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n-3 fatty acids reduce proteinuria in patients with chronic glomerular disease

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n-3 fatty acids reduce proteinuria in patients with chronic glomerular disease. Dietary supplementation with n-3 polyunsaturated fatty acids (n-3 PUFA) has been shown to reduce proteinuria in experimental models of renal diseases, but their potential role in the treatment of human renal disease is unknown. We administered n-3 PUFA in the form of triglycerides [with eicosapentenoic (EPA) + docosahexaenoic (DHA) = 3 g/day into 4 patients] and of ethyl esters (EPA + DHA = 7.7 g/day) into 10 patients (one patient twice) with chronic glomerular disease (membranous glomerulonephritis and focal glomerular sclerosis), all diagnosed histologically. Serum albumin was >2.4 g/dl and serum creatinine <2.5 mg/dl in all patients. Treatment was given for periods of six weeks, followed by a prolonged follow-up for 27 weeks in 10 cases. Dietary supplementation with n-3 PUFA caused the expected reduction in platelet generation of thromboxane B_2 (mean ± SEM, from 490 ± 70 ng/ml at baseline, to 342 ± 147 ng/ml at 6 weeks, $P < 0.05$) of serum triglycerides (from 236 ± 60 to 170 ± 43, $P < 0.01$), and a prolongation of the bleeding time (from 5.8 ± 0.4 min to 7.7 ± 0.4 min, $P < 0.01$) in patients treated with ethyl esters. A modest but significant reduction in serum total cholesterol was noticed (from 275 ± 27 to 252 ± 24 mg/dl). Urinary thromboxane B_2 excretion (as a reflection of renal TXA_2 production) and urinary 6-keto-$PGF_{1\alpha}$ excretion (as a reflection of renal PGI_2 production), assayed by extraction, chromatographic separation and RIA with antibodies cross-reacting with metabolites of the n-3 series, were both similarly reduced by the ethyl ester treatment (for TXB_2: 7.8 ± 1.7 ng/hr at week 0; 4.2 ± 0.6 ng/hr at week 6, $P = 0.06$; for 6-keto-$PGF_{1\alpha}$: 24.4 ± 5.1 ng/hr at week 0; 16.4 ± 2.9 ng/hr at week 6, $P = 0.09$). Serum albumin, creatinine and creatinine clearance did not change significantly throughout the study. The high dose regimen, however, caused a significant reduction in proteinuria (from 3.7 ± 1.0 g/24 hr at week 0 to 2.6 ± 0.7 g/24 hr at week 6, $P < 0.05$). This was sustained at weeks 10 and 16 and returned to baseline values at week 27. A modest reduction in blood pressure was also noticed, but it did not correlate with the change in proteinuria. Thus, n-3 PUFA may have a therapeutic role in reducing proteinuria in patients with chronic glomerular disease. The relationship of modifications in renal eicosanoids with this effect is uncertain.

There has been considerable recent interest about the effects of n-3 polyunsaturated fatty acids (n-3 PUFA) in human physiology and human diseases [1, 2]. Dietary supplementation with these fatty acids leads to a number of biological changes. These include effects on plasma lipids and lipoproteins [3], eicosanoid metabolism [4–7], platelet-vessel wall interactions [8], blood viscosity [9], arterial blood pressure [10–12], coagulation [13], cytokines [14, 15], and growth factors [16]. Most of these changes are of potential therapeutic benefit to patients with renal disease, either by ameliorating kidney function or slowing the progression of chronic renal failure.

Animal models of both inflammatory renal diseases [17–24] and reduced renal mass [25–29] mostly show improvement of kidney function following administration of n-3 fatty acids. However, to date there has been little focus on the potential application of these substances in a clinical framework. Reports have appeared for use in transplantation patients to limit cyclosporine-induced nephrotoxicity [30, 31], in lupus nephritis [32] and IgA nephropathy [33–35]. We therefore decided to investigate the effects of n-3 PUFA administration in human chronic glomerular disease, an important cause of chronic renal failure and for which no satisfactory conservative therapy is known.

Methods

Patients

Between December 1989 and March 1992, all patients admitted to the Nephrology Division of Spedali Riuniti di Santa Chiara in Pisa, Italy were included if they met the following criteria: (a) membranous or membrano-proliferative glomerulonephritis or idiopathic focal glomerular sclerosis characterized by renal biopsy within the previous six months; (b) albuminemia >2.4 g/dl; (c) serum creatinine <2.5 mg/dl. Exclusion criteria were: (1) intake of steroidal or non-steroidal anti-inflammatory agents, antiplatelet agents or anticoagulants; and (2) acute or subacute liver or pancreas disease. All patients were chronically on a low-sodium diet (2 g/day of sodium chloride were provided to patients to be distributed at patient's will throughout the meals). No other medical therapy was allowed, with the exception of antihypertensive therapy (nitrendipine), given to one patient (MM) chronically, and never altered throughout the study.

Protocol approval was obtained by an Internal Review Board and patients' consent was obtained in all cases. Ten patients were recruited: three of them were studied twice (Study A and B), and one patient was studied three times (once on Treatment

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Table 1. Clinical and baseline laboratory data of patients

Patient initials	Sex	Age *years*	Weight *kg*	Height *cm*	Histologic diagnosis	Baseline		
						Albumin *g/dl*	Creatinine *mg/dl*	Creatinine clearance *ml/min*
n-3 triglyceride study								
VP	M	56	67	162	membranous GN	3.0	1.1	89
LM	M	70	55	170	membranous GN	2.6	1.0	80
MM	M	39	73	180	focal GS	3.9	1.2	114
PMR	F	28	64	162	focal GS	4.2	0.6	105
n-3 ethyl ester study								
VP	M	56	67	162	membranous GN	2.4	0.9	80
LM	M	70	55	170	membranous GN	2.8	1.0	73
MM	M	39	73	180	focal GS	3.9	1.4	87
MM-II	M	39	73	180	focal GS	4.0	1.5	64
PMR	F	28	64	162	focal GS	3.9	0.6	108
BS	M	27	91	176	focal GS	3.9	1.4	90
LA	F	41	55	160	focal GS	2.5	0.6	155
GA	M	43	77	172	membranous GN	2.5	1.8	50
BM	M	22	91	187	focal GS	3.3	1.2	148
DG	F	38	62	160	focal GS	3.7	0.5	125
CE	F	43	79	161	focal GS	3.4	0.7	120

Pt. MM underwent two cycles with the ethyl ester preparation. Abbreviations are: GN, glomerulonephritis; GS, glomerulosclerosis.

A and twice on Treatment B). Patients' characteristics are listed in Table 1.

Study design

This was a prospective, open-label study. Patients received trial medications from week 0 to week 6, and were followed-up at weeks 10 and 16 after discontinuation of trial medication. In nine cases (3 on Treatment A and 6 on treatment B) a prolonged follow-up at week 27 was available. Long follow-up periods after discontinuation of trial treatment were chosen due to past observations of long-lasting carry-over effects of n-3 PUFAs [14, 15].

Trial treatments were: for treatment A, MaxEPA (provided by Fresenius, Bad Homburg, Germany), capsules of triglycerides of n-3 PUFA, each containing 750 mg of fish oil, yielding 33% of eicosapentaenoic acid (EPA) + docosahexaenoic acid (DHA), given as 12 capsules/day, with meals, for a total amount of n-3 PUFA supplementation of about 3 g/day; for treatment B, K-85 (provided through Fresenius), capsules of ethyl-esters of n-3 PUFA, each containing 1000 mg of fish oil, yielding 85% of EPA + DHA, given as 9 capsules/day, with meals, for a total amount of n-3 PUFA supplementation of 7.7 g/day.

Thus, treatment A was a low-dose regimen of 3 g n-3 PUFAs daily and treatment B a high dose regimen of 7.7 g n-3 PUFAs daily.

Patients were advised not to change their usual dietary habits, including remaining on a low-salt diet (approximately 2 g of NaCl supplement to be distributed, at patient's will, throughout the meals) and not altering the usual protein intake. This last point, specifically, was objectively monitored by measurements of urinary nitrogen excretion (see below).

Laboratory and clinical analyses included the following assays:

Incorporation of n-3 PUFAs into plasma lipids was evaluated through the analysis by gas liquid chromatography of fatty acids in plasma total lipids after preparation of methyl ester derivatives. After acidic transmethylation, fatty acid methyl esters were separated on a Dani GC-85.10 gas chromatograph equipped with a 30 m Supelco Omegawax capillary column (0.32 mm I.D., 0.25 μm film thickness) and a flame ionization detector. A discontinuous temperature gradient from 130 to 220°C was used. The H_2, air and carrier (helium) pressures were 0.8, 0.9 to 1, and 0.6 bar, respectively. Individual peaks were integrated automatically using a Shimadzu C-R6A Chromatopac integrator.

Serum triglycerides and total serum cholesterol were measured by enzymatic colorimetric methods (GDP-PAP and Monotest cholesterol CHOD-PAP, respectively, Boehringer-Mannheim, Mannheim, Germany). High-density lipoprotein (HDL) cholesterol was measured in the supernatant after precipitation of apolipoprotein B-containing lipoproteins with $MgCl_2$ by a similar enzymatic colorimetric method (Carlo Erba, Milan, Italy).

Bleeding time was measured according to Mielke's modification of the method of Ivy, using a disposable device (Surgicutt, Ortho Diagnostics, Raritan, New Jersey, USA) and a transverse incision on the lateral aspect of the volar surface of the forearm and mantaining venous pressure at 40 mm Hg by the inflation of a sphygmomanometric cuff.

Serum thromboxane B_2 (TXB_2) was measured in clotted blood prepared by placing duplicate 1 ml samples of freshly drawn, unanticoagulated blood into clean glass tubes that were kept at 37°C for 90 minutes. The serum was then promptly separated by centrifugation and was frozen at −30°C until assay. Serum TXB_2 levels were measured by radioimmunoassay [36].

Urinary excretion of renal eicosanoids (TXB_2, 6-keto-$PGF_{1\alpha}$ and PGE_2, as a reflection of renal production of TXA_2, PGI_2 and PGE_2, respectively) was determined in urine collected at night (about 8 hrs) from subjects in the treatment group at each time of examination. Eicosanoids were purified from urinary matrix by extraction with ethyl acetate and chromatography on

small columns of silicic acid [37]. The fraction containing TXB_2, 6-keto-$PGF_{1\alpha}$ and PGE_2 was dried, resolubilized in buffer, and aliquots corresponding to 125 ml of urine assayed by specific radioimmunoassays [37, 38].

Serum (S) and urine (U, on 24 hr urine collection in duplicate samples) creatinine were determined by standard colorimetric methods, based on the development of color in alkaline environment, in the presence of picric acid (Jaffé's reaction);

Creatinine clearance expressed in ml/min was determined by the standard formula

$$\frac{U \times V}{S}$$

where V is urinary volume.

Urinary daily protein excretion (proteinuria) was measured by the Red-Ponceau colorimetric method, and total serum proteins by the biuret method; such measurements were carried out in samples from three consecutive days for each time point of the study; patients were carefully instructed about the importance of a thorough 24 hour urine collection, crucial for all urinary determinations. Short-term (day-to-day) variability of the proteinuria measurement, as evaluated by the coefficient of variation (mean ± SD) of the three replicates at each time point of the study, was 8.7%. Long-term baseline variability of proteinuria, as determined by the coefficient of variation of the replicates in the five cases (4 patients) undergoing repeated studies was 13.7%.

Serum albumin was estimated from a fractional analysis of serum protein electrophoretogram (on standard paper electrophoresis).

Protein intake, in order to evaluate a possible spurious source of proteinuria variability during the study, was evaluated calculating the protein catabolic rate, as reflected by the Urea Nitrogen Appearance rate. This was derived from the formula:

$$PCR = (U_UN + 0.031 \times BW) \times 6.25,$$

where PCR (protein catabolic rate) is the amount of degraded protein per day, in g/day, U_UN is urinary urea nitrogen, in g, and BW is body weight, in kg, as described [39]; measurements of this parameter were obtained in triplicate for each point of the study, and normalized for body weight.

Blood pressure measurements (standard Riva-Rocci sphygmomanometric method) were taken in duplicate on every day of hospitalization by registered nurses, and averaged for each phase of the study.

Statistical evaluation

Comparisons of all baseline values to post-treatment (week 6) values were performed using the two-tailed Student's *t*-test for paired values. Significance level was set at $P < 0.05$. For proteinuria values, analysis of variance for repeated measures was also performed. Results are expressed as mean ± SEM.

Results

All patients initially entered the trial successfully completed the six weeks of treatment, with the exception of one who decided to suspend the treatment because of gastric discomfort and diarrhea after the intake of the first capsules. No other patient reported any major adverse effect, including one patient (LM) who had previously undergone partial gastrectomy because of peptic ulcer. A fishy aftertaste was, on the other hand, commonly reported.

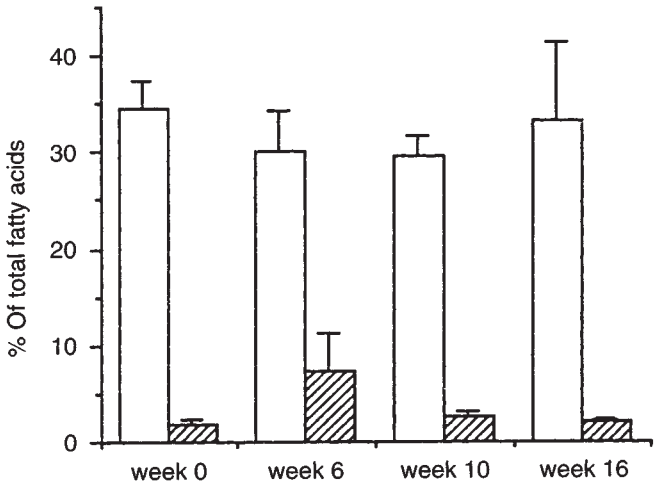


Fig. 1. *n-6 (□) and n-3 (▨) PUFA, as percent of total plasma lipids in patients treated with K-85.* Analysis of variance for repeated measures shows significant F values ($P < 0.05$). Student's *t*-test for paired values indicates significant differences ($P < 0.01$) in percent of both n-3 and n-6 PUFAs between week 0 and 6, and borderline significances ($P < 0.07$) between week 0 and week 10, due to the limited number of observations ($N = 8$ at weeks 0 and 6, and $N = 3$ at weeks 10 and 16).

All patients, when specifically questioned, reported to have had >90% intake of prescribed treatment capsules. This was objectively reflected in laboratory analyses of n-3 PUFA incorporation into plasma lipids, reduction in triglycerides and reduction in serum thromboxane.

The administration of n-3 PUFA as triglycerides (treatment A) resulted in significant changes of the fatty acid profiles of the n-3 and n-6 PUFA. The percentage levels of the n-3 PUFA in plasma were increased sixfold for 20:5 (EPA) and 22:5 (docosapentaenoic acid, DPA) and twofold for 22:6 (DHA), respectively, at the end of treatment. These fatty acids returned practically to basal levels after four weeks of washout (week 10 of the study). Arachidonic acid, unchanged at the end of treatment, was instead markedly reduced at the end of washout. The percent of total fatty acids given by n-3 and n-6 PUFAs during various phases of treatment B is shown in Figure 1. n-3 PUFA were about 2% at baseline, and increased to >7% at week 6. This change was not sustained subsequently, being on the average 3.3% at week 10. Reverse changes of n-6 PUFAs of the same magnitude were observed; however percent of total n-6 was still somewhat depressed at week 10, that is, four weeks after drug discontinuation, despite total n-3 having already returned to baseline by that time (Fig. 1). Interestingly, plasma levels of EPA and DHA at the end of both types of treatment were not statistically different, in spite of the higher dose of n-3 given as ethyl esters in treatment B.

Serum triglycerides consistently decreased in all patients during treatment. Reduction was, on the average, 26% (NS) for treatment A (not shown) and 28% for treatment B ($P < 0.01$; Fig. 2). The decrease was more pronounced in patients exhibiting initially high triglycerides.

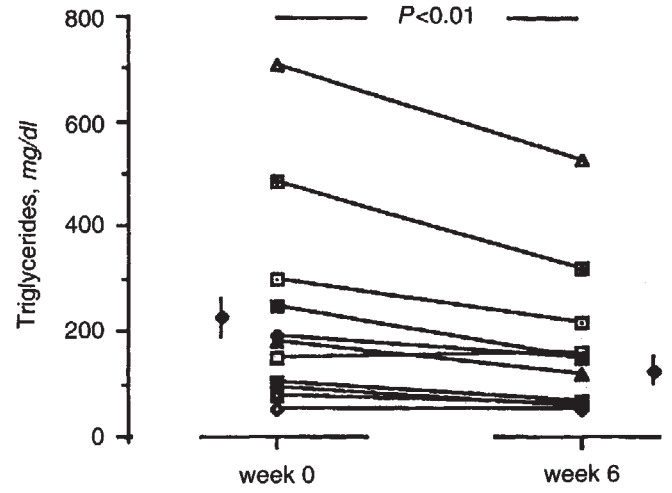


Fig. 2. *Serum triglycerides in patients treated with K-85, at week 0 and week 6.* Mean and SEM of the observations at the two time points are also shown.

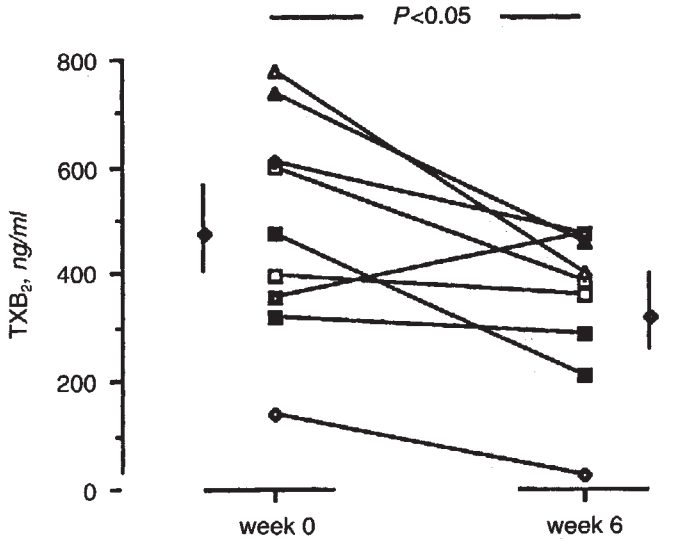


Fig. 3. *Serum thromboxane B_2 in patients treated with K-85, at week 0 and week 6.* Mean and SEM of the observations at the two time points are also shown.

Serum cholesterol also showed a minor reduction on the average (from 274 ± 27 mg/dl at week 0 to 252 ± 24 mg/dl at week 6 for treatment B), which was of borderline significance ($P = 0.05$) for the relatively high consistency among cases. HDL-cholesterol did not change (42.3 ± 3.6 mg/dl at week 0; 41.2 ± 4.7 at week 6 for treatment B). No significant change was detected with the lower dose treatment (not shown).

As expected, there was a significant reduction in serum thromboxane B_2. This was about 30% in patients treated with the high dose of n-3 FA (Fig. 3). A reduction of slightly lesser magnitude (23%) occurred in patients treated with the low dose.

Bleeding time increased significantly. The increase was 21% for treatment A (not shown) and 33% for treatment B ($P < 0.01$) (Fig. 4).

Urinary thromboxane B_2 excretion (as a reflection of renal

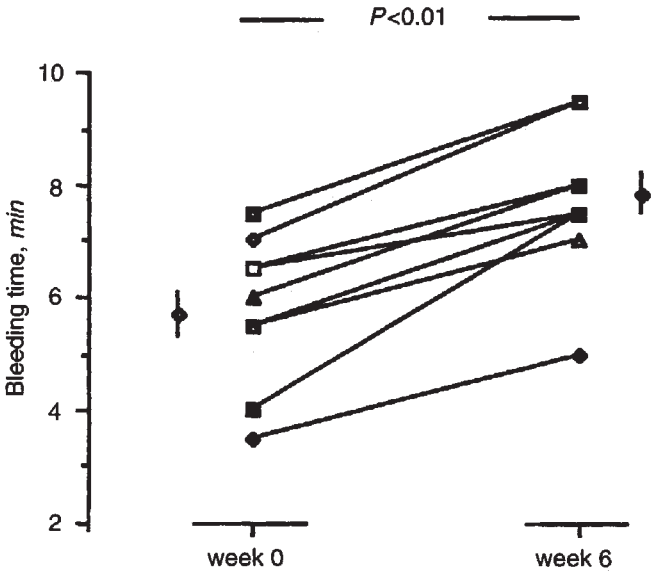


Fig. 4. *Bleeding time in patients treated with K-85, at week 0 and week 6.* Mean and SEM of the observations at the two time points are also shown.

TXA_2 production) and urinary 6-keto-$PGF_{1\alpha}$ excretion (as a reflection of renal PGI_2 production) were both similarly reduced by the ethyl ester treatment (for TXB_2: 7.8 ± 1.7 ng/hr at week 0; 4.4 ± 0.6 ng/hr at week 6, $P = 0.06$; for 6-keto-$PGF_{1\alpha}$: 24.3 ± 5.1 ng/hr at week 0; 15.2 ± 2.8 ng/hr at week 6, $P = 0.09$). A similar reduction also occurred for urinary PGE_2. Urinary eicosanoids were quickly back at baseline values already at week 10 (for urinary TXB_2: 8.4 ± 4.8 ng/hr) at week 10. Treatment with low-dose triglycerides was not associated, however, with any directional trend towards the reduction (not shown).

No significant changes were seen throughout the study for serum creatinine and serum albumin (data not shown). Creatinine clearance was 97 ± 8 ml/min and 113 ± 8 ml/min at weeks 0 and 6, respectively, for treatment A (NS); 104 ± 12 ml/min and 103 ± 12 ml/min at weeks 0 and 6, respectively, for treatment B (NS).

For both regimens some reduction in proteinuria was observed concomitantly with fish oil treatment. This was marginal for the low-dose treatment (from 3.8 ± 0.6 g/24 hr to 3.3 ± 0.9 g/24 hr, P = NS), but significant for the high-dose treatment (from 4.1 ± 1.1 g/24 hr to 2.9 ± 0.8 g/24 hr, $P < 0.05$; Fig. 5). This reduction was sustained, at week 10 and 18, and returned towards baseline values only at week 27 (Fig. 6). The one subject who received K-85 treatment twice showed similar reductions in proteinuria on both occasions (28% and 33% after the first and the second course of treatment, respectively).

Daily protein intake, as evaluated by the urea nitrogen appearance rate was remarkably stable throughout the study, being 1.100 ± 0.03 and 1.080 ± 0.04 g/kg/day before and after the triglyceride treatment, and 1.042 ± 0.03 and 1.043 ± 0.04 g/kg/day before and after the ethyl ester treatment, respectively, therefore not accounting for changes in proteinuria.

Some reduction in blood pressure was observed only with the higher dose regimen: during treatment B systolic blood pressure decreased from 143 ± 4 to 134.5 ± 3 mm Hg (P = NS) and

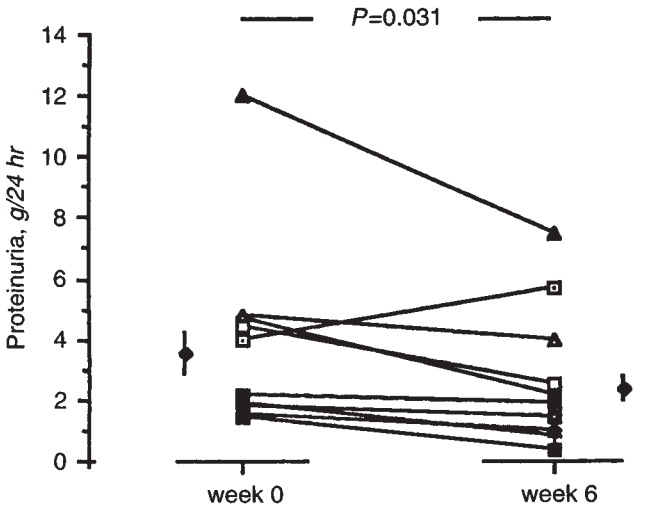
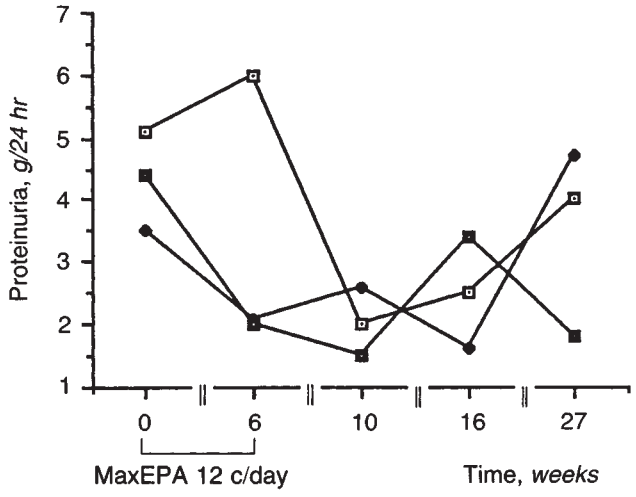


Fig. 5. *Proteinuria in patients treated with K-85, at week 0 and week 6.* Mean and SEM of the observations at the two time points are also shown.



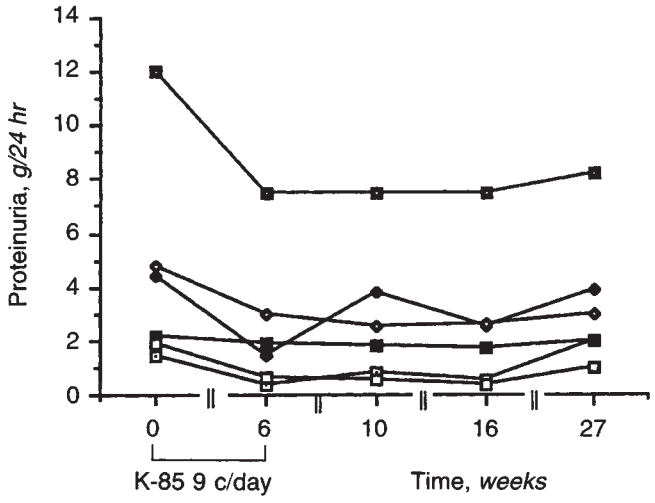


Fig. 6. *Time course of proteinuria in the 7 patients (3 on MaxEPA, 4 on K-85) who were followed up to the 27th week (21 weeks after drug discontinuation).*

diastolic blood pressure from 90 ± 2.4 to 85 ± 2.1 ($P < 0.05$, for the higher consistency among cases). No change occurred, on the other hand, in patients treated with the low-dose triglyceride regimen (from 132.5 ± 7.5 to 136.3 ± 11 mm Hg for systolic blood pressure; from 75 ± 7 to 55 ± 5 mm Hg for diastolic blood pressure). In the high-dose treatment group, however, changes in proteinuria were apparently unrelated to changes in blood pressure: at linear regression analysis, correlation coefficient (r) was 0.23 for systolic and 0.08 for diastolic blood pressure, respectively (for both P = NS). Reduction in blood pressure at least additive to that of calcium antagonist therapy (nitrendipine) was observed in patient MM with the high-dose regimen.

No difference in results in relationship with histology (membranous glomerulonephritis versus focal glomerular sclerosis) was observed.

Discussion

This study shows that, in patients with chronic glomerular disease, two different dose regimens of n-3 PUFAs may reduce proteinuria, and the high dose regimen did it to a statistically significant extent. The present study is, to the best of our knowledge, the first report of beneficial effects of n-3 FA supplementation in this patient category; however, it confirms a number of previous observations in experimental animals [17–29], mostly suggestive of a beneficial effect.

Patient compliance to treatment was monitored by questioning and, more objectively, by laboratory measurements of incorporation of n-3 FA in plasma phospholipids, monitoring of serum lipids and of serum TXB_2. Both treatment regimens used resulted in a significant increase in n-3 FA, as percentage of total plasma fatty acids, and in a corresponding decrease in the percentage of n-6 FA. The very consistent decreases in triglycerides and serum TXB_2, observed in the vast majority of our patients, confirm previous findings in healthy volunteers and non-renal patients, and extend them to a situation of chronic glomerular disease in which such changes might exert, by themselves, beneficial effects [40–43]. Also, the increase in bleeding time by fish oil, which we report in the present study, is consistent with previously reported data in other patient categories. This effect highlights the influence of fish oil on platelet-vessel wall interactions, one potential mechanism of improvement of renal function by fish oil [44].

Spontaneous variations of proteinuria during the course of renal disease are an obvious potential cause for false positive findings. However, the coefficient of variation of proteinuria during treatment was almost double than the one reflecting the long-term variability of proteinuria measurements in baseline conditions in the same patients, pointing to a specific effect of the treatment. Also, variations in proteinuria observed throughout this study could certainly not be accounted for by variation of protein intake, which remained remarkably stable. Also the time course of proteinuria suggests a time relationship with the treatment.

Changes in blood pressure are, in addition to favorable effects

on hemostasis, a potential mechanism by which n-3 FA may ameliorate proteinuria. These compounds have indeed been shown, in at least two prospective double-blind randomized trials, to reduce blood pressure in essential hypertension [10, 11]. A trend towards blood pressure reduction was indeed evident for the high-dose treatment also in our study. However the relationships of the blood pressure changes to the amelioration of proteinuria are not clear and certainly need further investigation. In our limited series of observations no relationship was found between the decrease in blood pressure and the decrease in proteinuria. It may also be pointed out that for only some antihypertensive agents (mostly some ACE inhibitors) there have been reports of beneficial effects on proteinuria (see below). Finally, n-3 FA may be additive or synergistic to antihypertensive medications in reducing blood pressure [45], but nothing is known about the potential additive or synergistic effect on other correlates of blood pressure such as proteinuria in patients with renal disease.

In terms of metabolism of n-3 PUFAs, it is especially noteworthy that the decrease in proteinuria in our series appears sustained for some 12 weeks after discontinuation of treatment. Only during the treatment period did the low proteinuria levels correspond well with the levels of EPA and DHA in plasma lipids. The effects on proteinuria were even more pronounced after discontinuation of treatment, whereas this was not reflected by EPA and DHA levels in plasma lipids. The time course of effects on proteinuria is indeed quite similar to the time course of reduction observed for interleukin-1, 2 and tumor necrosis factor [14, 15], another well-documented effect of n-3 FA for which there appears to be a dissociation from estimates of incorporation. This suggests, on the one hand, that n-3 FA effects require saturation of intracellular lipid compartments, which may only loosely reflected by the crude estimate of incorporation of n-3 PUFAs into plasma lipids or total cellular lipids; on the other hand, it suggests different time courses for different effects, some of which may reflect only very remotely the actual intake period of fish oil.

Treatment with n-3 FA was associated with a clear-cut reduction in both serum and urinary thromboxane, a reflection of platelet and renal production of thromboxane, respectively [38]. Theoretically, the reduction in renal thromboxane production may be linked mechanistically to the observed reduction in proteinuria [46]. However, the reduction in thromboxane production was transient, being already back to pre-treatment values four weeks after the discontinuation of treatment, and therefore correlating only partially with the time course of proteinuria reduction. Further, and in keeping with previously reported data [47], n-3 PUFAs reduced also both urinary PGE_2 and PGI_2. This last effect could therefore potentially counterbalance the potential benefit exerted by the reduction in renal thromboxane, and actually reduce the renal vasodilatory reserve in an already diseased kidney. Detrimental effects due to the reduction of vasodilatory prostanoids have been demonstrated for non-steroidal antiinflammatory drugs due, at least in part, to their massive reduction of PGE_2 and PGI_2 [48]. However, in the case of n-3 PUFAs, PGE_2 and PGI_2 reductions appear much less dramatic than with NSAIDs. Also, with n-3 PUFAs, PGI_3 [5] and PGE_3 [49] are alternatively formed. The antibodies used in our assays certainly cross-reacted to some extent with the PGI_3 derivative (about 20%), and, likely, with PGE_3. However, these assays certainly underestimated the amounts of PGI_3 and PGE_3 produced. Although the vasodilatory properties of PGI_3 are well known, much less is known about the potential vasodilatory effects of PGE_3; furthermore, their effects on kidney physiology and pathophysiology are not well understood. Indirectly, data on renal plasma flow and glomerular filtration rates in other human studies indicate that there can be improvement in these clinical indicators, and thus no deterioration of the renal vasodilatory reserve in patients with chronic kidney diseases who take n-3 PUFA [50].

Additionally, other effects, not measured in this trial, could have contributed to the clinical improvement observed. Among them are possible immunomodulatory effects due to depression of interleukins 1 and 2 and tumor necrosis factor [14, 15], antiproliferative effects, due to decrease in production of growth factors [16], possibly contributing to mesangial proliferative phenomena, and effects on the filtrability of macromolecules [51].

Our study did not show directional changes in other parameters of renal function, such as creatinine and creatinine clearance. This could be noteworthy in view of the fact that another drug category, ACE inhibitors, have also been shown to reduce proteinuria in similar patients, probably altering intraglomerular hemodynamics by different effects on the tone of the afferent versus the efferent arteriole [52]. The counterpart of this effect, for this drug category, is a detrimental and potentially dangerous increase in serum creatinine and a decrease in glomerular filtration rate. Whatever the mechanism of fish oil in affecting proteinuria may be, such mechanism has to be different from that of ACE inhibitors.

Most parameters showing changes concomitant with treatment, namely proteinuria, serum thromboxane, urinary thromboxane and prostacyclin, and serum triglycerides, showed more changes with the higher than with the lower dose treatment, suggesting the existence of a dose-effect relationship. This was not so, however, for changes in the incorporation in plasma lipids. The reason for this is uncertain, but may reflect, gain, the partitioning of long-chain fatty acids into functionally different intracellular pools, not reflected by the relatively crude estimate of total plasma lipids (refer to previous text).

Limitations of our study are the lack of a placebo control and the relatively small number of patients studied. A parallel-group, placebo-controlled study would have required many more subjects, and a crossover design was partially precluded by the long-term carryover effects of fish oil on many biological parameters, including, possibly, proteinuria. The relatively prolonged follow-up of some of our patients, and the reproducibility of proteinuria measurements both in the short-term and in the long-term in baseline conditions, in our series, reinforce our conclusions about beneficial effects on proteinuria. Because of these limitations, however, a larger study including more patients appears opportune, and is currently being planned; the influence of long-term supplementation must still also be determined in this group of patients.

In conclusion, high-dose n-3 PUFA for six weeks reduced proteinuria in non-dialyzed patients with chronic glomerular diseases. This effect was observed for up to 12 weeks after discontinuation of treatment and returned to baseline by week 21 after stopping therapy. Our observations indicate a potential therapeutic benefit of n-3 PUFA for patients with renal disease,

with an improvement of kidney function. Further investigations on long-term supplementation periods in more such patients are merited. The very favorable safety profile of these natural substances should stimulate further trials of n-3 PUFA as an adjuvant treatment for chronic glomerular disease.

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References

1. Leaf A, Weber PC: Cardiovascular effects of n-3 fatty acids. *N Engl J Med* 318:549–557, 1988
2. Simopoulos AP: Omega-3 fatty acids in health and disease and in growth and development. *Am J Clin Nutr* 54:438–463, 1991
3. Harris WS: Fish oil and plasma lipid and lipoprotein metabolism in humans: A critical review. *J Lipid Res* 30:785–807, 1989
4. Fischer S, Weber PC: Thromboxane A_3 (TXA_3) is formed in human platelets after dietary eicosapentaenoic acid (20:5 ω3). *Biochem Biophys Res Commun* 116:1091–1099, 1983
5. Fischer S, Weber PC: Prostaglandin I_3 is formed *in vivo* in man after dietary eicosapentaenoic acid. *Nature* 307:165–168, 1984
6. von Schacky C, Fischer S, Weber PC: Long-term effects of dietary marine omega-3 fatty acids upon plasma and cellular lipids, platelet function, and eicosanoid formation in humans. *J Clin Invest* 76:1626–1631, 1985
7. Knapp HR, Reilly IAG, Alessandrini P, FitzGerald GA: *In vivo* indexes of platelet and vascular function during fish oil administration in patients with atherosclerosis. *N Engl J Med* 314:937–942, 1986
8. De Caterina, Giannessi D, Mazzone A, Bernini W, Lazzerini G, Maffei S, Cerri M, Salvatore L, Weksler B: Vascular prostacyclin is increased in patients ingesting ω-3 polyunsaturated fatty acids before coronary artery bypass graft surgery. *Circulation* 82:428–438, 1990
9. Terano T, Hirai A, Hamazaki T, Kobayashi S, Fujita T, Tamura Y, Kumagai A: Effects of oral administration of highly purified eicosapentaenoic acid on platelet function, blood viscosity and red cell deformability in healthy human subjects. *Atherosclerosis* 46:321–331, 1983
10. Knapp HR, FitzGerald GA: The antihypertensive effects of fish oil: A controlled study of polyunsaturated fatty acid supplements in essential hypertension. *N Engl J Med* 320:1037–1043, 1989
11. Bønaa KH, Bjerve KS, Straume B, Gram IT, Thelle D: Effect of eicosapentaenoic and docosahexaenoic acids on blood pressure in hypertension: A population-based intervention trial from the Tromsø study. *N Engl J Med* 322:795–801, 1990
12. Singer P, Wirth M, Gödicke W, Heine H: Blood pressure lowering effect of eicosapentaenoic acid-rich diet in normotensive, hypertensive and hyperlipidemic subjects. *Experientia* 41:462–464, 1985
13. Hansen J-B, Olsen JO, Wilsgård L, Østerud B: Effects of dietary supplementation with cod liver oil on monocyte thromboplastin synthesis, coagulation and fibrinolysis. *J Int Med* 225 (Suppl 1):133–139, 1989
14. Endres S, Ghorbani R, Kelley VE, Georgilis K, Lonneman G, van der Meer JWM, Cannon JG, Rogers TS, Klemper MS, Weber PC, Schaefer EJ, Wolff SM, Dinarello CA: The effect of dietary supplementation with n-3 polyunsaturated fatty acids on the synthesis of interleukin-1 and tumor necrosis factor by momonuclear cells. *N Engl J Med* 320:265–271, 1989
15. Meydani SN, Endres S, Wood MM, Goldin BR, Soo C, Morrill-Labrode A, Dinarello CA, Gorbash SL: Oral n-3 fatty acid supplementation suppresses cytokine production and lymphocyte proliferation: Comparison in young and older women. *J Nutr* 121:547–555, 1991
16. Fox PL, DiCorleto PE: Fish oils inhibit endothelial cell production of platelet-derived growth factor-like protein. *Science* 241:453–456, 1988
17. Prickett JD, Robinson DR, Steinberg AD: Dietary enrichment with the polyunsaturated fatty acid ecosapentaenoic acid prevents proteinuria and prolongs survival in NZB × NZW F_1 mice. *J Clin Invest* 68:556–559, 1981
18. Kelley VE, Kirkman RL, Bastos M, Barret LV, Strom TB: Enhancement of immunosuppression by substitution of fish oil for olive oil as a vehicle for cyclosporin. *Transplantation* 48:98–102, 1989
19. Barcelli U, Kher V, Gallon L, Weiss M, Pahel P, Pollak VE: Effects of dietary polyunsaturated fatty acids on murine apoferritin induced glomerulonephritis. (abstract) *Clin Res* 32:769, 1984
20. Barcelli UO, Beach DC, Thompson B, Weiss M, Pollak VE: A diet containing n-3 and n-6 fatty acids favorably alters the renal phospholipids, eicosanoid synthesis and plasma lipids in nephrotic rats. *Lipids* 23:1059–1063, 1988
21. Robinson DR, Tateno S, Patel B, Hirai A: Dietary marine lipids after the course of autoimmune disease. *Prog Clin Biol Res* 282:295–303, 1988
22. Scharschmidt L, Miller M, Holthofer H, Sinha A, Schlondorff D, Gibbons N, Santiago A: A fish oil diet preserves renal function in nephrotoxic serum nephritis. *J Lab Clin Med* 115:405–414, 1990
23. Kher V, Barcelli U, Weiss M, Gallon L, Pajel P, Laskarzewski P, Pollak VE: Protective effect of polyunsaturated fatty acid supplementation in apoferritin-induced murine glomerulonephritis. *Prostaglandins Leukot Med* 22:323–334, 1986
24. Kee-Ching G, Jeng G, Fernandes G: Effect of fish oil diet on immune response and proteinuria in mice. *Proc Natl Sci Counc B ROC* 15:105–110, 1991
25. Scharschmidt LA, Gibbons NB, McGarry L, Berger P, Axelrod M, Janis R, Ko YH: Effects of dietary fish oil on renal insufficiency in rats with subtotal nephrectomy. *Kidney Int* 32:700–709, 1987
26. Barcelli U, Pollak VE: Is there a role for polyunsaturated fatty acids in the prevention of renal disease and renal failure? *Nephron* 41:209–212, 1985
27. Neumayer HH, Heinrich M, Schmissas T, Haller H, Wagner K: Prävention des postischämischen akuten Nierenversangens (ANV) durch Omega-3-Fettsäuren. (abstract) *Nieren und Hochdruck-Krandheiten* 18:394, 1989
28. Logan JL, Michael UF, Benson B: Effects of dietary fish oil on renal growth and function in uninephrectomized rats. *Kidney Int* 37:57–63, 1990
29. Clark WF, Parbtani A, Philbrick DJ, Holub BJ, Huff M: Chronic effects of omega-3 fatty acids (Fish Oil) in a rat 5/6 renal ablation model. *J Am Soc Nephrol* 1:1343–1353, 1991
30. Hess AD, Colombani PM, Esa A: Cyclosporine: Immunologic aspects in transplantation, in *Kidney Transplant Rejection: Diagnosis and Treatment*, edited by Williams GM, Burdick JF, Solez K, New York, Marcel Dekker 1986, pp. 353–382
31. van der Heide JJH, Bilo HJG, Teggzess AM, Donker AJM: The effects of dietary supplmentation with fish oil on renal function in cyclosporine-treated renal transplant recipients. *Transplant* 49: 523–527, 1990
32. Clark WF, Parbtani A, Huff MW, Reid B, Holub BJ, Falardeau P: Omega-3 fatty acid dietary supplementation in systemic lupus erythematosus. *Kidney Int* 36:653–660, 1989
33. Hamazaki T, Tateno S, Shishido H: Eicosapentaenoic acid and IgA nephropathy. *Lancet* 1:1017–1018, 1984
34. D'Amico G: The commonest glomerulonephritis in the world: IgA nephropathy. *Q J Med* 64:709–727, 1987

35. Donadio JV, Holman RT, Holub BJ, Bergstralh EJ: Effects of omega-3 polyunsaturated fatty acids (PUFA) in mesangial IgA nephropathy (abstract). *Kidney Int* 37:255, 1990
36. Giannessi D, De Caterina R, Gazzetti P, Bernini W, Masini S, Zucchelli GC: Methodological assessment and applications of a radioimmunoassay for thromboxane (TX) B_2 release by platelets. *J Nucl Med All Sci* 26:25–33, 1982
37. Jaffe BM, Berhman HR, Parker EW: Radioimmunoassay measurement of prostaglandin E, A and F in human plasma. *J Clin Invest* 52:398–405, 1973
38. Patrono C, Ciabattoni G, Remuzzi G, Gotti E, Bombardieri S, Di Munno O, Tartarelli G, Cinotti G, Simonetti B, Pierucci A: Functional significance of renal prostacyclin and thromboxane A_2 production in patients with systemic lupus erithematosus. *J Clin Invest* 76:1011–1018, 1985
39. Maroni BJ, Steinman TI, Mitch WE: A method for estimating nitrogen intake of patients with chronic renal failure. *Kidney Int* 27:58–65, 1985
40. Kasiske BL, O'Donnel MP, Cleary MP, Keane WF: Treatment of hyperlipidemia reduces glomerular injury in obese Zucker rats. *Kidney Int* 33:667–672, 1988
41. Kasiske BL, O'Donnel MP, Garvis WJ, Keane WF: Pharmacologic treatment of hyperlipidemia reduces glomerular injury in rat 5/6 nephrectomy model of chronic renal failure. *Circulation Res* 62:367–374, 1988
42. Pierucci A, Bianca MM, Simonetti D, Pecci G, Mavrikakis G, Feriozzi S, Cinotti G, Patrignani P, Ciabattoni G, Patrono C: Improvement of renal function with selective thromboxane antagonism in lupus nephritis. *N Engl J Med* 320:421–425, 1989
43. Storck JE, Dunn M: Hemodynamic roles of thromboxane A_2 and prostaglandin E_2 in glomerulonephritis. *J Pharmacol Exp Ther* 233:672–677, 1985
44. Donadio J, Anderson CF, Mitchell JC, Holley KE, Ilstrup DM, Fuster V, Chesebro JH: Membranoproliferative glomerulonephritis. A prospective clinical trial of platelet-inhibitor therapy. *N Engl J Med* 310:1421–1426, 1984
45. Singer P: n–3 fatty acids in comparison and in combination with other drugs in the management of mild hypertension, in *Fish Oil and Vascular Disease*, edited by De Caterina R, Kristensen SD, Schmidt EB, Springer-Verlag, London, Berlin, Heidelberg, 1992, pp. 111–118
46. Remuzzi G, Imberti L, Rossini M, Morelli C, Carminati C, Cattaneo GM, Bertani T: Increased glomerular thromboxane synthesis as a possible cause of proteinuria in experimental nephrosis. *J Clin Invest* 75:94–101, 1985
47. Düsing R, Stuck A, Göbel B, Weisser B, Vetter H: Effects of n-3 fatty acids on renal function and renal prostaglandin E metabolism. *Kidney Int* 38:315–319, 1990
48. Kimberly RP, Bowden R, Keiser HR, Plotz PH: Reduction of renal function by newer non-steroidal antiinflammatory drugs. *Am J Med* 64:804–810, 1978
49. Fischer S, von Schacky C, Schweer H: Prostaglandins E_3 and $F_{3\alpha}$ are excreted in human urine after ingestion of n-3 polyunsaturated fatty acids. *Biochim Biophys Acta* 963:501–508, 1988
50. Schapp HJG, Bilo C, Popp-Snijders PL, Muilder C, Donker JM: Effects of protein intake variation and omega-3 polyunsaturated fatty acids on renal function in chronic renal disease. *Life Sciences* 41:2759–2765, 1987
51. Jensen T, Stender S, Goldstein K, Hølmer G, Deckert T: Partial normalization by dietary cod liver oil of increased macrovascular albumin leakage in patients with insulin-dependent diabetes and albuminuria. *N Engl J Med* 321:1572–1577, 1989
52. Atlas SA, Niarchos AP, Case DB: Inhibitors of the renin-angiotensin system. Effects on blood pressure, aldosterone secretion and renal function. *Am J Nephrol* 3:118–127, 1983