The effect of n–3 long-chain polyunsaturated fatty acid supplementation on urine protein excretion and kidney function: meta-analysis of clinical trials1–3

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ABSTRACT

Background: Chronic kidney disease is a major worldwide problem. Although epidemiologic and experimental studies suggest that n–3 long-chain polyunsaturated fatty acid (n–3 LCPUFA) supplementation may prevent or slow the progression of kidney disease, evidence from clinical trials is inconsistent.

Objective: The objective was to combine evidence from controlled clinical trials to assess the effect of n–3 LCPUFA supplementation on the change in urine protein excretion (UPE) and on glomerular filtration rate (GFR).

Design: We performed a meta-analysis of clinical trials that tested the effect of n–3 LCPUFA supplementation on UPE, a marker of kidney damage, and on GFR, a marker of kidney function. A random-effects model was used to pool SD effect size (Cohen’s d) across studies.

Results: Seventeen trials with 626 participants were included in the meta-analysis. Most trials focused on patients with a single underlying diagnosis: IgA nephropathy (n = 5), diabetes (n = 7), or lupus nephritis (n = 1). The dose of n–3 LCPUFAs ranged from 0.7 to 5.1 g/d, and the median follow-up was 9 mo. In the pooled analysis, there was a greater reduction in UPE in the n–3 LCPUFA group than in the control group: Cohen’s d for all trials was 0.19 (95% CI: 0.64, 0.03; P = 0.01). In a patient with 1 g UPE/d, this corresponds to a reduction of 190 mg/d. Effects on GFR were reported in 12 trials. The decline in GFR was slower in the n–3 LCPUFA group than in the control group, but this effect was not significant (0.11; 95% CI: 0.07, 0.29; P = 0.24).

Conclusions: In our meta-analysis, use of n–3 LCPUFA supplements reduced UPE but not the decline in GFR. However, small numbers of participants in trials, different methods of assessing proteinuria and GFR, and inconsistent data reporting limit the strength of these conclusions. Large, high-quality trials with clinical outcomes are warranted. Am J Clin Nutr 2009;89:1937–45.

INTRODUCTION

Chronic kidney disease (CKD) is increasing in prevalence in the United States. The most common causes of CKD are diabetes and hypertension. However, epidemiologic and clinical studies examining nutrient-based risk factors suggest that the dietary intake of fatty acids may prevent CKD and slow its progression (1). Because the intake of essential fatty acids is low in the US diet, many trials have been conducted to determine the effects of dietary supplementation with long-chain fatty polyunsaturated fatty acids (n–3 LCPUFAs), also known as omega-3 fatty acids, including docosahexaenoic acid (DHA, 22:6n–3) and eicosapentaenoic acid (EPA, 20:5n–3) on CKD progression.

In animal models, dietary supplementation with n–3 LCPUFAs at low doses reduces the severity of kidney disease after exposure to nephrotoxic agents (2) and slows the progression of kidney disease (3). In human studies, n–3 LCPUFA supplementation has been shown to modify intermediaries in the pathogenesis of kidney disease, ie, lowering blood pressure, oxidative stress, and inflammation and improving endothelial function (4). In cross-sectional and longitudinal observational studies in humans, n–3 LCPUFA intake was associated with a lower risk of macroalbuminuria in adults with diabetes (5) and with a lower age-related decline in kidney function (6).

Randomized trials of n–3 LCPUFA supplementation on the progression of kidney disease have been conducted, but the results have been inconsistent (7). Potential reasons for the inconsistencies include differences in the pathology of underlying kidney disease or in the prevalence of risk factors for disease progression, differences in outcome assessment, small sample sizes, and limitations in study quality (eg, noncompliance or use of concurrent medications).

The objective of this meta-analysis was to combine evidence from controlled clinical trials to assess the effect of n–3 LCPUFA supplementation on change in urine protein excretion, a well-established clinical marker of kidney damage that is a common outcome in most trials, and on glomerular filtration rate (GFR), a marker of kidney function.

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METHODS

We searched the published literature for clinical trials that tested the effects of n–3 LCPUFA supplementation on urine protein excretion. We performed a Medline search using the medical subject headings *fish oil*, *omega 3 fatty acids*, *kidney (renal) disease*, and *clinical trials*. Details of the search terms are provided in Appendix A. The search period was from 1966 to October 2008. The Medline search included a search of the Cochrane database of randomized controlled trials, review of reference lists from original research, and a search of review articles.

Our prespecified inclusion criteria were as follows: 1) controlled clinical trials; 2) use of n–3 LCPUFAs, including eicosapentaenoic acid (EPA) and/or docosahexaenoic acid (DHA) or fish oils as the active treatment; 3) presence of a control or placebo group; and 4) reported effects on urine protein (or albumin) excretion. We excluded trials of patients who had undergone organ transplant or had end-stage kidney disease.

Three investigators (ERM, MM, and SPJ) independently abstracted the articles. Disagreements or uncertainties were adjudicated by consensus. From each article, we abstracted 1) characteristics of the study population, including sample size, country, age, sex, and targeted kidney disease condition; 2) characteristics of the trial design, including dose and duration of n–3 LCPUFA supplementation; 3) measurements of urine protein excretion; and 4) measured GFR or estimated GFR (eGFR) from creatinine-based estimating equations or 24-h urine creatinine clearance. The sum of the amount of EPA and DHA within intervention capsules was used to reflect the daily supplemental dose of n–3 LCPUFAs (mg/d) in each trial.

The method for evaluating urine protein excretion and GFR varied across studies. As a result, we calculated effect sizes as standardized measures of change (SD units) that are independent of the measurement unit used in individual trials. Effect sizes (Cohen’s d) were computed by dividing the difference between mean change in the intervention and the mean change in the control group by their pooled SDs (8). Effect sizes are interpreted as the relative effect of n–3 LCPUFA supplementation on change in urine protein excretion compared with the change in the control group. Pooled estimates and 95% CIs were calculated by using a random-effects meta-analysis model using STATA 9.2 (STATA Corp, College Station, TX).

In each study, we used intention-to-treat analyses, if available; otherwise we used data reported on trial participants that returned for follow-up. For trials with a crossover design, change in protein excretion was determined between baseline and end of first period. In a sensitivity analysis, randomized controlled double-blind studies (high-quality trials) were analyzed separately from unblinded trials (eg, the control group did not receive capsules). Retention rates are reported as the percentage of participants within each trial who came in for end-of study measurements. We report participant retention as the percentage of participants for whom end-of study measurements were made.

RESULTS

The trial selection process that resulted from our systematic literature review is shown in Figure 1. Seventeen clinical trials met our inclusion criteria. These trials, reported between 1986 and 2007, included a total of 626 participants (Table 1). The mean age in the trials ranged from 33 to 64 y. Ten trials were double-blinded randomized controlled trials, whereas the remainder did not use a placebo capsule, used concurrent controls (not randomized), or a blinded assessment of outcomes was not reported. Follow-up ranged from 6 wk to 48 mo (median: 9 mo). Most trials focused on patients with a single underlying diagnoses: IgA nephropathy (5 trials), diabetes (7 trials), and lupus nephritis (1 trial). Three trials enrolled patients with kidney disease of mixed etiologies, and one trial did not report the underlying kidney disease of the study participants. The dose of n–3 LCPUFAs (EPA and/or DHA) ranged from 0.7 to 5.1 g/d. The eGFR ranged from 24 to 116 mL · min⁻¹ · 1.73 m⁻² across studies (median: 73 mL · min⁻¹ · 1.73 m⁻²).

FIGURE 1. Flow diagram of trial selection process resulting from systematic search. ESRD, end-stage renal disease; LCPUFA, long-chain polyunsaturated fatty acids.

References identified
N = 242
Medline: 178
Cochrane Central: 52
Bibliographies: 12

Clinical trials retrieved for more detailed evaluation
N = 32

Excluded: N = 210
Not relevant
Duplicates
Review articles
No n-3 LCPUFA supplementations
No measure of renal outcomes
Trials involving ESRD
Trials involving renal transplant

Excluded N = 15
Single arm studies: 8
Urine protein excretion not reported: 4
Study compared two doses of “fish oil” (no placebo): 1
Same study reported twice: 1
No use of n-3 LCPUFA (used flax seed): 1

Included trials
N = 17

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Retention rates within trials were high (median: 97% of participants had end-of-study measurements). Five of 17 trials reported side effects from supplementation. Three of these trials did not quantify the proportion of patients experiencing these symptoms [e.g., “fishy aftertaste” in some patients (25), “occasional smell of fish on belching” (20), “eructation with fish taste” (27)]. One trial reported gastrointestinal side effects in 11% of the intervention group (28), and one trial reported that 17% of those assigned to fish-oil supplements had nausea at some point in the trial (22). There was no evidence of a differential dropout rate between the n–3 LCPUFA and control groups within trials.

The outcome variable of urine protein excretion was reported differently in trials (Table 2): protein in g/d (5 studies), albumin in g/L (3 studies), albumin as a percentage of excretion per hour, albumin as mg/d, protein as g/creatinine clearance, protein as g/L, albumin as mg/L, and albumin as mg/creatinine mmol (one study each).

The method for assessing GFR varied across trials (Table 3). Several studies measured GFR using one of several “gold standard” radioisotope determinations of kidney function (10, 19, 21, 26). Other studies used either 24-h creatinine clearance rates (15, 16, 23, 25) or creatinine-based estimating...
Effects of n–3 LCPUFA supplementation on urine protein excretion was greater in the n–3 LCPUFA supplementation group than in the control group in 13 of 17 trials. Only 5 trials (17, 18, 21, 27, 28), however, reported significant reductions in urine protein excretion. In the pooled analysis, n–3 LCPUFA supplementation significantly reduced urine protein excretion compared with the control. The pooled effect size (Cohen’s d) for all trials was −0.19 (95% CI: −0.34, −0.04; P = 0.01). The test for heterogeneity was nonsignificant (P = 0.84). The effect sizes (Cohen’s d) in studies of patients with IgA nephropathy (n = 5), diabetes (n = 7), and other conditions (n = 5) were −0.05 (95% CI: −0.31, 0.21; P = 0.71), −0.21 (95% CI: −0.46, 0.04; P = 0.10),
Effects of n–3 LCPUFA supplements on GFR or eGFR were reported in 12 of 17 trials (Table 3). The GFR decline was slower in the n–3 LCPUFA supplementation group than in the control group in 9 of 12 trials. Only 2 trials (24, 26), however, reported significant protective effects of n–3 LCPUFA supplementation in preventing GFR decline. Nonetheless, the pooled effect size and −0.33 (95% CI: −0.60, −0.06; \( p = 0.02 \)), respectively (Figure 2). In meta-regression analyses, trial characteristics including trial duration, n–3 LCPUFA dose, use of olive oil as placebo, randomized controlled clinical trial compared with other trial designs, and type of underlying kidney disease were not associated with the efficacy of n–3 LCPUFA supplements (data not shown).
(Cohen’s d) was not significant (0.11; 95% CI: -0.07, 0.29; \( P = 0.24 \)) (Figure 3). The test for heterogeneity was non-significant (\( P = 0.98 \)). In a meta-regression analysis, the change in urine protein excretion was not associated with change in GFR (-0.03; 95% CI: -0.98, 0.28; \( P = 0.24 \)).

**DISCUSSION**

In pooled analyses of clinical trials of patients with kidney disease, n-3 LCPUFA supplementation significantly reduced urine protein excretion but had no significant effect on decline in GFR. Whereas most individual trials (13 of 17) showed greater reductions in urine protein excretion in the n-3 LCPUFA supplementation groups than in the control groups, only 5 of 17 trials reported significant reductions. This likely reflects the fact that most trials in this meta-analysis were underpowered to examine the effects of n-3 LCPUFA supplementation on urine protein excretion. Whereas pooling results from these small trials increased our ability to estimate an overall effect, precise estimates of the effect of n-3 LCPUFA supplementation on urine protein excretion require adequately powered high-quality trials. Future studies should determine whether n-3 LCPUFAs reduce kidney damage by studying effects on other biomarkers of kidney injury as well as effects on clinically relevant endpoints.

n-3 LCPUFAs may reduce urine protein excretion through effects on both renal disease activity and glomerular hemodynamics. Previous meta-analyses examining the effects of n-3 LCPUFA supplements on blood pressure reported that supplementation with a relatively high dose of n-3 LCPUFAs, generally >3 g/d, results in clinically relevant, albeit modest, reductions in blood pressure (8, 9). Lower blood pressure may reduce renal perfusion and reduce GFR; lower urine protein excretion may simply parallel these renal hemodynamic effects. The trial by Clark et al (11) provides indirect evidence of a transient, potentially hemodynamic effect of n-3 LCPUFA supplements that may account for the observed reduced protein excretion in patients with lupus nephritis. In this trial, n-3 LCPUFA supplementation (4.4 g/d) reduced urine protein excretion by 37% at 1 y, but the effect disappeared after a 10-wk washout period. However, because the effect of n-3 LCPUFA supplementation on blood pressure is modest even at high doses (9), it is unlikely that this effect will completely explain the effects of n-3 LCPUFAs on urine protein excretion.

Changes in urine protein excretion do not appear to simply reflect the effects of n-3 LCPUFAs on GFR. In all studies, GFR declined in both the intervention and control arms of each study. The rate of decline was slower in the n-3 LCPUFA supplementation group, but this effect was not statistically significant. Hence, the observed significant effects of n-3 LCPUFA supplementation on urine protein excretion are not likely the result of renal hemodynamic effects (blood pressure or renal perfusion effects), because we would have expected parallel changes in GFR. Alternatively, n-3 LCPUFAs may have beneficial effects on
intermediaries in the pathophysiology of kidney disease, such as inflammation or reduced oxidative stress (4). Zeman et al (12) reported that n–3 LCPUFA supplementation lowered urine protein excretion while lowering circulating conjugated dienes—an in vivo marker of oxidative stress. In a mouse model of type 2 diabetes and obesity (KKA/T), n–3 LCPUFA supplementation reduced urine albumin excretion and inhibited the up-regulation of the inflammatory marker monocyte chemoattractant protein (MCP-1) expression without having measurable effects on blood pressure or GFR, results that suggest benefits beyond hemodynamic mechanisms (3).

Variability in the effects of n–3 LCPUFA supplementation on urine protein excretion rates may be due to differences in underlying etiologies of kidney disease in the patient populations. Five trials were conducted in patients with IgA nephropathy, which is often a very slowly progressive disease with alternating periods of activity and quiescence. In all 5 of the trials in patients with IgA nephropathy and in the lupus nephritis trial, the placebo groups had significant reductions in urine protein excretion from baseline, which suggests that participants were randomly assigned during a period of high disease activity. In the other trials that enrolled participants with diabetic kidney or hypertensive nephrosclerosis, a reversal of the underlying pathology responsible for progression is less likely. Whereas this may be an argument to pool results of trials that include participants with only a single underlying disease diagnosis, our results showed a similar magnitude of effect of n–3 LCPUFA supplementation on urine protein excretion across the trials pooled by diagnoses (IgA nephropathy, diabetic nephropathy, and mixed etiologies). Future trials will need to clearly define the patient population because findings in kidney diseases with a specific pathology may not be generalizable to other causes of urine protein excretion.

Whereas our meta-analysis offers insight into the possibility of a beneficial effect of n–3 LCPUFA supplements on urine protein excretion, a well-accepted marker of kidney damage and strong predictor of kidney disease progression, the included studies were small and many had methodologic limitations. In addition, these trials typically used very high doses (up to 5.1 g/d) and did not consistently collect measurements of physiologic explanatory variables, including GFR, blood pressure, or measurements of inflammation or oxidation.

As an additional limitation, our results were based on urine protein (albumin) excretion, a surrogate endpoint. There is interest and controversy associated with the use of urine protein excretion as a surrogate marker in kidney disease (13). There are many examples in which therapies that lower urine protein excretion are thought to be renoprotective (eg, angiotensin-converting enzyme inhibitors) and provide evidence of concordance between reduced proteinuria and a lower risk of kidney disease progression. However, there are counterexamples with discordant results. For instance, nonsteroidal antiinflammatory drugs acutely decrease proteinuria but increase the risk of kidney injury, whereas dietary protein restriction or lower therapeutic blood pressures lower urine protein excretion but have not been linked to a slower progression of kidney disease (14). Many trials included in this meta-analysis did not report effects
on renal glomerular function, and the correlation between changes in urine protein excretion and eGFR in those trials that did report or in which GFR could be estimated did not show a consistent pattern.

In summary, our meta-analysis suggests that n–3 LCPUFA supplementation lowers urine protein excretion. However, because many of the trials included in this meta-analysis were small, future studies designed to examine the potential benefits of n–3 LCPUFA therapy must improve on the quality of prior trials in several ways. First, trials should be adequately powered and use high-quality measures to estimate changes in urine protein excretion. Second, the trials should include measurements of explanatory variables in the pathophysiology of the underlying disease, including effects on GFR, blood pressure, markers of oxidation and inflammation, and urine protein fingerprinting, which may provide insight into whether glomerular or tubulointerstitial injury has been modified. Finally, the efficacy of n–3 LCPUFA supplements in population groups with different underlying causes of kidney disease needs to be determined.

The authors’ responsibilities were as follows—ERM and EG: concept and design of the study, critical revision of the article for important intellectual content, and draft of the manuscript; ERM, SPJ, JB, CAMA, LJA, and EG: analysis and interpretation of the data; EG and JB: statistical expertise; and ERM, MM, and SPJ: collection and assembly of the data. All authors were responsible for final approval of the manuscript. None of the authors declared a conflict of interest.

REFERENCES


APPENDIX A

MESH terms used to search for trials within MEDLINE (PUBMED):
