Intake of *n*-3 fatty acids and long-term outcome in renal transplant recipients: a *post hoc* analysis of a prospective cohort study

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Abstract

Supplementation with *n*-3 fatty acids may improve long-term outcomes of renal transplant recipients (RTR). Recent evidence suggests that EPA and DHA have different outcomes compared with α-linolenic acid (ALA). We examined the prospective associations of EPA–DHA and ALA intakes with graft failure and all-cause mortality in 637 RTR. During 3·1 years (interquartile range 2·7, 3·8) of follow-up, forty-one developed graft failure and sixty-seven died. In age- and sex-adjusted analyses, EPA–DHA and ALA intakes were not associated with graft failure. EPA–DHA intake was not significantly associated with mortality (hazard ratio (HR) 0·79; 95% CI 0·54, 1·15 per 0·1 energy% difference). ALA intake was significantly associated with mortality (HR 1·17; 95% CI 1·04, 1·31 per 0·1 energy% difference). This association remained following adjustments for BMI, proteinuria and intakes of fat, carbohydrate and protein. RTR in the highest tertile of ALA intake exhibited about 2-fold higher mortality risk (HR 2·21; 95% CI 1·23, 3·97) compared with the lowest tertile. In conclusion, ALA intake may be associated with increased mortality in RTR. Future RCT are needed to confirm these results.

Key words: *n*-3 Fatty acids: Renal transplant recipients: Mortality: Graft failure

The prevalence of end-stage renal disease (ESRD) is increasing worldwide[1]. In the USA, the incidence of ESRD in 2012 was as high as 400 cases per million residents per year[2]. The preferred treatment for most ESRD patients is renal transplantation. Compared with dialysis, treatment is less expensive, improves quality of life and increases survival[3,4]. Although 90% of allografts last for at least 1 year[5,6], more than half of these grafts do not last for longer than 10–12 years[6]. This is largely due to graft failure and premature mortality[6]. Thus, there is a great need for approaches to improve survival.

Many randomised-controlled trials have investigated whether *n*-3 fatty acid supplements reduce cardiovascular risk and improve graft survival in renal transplant recipients (RTR). A meta-analysis from 2007 concluded that it cannot be excluded that RTR may benefit from such supplements[7]. In this respect, it is important to realise that the effects of marine-derived *n*-3 fatty acids such as EPA and DHA and plant-derived *n*-3 fatty acids such as α-linolenic acid (ALA) may differ. For example, in the general population, it has been found that supplementation with EPA and DHA was protective, whereas supplementation with ALA was not[8]. Importantly, in a large Swedish study including more than

2000 men aged 50 years or older, it was suggested that exposure to ALA has an adverse effect on mortality[9]. In RTR, it is unclear whether marine-derived and plant-derived *n*-3 fatty acids differ in effects on long-term outcome.

In the present study, we aimed to investigate the association of marine-derived and plant-derived *n*-3 fatty acids with long-term outcome in stable outpatient RTR. For this purpose, we examined EPA–DHA and ALA intakes separately, and analysed whether the intakes of these *n*-3 fatty acids are associated with graft failure and all-cause mortality.

Methods

Design and study population

We performed a *post hoc* analysis in an existing prospective cohort study on RTR in which baseline FFQ data were available that were suitable to address our research question. The study took place at the University Medical Center Groningen between November 2008 and March 2011[10]. All RTR (≥18 years of age) had a functioning graft for at least 1 year, no alcohol or drug addiction and

sufficient knowledge of the Dutch language. In total, 817 RTR were invited for the study, of whom 707 (87 %) provided written consent to participate. In the present study, patients with missing dietary data were excluded from the analyses, resulting in 637 RTR for analysis. The present study was approved by the Institutional Review Board (METc 2008/186). Dietary intake and clinical parameters were assessed once at baseline. Outcome assessors were blinded to dietary intake measurements and other baseline parameters. RTR did not receive dietary recommendation regarding *n*-3 fatty acid intake. Patients were taking standard immunosuppressive and antihypertensive drugs.

Outcome parameters

The primary long-term outcomes of the study were graft failure and all-cause mortality. Graft failure was defined as return to dialysis or re-transplantation. The patients were followed-up until 30 April 2013, with no loss to follow-up.

Dietary assessment

Dietary intake was assessed using a semi-quantitative, validated FFQ that was developed at Wageningen University[11]. The questionnaire consisted of 177 food items to record intake during the previous month, expressed in times per day, week or month. The number of servings was recorded in natural units (e.g. slice of bread or apple) or household measures (e.g. cup or spoon). Patients had to fill in the questionnaire at home. All FFQ were checked by a trained researcher and verified with the patients in case of inconsistent answers. The results of the FFQ were converted into total energy and nutrient intake by using the Dutch Food Composition Table of 2006[12].

Clinical parameters

All measurements were obtained during a morning visit to the outpatient clinic. Patients were instructed to collect a 24-h urine sample the day before the visit. In the morning, blood was drawn after a fasting period of 8–12 h. Body weight and height were measured with patients wearing indoor clothing without shoes. BMI was calculated as weight in kilograms divided by height in metres squared (kg/m^2), and body surface area was measured as metres squared (m^2). Diabetes was defined as use of antidiabetic medication or fasting serum glucose $\geq 7\cdot 0$ mmol/l[13]. Fasting blood pressure, heart rate and mean arterial pressure were determined using a semi-automatic device (Dinamap 1846; Critikon). Blood pressure and heart rate were measured every minute for 15 min, and the last three measures were averaged[14]. Renal function was assessed by estimated glomerular filtration rate (eGFR)[15]. Serum creatinine levels were measured with a modified version of the Jaffé method (MEGA AU 510; Merck Diagnostica). Total urinary albumin concentration was analysed by nephelometry (Dade Behring Diagnostics), whereas total urinary protein concentration was determined using the Biuret reaction (MEGA AU 150). Serum cholesterol, glycated Hb and high-sensitive C-reactive protein were measured according to routine laboratory methods. Proteinuria was defined as urinary protein excretion $\geq 0\cdot 5$ g/24 h.

Information on patient health status, medical history and medication use was obtained from patient records. Information on smoking habits and alcohol intake was collected as well. Patients were divided into current smokers, former smokers or never smokers. In addition, patients were also divided into four groups according to their alcohol intake (g ethanol/d) (no alcohol, 0–10, 10–30 and >30 g/d). Smoking behaviour and alcohol intake were based on self-report.

Statistical analyses

Data analysis was performed using SPSS version 22.0 software (SPSS, Inc.) and R version 3.2.3. Data are presented as mean values and standard deviations or as medians and interquartile ranges (IQR) unless stated otherwise. *n*-3 Fatty acid intake was measured as a percentage of total energy intake (En%). For initial exploration, EPA–DHA and ALA intakes were divided into tertiles, which were compared by means of ANOVA, Kruskal–Wallis or χ^2 tests depending on whether the distribution of data was normal, skewed or categorical, respectively. Linear regression analyses with intake of *n*-3 fatty acids as a dependent variable were carried out to compare strengths of associations of different baseline variables with *n*-3 fatty acids. For these analyses, categorical variables were divided into two or more dummy variables. Baseline characteristics with a *P*-value <0·10 for the association with intake of *n*-3 fatty acids were selected as potential explanatory variables in subsequent Cox regression analyses. Cox regression analyses were also used to test whether there were interactions between *n*-3 fatty acid intake and potential explanatory variables (age, sex, BMI, smoking habits, alcohol intake, intakes of protein, fat and carbohydrates, proteinuria, blood pressure). In prospective analyses, we investigated the associations of EPA–DHA and ALA intake with graft failure and all-cause mortality in RTR. Analyses were performed with adjustment for age and sex (model 1) and further cumulative adjustments for BMI (model 2), alcohol intake, smoking behaviour (model 3), total fat intake, total protein intake, total carbohydrate intake (model 4), proteinuria and diastolic blood pressure (DBP) (model 5). Analyses were each time initially performed for EPA–DHA and ALA intakes as continuous variables and repeated for EPA–DHA and ALA intake as tertiles. Cox regression analyses with restricted cubic splines were carried out to show the association between ALA intake and all-cause mortality. A two-sided *P*-value <0·05 was considered statistically significant. As there were only a small number of statistical tests related to our *a priori* hypotheses, correction for multiple testing was not required[16,17].

In secondary analyses, *n*-3 fatty acid intake was measured in grams per day instead of En%. For this analysis, *n*-3 fatty acid intake was adjusted for total energy intake according to the residual method, which is based on the isoenergetic principle[18]. In addition, we calculated both the intake of EPA–DHA and the intake of ALA as a proportion of total intake of fatty acids. As sensitivity analyses, we repeated the Cox regression analyses after elimination of patients with extreme intakes of *n*-3 fatty acids outside of 3 SD from the mean value – that is, an EPA–DHA intake >0·30 En% or an ALA intake >1·10 En%.

Results

Population characteristics of RTR are presented in Table 1. Median time after transplantation was 5·6 (IQR 1·8–12·1) years. The mean age of the study population was 53 (SD 13) years, and 57 % was male. The participants were slightly overweight (mean BMI of 26·6 kg/m²) and 24 % had diabetes. The mean blood pressure of RTR was within normal boundaries (136/83 mmHg). It should, however, be noted that 88 % of the 637 participants were using antihypertensive drugs. Mean serum total cholesterol was 5·1 (SD 1·1) mmol/l, whereas statins were taken by 53 % of the recipients. Calcineurin inhibitors were used by 57·1 % of the RTR and 83·3 % were using proliferation inhibitors. Mean eGFR was 49 ml/min per 1·73 m². The median urinary protein excretion was 0·19 (IQR 0–8·91) mg/24 h, with 139 (22 %) RTR having proteinuria.

Energy intake was 9117 (SD 2664) kJ/d (2175 (SD 637) kcal/d). Of the total intake, 36 En% was derived from fat (SFA: 13 En%; MUFA: 12 En%; PUFA: 8 En%), 15 En% from proteins and 46 En% from carbohydrates. Median intakes of EPA–DHA and ALA were 92 (IQR 28–205) mg/d (0·04 (IQR 0·01–0·09) En%) and 1·2 (IQR 0·9–1·7) g/d (0·5 (IQR 0·4–0·7) En%), respectively. According to the European Food Safety Authority (EFSA), intake of EPA and DHA was below the recommended combined intake of 250 mg of EPA plus DHA/d in 524 RTR (82 %), whereas intake of ALA was in accordance with the guidelines (0·5 En%) in 352 RTR (55 %)[19]. Patient characteristics according to tertiles of EPA–DHA and ALA intakes (En%) are shown in online Supplementary Tables S1 and S2, respectively.

The associations of baseline characteristics with intake of *n*-3 fatty acids are shown in Table 2. Age was found to be positively associated with the marine-derived *n*-3 fatty acids ($\beta = 0·094$, $P = 0·02$), whereas age was inversely associated with intake of plant-derived ALA ($\beta = -0·093$, $P = 0·02$). In addition, moderate alcohol intake ($\beta = 0·197$, $P < 0·001$) and former smoking habits ($\beta = 0·128$, $P = 0·01$) were positively associated with EPA–DHA intake, whereas there was no association with ALA intake. Furthermore, total fat intake ($\beta = -0·157$, $P < 0·001$) and total carbohydrate intake ($\beta = -0·272$, $P < 0·001$) were inversely associated with EPA–DHA intake, whereas total fat intake ($\beta = 0·318$, $P < 0·001$) had a positive association with ALA intake. Carbohydrate intake was not associated with ALA intake. We found no significant interaction of *n*-3 fatty acid intake with potential explanatory variables for the association with graft failure and all-cause mortality.

For prospective analyses, the median follow-up was 3·1 (IQR 2·7–3·8) years. During the follow-up period, forty-one RTR developed graft failure, whereas sixty-seven RTR died.

Results for Cox regression analyses for graft failure are shown in Table 3. Neither EPA–DHA nor ALA intake appeared to be associated with graft failure (hazard ratio (HR) 0·83; 95 % CI 0·50, 2·27; and HR 0·90; 95 % CI 0·74, 1·09, respectively, per 0·1 En% difference) while treating intakes as continuous variables. Findings were similar when intakes were treated as tertiles.

Results of Cox regression analyses for all-cause mortality are shown in Table 4. In age- and sex-adjusted analyses, with EPA–DHA intake treated as a continuous variable, there was no association with all-cause mortality (HR 0·79; 95 % CI 0·54,

Table 1. Baseline characteristics of 637 renal transplant recipients
(Mean values and standard deviations; medians and interquartile ranges (IQR); numbers and percentages)

Characteristics	Mean	SD	Range (min–max)
Demographics			
Age (years)	53	13	18–78
Sex (% male)	57		
BMI (kg/m²)	26·6	4·8	15·7–45·0
BSA (m²)	1·9	0·2	1·1–2·8
Smoking behaviour (%)			
No	41·3		
Former	45·8		
Current	12·9		
Diabetes (yes)			
n	154		
%	24·2		
Haemodynamic parameters			
SBP (mmHg)	136	17	80–200
DBP (mmHg)	83	11	54–125
MAP (mmHg)	101	12	66–145
Heart rate (bpm)	69	12	41–122
Medication use			
Antihypertensives (%)	87·9		
Statins (%)	52·8		
CNI (%)	57·1		
Proliferation inhibitors (%)	83·8		
mTor (%)	1·6		
Prednisolone dose (mg/d)			0–20·0
Median	10		
IQR	7·5–10		
Renal function parameters			
Time after transplantation (years)			0·2–40
Median	5·6		
IQR	1·8–12·1		
Serum creatinine (μmol/l)			50–591
Median	124		
IQR	100–160		
eGFR (ml/min per 1·73 m²)	49	20	7–115
Proteinuria ≥0·5 g/d (%)			
n	139		
%	22		
Protein excretion (mg/24 h)			0·00–8·91
Median	0·19		
IQR	0·02–0·35		
Albumin excretion (mg/24 h)			0·02–6258
Median	42·5		
IQR	10–172		
Serum parameters			
Albumin (g/l)	43·0	3·0	28–51
Cholesterol (mmol/l)	5·1	1·1	2·3–9·7
LDL-cholesterol (mmol/l)	2·9	0·9	0·7–6·6
HDL-cholesterol (mmol/l)	1·4	0·5	0·4–3·5
TAG (mmol/l)			0·3–8·5
Median	1·7		
IQR	1·2–2·3		
HbA1c (%)	6·0	0·8	4·5–11·8
Hs-CRP (mg/l)			0·1–114·0
Median	1·6		
IQR	0·7–4·5		
Dietary intake (FFQ)			
Energy intake			
kJ/d	9117	2664	2770–27 428
kcal/d	2175	637	657–6575
Total fat intake (g/d)	88	34	21–412
Total protein intake (g/d)	82	20	25–177
Total carbohydrates (g/d)	249	78	16–539
Alcohol intake (g/d)			0·0–75·8
Median	2·6		
IQR	0·03–11·2		
n-3 Fatty acid intake			
EPA–DHA intake (mg/d)			0·0–1370
Median	92·1		
IQR	28·2–204·8		

Table 1. *Continued*

Characteristics	Mean	SD	Range (min–max)
Relative amount (En%)	0·04		
ALA intake (g/d)			0·3–6·4
Median	1·2		
IQR	0·9–1·7		
Relative amount (En%)	0·5		

BSA, body surface area; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; bpm, beats per minute; CNI, calcineurin inhibitor; mTor, mammalian target of rapamycin; eGFR, estimated glomerular filtration rate; HbA1c, glycated Hb; Hs-CRP, high-sensitive C-reactive protein; En%, energy percentage, ALA, *α*-linolenic acid.

Table 2. Association of baseline EPA–DHA and ALA intakes with baseline parameters (Regression coefficients (*β*) per standard deviation of EPA–DHA and ALA intakes)

	EPA–DHA		ALA	
Dependent variables	*β*	*P*	*β*	*P*
Demographics				
Age (years)	0·094	0·02	− 0·093	0·02
Sex	0·033	0·41	−0·068	0·09
BMI (kg/m²)	0·068	0·09	0·065	0·10
BSA (m²)	−0·025	0·53	0·095	0·02
Smoking habits				
Former	0·128	0·003	−0·017	0·69
Current	0·062	0·15	−0·061	0·16
Diabetes (% yes)	0·005	0·90	−0·002	0·96
Haemodynamic factors				
SBP (mmHg)	−0·045	0·26	−0·032	0·42
DBP (mmHg)	0·002	0·96	0·073	0·07
MAP (mmHg)	−0·036	0·37	0·005	0·89
Heart rate (bpm)	0·063	0·12	0·061	0·14
Dietary intake (FFQ)				
Total fat intake (g/d)	−0·157	<0·001	0·318	<0·001
Total protein intake (g/d)	−0·035	0·38	0·066	0·09
Total carbohydrates (g/d)	−0·273	<0·001	0·009	0·83
Alcohol intake (g/d)				
0–10	0·116	0·05	0·149	0·01
10–30	0·197	0·001	0·064	0·27
>30	0·113	0·01	0·048	0·29
Medication use				
Antihypertensives (% yes)	−0·015	0·70	0·019	0·63
Statins (% yes)	0·045	0·26	−0·062	0·12
Renal function factors				
Serum creatinine (μmol/l)	0·021	0·59	0·004	0·91
eGFR (ml/min per 1·73 m²)	−0·063	0·11	0·039	0·33
Albuminuria* (mg/24 h)	0·008	0·84	−0·082	0·04
Proteinuria* (≥0·5 g/d)	−0·004	0·91	−0·072	0·07
Serum parameters				
Albumin (g/l)	−0·022	0·58	0·011	0·79
Cholesterol (mmol/l)	0·048	0·23	−0·022	0·58
TAG (mmol/l)	0·004	0·91	0·002	0·95
Hs-CRP (mg/l)*	0·068	0·10	0·052	0·21
HbA1c (%)	0·042	0·30	0·007	0·86

BSA, body surface area; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; bpm, beats per minute; eGFR, estimated glomerular filtration rate; Hs-CRP, high-sensitive C-reactive protein; HbA1c, glycated haemoglobin; ALA, *α*-linolenic acid.
* Log-transformed for analysis.

1·15 per 0·1 En% difference), whereas ALA intake was significantly associated with increased all-cause mortality risk (HR = 1·17; 95 % CI 1·04, 1·31 per 0·1 En% difference) (model 1) (Fig. 1(a)). Moreover, RTR in the highest tertile of ALA intake (>0·60 En%) were at 2-fold higher risk of all-cause mortality (HR = 2·21; 95 % CI 1·23, 3·97) than those in the lowest tertile (reference tertile) (<0·45 En%). Associations remained materially unchanged after cumulative adjustment for potential explanatory variables, including BMI, DBP, alcohol intake, smoking behaviour, fat, protein and carbohydrate intake, and proteinuria.

In secondary analyses, the association of ALA intake with all-cause mortality was analysed using g/d instead of En%. In age- and sex-adjusted analyses, RTR in the highest tertile of ALA intake (>1·45 g/d) were at almost 2-fold higher risk of all-cause mortality (HR 1·90; 95 % CI 1·07, 3·37) compared with the lowest tertile (reference tertile) (<1·14 g/d). Our findings did not change after further cumulative adjustment for potential explanatory variables. We also calculated the intake of ALA as a proportion of the total intake of fatty acids. It remained that there was no association of ALA intake (now expressed as proportion of total fatty acid intake) with graft failure (HR 0·93; 95 % CI 0·80, 1·08 per 0·1 % difference). It also remained that there was an association of ALA intake (now expressed as proportion of total fatty acid intake) with increased risk for mortality (HR 1·14; 95 % CI 1·02, 1·27 per 0·1 % difference, *P* = 0·02).

In sensitivity analyses, we excluded patients with extreme intake of *n*-3 fatty acids. HR of EPA–DHA and ALA intakes for graft failure and all-cause mortality remained materially unchanged, with no associations for graft failure, and HR = 0·78 (95 % CI 0·50, 1·22) and HR = 1·21 (95 % CI 1·06, 1·37) per 0·1 En% difference for the associations of EPA–DHA and ALA intake with all-cause mortality, respectively (Fig. 1(b)).

Discussion and conclusion

Our study is, to the best of our knowledge, the first to separately investigate the associations of the marine-derived *n*-3 fatty acids EPA and DHA and the plant-derived *n*-3 fatty acid ALA with long-term outcome in a large cohort of RTR. Both EPA–DHA and ALA intakes were not associated with graft failure. In addition, EPA–DHA intake was not associated with all-cause mortality, whereas ALA intake was positively associated with all-cause mortality.

The *n*-3 fatty acids EPA, DHA and ALA are long-chain PUFA. EPA and DHA are mainly found in fatty fish, whereas vegetable oils such as linseed, soyabean and rapeseed oils are the main sources of ALA[20,21]. According to the EFSA, the combined intake of EPA plus DHA was found to be below the recommended intake in 82 % of RTR, while 45 % had an inadequate intake of ALA. Various recommendations for PUFA intakes are circulating around the world[22]. For instance, the ALA intake recommendation in Australia is 2·0 g/d (1 En%), whereas in the USA and Canada the recommended ALA intake is 1·35 g/d (0·7 En%)[23]. According to these international guidelines, the number of RTR with an ALA intake below the recommended daily intake increases enormously.

At least two meta-analyses of randomised-controlled trials have investigated whether fish oil supplementation may improve renal function, rejection rates, and patient and graft survival in RTR[7,24]. Both these meta-analyses reported that

Table 3. Cox regression analyses for prediction of graft failure based on *n*-3 fatty acid intakes (En%)
(Hazard ratios (HR) and 95 % confidence intervals)

| | Tertiles of *n*-3 fatty acid intake (En%) | | | | | | | Continuous (En% per 0·1 En% difference) | | |
| | I | II | | | III | | | | | |
Models*	Ref.	HR	95 % CI	P	HR	95 % CI	P	HR	95 % CI	P
EPA–DHA intake										
1	1·00	1·08	0·53, 2·20	0·84	0·79	0·36, 1·73	0·56	0·83	0·50, 2·27	0·46
2	1·00	1·09	0·53, 2·24	0·81	0·81	0·37, 1·77	0·59	0·98	0·92, 1·05	0·53
3	1·00	1·29	0·61, 2·72	0·51	0·95	0·42, 2·13	0·89	0·92	0·56, 1·49	0·72
4	1·00	1·27	0·60, 2·69	0·54	0·91	0·40, 2·11	0·83	0·89	0·53, 1·49	0·65
5	1·00	1·02	0·47, 1·23	0·96	0·72	0·30, 1·72	0·45	0·77	0·43, 1·39	0·39
ALA intake										
1	1·00	0·97	0·45, 2·09	0·95	1·10	0·51, 2·36	0·81	0·90	0·74, 1·09	0·27
2	1·00	0·99	0·46, 2·12	0·98	1·13	0·52, 2·44	0·75	0·90	0·75, 1·09	0·29
3	1·00	1·26	0·56, 2·83	0·58	1·47	0·65, 3·28	0·35	0·95	0·78, 1·14	0·56
4	1·00	1·34	0·58, 3·08	0·49	1·79	0·73, 4·39	0·20	0·96	0·77, 1·19	0·70
5	1·00	1·16	0·47, 2·85	0·75	1·99	0·80, 4·98	0·14	1·02	0·81, 1·30	0·86

En%, energy percentage; Ref., referent values; ALA, *α*-linolenic acid.
* Model 1, adjusted for age and sex; model 2, as model 1, additionally adjusted for BMI; model 3, as model 2, additionally adjusted for alcohol intake and smoking habits; model 4, as model 3, additionally adjusted for fat, protein and carbohydrate intakes; model 5, as model 4, additionally adjusted for proteinuria and diastolic blood pressure. Proteinuria was log-transformed for analyses.

Table 4. Cox regression analyses for the prediction of all-cause mortality based on *n*-3 fatty acid intakes (En%)
(Hazard ratios (HR) and 95 % confidence intervals)

| | Tertiles of *n*-3 fatty acid intake (En%) | | | | | | | Continuous (En% per 0·1 En% difference) | | |
| | I | II | | | III | | | | | |
Models*	Ref.	HR	95 % CI	P	HR	95 % CI	P	HR	95 % CI	P
EPA–DHA intake										
1	1·00	1·25	0·71, 2·20	0·44	0·72	0·39, 1·35	0·31	0·79	0·54, 1·15	0·22
2	1·00	1·35	0·71, 2·20	0·44	0·72	0·39, 1·35	0·31	0·79	0·54, 1·16	0·23
3	1·00	1·41	0·77, 2·54	0·26	0·89	0·46, 1·71	0·71	0·88	0·61, 1·29	0·52
4	1·00	1·56	0·86, 2·86	0·15	1·02	0·52, 2·00	0·97	0·95	0·64, 1·40	0·79
5	1·00	1·56	0·85, 2·86	0·15	0·96	0·48, 1·93	0·91	0·90	0·60, 1·34	0·60
ALA intake										
1	1·00	1·06	0·55, 2·07	0·86	2·21	1·23, 3·97	0·01	1·17	1·04, 1·32	0·01
2	1·00	1·07	0·55, 2·07	0·85	2·22	1·24, 3·98	0·01	1·17	1·04, 1·32	0·01
3	1·00	1·13	0·56, 2·25	0·74	2·20	1·20, 4·03	0·01	1·17	1·03, 1·32	0·02
4	1·00	1·05	0·52, 2·14	0·88	2·08	1·07, 4·05	0·03	1·16	1·01, 1·35	0·04
5	1·00	0·92	0·45, 1·90	0·83	2·04	1·05, 3·98	0·04	1·19	1·02, 1·38	0·03

En%, energy percentage; Ref., referent values; ALA, *α*-linolenic acid.
* Model 1, adjusted for age and sex; model 2, as model 1, additionally adjusted for BMI; model 3, as model 2, additionally adjusted for alcohol intake and smoking habits; model 4, as model 3, additionally adjusted for fat, protein and carbohydrate intakes; model 5, as model 4, additionally adjusted for proteinuria and diastolic blood pressure. Proteinuria was log-transformed for analyses.

there was insufficient evidence to conclude that fish oil has a beneficial effect on either of these outcomes. It was mentioned that the meta-analyses were hampered by methodological problems, including different doses of fish oils, different duration and timing of treatment, studies with small numbers of patients, inadequate randomisation and lack of blinding[7]. Recently, two studies in a large observational cohort, with 569 allograft failures and 406 deaths in 1990 RTR that were followed-up for 6·8 years, showed significant inverse associations of plasma phospholipid concentrations of marine-derived *n*-3 fatty acids with graft failure and mortality in RTR[25,26]. Interestingly, in a large systematic review of the effects of *n*-3 fatty acids on CVD in the general population it was concluded that EPA–DHA intake reduced the rates of

all-cause mortality, cardiac and sudden death and possibly stroke[8]. These authors analysed ALA separately from EPA–DHA and found that ALA was not associated with these positive findings[7,8]. In another systematic review, it was concluded that supplementation of EPA and DHA or fish oil has a consistent beneficial effects on serum lipids, whereas this is not the case for supplementation with ALA[27]. This pattern of decrease in risk associated with high intake of EPA–DHA and increase in risk associated with high intake of ALA are also present in studies including biomarkers. A recent study in which circulating levels of EPA, DHA and ALA were measured in 60-year-old adults showed that high circulating concentrations of EPA and DHA were found to be associated with low risk of all-cause mortality, whereas it was found that there was a trend
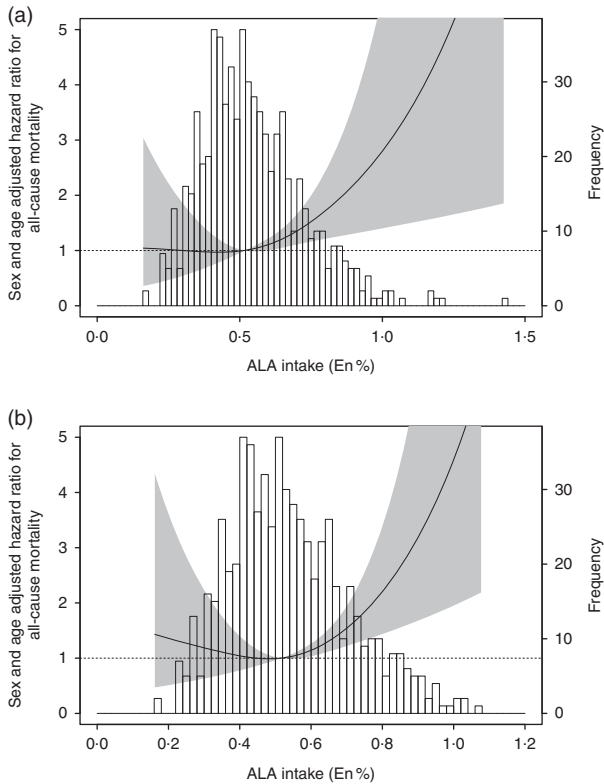
Fig. 1. (a) Restricted cubic spline showing the association between *a*-linolenic acid (ALA) intake (En%) and all-cause mortality in 637 renal transplant recipients (RTR). (b) Restricted cubic spline showing the association between ALA intake (En%) and all-cause mortality in 632 RTR. En%, energy percentage. ———, Age- and sex-adjusted risk for all-cause mortality; ▓, 95% CI of the HR.

towards an association of high circulating levels of ALA with an increased risk for all-cause mortality[28].

In our cohort, there was no association of EPA–DHA intake with all-cause mortality, whereas a high-ALA intake was associated with a significantly increased risk of all-cause mortality. The fact that the association between the marine-derived *n*-3 fatty acid intakes and all-cause mortality did not reach significance may have been the consequence of the relatively small number of cases (sixty-seven deaths in a cohort of 637 RTR during a follow-up period of 3·1 years). Indeed, in a recent analysis of a cohort study, with 406 deaths in 1990 RTR followed-up for 6·8 years, it was found that circulating levels of marine *n*-3 fatty acid in plasma phospholipids were inversely associated with all-cause and cardiovascular mortality after renal transplantation[26]. Other than in size and duration of follow-up, this study differed from our study with respect to percentage of males (67% in the study by Eide *et al.* *v.* 57% in our study), percentage of diabetics (18·2 *v.* 24·2%), percentage of former smokers (36·2 *v.* 45·8%) and eGFR (57 *v.* 49 ml/min per 1·73 m²), whereas age (52 *v.* 53 years), BMI (24·8 *v.* 26·6 kg/m²) and percentage of current smokers (16·0 *v.* 12·9%) were less dissimilar. Another difference between the studies is the way in which *n*-3 fatty acid exposure was assessed (i.e. a status marker (*n*-3 fatty acids in plasma phospholipids) in the study by Eide *et al.* *v.* intake (FFQ) in our study).

Results regarding the effect of EPA–DHA and ALA intake on graft failure are inconsistent. As mentioned earlier, meta-analyses of randomised controlled trials on this subject found no effect of EPA–DHA on graft failure[7,24], which is in line with our finding of a virtual absence of an association of EPA–DHA and ALA intakes with graft failure in RTR. An intervention study in 2344 post-myocardial infarction patients suggests, however, that a combined intake of 400 mg/d of EPA and DHA provides a small beneficial effect on renal function, whereas this was not the case for ALA[29]. A positive effect of EPA–DHA was also suggested by a study of circulating levels of EPA and DHA. In an observational cohort study from Norway, it was described that high levels of marine-derived *n*-3 fatty acids in plasma phospholipids were associated with better allograft survival. The authors showed an 11% reduction in risk of graft loss for every 1·0 wt% increase in EPA–DHA levels[25].

Recently, two authoritative meta-analyses in the field of *n*-3 fatty acid intake and mortality investigated whether there is a threshold for the relation of EPA–DHA intake with outcome[30,31]. These studies found that there was a decline in CHD[30,31] and total mortality[31] with increasing EPA–DHA intake in the range of EPA–DHA intake from 0 to 250 mg/d, whereas there was no further decline in CHD and total mortality with increasing intake beyond 250 mg/d. In our cohort, the median EPA–DHA intake was 92·1 (IQR 28·2–204·8) mg/d, with only 18% of the population consuming >250 mg of EPA–DHA/d. Therefore, the EPA–DHA intake in our cohort was rather low compared with the threshold determined in the previous meta-analyses.

The reason why a higher ALA intake is associated with increased risk of all-cause mortality in RTR is unknown. ALA can be metabolised via at least two pathways. First, ALA is a preferred substrate for β-oxidation, with either complete oxidation to CO_2 and water or incomplete conversion to intermediates that may subsequently be used for the synthesis of fatty acids and cholesterol[32,33].

A second route is by conversion of ALA into EPA and subsequently into DHA[32,34]. Rate limiting steps in this pathway include the activities of Δ5 and Δ6 desaturases[35,36]. In RTR, this route, but also β-oxidation, could be affected by glucocorticoids, a class of immunosuppressive drugs used to protect the transplanted kidney from rejection[37,38]. In our cohort, 99% of the RTR were treated with prednisolone. Glucocorticoids can depress the activities of Δ5 and Δ6 desaturases, giving rise to altered fatty acid precursor/metabolic ratios, possibly including the ALA:EPA–DHA ratio[35,39].

Finally, it is also possible that the association of ALA intake with high mortality risk might not be through the intake of ALA itself but rather via its decomposed products produced during baking or frying of ALA-rich foods[40]. ALA can be found in various widely used vegetable oils and is highly susceptible to peroxidation during heating[20,40]. The degraded products are hazardous to health and adverse effects may possibly become amplified in susceptible patients, such as RTR[40].

Strengths of the present study include its large population size. In total, data of 637 RTR were used for analyses, which made the results reliable. Another strength of the present study is the prospective analyses carried out with Cox regression in RTR using 3-year follow-up information of patients. At present, studies on the long-term effects of *n*-3 fatty acids are especially

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carried out in the general population. In our study, we were able to identify specific long-term association in our target group that nevertheless needs further investigations of causality. The third strong point is the inclusion of intake of ALA in the prospective study. Most studies in the field of *n*-3 fatty acids predominantly focus on EPA–DHA intake, whereas ALA intake might be important as well. We analysed EPA–DHA and ALA intakes separately, to investigate the specific effects of the marine-derived and plant-derived *n*-3 fatty acids, and found a difference between the two.

We acknowledge that our study also has some limitations. The present study is an observational epidemiological study, which may only suggest biological significance. Another limitation of our study is the use of an FFQ for measuring dietary intake. Measurements of intake were based on self-report, which may lead to misclassification, under-reporting and over-reporting. Furthermore, intakes were only measured once at baseline, whereas changes in intake and clinical parameters may appear during the 3 years of follow-up. Finally, dietary intake and status form complementary information on *n*-3 fatty acid exposure. The present study made use of dietary *n*-3 fatty acid intake, but not status. Future studies should therefore focus on *n*-3 fatty acid biomarkers also.

In conclusion, in RTR, EPA–DHA and ALA intakes showed no clear association with graft failure. EPA–DHA intake showed no association with all-cause mortality, whereas ALA intake was associated with increased all-cause mortality. Although the association between ALA intake and all-cause mortality was strong, a causal relationship in RTR is as yet questionable. Further investigation on the long-term outcome of *n*-3 fatty acids, particularly ALA, in RTR is warranted.

Supplementary material

For supplementary material/s referred to in this article, please visit https://doi.org/10.1017/S0007114516004207

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