# Fish Oil in Diabetic Nephropathy

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**OBJECTIVE** — Recent studies in nondiabetic kidney diseases suggest that dietary supplementation with n-3 polyunsaturated fatty acids (fish oil) may have beneficial effects on albuminuria, kidney function, arterial blood pressure, and dyslipidemia. Therefore, we evaluated the long-term effect of fish oil in diabetic nephropathy.

**RESEARCH DESIGN AND METHODS** — A 1-year double-blind randomized controlled study comparing fish oil (4.6 g n-3 fatty acids/day) with placebo (olive oil) was performed in an outpatient clinic in a tertiary referral center. Thirty-six normotensive IDDM patients with diabetic nephropathy were included; 18 were treated with fish oil. Seven patients dropped out (four received fish oil), and results for the remaining 29 are presented. Albuminuria (enzyme immunoassay), glomerular filtration rate ($^{51}$Cr-labeled EDTA plasma clearance), 24-h ambulatory blood pressure, and lipid profile were determined every 6 months.

**RESULTS** — Albuminuria increased by 22% (1–46%) (mean [95% CI]) in the fish oil group vs. 15% (−11–49%) in the placebo group (NS). Glomerular filtration rate decreased from 116 ± 7 to 105 ± 7 ml · min$^{-1}$ · 1.73 m$^{-2}$ (mean ± SE) vs. from 108 ± 6 to 103 ± 7, fish oil and placebo, respectively (NS). No significant changes occurred in 24-h ambulatory blood pressure: from 141 ± 4/82 ± 2 mmHg to 142 ± 5/83 ± 2 vs. from 140 ± 4/78 ± 2 to 144 ± 4/80 ± 3, fish oil and placebo, respectively (NS). In the fish oil group, serum triglycerides (median [range]) decreased from 0.97 (0.5–4.0) mmol/l to 0.8 (0.4–3.0) vs. from 1.01 (0.4–2.0) to 1.09 (0.4–2.0) in the placebo group ($P < 0.05$) and VLDL cholesterol decreased from 0.45 (0.23–1.88) to 0.37 (0.21–1.43) mmol/l vs. from 0.44 (0.21–0.94) to 0.41 (0.17–1.94)($P < 0.05$), but total and LDL cholesterol rose in the fish oil compared with the placebo group.

**CONCLUSIONS** — Our study does not suggest that fish oil has beneficial effects on albuminuria, kidney function, blood pressure, and dyslipidemia in normotensive IDDM patients suffering from diabetic nephropathy.

About 35% of all IDDM patients develop the clinical syndrome of diabetic nephropathy characterized by persistent albuminuria associated with a relentless decline in the glomerular filtration rate, raised arterial blood pressure, and varying degrees of dyslipidemia (1–4). Diabetic nephropathy is the main cause of the increased mortality and morbidity in IDDM patients (1–3,5). The excess mortality is mainly due to end-stage renal failure and cardiovascular events (1–3,6). During the last 2 decades several studies have clearly documented a beneficial effect of aggressive antihypertensive treatment with or without ACE-inhibitors on albuminuria, rate of decline in glomerular filtration rate and prognosis in hypertensive IDDM patients with diabetic nephropathy (7–13). The potential efficacy of this treatment in normotensive IDDM patients suffering from diabetic nephropathy has not been established (10,14). Various other treatment modalities have failed to influence outcome of diabetic nephropathy, including strict glycemic control (15) and lipid-lowering agents (16). The effect of a low-protein diet has been controversial (17,18). Some short- and long-term studies in different nondiabetic kidney diseases have suggested that dietary supplementation with n-3 polyunsaturated fatty acids (fish oil) has beneficial effects on albuminuria (19,20), kidney function (20,21), arterial blood pressure (19,21), and dyslipidemia (19,22).

The aim of our double-blind randomized controlled study in normotensive IDDM patients with diabetic nephropathy was to evaluate the potential beneficial effect of fish oil on albuminuria, glomerular filtration rate, systemic blood pressure, and dyslipidemia.

## RESEARCH DESIGN AND METHODS

— We performed a randomized double-blind parallel study for 1 year comparing the effect of a dietary supplementation with either 21 ml of cod-liver oil or 21 ml of olive oil. The patients were randomized after a run-in period of 4 weeks, during which 21 ml of olive oil was given. The cod-liver oil was given as an Eskisol Fish Oil emulsion (Pharma-Vinci A/S Frederiksværk, Denmark; 35% oil-water emulsion), which has a low content of A and D vitamins. The olive oil was also given in a 35% oil-water emulsion obtained from the same manufacturer. Vitamin C (3 mg/ml with acerola flavoring) and vitamin E (0.8 mg/ml) were added to both emulsions as antioxidants. The cod-liver oil emulsion provided 2.0 g of eicosapentaenoic acid and 2.6 g of docosahexaenoic acid daily. The olive oil contained no eicosapentaenoic acid or docosahexaenoic acid. The cod-liver oil contained 24.1% saturated fatty acids, 45.6% monounsaturated fatty acids, 9.4% eicosapentaenoic acid, 14.2% docosahexaenoic acid, and 6.7% other fatty acids. The olive oil contained 15.1% saturated fatty acids, 76.9% monounsaturated fatty acids, and 8.0% other fatty acids.

We examined the records of all IDDM patients with persistent albuminuria (>300 mg/day) due to diabetic nephropathy visiting the outpatient clinic at Steno Diabetes Center during 1992. Diabetic nephropathy was diagnosed clinically according to established criteria (8). All patients aged 18–55 years with arterial blood pressure <160/90 mmHg without antihypertensive medication and a glomerular filtration rate >25 ml · min$^{-1}$ · 1.73 m$^{-2}$ who had developed diabetes before the age of 40 were invited to enter the study. Forty-eight patients fulfilled

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these criteria, 4 were excluded because of diuretic treatment for edema, 1 was excluded because of a connective tissue disease, and 3 refused to participate. Of the 40 patients who accepted to participate 3 withdrew their acceptance at the first visit and 1 dropped out after 2 weeks in the placebo run-in period.

Thus, 36 patients were randomized using concealed randomization to receive either fish oil or olive oil in blocks of 4 according to their glomerular filtration rate. Seven patients dropped out during the intervention period: 4 stopped because of nausea (3 receiving fish oil), 1 became pregnant (receiving placebo), 1 developed glomerulonephritis (biopsy proven, receiving placebo), and 1 developed breast cancer (receiving fish oil). Four patients dropped out before any measurements on treatment were performed. Two patients, receiving fish oil, had measurements performed after 3 months, and 1 patient receiving fish oil completed 6 months follow-up. These three patients were included in an intention to treat analysis of the effect on albuminuria. Twenty-nine patients (14 on fish oil, 15 on placebo oil) completed the study (Table 1).

**Table 1—*Clinical characteristics of normotensive IDDM patients with diabetic nephropathy***

| | Fish oil | Olive oil |
|---|---|---|
| Sex (men/women) | 9/5 | 10/5 |
| Age (years) | 32 ± 7 | 34 ± 10 |
| Height (m) | 1.72 ± 0.09 | 1.74 ± 0.07 |
| Weight (kg) | 72.4 ± 12.8 | 70.1 ± 10.2 |
| BMI (kg/m$^2$) | 24.5 ± 3.8 | 23.2 ± 2.2 |
| Duration of diabetes (years) | 20 ± 4 | 20 ± 6 |
| Retinopathy (background/proliferative) | 7/7 | 7/8 |
| Smokers (%) | 50 | 47 |
| Insulin dose (U/kg) | 0.65 ± 0.18 | 0.66 ± 0.19 |

Data are means ± SD, *n*, or %..

All patients were insulin-dependent from the time of diagnosis and received at least 3 daily injections of human insulin. Except for one patient taking levothyroxine, none of the patients were taking other drugs. All patients were asked to reduce the overall calorie intake of their usual diabetic diet with ~800 kJ corresponding to the energy in the dietary oil supplement. The usual diet contained 45–55% carbohydrate, 30–35% fat, and 15–20% protein, without sodium or protein restriction. Retinopathy was scored after fundus photography after pupillary dilatation, as none, background, or proliferative retinopathy. Smokers were defined as subjects smoking >1 cigarette/day; all others were classified as nonsmokers. The study was approved by the local ethical committee, and the investigated patients gave fully informed consent.

Clinical investigations were carried out on one day between 0800 and 1300. Patients were studied after an overnight fast. After 30 minutes rest, a cannula was inserted into an antecubital vein in each arm. After collection of fasting plasma samples, patients had their normal breakfast and morning dose of insulin. During the investigations the patients rested supine and stood up only to pass urine. They drank 150–200 ml tap water/h during the study. The investigations were carried out at baseline and after 6 and 12 months of treatment.

To measure the glomerular filtration rate, we gave the patients a single intravenous injection of edetic acid labeled with 3.7 MBq sodium chromate-51 at 0900 and determined the radioactivity in plasma from samples of venous blood taken from the other arm 180, 200, 220, and 240 minutes after the injection (23,24). The results were standardized for 1.73 m$^2$ body surface area, using the patient's surface area at the start of the study. The mean coefficient of variation in the glomerular filtration rate from day to day in our laboratory is 4%. During the 4-h clearance period, urinary excretion of albumin, IgG, and $IgG_4$ were determined by enzyme immunoassay methods (25,26). Urine volume was corrected for residual urine determined by ultrasound at the beginning and end of the clearance period. Fractional clearances of albumin, IgG, and $IgG_4$ were obtained by dividing the clearance of the proteins (calculated as UV/P where *U* is urine protein concentration, *V* is urine flow, and *P* is plasma protein concentration) with the simultaneously measured glomerular filtration rate. Fractional clearances were calculated to adjust albuminuria for changes in plasma albumin concentration and glomerular filtration rate.

Twenty-four–hour ambulatory blood pressure was measured with the Takeda TM 2420 device, version 6 and 7 (A&D, Tokyo, Japan) the day before the clinical investigations (27). Blood pressure was measured every 15 min during the day (0700 to 2300) and every 30 min during the night (2300 to 0700). Values were averaged for each hour before calculating the 24-h average blood pressure. Twenty-four–hour blood pressure measurements were accepted if at least 50% of the programmed pressures were measured successfully for each hour during the whole 24-h monitoring interval. The coefficients of variation in the placebo group for 24-h mean systolic/diastolic blood pressure were 8/6%.

All patients collected four 24-h urine specimens at baseline and at 3, 6, 9, and 12 months for measurement of albumin, IgG, $IgG_4$, retinol-binding protein (28), sodium, phosphorous, creatinine, and urea. The urea excretion was used to calculate protein intake from the nitrogen content of the urea and an estimated value of non-urea nitrogen of 31 mg · kg$^{-1}$ · day$^{-1}$. Assuming a constant nitrogen balance, nitrogen intake equals the nitrogen intake of urinary urea plus non-urea nitrogen; protein intake (g/day) = nitrogen intake × 6.25 (29,30).

Blood glucose concentration was measured every 2 h during the clearance period and at each visit to the outpatient clinic by means of One Touch II (Lifescan, Milpitas, CA). Serum albumin, electrolytes, urate, hemoglobin, bilirubin, aspartate aminotransferase, total cholesterol, HDL cholesterol, triglyceride concentrations, and leukocyte and platelet counts were measured using conventional laboratory techniques. Serum creatinine was measured using a reaction rate kinetic technique eliminating pseudo creatinines (31). $HbA_{1c}$ was measured by high pressure liquid chromatography (HPLC) (Bio-Rad DIAMAT, Richmond, CA) (normal range 4.1–6.4%). Apolipoprotein(a), apolipoprotein(A-1) and apolipoprotein B were determined as previously described (4). The LDL cholesterol and VLDL cholesterol were calculated with use of Friedewald's formula (32). Platelet fatty acids, measuring long-term compliance as it takes ~8 weeks to obtain steady-state, were measured as previously described (33).

The patients visited the outpatient clinic after 3 and 9 months. At each visit, post-

**Table 2**—*Urinary excretion of albumin, IgG, $IgG_4$, and retinol binding protein, and glomerular filtration rate in normotensive IDDM patients with diabetic nephropathy treated with fish oil or olive oil*

| | Fish oil | | | Olive oil | | |
|---|---|---|---|---|---|---|
| | Baseline | 6 months | 12 months | Baseline | 6 months | 12 months |
| Fractional albumin clearance ($\times 10^{-6}$) | 127 ×/÷ 1.2 | 158 ×/÷ 1.3† | 169 ×/÷ 1.3 | 134 ×/÷ 1.4 | 107 ×/÷ 1.4 | 165 ×/÷ 1.5 |
| Fractional clearance of IgG ($\times 10^{-6}$) | 39 ×/÷ 1.3 | 40 ×/÷ 1.4 | 32 ×/÷ 1.6 | 35 ×/÷ 1.5 | 26 ×/÷ 1.3 | 40 ×/÷ 1.5 |
| Fractional clearance of $IG_4$ ($\times 10^{-6}$) | 53 ×/÷ 1.4 | 60 ×/÷ 1.4 | 42 ×/÷ 1.8 | 39 ×/÷ 1.4 | 38 ×/÷ 1.4 | 44 ×/÷ 1.5 |
| Selectivity index (IgG/albumin)* | 0.30 ± 0.02 | 0.28 ± 0.03 | 0.26 ± 0.03 | 0.28 ± 0.03 | 0.32 ± 0.05 | 0.24 ± 0.02 |
| Selectivity index ($IgG/IgG_4$)* | 0.81 ± 0.07 | 0.72 ± 0.07 | 0.91 ± 0.16 | 0.95 ± 0.09 | 0.84 ± 0.07 | 0.83 ± 0.07 |
| U-retinol–binding protein (μg/24 h) | 127 ×/÷ 1.4 | 104 ×/÷ 1.6 | 185 ×/÷ 1.5‡ | 138 ×/÷ 1.5 | 146 ×/÷ 1.5 | 232 ×/÷ 1.3‡ |
| Glomerular filtration rate (ml · $min^{-1}$ · 1.73 $m^{-2}$)* | 116 ± 7 | 111 ± 7‡ | 105 ± 7‡ | 108 ± 6 | 103 ± 6 | 103 ± 7 |

Data are geometric means ×/÷ antilog SE or means ± SE*. †$P < 0.05$ comparing changes in measured variables between the two groups; ‡$P < 0.05$ compared with baseline. $n = 14$ for fish oil; $n = 15$ for olive oil.

prandial blood glucose, $HbA_{1c}$, and serum creatinine concentration were measured.

### Statistical analysis

Clinical characteristics at baseline are given as means ± SD. Urinary excretion of albumin, IgG, and $IgG_4$, and the fractional clearances of these proteins were logarithmically transformed before statistical analysis because of their positively skewed distribution and are given as geometric means ×/÷ antilog SE. Plasma triglyceride, VLDL cholesterol and apolipoprotein(a) concentrations are given as median (range). All other data are given as means ± SE. All comparisons of normally or log-normally distributed parameters were done with a Student's *t* test, intergroup comparisons were done using unpaired and intragroup comparisons using paired design. Non-normally distributed parameters were compared using the Wilcoxon's rank-sum test for intragroup comparisons and the Mann-Whitney *U* test for comparisons between groups. A pre-study calculation of the required sample size was performed revealing that at least 34 patients were required to detect a 30% difference in urinary albumin excretion, our primary endpoint, between patients treated with fish oil and those treated with olive oil, assuming a coefficient of variation of 30% for urinary albumin excretion ($\alpha$ = 5% [two-sided], $\beta$ = 20%). All calculations were made with Statgraphics (STSC, Rockville, MD). $P \leq 0.05$ was considered significant (two-tailed).

**RESULTS** — The two groups were comparable regarding age, sex, duration of diabetes, retinopathy status, smoking habits, and insulin dosage, and albuminuria, arterial blood pressure, and glomerular filtration rate (Tables 1 and 2, and Fig. 1). The compliance was evaluated by determination of incorporation of n-3 fatty acids in the platelets. The proportion of eicosapentaenoic acid in platelet fatty acids increased at least by a factor 2 (mean 4) in all patients in the fish oil group from an average of 0.49 ± 0.05% at baseline to 2.19 ± 0.16 and 2.70 ± 0.29% at 6 and 12 months, respectively, compared with stable values in the placebo-treated group 0.52 ± 0.05, 0.60 ± 0.07, and 0.59 ± 0.07% at baseline and 6 and 12 months, respectively ($P < 0.001$). The proportion of docosahexaenoic acid was also increased from 1.75 ± 0.12 vs. 1.74 ± 0.12% at baseline to 3.57 ± 0.18 vs. 1.99 ± 0.13% at 12 months in the fish oil and placebo oil groups, respectively ($P < 0.001$).

Albuminuria and fractional albumin clearance were increased in both groups during the study period; however, the changes were only significant in the fish oil group after 12 months (Fig. 1 and Table 2). After the 12-month study period, albuminuria was increased (mean [95% CI]) 22% (1–46) in the fish oil group and 15 (−11–49) in the olive oil group. The change in the fish oil group was 1.05 (0.78–1.43) times the change in the olive oil group (NS, comparing changes in the two groups). Intention to treat analysis including all patients having measurements performed on treatment revealed the same result. Fractional albumin clearance was increased 33% (−10–95%) in the fish oil group and 23% (−12–71%) in the olive oil group (NS, comparing changes in the two groups). Furthermore, there were no changes within or between groups regarding 24-h urinary excretion of IgG, or $IgG_4$ (data not shown), or in fractional clearance of IgG, or $IgG_4$, or in the selectivity indexes (ratio of fractional clearances of: IgG/albumin or $IgG/IgG_4$). Urinary excretion of retinol-binding protein was significantly increased in both groups after 12 months (Table 2).

Rate of decline in glomerular filtration rate was 10.6 ± 9.7 ml · $min^{-1}$ · $year^{-1}$ in the

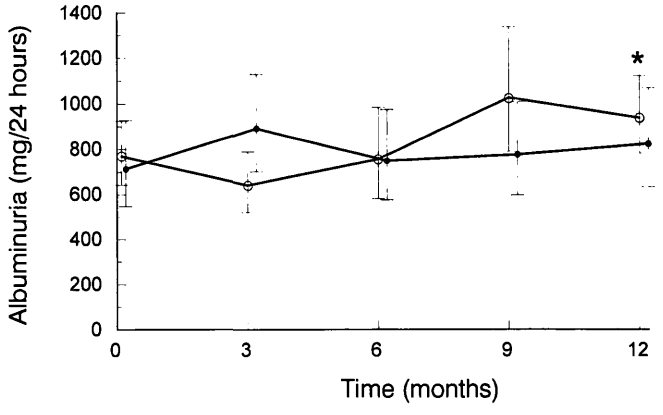


**Figure 1**—*Urinary excretion of albumin (geometric mean ×/÷ antilog SE) in normotensive IDDM patients with diabetic nephropathy treated with fish oil (○, n = 14) or olive oil (●, n = 15) (*P < 0.05 compared with baseline in the fish oil group, no difference between the two groups when changes in albuminuria were compared).*

**Table 3—*24-h ambulatory blood pressure, serum lipid concentrations, and hemoglobin $A_{1c}$ in normotensive IDDM patients with diabetic nephropathy treated with fish oil or olive oil***

| | Fish oil | | | Olive oil | | |
|---|---|---|---|---|---|---|
| | Baseline | 6 months | 12 months | Baseline | 6 months | 12 months |
| Blood pressure (mHg) | 141 ± 4/82 ± 2 | 140 ± 3/82 ± 2 | 142 ± 5/83 ± 2 | 140 ± 4/78 ± 2 | 140 ± 4/79 ± 2 | 144 ± 4/80 ± 3 |
| Triglycerides (mmol/l)* | 0.97 (0.5–4.0) | 0.67(0.4–3.1)†,‡ | 0.80(0.4–3.0)†,§ | 1.01(0.44–2.0) | 1.12 (0.5–2.0) | 1.09 (0.4–2.0) |
| Cholesterol (mmol/l) | 5.05 ± 0.2 | 5.39 ± 0.2†,§ | 5.51 ± 0.3‖ | 5.07 ± 0.3 | 5.05 ± 0.3 | 5.2 ± 0.3 |
| HDL cholesterol (mmol/l) | 1.55 ± 0.1 | 1.56 ± 0.1 | 1.55 ± 0.1 | 1.39 ± 0.1 | 1.28 ± 0.1 | 1.25 ± 0.1 |
| LDL cholesterol (mmol/l) | 2.93 ± 0.2 | 3.41 ± 0.22†,§ | 3.52 ± 0.24‖ | 3.23 ± 0.25 | 3.26 ± 0.26 | 3.40 ± 0.26 |
| VLDL cholesterol (mmol/l) | 0.45 (0.23–1.88) | 0.31 (0.20–1.43)†,‡ | 0.37 (0.21–1.43)†,§ | 0.44 (0.21–0.94) | 0.47 (0.24–0.96) | 0.41 (0.17–0.94) |
| Apolipoprotein A1 (g/l) | 1.89 ± 0.07 | 1.76 ± 0.08 | 1.67 ± 0.11 | 1.70 ± 0.12 | 1.60± 0.10 | 1.67 ± 0.09 |
| Apolipoprotein B (g/l) | 1.26 ± 0.08 | 1.26 ± 0.08 | 1.34 ± 0.09 | 1.29 ± 0.1 | 1.24 ± 0.1 | 1.31 ± 0.1 |
| Apolipoprotein (a) (U/l)* | 201 (10–867) | 182 (10–1,080) | 211 (10–1,220) | 473 (27–2,000) | 413 (28–2,060) | 404 (21–2,410) |
| $HbA_{1c}$ (%) | 8.8 ± 0.4 | 9.2± 0.4 | 8.8 ± 0.4 | 9.2± 0.3 | 9.6± 0.3 | 9.5 ± 0.2 |
| s-Creatinine (μmol/l) | 72 ± 6 | 80 ± 8† | 80 ± 7† | 75 ± 4 | 82 ± 4† | 86 ± 6‖ |

Data are means ± SE or median (range)*. †$P \leq 0.01$ compared with baseline; ‡$P = 0.001$ comparing changes in measured variables between the two groups; §$P < 0.05$ comparing changes in measured variables between the two groups; ‖$P < 0.05$ compared with baseline. $n = 14$ for fish oil, and $n = 15$ for olive oil.

fish oil group vs. 4.5 ± 9.7 in the olive oil group ($P = 0.10$); the mean (95% CI) difference in decline was 6.1 (−1.4–13.5) ml · $min^{-1}$ · $year^{-1}$. Conversely the rate of decline in creatinine clearance (24-h measurements taken at 0, 3, 6, 9, and 12 months) were 0.8 ± 8.7 and 14.7 ± 8.0 ml · $min^{-1}$ · $year^{-1}$ in the fish oil group and the olive oil group, respectively (NS). The slope of 1/serum creatinine with time was $-13.1 \pm 12 \times 10^{-4}$ and −14.7 ± 18 (l · $\mu mol^{-1}$ · $year^{-1}$ in the fish oil and olive oil groups, respectively [NS]). Serum creatinine rose significantly in both groups from 72 ± 6 vs. 75 ± 4 μmol/l at baseline to 80 ± 7 vs. 86 ± 6 after 12 months in the fish oil and olive oil groups, respectively ($P < 0.05$ compared with baseline).

There were no significant changes within or between groups in 24-h ambulatory blood pressure during the study period (Table 3). The mean (95% CI) difference in change in systolic pressure was 2 (−5–9) mmHg and in diastolic pressure 0 (−5–5) mmHg. In three patients a second measurement was performed in order to fulfill the previously stated criteria.

Plasma triglycerides and VLDL cholesterol decreased and total cholesterol and LDL cholesterol rose in the fish oil group. When the two groups were compared, the changes in plasma triglycerides and VLDL cholesterol after 6 and 12 months and in plasma total cholesterol and LDL cholesterol after 6 months were significant (Table 3). At 12 months the mean (95% CI) difference in change in triglycerides were 0.36 (0 to 0.72) mmol/l, and in cholesterol −0.34 (−0.83 to 0.15) mmol/l.

Protein intake was stable in the two groups and was on average 1.07 ± 0.10 and 1.10 ± 0.07 g · $kg^{-1}$ · $day^{-1}$ in the fish oil and olive oil groups, respectively (NS). Urinary excretion of sodium and phosphorous were stable throughout the study period (data not shown). The mean body weight was increased slightly in all patients from 71.2 ± 2.1 kg at baseline to 72.3 ± 2.1 at 12 months ($P = 0.01$), probably due to lack of compliance with the advised reduction in dietary calorie intake.

In each treatment group univariate linear regression between change in albuminuria as the dependent variable and mean values during the study period of the following variables: glomerular filtration rate, mean ambulatory blood pressure, $HbA_{1c}$, total cholesterol, triglycerides, and increase in platelet eicosapentaenoic acid as independent variables, revealed no significant associations. When a similar analysis was performed with rate of decline in glomerular filtration rate as the dependent variable, a significant correlation with plasma total cholesterol was found in both groups ($r^2 = 0.24$, $P = 0.04$) and ($r^2 = 0.31$, $P = 0.02$) in the fish oil and olive oil groups, respectively.

There were no significant changes within or between the groups in the plasma levels of sodium, potassium, phosphate, calcium ion, urea, aspartate aminotransferase, bilirubin, urate, leukocytes, or the platelet count during the study period (data not shown). Hemoglobin decreased from 8.9 ± 0.2 to 8.4 ± 0.2 mmol/l at baseline after 12 months in the fish oil group ($P < 0.01$) and from 8.8 ± 0.2 to 8.4 ± 0.2 mmol/l in the olive oil group ($P < 0.01$). Serum albumin concentrations were slightly reduced in the two groups from 522 ± 14 vs. 517 ± 14 μmol/l at baseline to 479 ± 16 vs. 484 ± 19 after 12 months in the fish oil and olive oil groups, respectively ($P < 0.01$ compared with baseline in both groups).

No serious side effects were observed, but 17% of the patients receiving fish oil emulsion dropped out because of nausea. This side effect can probably be partly avoided by the use of encapsulated fish oil. None of the patients who completed the study reported any side effects. The power of the study is given in Table 4.

**CONCLUSIONS** — Our 1-year randomized double-blind parallel study in IDDM patients with diabetic nephropathy revealed that dietary supplementation with a rather high dose of fish oil was unable to arrest the progressive rise in albuminuria, preserve kidney function, and reduce arterial blood pressure measured during ordinary 24-h life conditions. The lack of beneficial effects could not be explained by lack of compliance with the fish oil supplementation regimen since all our measurements of eicosapentaenoic acid and docosahexaenoic acid in the pool of platelet fatty acids showed increased values in all actively treated patients during the whole study period. Furthermore, the present dose of fish oil (4.6 g n-3 fatty acids/day) was chosen because previous studies reporting a beneficial effect on arterial blood pressure, kidney function, and microvascular albumin extravasation (33) frequently have used similar or smaller doses of fish oil as reviewed by Appel et al. (34) and De Caterina et al. (22). Finally it should be mentioned that the most frequently used dietary placebo supplementation in the above-

**Table 4—*Differences in change of variables during the study between groups***

| | |
|---|---|
| Urinary albumin excretion (%) | 36 |
| Glomerular filtration rate (ml · min$^{-1}$ · year$^{-1}$) | 10 |
| Blood pressure (mmHg) | |
| Systolic | 9 |
| Diastolic | 6 |

The study has 80% power to track these differences; $\alpha$ = 5% (two-sided).

mentioned trials has been oil mixture, olive oil and safflower oil (22,34).

In most studies n-3 fatty acids improve renal hemodynamics and reduce proteinuria and arterial blood pressure in renal transplant recipients treated with cyclosporine (21,35). While fish oil supplementation in animal studies of lupus nephritis models have been highly encouraging (36), disappointing results have been obtained in randomized double-blind human studies in lupus nephritis (36). Recently Donadio et al. (20) demonstrated that dietary fish oil supplementation reduced proteinuria and slowed the rate of loss of renal function assessed by serum creatinine in patients with IgA nephropathy in contrast to other studies (37,38).

Several factors (so-called progression promoters) such as systemic and glomerular hypertension, albuminuria, dietary protein intake, and hyperlipidemia contribute to the progression of diabetic and nondiabetic glomerulopathies as reviewed by Jacobson (39). None of the above-mentioned progression promoters showed any major alterations during our study. Furthermore glycemic control, which also may influence kidney function (40), remained about the same during the study.

Albuminuria is a marker of kidney disease, a progression promoter, and a predictor of treatment efficacy on the rate of decline in glomerular filtration rate in diabetic and nondiabetic kidney disease (41,42). All treatment modalities having a beneficial effect on experimental or clinical diabetic kidney disease have always been associated with a reduction in proteinuria (surrogate endpoint). Thus, reduction in albuminuria shortly after onset of a new treatment modality suggest a long-term beneficial effect on glomerular filtration rate. Unfortunately albuminuria rose with ~20% in both groups in our 1-year study. Previous short-term (<6 months) studies of fish oil in small numbers of normo-, micro-, or macroalbuminuric IDDM patients have yielded conflicting results regarding urinary albumin excretion (33,43,44). Our study suggests a transient (nonsustained) effect of fish oil on albuminuria (Fig. 1) during the first 3 months, as previously reported in some other renal diseases.

A limitation of our study is that the number of patients completing the 12-month follow-up was less than determined in our pre-study calculation, but it is possible, with 95% confidence, to exclude a decrease in albuminuria of >22%, or an increase of >43%, in the fish oil group compared with the placebo group.

We found a tendency toward an accelerated rate of decline in glomerular filtration rate in the fish oil group. A reduction in glomerular filtration rate and increased proteinuria has been reported during fish oil treatment in remnant kidney models of renal disease (45), probably due to a reduction in prostaglandin $E_2$. Interestingly the changes in creatinine clearances and slope of 1/s creatinine were in favor of the fish oil or neutral, suggesting an effect on the renal creatinine handling. This could be of importance for the interpretation of studies using creatinine clearance when evaluating the effect of fish oil on renal disease (20). Some previous studies of fish oil in IDDM patients have demonstrated a reduction of office arterial blood pressure in hypertensive and normotensive patients (33), while other studies have failed to demonstrate this beneficial effect (43,46). By applying a more accurate and precise technique for blood pressure monitoring, we found no effect of n-3 fatty acids on 24-h blood pressure taken during ordinary life conditions in normotensive IDDM patients with diabetic nephropathy.

A recent meta-analysis documented that the antihypertensive effect of n-3 fatty acids was only present in hypertensive and not in normotensive nondiabetic individuals (34). Since the planning and onset of our study, the definition of arterial hypertension has changed, and blood pressure levels between 140–159/90–99 mmHg are now regarded as stage 1 hypertension according to the Joint National Committee on Detection, Evaluation and Treatment of High Blood Pressure (JNC V) (47). These criteria have recently been adopted by the American Diabetes Association (48). The prevalence of stage 1 hypertension was 36% in our patients at entry, solely because of elevated systolic blood pressure.

The reductions in plasma triglyceride and VLDL cholesterol observed in the fish oil group are in accordance with most other studies (22). Although it is difficult to exclude, we find it unlikely that a beneficial effect of the n-3 fatty acids was mitigated by other constituents of the fish oil preparation, as beneficial effects were observed with almost the same oil, except with a higher content of vitamins A and D, in a previous short term study (33). The length of the observation period may be of importance, as the previous study of 8 weeks duration with the similar fish oil preparation (33) resulted in an improved lipid profile with an increase in HDL cholesterol and unchanged LDL cholesterol compared to our findings of unchanged HDL cholesterol and increasing total cholesterol and LDL cholesterol.

In conclusion, our study does not suggest that fish oil has beneficial effects on albuminuria, kidney function, blood pressure, and dyslipidemia in normotensive IDDM patients suffering from diabetic nephropathy.

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