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a randomized controlled trial

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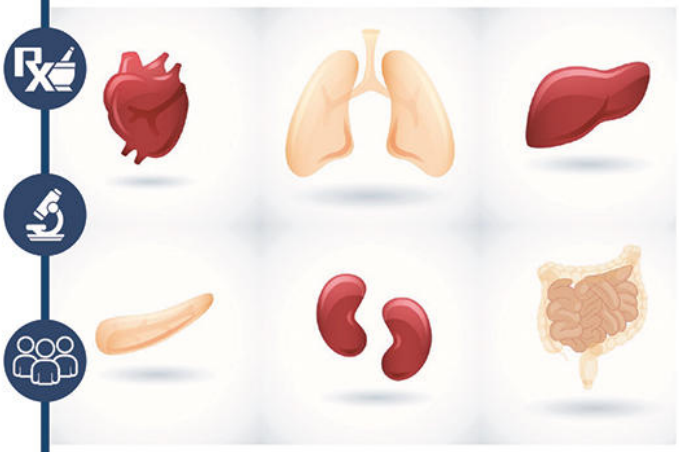

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Effects of marine n-3 fatty acid supplementation in renal transplantation: a randomized controlled trial

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Abbreviations:

CADI	Chronic Allograft Damage Index
DHA	Docosahexaenoic acid
eGFR	Estimated glomerular filtration rate
EPA	Eicosapentaenoic acid
FA	Fatty acid
FEPR	Fractional excretion of protein in urine
HDL	High density lipoprotein
hsCRP	High-sensitive C-reactive protein
IF	Interstitial fibrosis
ITT	Intention-to-treat
LDL	Low density lipoprotein
mGFR	Measured glomerular filtration rate

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PP	Per-protocol
RCT	Randomized controlled trial
wt%	Weight percentage

ABSTRACT

Marine n-3 fatty acids (FAs) may exert beneficial effects on inflammation, fibrosis, and endothelial function, which could preserve renal graft function. In this randomized controlled trial, 132 Norwegian renal transplant recipients received either 2.6 g of marine n-3 FAs or olive oil (control) daily for 44 weeks, in addition to standard care. Thirty patients did not complete the trial. The primary endpoint was change (Δ) in measured glomerular filtration rate (mGFR) during follow-up. We found no significant difference in Δ mGFR between the marine n-3 FA group and controls (6.7 vs 3.8 mL/min/1.73 m^2, p=0.15). Significant beneficial effects from marine n-3 FA supplementation were, however, seen in secondary endpoints plasma triglycerides, plasma high-sensitivity C-reactive protein and brachial artery flow mediated dilation. In the per-protocol population, also the renal graft indices percent interstitial fibrosis and Chronic Allograft Damage Index were significantly lower in the marine n-3 FA group. The cumulative incidence of adverse events did not differ between the marine n-3 FA group (n=218) and controls (n=240). In conclusion, marine FA supplementation did not improve renal function compared with controls, but was safe, lowered plasma triglyceride and high-sensitivity C-reactive protein levels and improved endothelial function (Clinical.Trials.gov identifier NCT01744067).

INTRODUCTION

During the last decades, improved surgical procedures and tailored immunosuppressive regimens have lowered the risk of graft loss during the first few years after renal transplantation, while long-term graft survival has remained virtually unchanged (1). Silent alloimmune mediated tissue injury and immunosuppressive drug side-effects are major contributors to progressive renal graft fibrosis, the key histological correlate for chronic allograft nephropathy and a predictor of graft survival (2, 3). Agents that may improve endothelial function or possess anti-inflammatory or anti-fibrotic properties would be attractive, since they might increase longevity of grafts. Experimental and clinical trials in various patient populations report anti-inflammatory effects by the major marine n-3 fatty acids (FAs) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), through synthesis of less pro-inflammatory compounds compared with those derived from arachidonic acid, inhibition of nuclear factor kappa B and synthesis of inflammation resolving mediators resolvins and protectins (4-7). Marine n-3 FAs improve FA oxidative phosphorylation, reduce triglyceride levels and possibly improve endothelial function (8-11). Marine n-3 FAs has also been linked to prevention of alloimmune mediated tissue injury and premature aging in renal grafts, production of regulatory T cells and inhibition of the pro-fibrotic transforming growth factor beta -1 / Smad signaling pathway (5, 12-15). A recent randomized clinical trial (RCT) in myocardial infarction survivors reported less development of myocardial fibrosis in patients who received high-dose marine n-3 FA supplements (16).

We have previously shown that higher plasma phospholipid marine n-3 FA levels were associated with better patient and graft survival in a large cohort (n=1990) of Norwegian renal transplant recipients (17, 18). In that observational cohort, plasma marine n-3 FA levels were inversely associated with plasma creatinine, inflammatory biomarkers and triglycerides, acute

rejections beyond the early post-transplant phase and development of interstitial fibrosis (IF) during the first year after transplantation (17-21).

The present study is the largest RCT investigating the effects of marine n-3 FA supplementation in renal transplantation performed to date, and the first to include data on renal graft histopathological indices and endothelial function. The main objective of this trial was to evaluate whether daily supplementation of 2.6 g marine n-3 FAs for 44 weeks, compared with controls, would improve renal function beyond standard care in renal transplant recipients.

MATERIALS AND METHODS

Study participants

Inclusion criteria of the present trials were age ≥ 18 years, a functional graft (estimated glomerular filtration rate [eGFR] according to the Chronic Kidney Disease Epidemiology Collaboration formula > 30 mL/min/1.73m^2 at 6 – 8 weeks post-transplant) and written informed consent (22). Exclusion criteria were allergy to fish, other seafoods or the study drug, a kidney donor > 75 years old, participation in another clinical trial and foreign citizenship (controls beyond the baseline visit at centers outside Norway, including the scheduled visit at one year post-transplant). From 298 patients, who received a renal transplant at Oslo University Hospital Rikshospitalet between 15th of June 2013 and 15th of June 2014, 176 patients were eligible for inclusion and 132 patients were included in the trial (Figure 1). All patients gave written informed consent to participate in the trial and a separate written consent was given for renal graft biopsy sampling. The study was approved by the Regional Committees for Medical and Health Research Ethics in Norway and The Norwegian Medicines Agency. The trial was performed in accordance with the Declaration of Helsinki (Clinical.Trials.gov identifier NCT01744067, ORENTRA).

Study design, randomization and monitoring

This study was an investigator initiated, single center, randomized, double blind, controlled trial. We used marine n-3 FA ethyl ester (460 mg/g EPA and 380 mg/g DHA [Omacor®, Pronova Biopharma] given as one capsule of 1 g three times a day ≈ 2.6 g effective dose of EPA plus DHA per day) and controls received extra virgin olive oil, 3 capsules of 1 g per day, both provided by Pronova Biopharma. Patients were instructed to take study drugs with meals to assure emulsification of the study drug for better gastrointestinal FA uptake and to avoid possible interference with concomitant medication (23). Most patients were treated according to the standard immunosuppressive protocol, including induction with basiliximab, followed by maintenance immunosuppression with prednisolone (n=132), mycophenolate (n=131) and the calcineurin inhibitor tacrolimus (n=128). One dose of methylprednisolone was given at the time of transplantation. Prednisolone dose was started at 20 mg daily (day 0-14) and tapered gradually via 15 mg (day 15-28), 10 mg (day 29-60) and 7.5 mg (day 61-180) to 5 mg per day from six months post-transplant. Tacrolimus 0.05 mg/kg twice daily was started at the time of transplantation, after which tacrolimus trough concentrations were monitored and tacrolimus dose adjusted accordingly with target 3 - 7 µg/L. We used mycophenolate mofetil 750 twice daily or mycophenolate sodium 540 mg twice daily. Prophylactic treatment with ganciclovir was given to cytomegalovirus seronegative recipients with seropositive donors and all patients received trimethoprim-sulfamethoxazole as prophylactic treatment against pneumocystis carinii pneumonia during the first six months after transplantation. Screening for polyoma virus and cytomegalovirus viremia was performed every month during the first six months post transplantation and thereafter every third month.

Patients were randomly allocated to receive either marine n-3 FAs (n=66) or control oil (n=66) by computer generated randomization codes (SAS, Cary, NC, US) provided by the Monitor. Allocation concealment was ensured by the randomization method, where study participants, care givers and investigators were kept blinded until the final monitoring visit at 25th May 2017, and by using hard capsules of similar shape and color in both study groups. At the final visit the electronic case report form and the study data file were locked and randomization codes were made available. There were no code breaks before unblinding.

Study visits, endpoints and procedures

Study duration was 44 weeks. Study measurements were performed at baseline (8 weeks post-transplant) and at the end of study (one year after transplantation). Data were entered into an electronic case report form at the baseline visit, at scheduled telephone interviews during follow-up and four weeks after the final visit, at additional visits in case of serious adverse events and at the final visit.

The primary endpoint was absolute change during follow-up (Δ) in measured glomerular filtration rate (mGFR), which was measured by iohexol clearance. Secondary renal endpoints were renal graft histopathological indices Chronic Allograft Damage Index (CADI), percent graft inflammation and percent IF. Additional renal endpoints were fractional excretion of protein in urine (FEPR) as a measure of proteinuria and plasma high-sensitive C-reactive protein levels (hsCRP) as a measure of systemic inflammation. Secondary cardiovascular endpoints were flow mediated dilation as a measure of endothelial function and plasma triglyceride, high density lipoprotein (HDL), low density lipoprotein (LDL) and total cholesterol levels. Additional cardiovascular endpoints were systolic and diastolic blood

pressure, pulse wave velocity as a measure of arterial stiffness and plasma fasting glucose levels and two hours post-challenge plasma glucose during an oral glucose tolerance test.

Study procedures are described in detail in the supplemental material. In short, blood samples were drawn on the morning of the baseline and final visits with the patient in a fasting state. Gas chromatography was used to determine individual plasma phospholipid FA (hereafter plasma FA) levels, quantified as weight percentage (wt%) of plasma total FAs (24, 25). Marine n-3 FA level was defined as the sum of EPA and DHA. Flow mediated dilation was measured by high-frequency ultrasonography examination of the brachial artery diameter. The ultrasound probe was placed over the brachial artery 5 cm proximal to the antecubital fossa and resting measurements were performed after a minimum of 10 minutes relaxation. Thereafter, we inflated a sphygmomanometer cuff on the proximal forearm to stop blood flow in the brachial artery for 5 minutes. From continuous video sessions, end-diastole measurements were performed before, during occlusion and the first two minutes post-occlusion. Flow mediated dilation was defined as the change in arterial diameter between the largest post-occlusion diameter and the resting diameter and was expressed as absolute (mm) and percent change. Carotid femoral pulse wave velocity was measured by use of the SphygmoCor® apparatus (AtCor Medical, West Ryde, NSW, AUS). Fasting and two hours post challenge plasma glucose during an oral glucose tolerance test were performed in patients without overt diabetes. Patients were diagnosed with post transplantation diabetes mellitus if they met with one of the following criteria: fasting plasma glucose ≥126 mg/dL (≥7.0 mmol/L), two hours post-challenge plasma glucose ≥200 mg/dL (≥11.1 mmol/L) or random plasma glucose ≥200 mg/dL (≥11.1 mmol/L) in combination with symptoms of hyperglycemia. Ultrasonography guided percutaneous renal graft core biopsies with 18 G needle were obtained at baseline and at the final visit. Light microscopical evaluation of the

biopsies was performed by two investigators (F.P.R. and I.A.E.) who were blinded for the patient's identity and clinical data. We performed a semi-quantitative estimation of percent graft inflammation and IF in the renal cortex, outside scar tissue (26). CADI was defined as in the original definition (27), with one adjustment made for degree of IF, making it count for each 10% step (0-9) as opposed to the 0-3 scale used in the original definition.

Sample size

Power estimation was based on an absolute difference in Δ mGFR of 8.0 mL/min/1.73m^2 between the study groups and a 20% drop-out rate. To ensure adequate statistical power, we calculated that 132 patients had to be included in the trial.

Statistical analysis

Primary and secondary endpoints were analyzed in intention-to-treat (ITT) and per-protocol (PP) populations. Kolmogorov-Smirnov test and normality plots were used to assess normality. Difference in Δ values for study endpoints between the marine n-3 FA and control group was evaluated by Students t-test for normally distributed data and Mann-Whitney U-test for non-normally distributed data. A similar statistical approach was used for evaluation of baseline and end of study endpoint values. We used Fishers exact test to evaluate differences in the cumulative incidence of adverse events between the study groups. A two-sided p-value of < 0.05 was considered statistically significant. We used SPSS® version 24.0 (IBM, New York, NY, US) and STATA® version 14.0 (Stata Corp, College Station, Texas, US) for statistical analyses.

RESULTS

Demographics

Patient characteristics are presented in Table 1. In general, the groups were well matched, but there was a higher prevalence of hypertension in the marine n-3 FA group. Median baseline plasma marine n-3 FA level was 6.0 wt% in both study groups (interquartile range 4.7 – 7.3 wt%). In patients who received marine n-3 FA supplements and completed the trial, we found a mean absolute increase in plasma marine n-3 FA levels of 4.0 wt%, while it remained unchanged in the control group (Table 2).

Renal function

Renal function improved during follow-up in both study groups (Table 2). In the marine n-3 FA group mGFR increased from 56.2 ± 15.3 mL/min/1.73m^2 at baseline to 61.2 ± 14.4 mL/min/1.73m^2 at the end of study, while in controls mGFR increased from 54.9 ± 16.8 to 59.0 ± 17.4 mL/min/1.73m^2 during follow-up (Table S1). There was no significant difference in Δ mGFR between the marine n-3 FA group and controls (6.7 ± 11.7 vs 3.8 ± 10.3 mL/min/1.73m^2, p=0.15, Figure S1).

Renal graft fibrosis and chronic allograft damage

In patients who completed the trial, the degree of IF in the renal cortex remained stable in the marine n-3 FA group (12.9 ± 11.5% at baseline and 12.5 ± 10.3% at the end of study) while it increased in controls (from 12.6 ± 10.2% at baseline to 16.0 ± 13.0% at the end of study). Difference between the study groups for Δ percent IF was statistically significant in PP analysis (-0.6 ± 9.0 % in the marine n-3 FA group compared with 3.6 ± 11.1 % in controls, p=0.04), but not in ITT analysis (Table 2 and Figure S1). Similarly, in PP analysis, CADI

score remained stable during follow-up in the marine n-3 FA group, while it increased in controls (Table S1) and Δ CADI differed significantly between the groups (Table 2).

Proteinuria and inflammation

FEPR was reduced during follow-up in both groups and there was no significant difference in Δ FEPR between the study groups (Table 2). The degree of graft inflammation was slightly higher at the end of study than at baseline for both study groups (Table S1). There was no significant difference in Δ percent graft inflammation between the groups (Table 2 and Figure S1). Marine n-3 FA supplementation reduced hsCRP from 4.6 ± 10.2 mg/L at baseline to 3.5 ± 4.0 mg/L at the end of study, as opposed to an increase in the control group from 3.2 ± 4.3 mg/L at baseline to 5.9 ± 8.1 mg/L at the final visit (Table S1). Thus Δ hsCRP differed significantly between the study groups (Table 2).

Endothelial function

Mean baseline post-occlusion brachial artery diameter was 0.2 mm (~5%) larger than the corresponding resting value. During follow-up the difference between post-occlusion and resting values increased by 0.1 mm (~2%) on average in the marine n-3 FA group, while it remained stable in controls. We found a corresponding significant increase in Δ percent flow mediated dilation in the marine n-3 FA group compared with controls (2.0 ± 3.8 % vs 0.5 ± 2.4 %, p=0.02, Figure S1).

Blood lipids and glucose

Plasma triglyceride levels decreased significantly in the marine n-3 FA group during follow-up as opposed to controls (-39.8 ± 74.2 vs -7.8 ± 77.2 mg/dL, p=0.02). Marine n-3 FA

supplementation had no effect on plasma HDL, LDL or total cholesterol levels, fasting plasma glucose or two hours post-challenge plasma glucose (Table 3).

Blood pressure and arterial stiffness

There was a slight reduction in systolic blood pressure and pulse wave velocity during follow-up in both study groups, while diastolic blood pressure remained unchanged (Table S2). Marine n-3 FA supplementation had no effect on pulse wave velocity or blood pressure (Table 3).

Safety and study drug adherence

A detailed description of adverse events, reasons for withdrawal from the trial and study drug adherence are given in the supplemental material. In short, 30 patients (of whom 16 belonged to the marine n-3 FA group) did not complete the trial. Two patients were termed screening failure by the Monitor and two patients did not meet at the final visit (Figure 1). During follow-up, there were 218 adverse events in the marine n-3 FA group compared with 240 adverse events in the control group (Table 4). Gastrointestinal discomfort was the most frequent complaint in both the marine n-3 FA group (26%) and the control group (32%), especially early after study drug initiation, and led to patient withdrawal in 17 cases (9 in the marine n-3 FA group and 8 in controls). One patient in the marine n-3 FA group died due to cancer. Graft failure occurred in one patient in the control group. There were 15 acute rejections in 11 patients in the marine n-3 FA group and 16 acute rejections in 14 patients in the control group.

There was no significant difference in tacrolimus trough levels at end of study between the groups (marine n-3 FA group: 6.19 ± 2.04 µg/L vs controls: 5.86 ± 1.66 µg/L, p=0.33). Also

tacrolimus doses were similar between the study groups (mean dose 4.8 ± 2.4 mg in the marine n-3 FA group vs 4.6 ± 2.6 mg in controls, p=0.45).

Adherence to study treatment was evaluated by plasma marine n-3 FA levels. In addition, we performed a pill count at the final visit. In the PP population, five patients who received marine n-3 FA supplements missed ≥ 5% of doses.

Plasma marine n-3 fatty acid levels and study endpoints

Subgroup and post-hoc analyses were performed in the ITT population and are presented in the supplemental material. End of study plasma marine n-3 FA level, assumed to reflect stable levels during follow-up, was significantly correlated with Δ percent IF (r = -0.27, Figure S2), Δ CADI (r = -0.32), Δ hsCRP (r = -0.21) and Δ triglycerides (r = -0.29), and a borderline significant correlation was found with Δ flow mediated dilation (r = 0.21, p=0.05, Figure S2). No significant correlation with end of study plasma marine n-3 FA level was shown with Δ mGFR (r = 0.10, p=0.36, Figure S2) or other study endpoints, including renal graft inflammation (r = -0.11, p=0.31, Figure S2).

Subgroup analysis showed that patients with lower than median baseline plasma marine n-3 FA level (<6.0 wt%, n=61), had a significant increase in Δ mGFR after marine n-3 FA supplementation compared with controls (8.2 ± 12.6 vs 1.6 ± 11.2 mL/min/1.73m^2, p=0.04). Change in plasma marine n-3 FA level during follow-up was higher in patients with lower baseline levels (r = -0.35, p=0.01, Figure S3).

Patients with acute or chronic acute rejections at the final visit (n=5) had higher percent graft inflammation, but the low number of cases made comparison between the study groups unreliable. Excluding these patients from the analysis did not influence results.

DISCUSSION

The main finding in this study was that no significant improvement in renal function could be demonstrated after 44 weeks of marine n-3 FA supplementation compared with controls. However, some beneficial findings were seen in secondary endpoints. Plasma triglyceride and hsCRP levels were significantly reduced and flow mediated dilation improved during follow-up in the marine n-3 FA group compared with controls. In the PP population, percent IF and CADI remained stable in the marine n-3 FA group, as opposed to an increase in these histopathological indices in controls during follow-up.

Previous RCTs investigating the effects of marine n-3 FA supplementation in renal transplant recipients were hampered by low sample sizes and short study duration (28, 29). Although renal function improved after marine n-3 FA supplementation in some studies, most trials reported a non-significant increase in Δ mGFR of 3 – 5 mL/min/1.73m^2 (29). Meta-analyses of these RCTs (n = 733 – 812 patients included) found no statistically significant effect on renal function (28, 29). The mean increase in Δ plasma marine n-3 FA level of 4.0 wt% in the marine n-3 FA group approximates the difference between the lower and upper plasma marine n-3 FA quartile in a large observational study of Norwegian renal transplant recipients. In that study, beneficial associations between plasma marine n-3 FA level and renal function was only shown beyond three years post-transplant, suggesting that potential renoprotective effects of marine n-3 FAs might prevent decline in renal function in the long run.

Most studies in renal transplant recipients report a significant reduction in plasma triglycerides after marine n-3 FA supplementation, but no significant effect on plasma total or LDL cholesterol levels, blood pressure or proteinuria (28, 29), in line with findings in the present study. The triglyceride lowering effect of marine n-3 FAs has been repeatedly shown in various patient populations (9). A small increase in HDL cholesterol level was reported in a meta-analysis of RCTs (29), but was not shown in the present study.

We found a reduction in plasma hsCRP levels in the marine n-3 FA group compared with controls, suggesting an effect on low-grade systemic inflammation. However, we found no significant effect of marine n-3 FA supplementation on percent renal graft inflammation. Hernandez and colleagues investigated the effects of marine n-3 FA supplementation (effective dose of 1.9 g EPA plus DHA / day) on renal graft inflammation during the first three months after transplantation (30). In that trial, markers of tumor necrosis factor pathway activation in renal graft tissue, obtained by fine needle aspiration, were lower during acute rejection episodes in patients who received marine n-3 FA supplements compared with controls. However, they found no difference in inflammatory biomarkers between the study groups in patients without rejection.

Chronic inflammation is linked to endothelial dysfunction (31). We found an improvement in percent flow mediated dilation in patients who received marine n-3 FA supplements, consistent with reports in patients with dyslipidemia and metabolic syndrome (8). Flow mediated dilation response indicates the availability of endothelial produced nitrogen monoxide to induce local vascular smooth muscle relaxation. The availability of local nitrogen monoxide in the vascular wall may be reduced when exposed to reactive oxygen species. Improved FA oxidative phosphorylation by marine n-3 FAs could reduce formation

of reactive oxygen species and preserve mitochondrial function, partly via increased long-chain FA and carnitine transporter capacity (10), which serves as a possible explanation for the observed improvement in endothelial function. Although endothelial dysfunction is linked to ischemia reperfusion injury of renal grafts and is associated with reduced patient survival after renal transplantation, the clinical relevance a small improvement in percent flow mediated dilation in the transplant setting is unclear (8, 32).

Chronic inflammation is also linked to development of fibrosis in native and transplanted kidneys (2). In the present study, we found a significant difference in Δ percent IF between the marine n-3 FA group and controls in PP analysis, but not in ITT analysis. The discrepant results might have been related to outlier values in the ITT analysis from patients who withdrew early from the trial due to serious adverse events. Plasma marine n-3 FA levels were associated with less increase in percent IF during follow-up, consistent with findings in a previous observational study of Norwegian renal transplant recipients (21). Nonetheless, the discrepant results in PP and ITT analyses questions the robustness of the finding. Moreover, the clinical relevance of a 3% lower percent IF or a 1 point lower of CADI is unclear (27, 33). The recent OMEGA-REMODEL trial in myocardial infarction survivors found less IF outside infarction scars and better left ventricular function in patients who received daily 3.4 g of EPA plus DHA for six months (16), suggesting that marine n-3 FAs possess anti-fibrotic properties.

RCTs investigating the effects of marine n-3 FA supplementation are scarce in patients with chronic kidney disease (34). Donadio and colleagues performed a study in an American IgA nephropathy cohort and found less decline in renal function after supplementation of 3.2 g of EPA plus DHA per day for two years compared with controls (35). However, later trials have

not reproduced this finding and there is currently not sufficient evidence to recommend marine n-3 FA supplementation in glomerulonephritis or chronic kidney disease (34). Patients in the present trial had high baseline plasma marine n-3 FA levels, typical of patients on a Nordic diet rich in fish and seafood and about five times higher than levels reported from American cohorts (17, 36, 37). Patients with low baseline levels (< 6.0 wt%) had a steeper increase in plasma marine n-3 FA level after supplementation and a statistically significant improvement of mGFR during follow-up compared with controls. These findings suggest that patients with low background marine n-3 FA consumption might be more likely to benefit more from marine n-3 FA supplementation. Most other RCTs focusing on marine n-3 FAs in renal transplant recipients have been performed in the Mediterranean countries or in the Netherlands, where consumption of fish and seafoods is also relatively high (28, 29). Thus, similar studies performed in regions with a low consumption of marine n-3 FAs are warranted. Also explorative studies investigating effects of marine n-3 FA consumption on mechanisms involved in development of renal graft fibrosis would be of interest (7, 12, 38, 39).

The cumulative incidence of adverse events during follow-up did not differ between the groups. Gastrointestinal discomfort was experienced by nearly a third of the patients and is common in renal transplant recipients receiving mycophenolate and tacrolimus (40). Discontinuation of the study drug resolved the problem in about half of these patients. Previous RCTs focusing on marine n-3 FA supplementation in renal transplant recipients report very limited data on adverse events (28, 29). Marine n-3 FA supplementation did not significantly influence tacrolimus trough concentrations. However, dedicated pharmacokinetic studies are needed to evaluate marine n-3 FAs interact with immunosuppressive drugs (23).

Based on RCTs performed to date, including the present study, there is not sufficient evidence to recommend the use of marine n-3 FA supplements in renal transplantation. However, the safety of marine n-3 FAs is well documented and clinical trials report beneficial effects on renal and cardiovascular risk factors that may converge into improved patient and graft survival, as suggested from observational data (17, 18). Thus, larger studies with longer duration are needed to investigate whether marine n-3 FAs preserve renal function and improve patient and graft survival.

Strengths of this study include a well described cohort, fatty acid analysis performed at baseline and end of study, gold standard measurement of renal function, flow mediated dilation and pulse wave velocity measurements, renal histopathological indices, measurement of several other cardiovascular risk factors and monitoring of adverse events and concomitant medication during follow-up. However, this study has also some important limitations. The study duration might have been too short for postulated effects on endothelial function and graft fibrosis to translate into improved renal function. The subgroup analyses were hampered by low sample sizes, increasing the risk of type II errors. Thirty patients did not complete the trial (23% drop-out rate). Most adverse events and patient withdrawals occurred during the first two months of follow-up. In retrospect, inclusion of patients at 4 - 6 months post-transplant might have been more appropriate. We used olive oil as the control oil in this study, which may not be inert (41). Finally, study participants were mainly Caucasian men, limiting generalizability.

In conclusion, this study demonstrated that supplementation with 2.6 g marine FAs / day for 44 weeks during the first year after renal transplantation was safe, but did not improve renal function compared with controls. Marine n-3 FA supplements lowered plasma triglyceride

and C-reactive protein levels and improved endothelial function. In patients who completed the trial on randomized treatment, marine n-3 FA supplementation prevented development of graft fibrosis. This finding may not be robust as a significant effect was not found in the ITT analysis. Data on long-term renal function and graft survival is necessary to determine the clinical relevance of these findings.

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DISCLOSURE

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

FIGURE LEGEND

Figure 1: Study flow-chart

CONSORT diagram presenting patient screening, enrollment, randomization and reasons for withdrawal from the trial.

Abbreviations: eGFR: Estimated glomerular filtration rate. FA: Fatty acids. w: Weeks after renal transplantation.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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Table 1: Baseline characteristics and plasma phospholipid fatty acid levels

Variables	All patients	Marine n-3 FA group	Control group	p
Number of patients	132	66	66	
Recipient age, *years*	53.4 (13.8)	52.8 (13.5)	54.1 (14.2)	0.61
Recipient gender, Female, *%*	25.8	28.8	22.7	0.43
Ethnicity, Caucasian, *%*	92.4	90.9	93.9	0.71
Marine n-3 fatty acids, *wt%*	6.3 (2.1)	6.4 (2.2)	6.3 (2.1)	0.78
Eicosapentaenoic acid, *wt%*	1.8 (1.1)	1.8 (1.1)	1.8 (1.1)	0.71
Docosahexaenoic acid, *wt%*	4.5 (1.3)	4.6 (1.3)	4.4 (1.3)	0.45
Alpha linolenic acid, *wt%*	0.3 (0.1)	0.3 (0.1)	0.3 (0.1)	0.44
Linoleic acid, *wt%*	23.9 (2.8)	23.6 (3.0)	24.2 (3.6)	0.22
Arachidonic acid level, *wt%*	8.5 (1.7)	8.5 (1.8)	8.5 (1.5)	0.61
eGFR, *mL/min/1.73m^2*	68.9 (21.5)	70.7 (21.1)	67.1 (21.8)	0.34
mGFR, *mL/min/1.73m^2*	55.5 (16.0)	56.2 (15.3)	54.9 (16.8)	0.64
Body mass index, *kg/m^2*	26.0 (3.9)	25.7 (3.8)	26.3 (4.0)	0.44
b-Hemoglobin, *g/L*	12.3 (1.5)	12.3 (1.6)	12.3 (1.3)	0.96
p-Total cholesterol, *mg/dL*	223.1 (50.2)	226.6 (55.2)	219.7 (44.8)	0.43
p-Triglycerides, *mg/dL*	163.4 (93.5)	172.6 (91.4)	154.3 (95.4)	0.26
Fasting plasma glucose, *mg/dL*	100.2 (14.4)	102.0 (16.2)	98.2 (11.8)	0.17
p-Albumin, *g/L*	42.4 (2.9)	42.1 (2.8)	42.7 (2.9)	0.21
hsCRP, *mg/L*	3.9 (7.8)	4.6 (10.2)	3.2 (4.3)	0.30
FEPR	3 (2 – 6)	3 (2 – 5)	3 (2 – 6)	0.42
Primary renal disease, *%*				
Diabetes nephropathy	12.9	10.6	15.2	
Hypertensive nephropathy	23.5	22.7	24.2	
Glomerulonephritis	30.3	33.3	27.3	0.71
Pre-transplantation disease, *%*				
Hypertension	71.2	78.8	63.6	0.05
Diabetes mellitus	16.7	13.6	19.7	0.35
Coronary disease	12.1	12.1	12.1	>0.99
Cancer	10.6	7.6	13.6	0.26
Drugs used at baseline, *%*				
Statins	24.2	22.7	25.8	0.69
Acetylsalicylic acid	42.4	40.9	43.9	0.73
ACEi or ARB	9.8	7.6	12.1	0.38
Tacrolimus	97.0	97.0	97.0	>0.99
Systolic blood pressure, *mmHg*	134 (15)	132 (14)	135 (17)	0.21
Diastolic blood pressure, *mmHg*	82 (10)	81 (11)	82 (9)	0.54
Pulse wave velocity, *m/sec*	10.2 (3.2)	9.8 (2.6)	10.5 (3.7)	0.16
Resting brachial artery diameter, *mm*	3.9 (0.6)	3.9 (0.7)	4.0 (0.6)	0.33
Maximum post-occlusion brachial artery diameter, *mm*	4.1 (0.6)	4.1 (0.7)	4.2 (0.6)	0.34

Flow mediated dilation, %	4.9 (2.3)	4.7 (2.3)	5.0 (2.3)	0.47
Nr. of antihypertensive drugs, %				
None	19.7	15.2	24.2	
One	29.5	28.8	30.3	
Two	29.5	30.3	28.8	
Three or more	21.2	25.7	16.7	0.12
Nr. of previous renal transplants, %				
None	86.4	89.4	83.3	
One	12.1	10.6	13.6	
Two or more	1.6	0.0	3.0	0.50
Dialysis vintage, *months*	8 (0 – 20)	7 (0 – 19)	9 (0 – 22)	0.18
Dialysis mode, %				
Hemodialysis	45.5	40.9	50.0	
Peritoneal dialysis	23.5	28.8	18.2	
Preemptive transplantation	31.0	29.3	31.8	0.35
ABO incompatible transplantation, %	7.6	6.1	9.1	0.51
Preformed donor specific antibodies, %	9.8	7.6	12.1	0.38
Living donor, %	24.2	21.2	27.3	0.42
Cold ischemia time, *hours*	11.2 (6.1)	10.8 (5.9)	11.4 (6.4)	0.59
Delayed graft function, %	11.4	9.1	13.6	0.41
CADI	4 (2 – 5)	4 (3 – 6)	4 (2 – 5)	0.82
Renal graft inflammation, %	3 (1 – 8)	3 (1 – 10)	4 (1 – 8)	0.84
Renal graft fibrosis, %	10 (6 – 17)	10 (6 - 16)	10 (5 – 18)	0.83
Number of HLA mismatches, %				
None or one	15.9	13.7	18.2	
Two or three	51.5	51.5	51.6	
Four or more	32.6	34.8	30.3	0.77
Daily use of low-dose marine n-3 fatty acid supplements (cod liver oil), %	14.4	12.1	16.7	0.46
Physical exercise, %				
High intensity ≥ twice per week	38.6	37.9	39.4	
High intensity once per week	3.8	7.6	0.0	
Low intensity ≥ twice per week	41.7	45.5	37.9	
Low intensity once per week	8.3	4.5	12.1	
None	7.6	4.5	10.6	0.28
Smoking habits, %				
Daily smoker	15.2	15.2	15.2	
Non-daily smoker	2.3	3.0	1.5	
Former heavy smoker	8.3	6.1	10.6	
Former light smoker	32.6	31.8	33.3	
Life-long non-smoker	41.7	43.9	39.4	0.85

Baseline patient characteristics and plasma phospholipid levels of major n-3 and n-6 polyunsaturated fatty acids are presented as percentage for categorical data, median (interquartile range) or mean value (standard deviation) for continuous variables. Differences between groups were evaluated with Chi square for dichotomous categorical data, Mantel-Haenzel linear trend for other categorical variables, Mann Whitney U-test for dialysis vintage, FEPR and renal graft indices and Student t-test for other continuous variables.

Abbreviations: ACEi: Angiotensin converting enzyme inhibitor. ARB: Angiotensin II receptor blocker. CADI: Chronic Allograft Damage Index. eGFR: Estimated glomerular filtration rate (CKD-EPI formula). FA: Fatty acid. FEPR: Fractional excretion of protein in urine. HLA: Human leukocyte antigen. hsCRP: Plasma high sensitive C-reactive protein. mGFR: Measured glomerular filtration rate. wt%: Weight percentage of total plasma phospholipid fatty acids.

Table 2: The effects of marine n-3 fatty acid supplementation on change in renal endpoints during follow-up

		Marine n-3 FA group		**Control group**	
Variables	n	Mean ± SD	n	Mean ± SD	p
Δ EPA + DHA, wt%					
ITT	61	4.0 ± 2.7	65	0.2 ± 2.2	<0.001
PP	49	4.4 ± 2.3	52	0.1 ± 1.8	<0.001
Δ mGFR, mL/min/1.73m^2					
ITT	58	6.7 ± 11.7	62	3.8 ± 10.3	0.15
PP	49	6.7 ± 11.6	49	4.3 ± 10.4	0.29
Δ CADI					
ITT	43	0.2 ± 2.7	47	0.9 ± 3.0	0.18
PP	37	-0.3 ± 2.4	40	1.0 ± 3.1	0.03
Δ Renal graft fibrosis, %					
ITT	50	0.7 ± 9.6	53	3.6 ± 11.0	0.18
PP	42	-0.6 ± 9.0	45	3.6 ± 11.1	0.04
Δ Renal graft inflammation, %					
ITT	50	0.9 ± 8.6	53	1.8 ± 9.2	0.53
PP	42	0.4 ± 8.1	45	2.0 ± 8.5	0.47
Δ hsCRP, mg/L					
ITT	61	-1.2 ± 11.0	65	2.8 ± 7.1	0.02
PP	50	-1.9 ± 11.6	52	2.3 ± 7.4	0.04
Δ FEPR					
ITT	61	-1.5 ± 4.6	65	-1.0 ± 3.9	0.18
PP	50	-1.8 ± 4.0	52	-1.1 ± 4.2	0.34

Differences between the marine n-3 fatty acid (FA) group and the control group (olive oil) were evaluated by Mann Whitney U-test (renal graft indices and FEPR) and Student t-test (other variables) and as appropriate.

Abbreviations: CADI: Chronic Allograft Damage Index. DHA: Docosahexaenoic acid. EPA: Eicosapentaenoic acid. FA: Fatty acid. FEPR: Fractional excretion of protein in urine. hsCRP: Plasma high sensitive C-reactive protein. ITT: Intention-to-treat population. mGFR: Measured glomerular filtration rate. PP: Per-protocol population. wt%: Weight percentage of total plasma phospholipid fatty acids.

Table 3: The effects of marine n-3 fatty acid supplementation on change in cardiovascular endpoints during follow-up

		Marine n-3 FA group		**Control group**	
Variables	n	Mean ± SD	n	Mean ± SD	p
Δ p-Triglycerides, mg/dL					
ITT	61	-39.8 ± 74.2	65	-7.8 ± 77.2	0.02
PP	50	-41.4 ± 68.3	52	-6.7 ± 83.8	0.02
Δ p-HDL cholesterol, mg/dL					
ITT	61	-0.6 ± 15.9	65	-2.9 ± 19.8	0.46
PP	50	-0.2 ± 15.9	52	-1.5 ± 20.2	0.71
Δ p-LDL cholesterol, mg/dL					
ITT	61	-33.9 ± 44.6	65	-30.3 ± 40.7	0.63
PP	50	-35.3 ± 45.9	52	-33.7 ± 38.8	0.85
Δ p-Total cholesterol, mg/dL					
ITT	61	-41.5 ± 50.6	65	-35.1 ± 41.8	0.46
PP	50	-43.0 ± 52.6	52	-37.7 ± 43.6	0.59
Δ p-Fasting glucose, mg/dL					
ITT	54	3.8 ± 20.3	45	1.2 ± 10.9	0.44
PP	45	4.9 ± 21.8	36	1.2 ± 10.9	0.35
Δ p-2hPG, mg/dL					
ITT	52	-8.3 ± 45.6	44	-6.8 ± 33.7	0.86
PP	43	-6.0 ± 48.6	35	-7.3 ± 31.6	0.89
Δ Flow mediated dilation, %					
ITT	52	2.0 ± 3.8	54	0.5 ± 2.4	0.02
PP	41	2.1 ± 4.0	43	0.7 ± 2.4	0.05
Δ Pulse wave velocity, m/sec					
ITT	58	-0.6 ± 2.1	58	-0.6 ± 2.4	0.99
PP	47	-0.7 ± 2.2	47	-0.7 ± 2.3	0.92
Δ Systolic BP, mmHg					
ITT	61	-5.5 ± 14.4	65	-3.9 ± 14.1	0.53
PP	50	-5.3 ± 15.0	52	-4.3 ± 13.2	0.72
Δ Diastolic BP, mmHg					
ITT	61	-2.6 ± 9.1	65	-2.7 ± 9.4	0.96
PP	50	-3.1 ± 9.3	52	-3.8 ± 9.0	0.69

Differences between the marine n-3 fatty acid (FA) group and the control group (olive oil) were evaluated by Student t-test. Abbreviations: BP: Blood pressure. HDL: High density lipoprotein. ITT: Intention-to-treat population. LDL: Low density lipoprotein. PP: Per-protocol population. 2hPG: Two hours post-challenge plasma glucose during an oral glucose tolerance test.

Table 4: Adverse events

Variables	**Marine n-3 FA**	**Controls**	p
Number of patients	66	66	
Number of adverse events	218	240	0.33
Death	1	0	
Graft failure	0	1	
Acute rejection episode	15	16	
Chronic antibody mediated rejection	1	1	
De novo donor specific antibodies	3	3	
Polyoma virus nephropathy	1	0	
Polyoma virus viremia	5	3	
Cytomegalovirus disease	2	4	
Cytomegalovirus viremia	4	9	
Recurrence of primary renal disease	2	3	
Post transplantation diabetes mellitus	10	7	
Transplant ureter stenosis	1	3	
Coronary artery disease	0	2	
Peripheral artery stenosis	0	2	
Transplant artery stenosis	1	2	
Stroke	0	1	
Atrial fibrillation	2	2	
Aortic or mitral valve disease	2	0	
Deep vein thrombosis	2	1	
Fracture	2	2	
Cancer (solid organ)	2	2	
Cancer (skin)	1	1	
Septicemia	4	4	
Lower respiratory tract infection	8	6	
Urinary tract infection	5	15	
Gastrointestinal discomfort (abdominal pain, diarrhea, nausea or regurgitation)	17	21	

Adverse events during 44 weeks of follow-up. Difference between the marine n-3 fatty acid (FA) group and the control group was evaluated by Fishers Exact test. In addition, the distribution of selected adverse events is given.