no associations between intake of total fish and plasma Kyn, KA, AA, or XA. Consumption of total fish was inversely correlated with the inflammatory markers neopterin ($\rho = -0.08$, $P < 0.001$) and KTR ($\rho = -0.09$, $P < 0.001$). Adding fasting status (dichotomous) as a covariate to the multivariate model did not change the results appreciably.

Correlation between intake of fatty and lean fish and kynurenines, neopterin, and KTR

Fatty fish intake was inversely correlated with HK, whereas lean fish intake was positively associated with HAA (Fig. 2). The association between intake of lean fish and HAA was no longer statistically significant after adjustment for fasting status. There were no associations between types of fish intake and plasma Kyn, KA, AA, or XA. There were inverse associations between fatty fish intake and KTR and between lean fish intake and neopterin. All the aforementioned correlations were generally weak ($\rho = -0.08$ to 0.07), but statistically significant ($P < 0.01$).

Correlation between n-3 LC-PUFA intake and kynurenines, neopterin, and KTR

Consumption of total n-3 LC-PUFA, EPA, and DHA was positively associated with Trp ($\rho = 0.09$, $P < 0.001$) and inversely associated with HK ($\rho = -0.08$, $P < 0.001$; Fig. 2). Total n-3 LC-PUFA consumption was inversely associated with neopterin ($\rho = -0.09$, $P < 0.001$) and KTR ($\rho = -0.12$, $P < 0.001$). There were similar results for EPA and DHA.

Correlations in patients with diabetes

Partial correlation analyses were performed in a subgroup of patients with diabetes. Adjusted correlation coefficients between intakes of total fish, types of fish, and n-3 LC-PUFA and plasma concentrations of Trp, kynurenines, neopterin, and KTR in 306 patients with diabetes

Table 1 Baseline characteristics of 2324 patients with coronary artery disease in total and by quartiles of energy-adjusted total fish intake

	Total fish intake (g/day)					
	Total ($n = 2324$)	1st ($n = 581$)	2nd ($n = 581$)	3rd ($n = 581$)	4th ($n = 581$)	P_{trend}
Total fish (g/day)	97.2 (65.2, 141)	50.8 (35.0, 71.9)	78.8 (61.0, 96.9)	112 (93.3, 131)	178 (148, 217)	
Age (years)	62 (55, 69)	57 (51, 66)	62 (56, 70)	64 (57, 70)	63 (56, 69)	<0.001
BMI (kg/m^2)	26.4 (24.5, 28.9)	26.2 (24.3, 28.4)	26.3 (24.4, 29.0)	26.3 (24.4, 28.7)	27.0 (24.8, 29.4)	<0.001
Male sex (%)	80.1	83.3	75.9	75.9	85.4	0.40
Current smokers[a] (%)	31.1	37.9	30.6	27.7	28.1	<0.001
Diabetes[b] (%)	13.2	12.0	12.9	12.7	15.0	0.17
Hypertension[c] (%)	46.1	41.0	43.5	50.9	48.9	<0.01
eGFR (mL/min/1.73 m^2)	92 (82, 100)	96 (87, 104)	91 (80, 100)	91 (81, 97)	92 (83, 99)	<0.001
Biomarkers related to the kynurenine pathway						
Tryptophan (µmol/L)	68.0 (59.2, 77.5)	67.1 (58.3, 76.9)	66.9 (56.8, 76.1)	68.0 (59.6, 77.5)	69.6 (61.7, 78.7)	<0.01
Kynurenine (µmol/L)	1.63 (1.35, 1.92)	1.56 (1.30, 1.89)	1.65 (1.36, 1.97)	1.68 (1.39, 1.97)	1.64 (1.36, 1.91)	<0.01
Kynurenic acid (nmol/L)	47.6 (37.0, 60.5)	45.4 (34.7, 57.6)	47.2 (37.0, 61.0)	49.6 (38.7, 61.6)	48.6 (37.9, 61.3)	<0.01
Anthranilic acid (nmol/L)	13.7 (10.9, 17.6)	13.2 (10.5, 16.9)	14.1 (11.2, 18.3)	14.0 (11.0, 18.1)	13.8 (11.1, 17.4)	0.12
Hydroxykynurenine (nmol/L)	28.8 (22.7, 36.9)	27.9 (22.5, 35.9)	29.1 (22.4, 38.4)	28.8 (23.1, 36.3)	29.0 (23.0, 36.8)	0.32
Xanthurenic acid (nmol/L)	13.9 (9.9, 19.0)	13.8 (9.8, 18.4)	13.3 (9.6, 18.7)	13.8 (9.9, 18.6)	14.7 (10.7, 20.5)	0.04
Hydroxyanthranilic acid (nmol/L)	34.0 (25.6, 44.5)	33.2 (24.8, 43.7)	33.4 (24.6, 43.6)	33.7 (25.5, 43.8)	35.9 (27.0, 47.0)	<0.01
Inflammation markers						
Neopterin (nmol/L)	8.0 (6.6, 9.9)	7.7 (6.5, 9.7)	8.1 (6.6, 10.3)	8.2 (6.7, 10.2)	7.8 (6.4, 9.5)	0.38
KTR (nmol/µmol)	23.6 (20.0, 28.5)	23.1 (19.6, 27.9)	24.4 (20.5, 29.8)	24.1 (20.3, 29.0)	23.0 (19.4, 27.5)	0.58
C-reactive protein (mg/L)	1.85 (0.88, 4.09)	1.97 (0.93, 4.42)	1.87 (0.90, 4.23)	1.84 (0.91, 4.00)	1.67 (0.82, 3.90)	0.05
Serum lipids						
Apolipoprotein A1 (g/L)	1.24 (1.08, 1.42)	1.23 (1.06, 1.40)	1.25 (1.09, 1.42)	1.25 (1.11, 1.45)	1.23 (1.09, 1.43)	0.08
Apolipoprotein B (g/L)	0.85 (0.71, 1.02)	0.85 (0.71, 1.02)	0.85 (0.71, 1.03)	0.86 (0.71, 1.02)	0.85 (0.73, 1.02)	1.00
Triglycerides (mmol/L)	1.52 (1.10, 2.20)	1.64 (1.14, 2.35)	1.52 (1.11, 2.20)	1.48 (1.14, 2.07)	1.46 (1.06, 2 17)	<0.01
Extent of coronary artery disease						
Three-vessel disease (%)	32.0	27.9	28.1	36.3	35.9	<0.001
Left ventricular ejection fraction <50 % (%)	10.5	9.3	12.4	10.7	9.8	0.98
Cardiovascular history						
Myocardial infarction (%)	41.0	39.6	40.1	43.4	41.0	0.42
Coronary artery bypass graft surgery (%)	14.1	10.2	13.6	16.4	16.4	<0.01
Percutaneous coronary intervention (%)	21.6	24.1	18.1	23.4	20.8	0.56
Clinical diagnosis						
Stable angina pectoris (%)	84.2	80.0	84.0	87.3	85.5	<0.01
Acute coronary syndrome (%)	14.2	17.9	14.1	11.4	13.3	0.01
Aortic valve stenosis (%)	1.6	2.1	1.9	1.4	1.2	0.19
Medication at baseline						
Acetylsalicylic acid (%)	76.9	75.7	75.4	77.3	79.0	0.14
Statins (%)	72.5	70.6	71.9	76.6	71.1	0.45
β-Blockers (%)	68.6	66.3	71.8	68.7	67.6	0.91
ACEI and/or ARB (%)	29.1	24.1	31.7	30.0	30.4	0.05
Calcium channel blockers (%)	22.4	19.3	21.2	24.8	24.3	0.02

Values are medians (25th, 75th percentiles). *P* for trend calculated using quantile regression for continuous variables and logistic regression for dichotomous variables. Missing data: TG ($n = 2$) and three-vessel disease ($n = 5$)

ACEI angiotensin-converting enzyme inhibitor, *ARB* angiotensin receptor blocker, *eGFR* estimated glomerular filtration rate, *KTR* kynurenine-to-tryptophan ratio

[a] Current smokers defined as self-reported current smokers, ex-smokers with cessation within the last month before baseline, or participants with cotinine ≥85 nmol/L

[b] Self-reported type 1 or 2 diabetes mellitus, fasting plasma glucose ≥7.0 mmol/L, or non-fasting plasma glucose ≥11.0 mmol/L

[c] Receiving antihypertensive medical treatment

Table 2 Baseline daily dietary intake among 2324 patients with coronary artery disease in total and by quartiles of energy-adjusted total fish intake

	Total fish intake (g/day)					
	Total ($n = 2324$)	1st ($n = 581$)	2nd ($n = 581$)	3rd ($n = 581$)	4th ($n = 581$)	P_{trend}
Total fish (g)	97.2 (65.2, 141)	50.8 (35.0, 71.9)	78.8 (61.0, 96.9)	112 (93.3, 131)	178 (148, 217)	
Energy intake (kJ)	8562 (6964, 10,478)	9340 (7745, 11,314)	8105 (6460, 9871)	8012 (6536, 9795)	8741 (7277, 10,806)	0.02
Carbohydrates (E%)	49.8 (45.4, 54.1)	50.8 (46.5, 55.1)	51.1 (46.8, 55.0)	50.1 (46.0, 53.8)	47.2 (42.8, 51.6)	<0.001
Fiber (E%)	2.3 (1.9, 2.7)	2.1 (1.8, 2.5)	2.3 (2.0, 2.7)	2.3 (2.0, 2.7)	2.3 (1.9, 2.7)	<0.001
Protein (E%)	16.7 (15.2, 18.4)	15.0 (13.8, 16.3)	16.2 (15.0, 17.8)	17.1 (15.9, 18.3)	18.6 (17.3, 20.2)	<0.001
Total fat (E%)	31.3 (27.9, 35.2)	31.7 (28.3, 35.9)	30.6 (27.7, 34.3)	31.2 (27.6, 34.8)	32.0 (28.2, 35.6)	0.62
SFA (E%)	11.5 (9.8, 13.1)	12.1 (10.5, 14.0)	11.6 (10.0, 13.0)	11.1 (9.6, 12.9)	11.1 (9.3, 12.8)	<0.001
MUFA (E%)	10.1 (8.8, 11.4)	10.1 (8.9, 11.6)	9.9 (8.6, 11.1)	10.0 (8.9, 11.3)	10.4 (8.9, 11.6)	0.07
PUFA (E%)	6.8 (5.7, 8.2)	6.6 (5.4, 8.1)	6.4 (5.4, 7.8)	6.8 (5.7, 8.1)	7.3 (6.2, 8.7)	<0.001
Alcohol (E%)	1.0 (0.0, 2.9)	1.0 (0.0, 3.0)	0.90 (0.0, 2.7)	1.0 (0.0, 2.8)	1.2 (0.1, 3.0)	0.04
n-3 PUFA						
n-3 PUFA[a] (E%)	1.3 (1.0, 1.6)	1.0 (0.84, 1.3)	1.2 (0.97, 1.4)	1.4 (1.1, 1.6)	1.6 (1.3, 2.0)	<0.001
n-3 LC-PUFA[b] (E%)	0.44 (0.25, 0.74)	0.21 (0.11, 0.36)	0.37 (0.24, 0.56)	0.53 (0.35, 0.77)	0.74 (0.51, 1.1)	<0.001
DHA (E%)	0.24 (0.14, 0.40)	0.12 (0.06, 0.19)	0.21 (0.14, 0.31)	0.29 (0.20, 0.42)	0.41 (0.29, 0.60)	<0.001
EPA (E%)	0.16 (0.08, 0.28)	0.07 (0.03, 0.13)	0.13 (0.08, 0.21)	0.19 (0.12, 0.29)	0.27 (0.18, 0.41)	<0.001
Supplement use						
Cod liver oil (%)	27.5	23.2	25.6	31.5	29.6	<0.01
Fish oil (%)	16.5	15.0	14.8	18.4	17.9	0.07
Food intake						
Fatty fish (g)	15.5 (4.6, 29.4)	5.5 (1.1, 12.7)	11.4 (4.5, 21.7)	18.3 (7.8, 30.6)	35.4 (18.7, 55.7)	<0.001
Lean fish (g)	32.5 (17.0, 52.3)	15.0 (5.6, 27.0)	27.5 (14.8, 38.8)	40.1 (27.5, 55.6)	64.1 (41.3, 89.8)	<0.001
Processed fish (g)	14.9 (7.0, 23.9)	11.0 (4.9, 18.9)	13.0 (7.0, 20.0)	15.0 (10.0, 25.0)	20.0 (11.0, 32.1)	<0.001
Fish on sandwich (g)	9.0 (4.0, 23.0)	4.0 (0.0, 9.5)	9.0 (2.0, 18.0)	13.0 (4.0, 24.0)	19.0 (9.0, 39.0)	<0.001
Shellfish (g)	2.0 (0.0, 11.0)	1.0 (0.0, 5.0)	2.0 (0.0, 7.0)	2.0 (0.0, 11.0)	5.0 (0.0, 21.6)	<0.001
Fruit and berries (g)	218 (133, 331)	207 (130, 313)	210 (124, 326)	221 (131, 332)	238 (150, 359)	<0.001
Vegetables (g)	182 (116, 269)	155 (93.0, 233)	164 (108, 240)	189 (124, 271)	228 (142, 315)	<0.001
Meat (g)	108 (71, 153)	121 (77.8, 173)	104 (68.6, 145)	100 (69.6, 136)	111 (71.3, 156)	0.54

Continuous variables are presented as medians (25th, 75th percentiles). *P* for trend is assessed using quantile regression for continuous variables and logistic regression for dichotomous variables

DHA docosahexaenoic acid, *DPA* docosapentaenoic acid, *EPA* eicosapentaenoic acid, *E%* percent of total energy intake, *LC-PUFA* long-chain polyunsaturated fatty acid, *MUFA* monounsaturated fatty acid, *n-3* omega-3, *SFA* saturated fatty acid

[a] Sum of α-linolenic acid, EPA, DPA, and DHA

[b] Sum of EPA, DPA, and DHA

are presented in Fig. 3. Intakes of total fish ($\rho = 0.17$, $P < 0.01$, P interaction $= 0.08$), processed fish ($\rho = 0.15$, $P < 0.01$, P interaction $= 0.15$), and fish on sandwich ($\rho = 0.26$, $P < 0.001$, P interaction < 0.001) were positively associated with plasma Trp. Lean fish intake was positively associated with HAA ($\rho = 0.22$, $P < 0.001$, P interaction $= 0.01$). The correlation coefficients between total fish, lean fish, and n-3 LC-PUFA intakes and plasma neopterin were slightly stronger in patients with diabetes, but these correlations did not reach statistical significance (Fig. 3). Intakes of total fish ($\rho = -0.17$, $P < 0.01$, P interaction $= 0.02$) and n-3 LC-PUFA ($\rho = -0.19$, $P < 0.001$, P interaction $= 0.09$) were inversely associated with KTR.

Multiple linear regression analysis

Results for the association between intake of total fish, fatty fish, lean fish, and n-3 LC-PUFA and plasma Trp, Kyn, HK, HAA, neopterin, and KTR further assessed by multiple linear regression analysis are shown in Table 3. Intake of fatty fish and n-3 LC-PUFA was inversely associated with

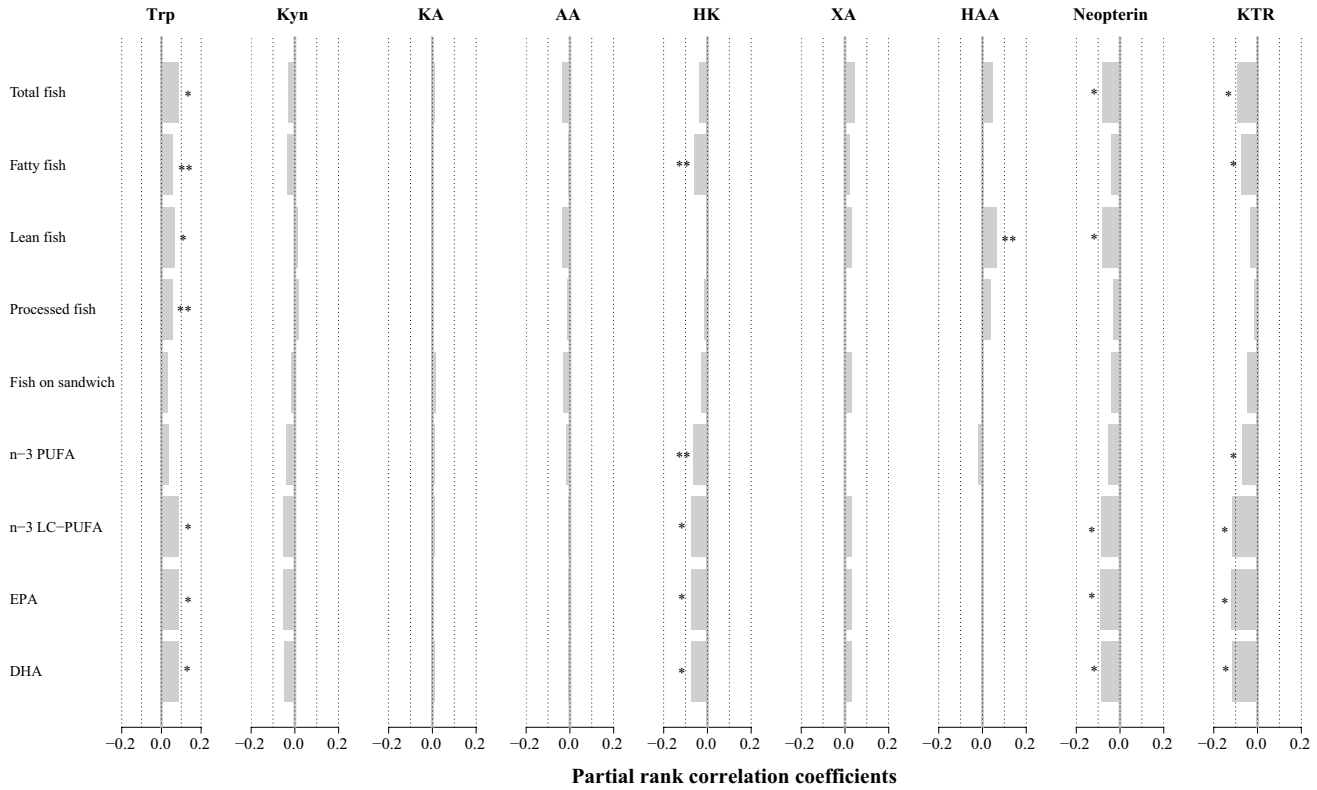

Fig. 2 Correlations between intake of fish and n-3 LC-PUFA and plasma concentrations of Trp, kynurenines, neopterin, and KTR in the total cohort of 2324 patients with coronary artery disease. Partial Spearman's rank correlations were adjusted for age, sex, estimated glomerular filtration rate, BMI, smoking, and energy intake. $^{*}P < 0.001$; $^{**}P < 0.01$. *AA* anthranilic acid, *DHA* docosahexaenoic acid, *EPA* eicosapentaenoic acid, *HAA* hydroxyanthranilic acid, *HK* 3-hydroxykynurenine, *KA* kynurenic acid, *KTR* kynurenine-to-tryptophan ratio, *Kyn* kynurenine, *LC-PUFA* long-chain polyunsaturated fatty acid, *n-3* omega-3, *Trp* tryptophan, *XA* xanthurenic acid

HK, whereas intake of lean fish was positively associated with HAA. Intake of fish and n-3 LC-PUFA was inversely associated with neopterin and KTR. Adding fasting status (dichotomous) to the model did not change the results substantially, except for the association between intake of lean fish and plasma HAA which was no longer statistically significant. The intake of fish on sandwich or processed fish was not associated with plasma Trp, Kyn, HK, HAA, neopterin, and KTR (data not shown). There were no associations between intake of total fish, any types of fish, or n-3 LC-PUFA and plasma KA, AA, and XA (data not shown).

Adjustment for consumption of alcohol, fiber, and vegetables attenuated the associations between intake of fatty fish (P for trend = 0.03) and n-3 LC-PUFA (P for trend = 0.04) and plasma HK (Supplementary Table 1). Adjustment for dietary intake did attenuate the regression coefficients slightly for intake of fish and n-3 LC-PUFA and plasma neopterin and KTR, but the associations remained statistically significant (P for trend <0.01) (Supplementary Table 1).

Discussion

Main observations

This study in individuals with CAD focused on correlations between intake of fish and n-3 LC-PUFA and plasma Trp, six plasma kynurenines, neopterin, and KTR. Intakes of fatty fish and n-3 LC-PUFA were inversely associated with HK. Fish and n-3 LC-PUFA intakes were inversely associated with neopterin and KTR. Associations were weak; however, some correlations were stronger in individuals with diabetes.

Possible mechanisms

Intakes of fish and n-3 LC-PUFA and kynurenines

More than 90 % of Trp is metabolized via the Kyn pathway, and thus dietary availability of Trp may affect the flux through this pathway and thereby the levels of kynurenines

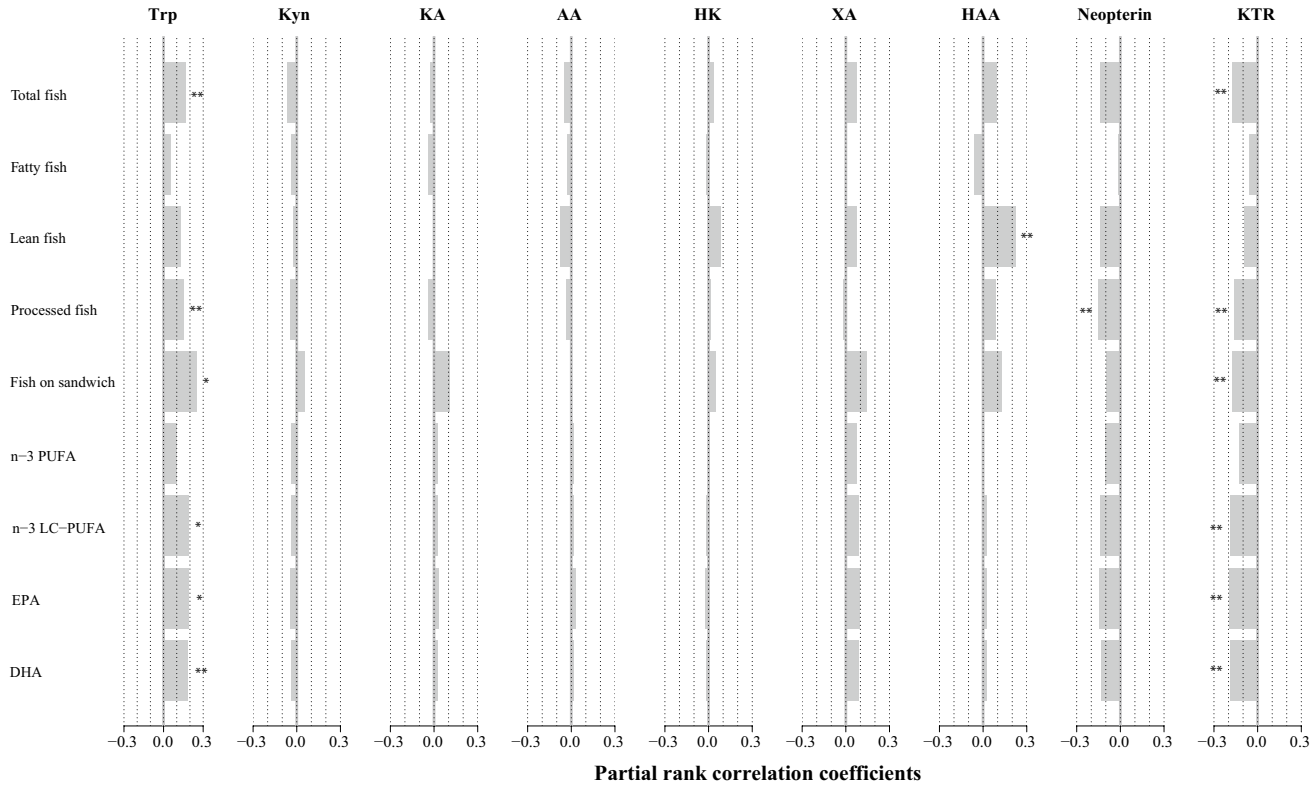

Fig. 3 Correlations between intake of fish and n-3 LC-PUFA and plasma concentrations of Trp, kynurenines, neopterin, and KTR in a subgroup of 306 patients with coronary artery disease and diabetes mellitus. Partial Spearman's rank correlations were adjusted for age, sex, estimated glomerular filtration rate, BMI, smoking, and energy intake. $^{*}P < 0.001$; $^{**}P < 0.01$. *AA* anthranilic acid, *DHA* docosahexaenoic acid, *EPA* eicosapentaenoic acid, *HAA* hydroxyanthranilic acid, *HK* 3-hydroxykynurenine, *KA* kynurenic acid, *KTR* kynurenine-to-tryptophan ratio, *Kyn* kynurenine, *LC-PUFA* long-chain polyunsaturated fatty acid, *n-3* omega-3, *Trp* tryptophan, *XA* xanthurenic acid

[1]. However, data on the effects of habitual tryptophan intake on circulating kynurenines, neopterin, and KTR in humans are scarce. Supplementation with 1–5 g/day of Trp had no effect on blood markers of Trp or kynurenines in healthy Japanese women [29]. In our study, intakes of fish and n-3 LC-PUFA were positively associated with plasma Trp. On the other hand, there were no associations between intake of fish or n-3 LC-PUFA and plasma Kyn, whereas the relationship with HK was negative. Thus, fish intake does not simply appear to be a marker of higher Trp flux through the Kyn pathway.

Tryptophan degradation is induced particularly by the cytokine IFN-γ, although other cytokines may also affect catabolism through the Kyn pathway [30]. The n-3 LC-PUFAs are considered to have anti-inflammatory properties and have been linked to decreased concentrations of inflammatory markers such as CRP, TNF-α, and IL-6, although not all studies report this association [31, 32]. Moreover, intakes of PUFA have been linked to decreased inflammation through their effects on leukocyte action, eicosanoid production, and T cell proliferation [32, 33]. In the present study, fatty fish and n-3 LC-PUFA intakes were inversely associated with HK, which may be explained by a lower level of inflammation with higher intake. The enzyme kynurenine 3-monooxygenase converting Kyn to HK has been induced by IFN-γ treatment in vitro [30]. Moreover, HK was positively correlated with CRP ($\rho = 0.20$, $P < 0.05$) [3]. In addition to n-3 LC-PUFA, other components of fish such as vitamin D [34] have been linked to lower levels of inflammatory markers possibly adding to the anti-inflammatory properties of fish intake. Furthermore, the associations between fatty fish and n-3 LC-PUFA intakes and plasma HK were attenuated when adjusted for consumption of alcohol, fiber, and vegetables, and the overall dietary pattern should be considered in future studies.

Intakes of fish and n-3 LC-PUFA and plasma neopterin and KTR

Increased plasma concentrations of neopterin and KTR may be viewed as markers of IFN-γ-mediated immune activation [4, 5], and associations with these markers may reflect relation to IFN-γ activity. Our findings of an inverse association between fatty fish and n-3 LC-PUFA intakes

Table 3 Unstandardized β coefficients (95 % CI) for plasma Trp, Kyn, HK, HAA, neopterin, and KTR by quartile of fish and n-3 LC-PUFA intakes (g/day) obtained by multiple linear regression in 2324 patients with coronary artery disease

	Trp	Kyn	HK	HAA	Neopterin	KTR
Total fish						
50.8 (35.0, 71.9)[a]						
78.8 (61.0, 96.9)	0.68 (−0.91, 2.27)	0.01 (−0.04, 0.06)	−0.69 (−2.44, 1.05)	0.81 (−1.00, 2.63)	−0.09 (−0.44, 0.27)	−0.12 (−0.93, 0.77)
112 (93.3, 131)	2.39 (0.78, 3.99)	0.01 (−0.03, 0.06)	−1.28 (−3.04, 0.47)	0.89 (−0.93, 2.71)	−0.23 (−0.59, 0.13)	−0.59 (−1.41, 0.23)
178 (148, 217)	2.83 (1.25, 4.42)	−0.03 (−0.08, 0.01)	−0.87 (−2.60, 0.87)	2.18 (0.38, 3.99)	−0.65 (−1.00, −0.29)	−1.55 (−2.37, −0.74)
P for trend	<0.001	0.20	0.26	0.02	<0.001	<0.001
Fatty fish						
1.2 (0.0, 5.5)						
8.6 (4.6, 13.4)	−0.04 (−1.62, 1.54)	0.02 (−0.02, 0.07)	−0.75 (−2.48, 0.99)	−1.09 (−2.90, 0.71)	0.02 (−0.33, 0.37)	0.23 (−0.58, 1.04)
20.9 (16.5, 25.4)	2.36 (0.77, 3.95)	0.03 (−0.02, 0.08)	−1.43 (−3.17, 0.31)	0.21 (−1.60, 2.02)	−0.16 (−0.51, 0.20)	−0.50 (−1.32, 0.31)
44.8 (35.3, 60.3)	1.92 (0.34, 3.49)	−0.03 (−0.08, 0.02)	−2.36 (−4.09, −0.64)	−0.12 (−1.91, 1.67)	−0.35 (−0.70, 0.01)	−1.24 (−2.05, −0.43)
P for trend	<0.01	0.23	<0.01	0.73	0.03	<0.01
Lean fish						
7.0 (2.5, 14.4)						
25.6 (19.6, 28.2)	1.65 (0.07, 3.23)	0.03 (−0.02, 0.07)	−1.30 (−3.03, 0.43)	1.19 (−0.60, 2.98)	−0.22 (−0.57, 0.13)	−0.29 (1.10, 0.52)
40.7 (35.7, 46.1)	0.99 (−0.60, 2.57)	0.01 (−0.03, 0.06)	−0.77 (−2.51, 0.97)	1.60 (−0.20, 3.41)	−0.24 (−0.59, 0.12)	−0.12 (−0.93, 0.70)
74.2 (59.5, 93.2)	2.33 (0.73, 3.93)	0.02 (−0.02, 0.07)	−1.09 (−2.84, 0.66)	2.85 (1.04, 4.67)	−0.73 (−1.09, −0.37)	−0.72 (−1.55, 0.10)
P for trend	0.02	0.47	0.34	<0.01	<0.001	0.13
n-3 LC-PUFA						
0.39 (0.22, 0.62)						
0.71 (0.48, 0.99)	2.45 (0.85, 4.05)	−0.05 (−0.10, −0.00)	−2.85 (−4.60, −1.09)	0.31 (−1.52, 2.14)	−0.68 (−1.04, −0.33)	−1.71 (−2.53, −0.89)
1.27 (1.01, 1.51)	2.87 (1.27, 4.47)	−0.01 (−0.06, 0.03)	−2.41 (−4.16, −0.66)	−0.42 (−2.24, 1.41)	−0.52 (−0.88, −0.17)	−1.24 (−2.06, −0.42)
2.50 (1.99, 3.14)	3.68 (2.11, 5.26)	−0.08 (−0.13, −0.04)	−2.71 (−4.44, −0.99)	0.37 (−1.43, 2.17)	−0.83 (−1.19, −0.48)	−2.69 (−3.50, −1.89)
P for trend	<0.001	<0.01	<0.01	0.89	<0.001	<0.001

Multiple linear regression was performed with all independent variables included in the model simultaneously [energy intake (continuous), age (continuous), gender, BMI (continuous), estimated glomerular filtration rate (continuous), and smoking (no/yes)]. Results are presented as unstandardized β coefficients (95 % CI). *P* for trend was calculated using quartiles as continuous variable in otherwise identical multiple linear regression models

HAA 3-hydroxyanthranilic acid, *HK* 3-hydroxykynurenine, *KTR* kynurenine/tryptophan ratio, *LC-PUFA* long-chain polyunsaturated fatty acid

[a] Median (25th, 75th percentiles). $N = 581$ per quartile

and plasma neopterin and KTR are in line with proposed anti-inflammatory effects of n-3 LC-PUFA intake. However, ex vivo studies in relation to the proposed effects of LC-PUFA intake on IFN-γ activity have not been straightforward. Different results have been reported, including a decrease [35] or no difference [36–38] in the production of IFN-γ with n-3 LC-PUFA supplementation compared with control treatment. In contrast, increased production of IFN-γ was found with a daily supplementation of 2 g EPA + DHA compared to baseline values [39]. In these trials, dosages from 720 to 5000 mg/day of n-3 LC-PUFA were used during a relatively short time period (10–12 weeks), and the effects of habitual dietary intake of n-3 LC-PUFA as explored in our study on IFN-γ production in vivo are unknown. Furthermore, other nutrients (besides n-3 LC-PUFA) in fish may also contribute to the inverse association with circulating neopterin and KTR, since circulating selenium, vitamin B_6, and vitamin E has been inversely associated with plasma neopterin and/or KTR [40–42].

Diabetic patients

In a subgroup of patients with diabetes, we found lean fish intake to be positively associated with HAA. The effects of dietary intake on the Kyn pathway are largely unknown, and what component of lean fish may account for higher plasma HAA is unclear. Furthermore, either whether lean fish consumption per se may be associated with an accumulation of HAA in plasma or whether the increased plasma HAA is indicative of a shift toward the niacin pathway via HAA is uncertain. Moreover, reported lean fish intake may well be a marker of other factors involved in the regulation of HAA that we were unable to adjust for in the current analysis. Nonetheless, considering that high plasma HAA has been associated with increased risk of myocardial infarction in patients with CAD [12], investigating effects of different types of fish intake on metabolites in the Kyn pathway, especially in individuals with diabetes, would be of interest in future studies.

Strengths and limitations

The main strengths of the current study include its large sample size, the range of plasma kynurenines analyzed, and the comprehensive information of both dietary and supplementation data. In addition to total fish intake, data also included type of fish and n-3 LC-PUFA. The extensive clinical characteristics enabled us to control for several factors known to effect circulating concentrations of kynurenines, neopterin, and KTR. There are some limitations that merit attention. The cross-sectional design does not address causality, and the risk of chance findings due to multiple testing cannot be excluded. Furthermore, the study cohort with CAD limits the generalizability of our findings.

Moreover, it is challenging to measure true dietary intake. Intake of fish and n-3 LC-PUFA based on the current FFQ has been compared with n-3 fatty acid composition in plasma phospholipids and with a dietary record [21], with similar results as others [43]. In addition, reported intake of n-3 LC-PUFA correlated well with serum concentrations in a subgroup of patients ($n = 723$) in our population ($\rho = 0.515$, $P < 0.001$) [24]. Furthermore, reported fish intake in our study was similar to that of other Norwegian studies [44, 45]. Thus, we consider that the FFQ used in our current study estimates fish and n-3 LC-PUFA intakes reasonably well, although the variation between individuals may be considerable [21]. The participants in our study had a high intake of both fish and supplements containing EPA and DHA. Eighty-nine percent of the participants reported to have an EPA and DHA consumption corresponding to or above the current recommendation of 250 mg/day [46], which limits the possibility to explore associations with low intakes. The temporal relationships between dietary intake and the measured compounds in our study are unknown. The FFQ measures habitual dietary intake, reflecting the average intake during the past year, and any effects with a shorter temporal relationship may be missed. Furthermore, fish intake by itself may be an indicator of a dietary pattern generally considered as healthy, and overall dietary pattern might be more important than single nutrients or foods. Therefore, dietary pattern analysis would be an additional model to further explore associations between diet and the Kyn pathway in future studies.

Conclusion

In conclusion, consumption of fish and n-3 LC-PUFA was only weakly associated with a few plasma kynurenines, suggesting that fish intake is not an important determinant of circulating kynurenines. Notably, some correlations between fish intake and kynurenines were stronger in diabetic patients. The inverse association between intake of fish and n-3 LC-PUFA and plasma neopterin and KTR may suggest a slightly lower immune activation with higher intakes.

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Compliance with ethical standards

Conflict of interest None of the authors reported a conflict of interest.

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