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Fish and omega-3 fatty acid intake in relation to circulating fibroblast growth factor 23 levels in renal transplant recipients

L.C. Baia [a,b,1], E. Van den Berg [a,1], M.G. Vervloet [c], I.P. Heilberg [b], G. Navis [a], S.J.L. Bakker [a], J.M. Geleijnse [d], D. Kromhout [d], S.S. Soedamah-Muthu [d], M.H. De Borst [a,*], on behalf of the NIGRAM consortium

[a] Department of Nephrology, University Medical Center Groningen and University of Groningen, The Netherlands
[b] Department of Nephrology, Federal University of São Paulo, São Paulo, Brazil
[c] Department of Nephrology, VU University Medical Center Amsterdam, The Netherlands
[d] Division of Human Nutrition, Wageningen University, The Netherlands

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Abstract *Background and aims:* A high circulating fibroblast growth factor 23 (FGF23) level is an independent risk factor for cardiovascular mortality in renal transplant recipients and the general population. N-3 fatty acids eicosapentaenoic (EPA) and docosahexaenoic acid (DHA) may contribute to cardiovascular risk reduction. We investigated whether fish and EPA-DHA intake are related to FGF23 levels in renal transplant recipients.
Methods and results: We performed a cross-sectional analysis in 619 stable renal transplant recipients (mean age 53 years, 57% male, estimated glomerular filtration rate [eGFR] 53 ± 20 mL/min/1.73 m^2). Dietary intake was assessed by a 177-item food frequency questionnaire. Serum intact FGF23 was measured by ELISA. We examined differences in FGF23 levels across categories of fish and EPA-DHA intake using analysis of variance models adjusted for age, sex, dietary and lifestyle factors and key determinants of FGF23. Patients consumed on average 15 g of fish and 139 mg EPA-DHA/day. Median FGF23 was 62 pg/mL (IQR 43–98 pg/mL). Higher dietary EPA-DHA and fish intake were associated with lower serum FGF23 levels. Subgroup analyses revealed that particularly in patients with reduced renal function (eGFR <60 mL/min/1.73 m^2), adjusted FGF23 levels (114, 79, 75 pg/mL, $P = 0.0001$) were inversely associated with tertiles of EPA-DHA intake. Similarly, we observed an inverse association between fish consumption and serum FGF23 levels in adjusted analyses.
Conclusion: A higher intake of fish and dietary *n*-3 fatty acids (EPA-DHA) is related to lower circulating FGF23 levels in renal transplant recipients. Further research is needed to assess the causality of this association and the clinical implications.

Introduction

Chronic kidney disease (CKD) is a worldwide health burden, affecting about 15% of the Western adult population. For most patients with end-stage renal disease, kidney transplantation is the preferred treatment. Although short-term graft and patient survival have improved

Abbreviations: CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; FGF23, fibroblast growth factor 23; PTH, parathyroid hormone.

* Corresponding author. University of Groningen, University Medical Center Groningen, Department of Nephrology, PO Box 30.001, 9700 RB Groningen, The Netherlands. Tel.: +31 50 3616161; fax: +31 50 3619310.
E-mail address: m.h.de.borst@umcg.nl (M.H. De Borst).

[1] Contributed equally.

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impressively over the past decades, cardiovascular disease limits long-term patient survival [1]. Both the incidence and prevalence of cardiovascular disease are several times higher in renal transplant recipients than in the general population [2].

In patients with impaired renal function, deregulated phosphorus metabolism characterized by elevated circulating levels of the phosphaturic hormone fibroblast growth factor 23 (FGF23) plays a specific role in the pathophysiology of cardiovascular disease. In response to – particularly inflammatory – renal injury, the renal expression of Klotho, a mandatory cofactor for the specific FGF23 receptor, is lost [3] which may contribute to increased circulating FGF23 levels [4]. Besides being an indicator of impaired renal function and disturbed phosphorus metabolism, high circulating FGF23 levels may also directly contribute to the development of left ventricular hypertrophy [5]. Many epidemiologic studies have identified a high FGF23 level as an independent risk factor of cardiovascular disease and all-cause mortality in the general population [6], across stages of CKD[7], and in renal transplant recipients [8,9]. Consequently, strategies to reduce FGF23 levels may have clinical impact.

Patients with impaired renal function have lower serum levels of the n-3 fatty acids eicosapentaenoic (EPA) and docosahexaenoic acid (DHA), mainly consumed through fish, which may be linked to a higher cardiovascular disease risk in these patients [10]. Randomized controlled trials in patients with chronic kidney disease (CKD) showed a decrease in serum inflammatory markers after EPA-DHA supplementation [11]. Given the role of FGF23 as a cardiovascular risk factor in renal patients and the potential cardioprotective and anti-inflammatory effects of *n*-3 fatty acids, we investigated whether intake of fish and EPA-DHA are related to serum FGF23 levels in a well-defined cohort of 619 renal transplant recipients.

Methods

Study population

We conducted a cross-sectional analysis in a large, single center renal transplant recipient cohort. We invited all renal transplant recipients ($\geq$18 years) with a functioning graft for at least one year, who visited our outpatient clinic between November 2008 and March 2011. Renal patients had all been transplanted in the University Medical Center Groningen. They had sufficient knowledge of the Dutch language and no history of drug or alcohol addiction. Of 817 initially invited patients, 707 (87%) signed written informed consent to participate in this study. After exclusion of patients with missing data on dietary n-3 fatty acids (n = 82), missing data on eGFR (n = 2) and FGF23 concentrations (n = 4), data from 619 patients were available for analyses. The Institutional Review Board on human experimentation approved the study protocol (METc 2008/186), which was in adherence to the Declaration of Helsinki. The routine regimen included no specific dietary counseling, except for discouraging excess sodium intake and encouraging losing weight in overweight individuals. Renal transplant recipients were on standard antihypertensive and immunosuppressive therapy, which was as previously described [12]. Data on current medication including vitamin D treatment (cholecalciferol, alfacalcidol or paricalcitol) was extracted from the medical records.

Dietary assessment

All patients adhered to their regular dietary habits during examination. Dietary intake was assessed using a semi quantitative food frequency questionnaire (FFQ) which has been validated as described previously [13]. The FFQ inquired about intake of 177 food items during the last month. For each item, the frequency was recorded in times per day, week, or month, and seasonal variations were taken into account. The number of servings was expressed in natural units (for example, slice of bread or apple) or household measures (for example, cup or spoon). The questionnaire was self-administered and filled out at home. At the day of the visit to the outpatient clinic, all FFQs were checked for completeness by a trained researcher and inconsistent answers were verified with the patients. Total energy and nutrient intake per day was calculated using Dutch Food Composition Tables [14]. Additionally, all participants were instructed to collect a 24-h urine sample according to a strict protocol. Sodium intake was estimated from 24-h urine sodium excretion and the accuracy of FFQ for protein intake estimation was inferred by correlating protein intake with the protein equivalent of nitrogen appearance (PNA) [15].

Clinical and biochemical parameters

Information on patient's health status and medical history was obtained from patient records. Patients received state-of-the art treatment (Table 1), and data on current medication was extracted from the medical records. Body weight and height were measured while participants wore indoor clothing without shoes. Body Mass Index (BMI) was calculated as weight divided by height squared (kg/m^2). Blood pressure (BP) was measured as described previously [16]. Hypertension was defined as BP $\geq$140/90 mmHg or use of antihypertensive medication. Diabetes mellitus was considered present when serum glucose was above 7 mmol/l (126 mg/dl) or when the patient used antidiabetic medication.

Blood was drawn after an 8–12 h overnight fasting period in the morning after completion of the 24 h urine collection. Urinary and plasma concentrations of sodium, chloride, potassium, calcium, phosphate and urea were measured using routine clinical laboratory methods as were plasma hsCRP, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides and albumin. Intact parathyroid hormone (PTH) was measured in EDTA plasma using radioimmunoassay. Serum creatinine level was determined using a modified version of the Jaffé method (MEGA

Table 1 Characteristics of 619 renal transplant recipients across tertiles of EPA-DHA.

	Tertiles of EPA-DHA intake (mg/d)			
	<39 (n = 206)	40–158 (n = 207)	≥159 (n = 206)	P value
Age (years)	51 ± 13	53 ± 12	54 ± 12	0.03
Sex, n (% males)	114 (55)	117 (56)	122 (59)	0.72
BMI (kg/m^2)	26 ± 5	26 ± 5	27 ± 4	0.31
Waist circumference (cm)	98 ± 15	98 ± 15	100 ± 14	0.19
Systolic BP (mmHg)	135 ± 16	138 ± 17	135 ± 18	0.27
Diastolic BP (mmHg)	82 ± 10	84 ± 11	83 ± 11	0.18
Time since transplantation (y)	6 [2–13]	6 [2–12]	6 [2–12]	0.80
Current smoker n (%)	27 [14]	21 [11]	28 [14]	0.49
Diabetes mellitus n (%)	40 [19]	31 [15]	42 [20]	0.21
Alcohol use (g/d)				
No	15 [8]	22 [12]	19 [11]	0.59
1-20 g/d	145 (78)	148 (78)	139 (77)	
>20 g/d	27 [14]	19 [10]	23 [13]	
Energy (kcal)	2156 ± 672	2196 ± 601	2197 ± 641	0.42
Medication use				
Antihypertensives n (%)	183 (89)	181 (87)	180 (88)	0.90
Proliferation inhibitor n (%)	166 (81)	169 (82)	183 (89)	0.06
Mycophenolate n (%)	135 (66)	137 (66)	138 (67)	
Azathioprine n (%)	31 [15]	32 [16]	45 [22]	
Calcineurin inhibitor n (%)	120 (58)	127 (61)	104 (51)	0.08
Cyclosporine n (%)	80 (39)	81 (39)	89 (43)	
Tacrolimus n (%)	40 [19]	41 [20]	32 [16]	
Diuretics n (%)	86 (42)	82 (40)	84 (41)	0.91
Vitamin D analogues n (%)	45 [22]	52 [25]	45 [22]	0.68
Serum parameters				
Creatinine (μmol/L)	121 [95–159]	130 [102–164]	124 [103–153]	0.29
eGFR(ml/min/1.73 m^2)*	55 ± 22	51 ± 20	52 ± 19	0.09
Urea (mmol/l)	9.3 [7.1–12.9]	9.5 [7.3–14.2]	9.8 [7.2–13.2]	0.42
PTH (pmol/l)	9.4 [5.6–14.7]	9.4 [6.3–14.5]	8.3 [5.5–13.4]	0.29
Albumin (g/l)	43 ± 3	43 ± 3	43 ± 3	0.98
hsCRP (mg/l)	1.4 [0.6–3.8]	1.6 [0.6–4.2]	1.7 [0.8–5.0]	0.61
Sodium (mmol/l)	141 ± 3	141 ± 3	141 ± 3	0.46
Potassium (mmol/l)	4.0 ± 0.4	4.0 ± 0.5	4.0 ± 0.5	0.92
Calcium (mmol/l)	2.4 ± 0.16	2.4 ± 0.15	2.4 ± 0.15	0.75
Phosphate (mmol/l)	1.0 ± 0.2	1.0 ± 0.2	1.0 ± 0.7	0.52
Total cholesterol (mmol/L)	5.1 ± 1.2	5.1 ± 1.0	5.2 ± 1.1	0.84
HDL cholesterol (mmol/L)	1.4 ± 0.4	1.4 ± 0.5	1.4 ± 0.5	0.30
LDL cholesterol (mmol/L)	3.0 ± 0.9	2.9 ± 0.9	3.0 ± 1.0	0.71
Triglycerides (mmol/L)	1.9 ± 1.0	1.9 ± 1.0	1.8 ± 1.0	0.63
Urinary parameters				
Creatinine (μmol/24 h)	114 ± 36	115 ± 33	119 ± 31	0.32
Albumin (mg/24 h)	42 [9–184]	34 [9–172]	43 [11–152]	0.93
Sodium (mmol/24 h)	150 ± 58	157 ± 61	162 ± 63	0.11
Calcium (mmol/24 h)	2.4 [1.1–3.7]	2.3 [1.0–3.9]	2.3 [1.2–3.9]	0.88
Phosphate (mmol/24 h)	24.1 ± 9.1	24.4 ± 8.5	25.8 ± 8.3	0.11

Values in Table 1 are mean ± SD, n(%) or median (p25–p75). P values were obtained from ANOVA for normally distributed continuous variables, from Kruskall–Wallis test for skewed continuous variables and from Chi-squared test for categorical variables.
EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; BP: blood pressure; *eGFR: estimated glomerular filtration rate, based on Chronic Kidney Disease-Epidemiology (CKD-EPI) Collaboration formula [17]; PTH: parathyroid hormone; hsCRP: high sensitivity C-reactive protein.

AU 510, Merck Diagnostica, Darmstadt, Germany). Renal function was assessed by calculating the estimated glomerular filtration rate (eGFR) applying the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [17]. Urinary albumin concentration was determined by nephelometry (Dade Behring Diagnostic, Marburg, Germany). Total urinary protein concentration was analyzed using the Biuret reaction (MEGA AU 510, Merck Diagnostica, Darmstadt, Germany). Serum intact FGF23 levels were determined using a commercially available ELISA kit (Kainos Laboratories, Inc., Tokyo, Japan). Intra- and inter-assay CVs are <10% and <14%, respectively [18], reference range in healthy subjects is 10–40 pg/mL.

Statistical analysis

Statistical analyses were performed using Stata (version 11.0, StataCorp, College Station, Texas, USA) and SAS statistical packages (version 9.3, SAS Institute, Cary, North Carolina, USA). A two sided P-value <0.05 was considered statistically significant. Because the intakes of EPA and

DHA were highly correlated (Spearman correlation coefficient: 0.99) the sum of EPA and DHA was used in the analyses.

Descriptive analyses were performed to calculate mean and standard deviations (SD) or medians and interquartile range (IQR: p25, p75). Differences in baseline variables among different categories of *n*-3 fatty acid consumers were evaluated by using the Kruskal–Wallis test for skewed variables, the analysis of variance (ANOVA) for normally distributed continuous variables and Chi-squared test for categorical data. The association between EPA-DHA intake and lipid profile (i.e. total, HDL, or LDL cholesterol or triglyceride levels) was studies using Spearman correlation analysis.

Backward linear regression was used to identify correlates of plasma FGF23 levels. The following covariates were tested: age, sex, donor type (deceased or living), warm and cold ischemia times, BMI, cardiovascular history, Framingham risk score factors, eGFR, albuminuria, serum phosphate, 24-h urinary phosphate excretion (representing phosphate intake), 24-h urinary urea excretion (representing protein intake), high-sensitivity C-reactive protein (hsCRP), serum albumin, hemoglobin, or NT-proBNP levels, use of angiotensin-converting enzyme inhibitor or angiotensin receptor blocker, and treatment with vitamin D analogues. Variables significantly associated with FGF23 levels using backward linear regression analysis were subsequently tested in a forward linear regression model. Variables that were significant in this model were considered independent correlates of FGF23 levels.

To assess whether mean FGF23 levels differed according to categories of EPA-DHA intake or fish intake ANOVA models were used. EPA-DHA intake was divided into tertiles and fish intake was categorized as 0, >0–14, and ≥15 g/d (because of 18% no fish eaters). Back transformation was performed to obtain geometric mean FGF23 levels and 95% confidence intervals in categories of fish intake (or EPA-DHA intake). Several multivariable models were applied, adjusting for age and sex (model 1), and for lifestyle factors, i.e. total energy intake, alcohol use (0, >0–20, >20 g/d), smoking status and BMI (model 2). *P*-values for the differences in dietary intake were obtained from the Kruskal–Wallis test for skewed variables. Based on the outcomes of this analysis, we additionally adjusted our data for fruit and vegetables intake (model 3) on the assumption that fish consumers are more likely than are fish non-consumers to follow a healthy diet rich in fruits and vegetables [19]. Finally, known determinants of FGF23, i.e. serum calcium, phosphate and PTH were added to the full model (model 4).

The association between fish or EPA-DHA intake and serum FGF23 was analyzed for an interaction by eGFR, either as a dichotomous or continuous variable, by adding an interaction term (eGFRxFGF23) to all ANOVA models. Because a significant interaction term was found ($P < 0.0001$), we repeated the analyses in strata of renal graft function (eGFR <60 vs. ≥60 mL/min/1.73 m^2).

Table 2 Multivariate analysis of FGF23 correlates.

Variable	Standardized beta	*P* value
eGFR (CKD-EPI)	−0.27	<0.001
Serum phosphate	0.24	<0.001
NTproBNP	0.24	<0.001
Urinary phosphate excretion	0.17	<0.001
Triglycerides	0.12	<0.001
Age	0.10	0.01
Gender (0 = male; 1 = female)	−0.10	0.01
Smoking (0 = never; 1 = ever)	0.08	0.01
Parathyroid hormone	0.07	0.045
Albuminuria	0.07	0.049

Excluded from the model: use of RAAS blockers or vitamin D analogues, cold and warm ischemia times, time since transplantation, hemoglobin, donor status (living or deceased), presence of diabetes mellitus, BMI, urinary urea excretion, serum cholesterol, hsCRP. Model fit: R^2 0.40.

Results

Patient characteristics

Patients were on average 53 ± 13 years old and 57% was male. The mean BMI was 26.7 ± 4.8 kg/m^2, with 59% of the patients being overweight. The median time since transplantation was 5.4 [1.9–12.2] years. Mean systolic blood pressure was 136/83 mmHg and 91% of the cohort had hypertension. The mean fish intake was 15 g/d, which corresponds to approximately one serving per week, and EPA-DHA intake was 139 mg/d. The median serum intact FGF23 level was 62 [IQR: 43–98] pg/mL. Patients with a reduced graft function (eGFR<60 mL/min/1.73 m^2, $n = 403$, 65%) had a serum FGF23 concentration of 78 [IQR: 54–121] pg/mL, which was higher than in those with a normal graft function ($n = 216$, 47 pg/mL [IQR: 35–61]; $P < 0.001$). Patient characteristics in tertiles of EPA-DHA intake are shown in Table 1. Those who had a higher EPA-DHA intake were slightly older ($P = 0.03$). No significant differences were observed in cardiovascular risk factors, medication use, or other clinical parameters. EPA-DHA intake was not associated with lipid profile (HDL, LDL, total cholesterol or triglycerides) or hsCRP. Multivariate regression analysis revealed independent determinants of FGF23 levels in our cohort (Table 2).

Fish consumption and circulating FGF23

Circulating FGF23 levels were inversely related to fish intake (Table 3), with geometric mean levels of 78.0 pg/mL for 0 g/d, 73.3 pg/mL for 1–14 g/d and 64.4 pg/mL for ≥15 g/d (*P*-trend = 0.06) after adjustment for age, sex, BMI and dietary and lifestyle factors (model 3). After adjustment for key determinants of FGF23 (serum calcium, phosphate and PTH), the association became statistically significant ($P = 0.03$, Table 3, model 4). The association between fish intake and FGF23 levels was influenced by eGFR (*P*-interaction <0.0001 in all models). In patients with a reduced graft function (eGFR <60 mL/min/1.73 m^2), FGF23 levels were 108.9 pg/mL, 89.5 pg/mL and 77.0 pg/

Table 3 Fish intake and serum FGF23 levels (pg/mL)[a] in 619 renal transplant patients, in the total cohort and stratified by renal graft function.

	Fish intake (g/d)			
	0	1–14	≥15	*P*-value
Total cohort				
N	108	234	277	
Model 1	72.3 (63.0–82.9)	72.0 (65.6–79.0)	64.5 (59.2–70.2)	0.167
Model 2	76.7 (66.3–88.8)	73.5 (66.4–81.4)	64.2 (58.5–70.5)	0.063
Model 3	76.9 (66.4–89.0)	73.4 (66.2–81.4)	64.3 (58.4–70.6)	0.067
Model 4*	76.7 (68.1–86.4)	75.5 (69.4–82.1)	62.8 (58.1–67.8)	0.002
Patients with eGFR <60 mL/min/1.73 m^2.				
N	62	149	192	
Model 1	99.5 (82.5–120.0)	91.4 (81.0–103.1)	75.8 (68.1–84.3)	0.015
Model 2	106.7 (87.3–130.4)	93.1 (81.4–106.4)	75.2 (67.1–84.4)	0.005
Model 3	107.6 (88.0–131.6)	92.2 (80.5–105.6)	75.3 (67.1–84.6)	0.005
Model 4	107.3 (89.7–128.3)	89.6 (79.4–101.2)	77.0 (69.5–85.3)	0.005
Patients with eGFR ≥60 mL/min/1.73 m^2.				
N	46	85	85	
Model 1	46.1 (40.4–52.5)	47.4 (43.1–52.1)	45.2 (41.1–49.7)	0.784
Model 2	49.1 (42.9–56.2)	48.8 (44.1–53.9)	42.2 (37.9–47.0)	0.104
Model 3	48.7 (42.5–55.7)	49.3 (44.6–54.5)	42.2 (37.9–47.0)	0.091
Model 4	48.1 (42.4–54.5)	49.5 (45.1–54.4)	42.3 (38.3–46.8)	0.071

[a] FGF23 levels are geometric means (95% confidence interval).
Model 1 = age and gender adjusted Model 2 = model 1 + energy intake, alcohol consumption, smoking status, BMI and use of vitamin D analogues Model 3 = model 2 + vegetables and fruit Model 4 = model 3 + serum calcium, phosphate and PTH Model 4* = model 3 + eGFR, serum calcium, phosphate and PTH.

mL ($P = 0.003$) in consecutive categories of fish intake, using the fully adjusted model. The association was not significant in those with normal graft function ($P > 0.2$, Table 3).

EPA-DHA intake and circulation FGF23

Circulating FGF23 levels decreased with increasing EPA-DHA intake (Table 4), with geometric mean levels of 81.9 pg/mL for <39 mg/d, 63.9 pg/mL for 40–158 mg/d and 65.6 pg/mL for ≥159 mg/d ($P = 0.003$) after adjustment for age, sex, BMI and dietary and lifestyle factors (model 3). After further adjustment for key determinants of FGF23, this inverse association remained ($P = 0.001$, Table 4). As for fish intake, the association between EPA-DHA intake and FGF23 was influenced by eGFR (P-interaction <0.0001 in all models). In patients with a reduced graft function (eGFR <60 mL/min/1.73 m^2), FGF23 levels were 109.6 pg/mL, 77.9 pg/mL and 78.2 pg/mL (P-trend = 0.0002) in consecutive tertiles of EPA-DHA, using the fully adjusted model 4 (Fig. 1). In those with an eGFR >60 mL/min/1.73 m^2, the association with EPA-DHA intake was also significant ($P = 0.004$), but FGF23 levels were 50% lower than in those with reduced graft function (Table 4). Further adjustments for urinary sodium and phosphate excretion did not alter the results.

Discussion

The main finding of the current study is the inverse association of fish and dietary intake of *n*-3 long-chain polyunsaturated fatty acids (EPA-DHA) with serum FGF23 levels in a large cohort of renal transplant recipients. Particularly in patients with reduced graft function (eGFR <60 mL/min/1.73 m^2) circulating FGF23 levels were reduced with increasing tertiles of EPA-DHA or categories of fish intake.

Daily intake of fish (~15 g/d) and EPA-DHA (~139 mg/d) in our cohort of renal transplant recipients was well below the recommended intake levels of two servings of fish per week, equaling 450 mg/d EPA-DHA [20]. Similar low levels of intake (15 g/d fish) were also observed in a recent clinical trial in post-myocardial infarction patients in The Netherlands [21]. A strong point of the present study is the large, well-characterized cohort of renal transplant recipients, with extensive dietary data collection, serum samples and 24-h urinary samples. This enabled us to adjust for many potential dietary confounders, including 24-h sodium and phosphate excretion. Nevertheless, since this is an observational study, we cannot exclude the possibility of residual confounding by (unmeasured) dietary or lifestyle factors, such as physical activity, that could potentially have influenced serum FGF23 in our patients. Furthermore, we did not measure a circulating biomarker of n-3 fatty acid content to confirm the association between n-3 fatty acid intake and FGF23 levels. Another limitation is the cross-sectional nature of our study, and patients may have intentionally changed their diets for health-related reasons. On the other hand, since fish is not a major contributor to total daily protein and mineral intakes, we consider it unlikely that patients have been advised to change their intake of fish. Finally, we did not adjust for serum vitamin D levels or dietary

Table 4 EPA-DHA intake and serum FGF23 levels (pg/mL)[#] in 619 renal transplant patients, in the total cohort and stratified by renal graft function.

	EPA-DHA intake (mg/d)			
	<39	40–158	≥159	*P*-value
Total cohort				
N	206	207	206	
Model 1	76.9 (69.6–84.9)	64.9 (58.8–71.6)	64.7 (58.6–71.4)	0.022
Model 2	81.2 (72.9–90.4)	64.1 (57.6–71.2)	63.3 (58.6–72.9)	0.004
Model 3	81.3 (72.9–90.5)	64.1 (57.6–71.4)	65.3 (58.4–72.9)	0.004
Model 4*	86.5 (79.3–94.3)	62.1 (57.0–67.6)	63.4 (58.0–69.3)	<0.0001
Patients with eGFR <60 mL/min/1.73 m^2.				
N	116	141	146	
Model 1	106.4 (92.9–121.9)	80.0 (70.8–90.4)	74.6 (66.2–84.2)	0.0004
Model 2	113.1 (97.4–131.2)	78.6 (68.9–89.7)	75.4 (66.1–86.1)	0.0001
Model 3	112.7 (97.0–131.0)	78.7 (69.0–89.8)	75.3 (65.9–86.0)	0.0002
Model 4	108.3 (94.7–123.8)	78.0 (69.3–87.8)	78.4 (69.6–88.0)	0.0003
Patients with eGFR ≥60 mL/min/1.73 m^2.				
N	90	66	60	
Model 1	50.3 (45.9–55.2)	41.6 (37.4–46.3)	45.7 (40.9–51.1)	0.030
Model 2	53.0 (48.3–58.2)	40.2 (36.0–44.8)	44.0 (38.9–49.7)	0.001
Model 3	52.7 (48.0–58.0)	40.0 (35.8–44.8)	44.9 (39.6–50.9)	0.001
Model 4	52.7 (48.2–57.5)	41.3 (37.2–45.9)	43.3 (38.5–48.7)	0.001

[a]FGF23 levels are geometric means (95% confidence interval).
Model 1 = age and gender adjusted Model 2 = model 1 + energy intake, alcohol consumption, smoking status, BMI and use of vitamin D analogues Model 3 = model 2 + vegetables and fruit Model 4 = model 3 + serum calcium, phosphate and PTH Model 4* = model 3 + eGFR, serum calcium, phosphate and PTH.

vitamin D intake as such parameters were lacking in the present cohort. Treatment with vitamin D analogues did however not contribute to FGF23 levels, nor did it influence the association between EPA-DHA intake and FGF23 levels.

Although our data do not allow us to draw conclusions on how fish or EPA-DHA could regulate FGF23 levels, we can speculate on a potential mechanism through anti-inflammatory effects increasing renal klotho expression. Pro-inflammatory cytokines have been linked to a reversible klotho down-regulation in the kidney [23]. In line, FGF23 levels have recently been linked with systemic inflammation in CKD [24]. The inverse association between EPA-DHA intake and FGF23 could therefore be explained by a partial restoration of renal klotho expression due to its anti-inflammatory effects on tubular epithelial cells [25]. Also, n-3 fatty acids may increase the expression of antioxidant genes [26], and oxidative stress has been associated with lower klotho expression [27]. Of interest, interventions that reduced oxidative stress partially restored renal klotho expression in animal models [28]. As an alternative explanation, n-3 fatty acids could act directly on the PTH receptor, a potent inducer of FGF23 [29]; yet adjustment for phosphate, calcium and PTH did not change the observed association between n-3 fatty acid or fish intake and FGF23.

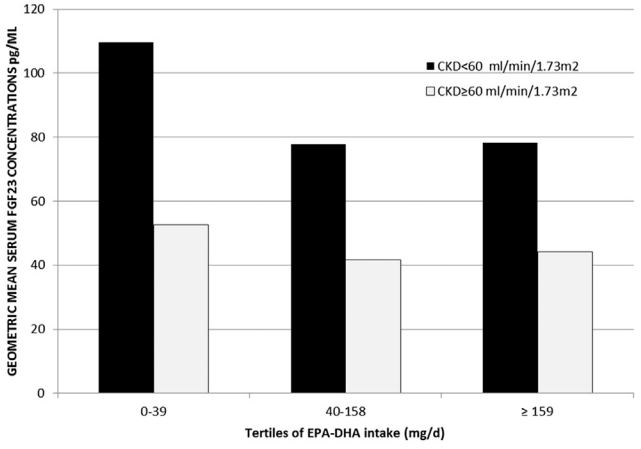

Figure 1 Serum FGF23 levels (pg/mL) according to tertiles of dietary EPA-DHA intake stratified by renal graft function (eGFR above or below 60 mL/min/1.73 m^2).

Serum FGF23 levels have been shown to be influenced by phosphate-rich foods, particularly by protein from animal sources. Yet the most important factor contributing to FGF23 levels is suggested to be inorganic phosphate [30].

In summary, the present study showed for the first time that fish and EPA-DHA intake are associated with serum FGF23 levels in a large cohort of renal transplant recipients. Future prospective studies should address whether increased fish or EPA-DHA intake could be new strategies to reduce cardiovascular morbidity and mortality after kidney transplantation through reduction of FGF23.

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