The Effects of n-3 Long-Chain Polyunsaturated Fatty Acid Supplementation on Biomarkers of Kidney Injury in Adults With Diabetes

Results of the GO-FISH trial

**OBJECTIVE**—Long-chain n-3 polyunsaturated fatty acid (n-3 PUFA) supplements may have renoprotective effects in patients with diabetes, but previous trials have been inconsistent. We performed a randomized controlled trial of n-3 PUFA supplementation on urine albumin excretion and markers of kidney injury in adults with type 2 diabetes.

**RESEARCH DESIGN AND METHODS**—We conducted a randomized, placebo-controlled, two-period crossover trial to test the effects of 4 g/day of n-3 PUFA supplementation on markers of glomerular filtration and kidney injury in adults with adult-onset diabetes and greater than or equal to trace amounts of proteinuria. Each period lasted 6 weeks and was separated by a 2-week washout. The main outcome was urine albumin excretion and, secondarily, markers of kidney injury (kidney injury molecule-1, N-acetyl β-D-glucosaminidase [NAG], neutrophil gelatinase-associated lipocalin [NGAL], and liver fatty acid–binding protein [LFABP]), serum markers of kidney function (cystatin C, β2-microglobulin, and creatinine), and estimated glomerular filtration rate (eGFR).

**RESULTS**—Of the 31 participants, 29 finished both periods. A total of 55% were male, and 61% were African American; mean age was 67 years. At baseline, mean BMI was 31.6 kg/m², median eGFR was 76.9 mL/min/1.73 m², and median 24-h urine albumin excretion was 161 mg/day. Compared with placebo, n-3 PUFA had nonsignificant effects on urine albumin excretion (−7.2%; 95% CI −20.6 to 8.5; P = 0.35) and significant effects on urine NGAL excretion (−16% [−29.1 to −0.5%]; P = 0.04). There was no effect on serum markers of kidney disease or eGFR. In subgroup analyses, there were significant decreases in 24-h urinary excretion of albumin, NGAL, LFABP, and NAG among participants taking medications that block the renin-angiotensin-aldosterone system (RAAS).

**CONCLUSIONS**—These results suggest a potential effect of n-3 PUFA supplementation on markers of kidney injury in patients with diabetes and early evidence of kidney disease. In the context of prior studies, these results provide a strong rationale for long-term trials of n-3 PUFA on chronic kidney disease progression.

Diabetes is a leading cause of chronic kidney disease (CKD) (1). Treatments to slow the progression of CKD in diabetes include blocking the renin-angiotensin-aldosterone system (RAAS), implementing lower blood pressure (BP) treatment goals, and treating hyperglycemia (2). These therapies can also reduce urine protein excretion, a marker of disease severity. Indeed, maximal reduction of urine protein excretion has been proposed as a goal of drug therapy (3). Long-chain n-3 polyunsaturated fatty acid (n-3 PUFA) supplements may improve endothelial function, lower BP, and have independent antiproteinuric effects (4). However, evidence of benefit from supplementation with n-3 PUFA on urine protein excretion in the setting of diabetic kidney disease is inconsistent (5–11).

New markers of kidney function and injury hold considerable promise as a means to evaluate the potential benefits of therapies designed to retard the progression of CKD. Biomarkers of tubulointerstitial kidney damage, including kidney injury molecule-1 (KIM-1), N-acetyl β-D-glucosaminidase (NAG), neutrophil gelatinase-associated lipocalin (NGAL), and liver fatty acid–binding protein (LFABP) may have greater sensitivity for identifying effects on kidney injury than total urine protein or albumin excretion, which reflect both kidney injury and hemodynamic effects (12,13). Novel serum markers, including β2-microglobulin and cystatin C, may provide greater sensitivity for determining short-term effects of therapies on estimated glomerular filtration rate (eGFR) than traditional markers of filtration. These new markers of kidney function and injury might be especially useful in guiding the design of subsequent long-term trials.

In this context, we conducted a randomized, controlled crossover trial to evaluate the efficacy of n-3 PUFA supplements on improving markers of kidney injury and function in adults with adult-onset diabetes and greater than or equal to trace amounts of proteinuria.

**RESEARCH DESIGN AND METHODS**

**Study design and study population**

This study was a single-center, randomized, controlled two-period crossover...
trial of n-3 PUFA supplements versus placebo (corn oil) supplements. Each period lasted 6 weeks. Participants were recruited at the Johns Hopkins ProHealth clinic, a community-based research clinic in Baltimore, MD. The primary recruitment process was mass mailings of invitations to persons with self-reported type 2 diabetes. Study brochures were mailed to zip codes near Johns Hopkins ProHealth. Participants underwent two screening visits to determine eligibility prior to enrollment.

Inclusion criteria were a self-reported diagnosis of diabetes, either treated with oral medication(s) or diet-controlled; age >21 years; an average systolic BP (SBP) <150 mmHg and diastolic BP (DBP) <90 mmHg during two screening visits; and a quantified level of proteinuria greater than or equal to trace but <4+ on urine dipstick analysis during screening. If participants were taking antihypertensive, hypoglycemic, or lipid-lowering medications, we required stable doses for a minimum of 2 months prior to randomization. Exclusion criteria were poorly controlled diabetes (self-reported hemoglobin A1c [HbA1c] >9%); use of insulin; being pregnant or planning on becoming pregnant during the study period; unwillingness to stop taking fish oil supplements 1 month prior to randomization for those on fish oil supplements; or an eGFR <30 mL/min (stage 4 or stage 5 CKD) based on the Modification of Diet in Renal Disease equation (14). The Johns Hopkins Institutional Review Boards approved the study protocol. All participants provided written informed consent.

Randomization and interventions
If participants were eligible based on their screening visits, they were asked to complete a 2–4-week run-in period during which they took four corn oil capsules per day. After run-in, participants who remained interested and eligible were randomized. Randomized assignments were generated from a random-numbers table, placed in sealed opaque envelopes, and opened by the study coordinator in consecutive order. An 8-week supply of capsules was provided to participants in preallocated monthly pill packs. Participants, all study staff, and analytic team members were masked to the randomization sequence.

Participants took four capsules per day during the intervention period. Each 1-g capsule of n-3 PUFA contained ~85% n-3 fatty acid ethyl esters sourced from fish oils, a combination of ethyl esters of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in an ~2:1 ratio. A 2-week washout separated the two periods. The placebo capsules were identical in appearance to the n-3 PUFA capsules and contained an isocaloric equivalent of corn oil. Pharmacokinetic studies suggested that a 6-week period would be adequate for serum levels of n-3 PUFA to reach steady state and that a 4-week period would provide sufficient time for serum levels to return to baseline levels following withdrawal (15,16). Participants were asked not to alter medication regimens of drugs that affect BP, diabetes, or lipids.

Data collection
Study participants completed visits at baseline and at the end of each treatment period. Weight, height, and waist circumference were measured. BP was measured by the oscillometric technique using an OMRON 907 machine (Omrorn, Vernon Hills, IL). A set of three readings (separated by 30 s) after 5 min of rest was performed following a standardized protocol.

Study participants provided fasting blood samples and 24-h urine collections at each of the three clinic visits. Blood samples were allowed to clot at room temperature for 15 min and centrifuged at 2°C. Serum aliquots were stored at −70°C. Aliquots of 24-h urine collections were collected and stored at −70°C. Fasting serum levels of total cholesterol, HDL cholesterol, triglycerides, fasting serum glucose, and liver function tests were measured by a local laboratory (Quest Diagnostics). LDL cholesterol was estimated using the Friedewald equation (17). Erythrocyte fatty acids including DHA and EPA were isolated by solid-phase extraction, identified, and quantitated by gas liquid chromatography–mass spectrometry in the Peroxisome Diseases section of the Kennedy Krieger Institute using standard methodologies (18).

Study outcomes
The primary outcome variable was 24-h urine albumin excretion. Secondary outcome variables were eGFR, cystatin C, and β2-microglobulin measured in fasting serum and NAG, LFABP, NGAL, and KIM-1 measured in 24-h urine samples. All outcome variables were measured at baseline and the end of each treatment period. Laboratory assays of outcome measures were performed at the Cincinnati Children's Hospital Medical Center by personnel who were masked to participant randomized sequence. Urine albumin was measured by immunoturbidimetry using Dimension Xpand Plus clinical chemistry system (Siemens). Serum cystatin C and β2-microglobulin were measured by particle-enhanced immunonephelometric assays (Dade Behring, Deerfield, IL). Urine NAG activity was measured using a colorimetric assay (Roche Diagnostics) as previously described (19). Urine NGAL was assayed using a human-specific commercially available ELISA (AntibodyShop, Grusbaken, Denmark), and LFABP was measured using commercially available ELISA kits (CMIC Co., Tokyo, Japan) per the manufacturer's instructions. The urine KIM-1 ELISA was constructed using commercially available reagents (R&D Systems, Minneapolis, MN). eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration equation (14).

Statistical considerations
Sample size for this study was based on estimates of the difference in change in 24-h urine albumin excretion (the primary outcome variable) and in eGFR. For urine protein excretion, we anticipated that a sample size of 30 participants would result in 80% power to detect a difference between the n-3 PUFA and placebo periods of 10% at an α of 0.05.

The primary statistical analysis was the comparison of the difference in change in markers of kidney injury between the n-3 PUFA supplementation period and placebo period. The primary variable was the mean change in outcome variables from baseline using repeated-measures analysis (i.e., generalized estimating equations with an exchangeable correlation structure). The distributions of data were checked for normality and transformed where appropriate. Other outcome variables were analyzed with a similar approach. Carryover effects of the n-3 PUFA supplement on the erythrocyte membrane fatty acids were evaluated using treatment by assignment-order interaction terms.

We used interaction terms to evaluate effect modification by prerandomization medication use, namely, diuretics, statins, glucose-lowering medications, and medications that block the RAAS (e.g., ACE inhibitors and angiotensin receptor blockers [ARBs]). Statistical significance was P < 0.05. All analyses were performed.
RESULTS

Study participants
Of 132 prescreened participants, we randomized 31 participants (Fig. 1). A lack of detectable urine dipstick proteinuria at screening was the most common reason for participants being ineligible. One randomized participant withdrew before data collection, and one completed only one period. Baseline characteristics are presented in Table 1, after excluding the participant who withdrew prior to data collection. Mean (SD) age of participants was 67.4 (11.5) years, 55% were male, and 61% were African American. Mean (SD) BMI was 31.6 (5.3) kg/m². At baseline, 68% of participants had microalbuminuria (30–300 mg/day), and 26% had macroalbuminuria (>300 mg/day). Most participants were taking lipid-lowering therapy (65%), RAAS inhibitors (68%), and/or hypoglycemic medications (81%).

Urine markers of kidney injury
Baseline values and changes in urine markers of kidney injury are reported in Table 2. Compared with the placebo period, n-3 PUFA had no significant effects on the 24-h urine excretion of albumin but significantly lowered NGAL (−16%; 95% CI −29.1 to −0.5%; P = 0.04). Although n-3 PUFA did not significantly lower other markers of kidney injury, there was a consistent trend of reduction during the n-3 PUFA supplementation period (Fig. 2).

In stratified analysis by use of chronic stable doses of diuretics, statins, oral hypoglycemic medications, and RAAS-inhibitor medications, interaction terms used to evaluate the effects of RAAS and n-3 PUFA supplementation use were significant for NGAL (P = 0.01), NAG (P < 0.01), and microalbuminuria (P = 0.04), borderline significant for LFABP (P = 0.06), and nonsignificant for KIM-1 (P = 0.35) (Table 3). Within the stratum of RAAS-inhibitor users, there were significant reductions in all of the urinary kidney injury markers except KIM-1. In contrast, among those not taking RAAS inhibitors, we observed nonsignificant increases in most markers and a significant increase in NAG (1.32 units/day; 95% CI 1.02–1.70). The other medication classes did not demonstrate a similar consistent pattern of effect modification (Fig. 3).

Serum markers of kidney function
At baseline, the mean (SD) eGFR, serum cystatin C, and β₂-microglobulin levels were 76.9 (22.3) mL/min/1.73 m², 0.95 (0.34) mg/L, and 2.33 (0.95) mg/L, respectively. eGFR was highly correlated with serum β₂-microglobulin (r = −0.69) and cystatin C (r = −0.76). n-3 PUFA had nonsignificant effects on serum markers of kidney function and eGFR. Specifically, the difference in eGFR comparing n-3 PUFA supplementation to control was −0.9 mL/min/1.73 m² (95% CI −3.1 to 1.4; P = 0.45). The differences in β₂-microglobulin and cystatin C were −0.01 mg/L (−0.12 to 0.09; P = 0.79) and 0.04 mg/L (−0.10 to 0.18; P = 0.55), respectively.

Other outcomes
Baseline change and treatment-arm comparisons of other outcomes are shown in Table 4. Compared with placebo, n-3 PUFA supplementation was associated with a nonsignificant change in SBP (−3.4 mmHg; 95% CI −7.7 to −1.0; P = 0.13) and DBP (−1.5 mmHg [−3.8 to 0.8]; P = 0.19) and significant reduction in triglycerides (−24.8 mg/dL [−43.7 to −5.8]; P = 0.01). In contrast, when compared with placebo, n-3 PUFA supplementation was associated with increases in serum glucose (mean difference 6.5 mg/dL [0.6–12.3]; P = 0.03). There was also a significant increase in alanine aminotransaminase of 2.6 units/L (0.4–4.8; P = 0.02).

Compliance and safety
Analyses of erythrocyte membrane fatty acids (Table 4) revealed significant increases in EPA, DHA, and total n-3 fatty acids during the n-3 PUFA intervention period compared with placebo, suggesting...
Figure 2—Mean percent difference in 24-h urinary excretion of KIM-1, NGAL, LFABP, NAG, and albumin (ALB) comparing the n-3 PUFA to the control period. (A high-quality color representation of this figure is available in the online issue.)
n-3 PUFA supplementation may also alter the balance of n-3 and n-6 fatty acids available for prostaglandin production, causing a favorable shift toward production of prostaglandins with more vasodilator effects, enhanced immune function, and reduced inflammation (16,23), mechanisms that may contribute to CKD progression in diabetic kidney disease. n-3 PUFA supplementation may also decrease renal ischemic effects through rheostatic changes on erythrocyte membranes. Stirban et al. (21) noted improved postprandial microvascular reactive hyperemia after ischemia in adults with non–insulin-dependent diabetes with 2 g/day of n-3 PUFA. This mechanism was believed to be largely independent of nitric oxide–mediated regulations of vascular tone and more related to the influence of n-3 PUFA on prostaglandin pathways.

In our trial, n-3 PUFA had nonsignificant effects on urine albumin excretion (−7.2%; 95% CI −20.6 to 8.5; P = 0.35). The nonsignificant overall effects of n-3 PUFA supplementation on the primary outcome variable of urine albumin excretion suggest a lack of benefit or reflect a trial is underpowered. Nonetheless, in subgroup analyses, n-3 PUFA significantly reduced urine albumin excretion only in participants on RAAS-inhibition therapy, representing 70% of participants in this trial. Indeed, the use of RAAS-inhibition therapy would be considered a standard of care for patients with diabetes and early CKD, fourth, the high rates of adherence and follow-up, and the use of high-quality laboratory methods enhance the internal validity of the trial. Fifth, novel markers of kidney injury are not susceptible to hemodynamic changes that may alter urine albumin excretion.

There are also several limitations. We used a short-term crossover trial design, selected to enhance power to detect differences. Although no carryover effects were detected in the analyses of outcome variables, we detected a carryover effect of the intervention on EPA levels in erythrocytes. Erythrocyte EPA levels did not return to baseline at the end of the study in the group first assigned to n-3 PUFA despite a 2-week washout period and a 6-week period on placebo. We note that this type of carryover effect will tend to mask the effects of n-3 PUFA in the crossover analysis. Hence, future short-term trials of n-3 PUFA would benefit from a longer washout period or a parallel-arm design. Another limitation pertains to our reporting a large number of secondary outcomes and subgroup analyses stratified by baseline medication use. With this approach, one must be cautious in interpreting statistical significance due to multiple comparisons and the risk of type 1 errors. For example, although we report significant reductions in urine excretion of albumin, NAG, NGAL, and LFABP in those participants on RAAS therapy at baseline, there was evidence of increased urine excretion of NAG in those not taking RAAS therapy. This finding can either reflect the truth or, because of type 1 error associated with post hoc subgroup

### Table 3—Test for interactions between concurrent medication use and n-3 PUFA effects on markers of kidney injury

|                      | n-3 PUFA vs. placebo | P value interaction |
|----------------------|----------------------|---------------------|
|                      | Yes                  | No                  |
| Statins              |                      |                     |
| KIM-1 (pg/day)       | 0.97 (0.85–1.11)     | 0.87 (0.72–1.06)    | 0.36 |
| NGAL (ng/day)        | 0.85 (0.67–1.07)     | 0.83 (0.64–1.06)    | 0.90 |
| LFABP (ng/day)       | 0.68 (0.43–1.06)     | 1.01 (0.64–1.62)    | 0.27 |
| NAG (units/day)      | 0.74 (0.59–0.94)     | 1.12 (0.80–1.56)    | 0.05 |
| Microalbuminuria (mg/day) | 0.86 (0.70–1.06) | 0.80 (0.90–1.28)    | 0.17 |
| Diuretics            |                      |                     |
| KIM-1 (pg/day)       | 0.96 (0.82–1.12)     | 0.92 (0.79–1.08)    | 0.76 |
| NGAL (ng/day)        | 0.78 (0.61–1.00)     | 0.87 (0.71–1.08)    | 0.52 |
| LFABP (ng/day)       | 0.65 (0.32–1.31)     | 0.87 (0.65–1.18)    | 0.39 |
| NAG (units/day)      | 0.94 (0.69–1.28)     | 0.80 (0.62–1.04)    | 0.45 |
| Microalbuminuria (mg/day) | 0.93 (0.75–1.14) | 0.93 (0.74–1.16)    | 0.98 |
| Diabetes medications |                      |                     |
| KIM-1 (pg/day)       | 0.91 (0.81–1.03)     | 1.05 (0.82–1.33)    | 0.32 |
| NGAL (ng/day)        | 0.80 (0.65–0.98)     | 1.01 (0.78–1.30)    | 0.28 |
| LFABP (ng/day)       | 0.74 (0.50–1.10)     | 0.94 (0.61, 1.47)   | 0.56 |
| NAG (units/day)      | 0.80 (0.64–0.99)     | 1.11 (0.70–1.74)    | 0.18 |
| Microalbuminuria (mg/day) | 0.87 (0.73–1.04) | 1.20 (0.97–1.49)    | 0.09 |
| RAAS inhibitors      |                      |                     |
| KIM-1 (pg/day)       | 0.91 (0.79–1.04)     | 1.02 (0.84–1.23)    | 0.35 |
| NGAL (ng/day)        | 0.73 (0.60–0.88)     | 1.16 (0.86–1.57)    | 0.009 |
| LFABP (ng/day)       | 0.63 (0.41–0.96)     | 1.24 (0.77–2.02)    | 0.06 |
| NAG (units/day)      | 0.70 (0.55–0.89)     | 1.32 (1.02–1.70)    | 0.002 |
| Microalbuminuria (mg/day) | 0.83 (0.69–1.00) | 1.19 (0.87–1.61)    | 0.04 |

RAAS inhibitors and n-3 PUFA may have important clinical implications and needs to be further investigated as a potential renoprotective combination in patients with diabetes and proteinuria.

Our trial has several strengths. First, the crossover design and relatively short-term intervention periods allowed for assessments of the acute effects on urine albumin excretion and eGFR in the setting of stable doses of medications that may affect urine protein excretion. Second, we enrolled diabetic participants with proteinuria, a common complication and early sign of CKD in patients with diabetes. Third, the diabetes, hyperlipidemia (if present), and hypertension were treated. Hence, supplementation with n-3 PUFA may have added benefit to conventional therapies in those with diabetes and early CKD. Fourth, the high rates of adherence and follow-up, the use of 24-h urine sample collections, and the use of high-quality laboratory methods enhance the internal validity of the trial.

There are also several limitations. We used a short-term crossover trial design, selected to enhance power to detect differences. Although no carryover effects were detected in the analyses of outcome variables, we detected a carryover effect of the intervention on EPA levels in erythrocytes. Erythrocyte EPA levels did not return to baseline at the end of the study in the group first assigned to n-3 PUFA despite a 2-week washout period and a 6-week period on placebo. We note that this type of carryover effect will tend to mask the effects of n-3 PUFA in the crossover analysis. Hence, future short-term trials of n-3 PUFA would benefit from a longer washout period or a parallel-arm design. Another limitation pertains to our reporting a large number of secondary outcomes and subgroup analyses stratified by baseline medication use. With this approach, one must be cautious in interpreting statistical significance due to multiple comparisons and the risk of type 1 errors. For example, although we report significant reductions in urine excretion of albumin, NAG, NGAL, and LFABP in those participants on RAAS therapy at baseline, there was evidence of increased urine excretion of NAG in those not taking RAAS therapy. This finding can either reflect the truth or, because of type 1 error associated with post hoc subgroup
Each intervention capsule was a highly concentrated source of DHA/EPA derived from marine fish oils, and the dose was 4.0 g/day. This high dose of n-3 PUFA is far above the daily average intake of U.S. adults (0.15 g/day) (26) and contributes minimally to total calorie intake (~40 kcal/day). This dose has therapeutic efficacy for triglyceride reduction (~25–40%) and is the dose currently sold by prescription for the treatment of hypertriglyceridemia. A lower dose of n-3 PUFA (1.0 g/day) has reduced cardiovascular events in some (27,28) but not all (29) clinical trials. In our prior meta-analysis of n-3 PUFA supplementation on urine protein excretion, the dose of n-3 PUFA did not modify the findings, but few trials used doses ≤1 g/day. Since 1 g/day is the currently recommended daily dose of EPA/DHA for adults with coronary heart disease (30), further research is also needed to evaluate the effects of n-3 PUFA across a wider range of doses. Within the Vitamin D and Omega-3 Trial, a National Institutes of Health–funded trial, 1,500 Vitamin D and Omega-3 Trial participants with diabetes, randomly assigned in a 2 × 2 factorial design to vitamin D3 (cholecalciferol) 1,600 IU daily versus placebo and to EPA 500 mg plus DHA 500 mg daily versus placebo, will be studied to ascertain effects of study interventions on albuminuria and GFR (31). Their trial is expected to finish in the year 2015.

Dose effects are also relevant to the observed adverse effects of n-3 PUFA supplements on fasting serum glucose. Increased fasting glucose with n-3 PUFA supplementation has been reported in many, but not all, trials. A recent Cochrane meta-analysis of 15 trials of fish oil supplementation on glucose homeostasis reported a nonsignificant pooled increase in fasting glucose of 2.9 mg/dL (95% CI −2.3 to 8.3 mg/dL), with no effects on HbA1c (32). The mechanisms involved in increasing fasting glucose are unclear, but n-3 PUFA reduce hepatic synthesis of triglycerides and increase hepatic fatty acid β-oxidation, resulting in increased hepatic glucose output through increased glycogenolysis and/or gluconeogenesis (33). Nonetheless, whether a lower dose of n-3 PUFA is sufficient to lower kidney injury but below a threshold to cause hyperglycemic effects needs to be tested.

In conclusion, in this randomized, controlled clinical trial, n-3 PUFA failed to reduce the primary outcome of urine albumin excretion. However, there was a consistent trend of benefit for all urine biomarkers and a significant reduction in NGAL. Furthermore, our post hoc subgroup findings raise the intriguing possibility that the greatest effects of n-3 PUFA are in individuals on RAAS inhibitors. There were small reductions in SBP and DBP, an increase in fasting glucose, but no apparent effect on eGFR with n-3 PUFA supplementation. Our results in the context of prior studies provide a strong rationale for larger trials that are adequately powered for smaller effects on urine albumin excretion or trials that are conducted in participants who take ACE/ARB therapy. In long-term trials of n-3 PUFA with clinical outcomes such as CKD progression, which are underway, careful consideration should be given for
n-3 PUFA supplement trial in diabetes

Table 4—Baseline, change from baseline, and difference in change between placebo and n-3 PUFA groups in BP, lipid profile, glucose, and erythrocyte membrane n-3 PUFA

| Symptom                      | Baseline mean (SD) | Change placebo to baseline (95% CI) | Change n-3 PUFA to baseline (95% CI) | Difference in change n-3 PUFA vs. placebo (95% CI) | P value |
|------------------------------|--------------------|-------------------------------------|--------------------------------------|---------------------------------------------------|---------|
| SBP (mmHg)                   | 130.6 (18.8)       | −0.7 (−5.1 to 3.7)                  | −4.1 (−8.4 to 0.3)                   | −3.4 (−7.7 to 1.0)                                | 0.13    |
| DBP (mmHg)                   | 68.2 (10.2)        | −1.1 (−3.5 to 1.2)                  | −2.7 (−5.0 to 0.4)                   | −1.54 (−3.9 to 0.8)                               | 0.20    |
| Heart rate (bpm)             | 66.1 (10.3)        | −1.1 (−3.4 to 1.4)                  | −1.4 (−3.8 to 1.0)                   | −0.4 (−2.8 to 2.0)                                | 0.77    |
| Total cholesterol (mg/dL)    | 156.9 (30.8)       | −2.8 (−4.8 to 10.5)                 | −0.4 (−7.9 to 7.2)                   | −3.2 (−10.8 to 4.4)                               | 0.41    |
| HDL cholesterol (mg/dL)      | 53.4 (14.5)        | 1.2 (−1.6 to 4.0)                   | 1.2 (−1.5 to 4.0)                    | 0.02 (−2.8 to 2.3)                                | 0.99    |
| LDL cholesterol (mg/dL)      | 77.7 (24.3)        | 0.6 (−5.8 to 7.0)                   | 1.6 (−4.7 to 7.8)                    | 1.0 (−5.9 to 7.4)                                 | 0.77    |
| Triglycerides (mg/dL)        | 128.8 (69.9)       | 9.0 (−9.9 to 28.0)                  | −15.7 (−34.3 to 3.0)                 | −24.8 (−43.7 to −5.8)                             | 0.01    |
| Glucose (mg/dL)              | 124.9 (40.7)       | −4.1 (−1.7 to 10.0)                 | 10.6 (4.8–16.4)                      | 6.5 (0.6–12.3)                                    | 0.03    |

Liver function

| Symptom                      | Baseline mean (SD) | Change placebo to baseline (95% CI) | Change n-3 PUFA to baseline (95% CI) | Difference in change n-3 PUFA vs. placebo (95% CI) | P value |
|------------------------------|--------------------|-------------------------------------|--------------------------------------|---------------------------------------------------|---------|
| α-glutamyl transpeptidase (units/L) | 42.0 (52.0)       | 2.1 (−2.2 to 6.4)                   | 0.8 (−5.1 to 6.7)                    | −1.3 (−7.0 to 4.4)                                | 0.65    |
| Alanine aminotransferase      | 10.0 (10.0)        | −0.3 (−2.1 to 1.5)                  | 2.3 (0.3–4.3)                        | 2.6 (0.4–4.8)                                     | 0.02    |
| Aspartate aminotransferase    | 19.7 (9.1)         | −1.4 (−3.9 to 1.2)                  | 0.2 (−2.5 to 2.9)                    | 1.6 (−0.4 to 3.6)                                 | 0.12    |

Erythrocyte membrane fatty acids

| Symptom                      | Baseline mean (SD) | Change placebo to baseline (95% CI) | Change n-3 PUFA to baseline (95% CI) | Difference in change n-3 PUFA vs. placebo (95% CI) | P value |
|------------------------------|--------------------|-------------------------------------|--------------------------------------|---------------------------------------------------|---------|
| C20:5 (n-3) EPA (μg/mL)      | 68.3 (3.4)         | 1.3 (−0.7 to 3.3)                   | 16.1 (14.1–18.1)                     | 14.8 (12.8–16.8)                                  | <0.01   |
| C22:6 (n-3) DHA (μg/mL)      | 59.0 (18.7)        | 5.7 (0.1–11.4)                     | 15.9 (10.3–21.4)                     | 10.1 (4.5–15.8)                                   | <0.01   |
| Total n-3 fatty acids (μg/mL)| 96.9 (25.7)        | 9.0 (−0.3 to 18.3)                 | 36.8 (27.5–46.0)                     | 27.8 (18.5–37.1)                                  | <0.01   |
| Total n-6 fatty acids (μg/mL)| 440.3 (78.0)       | −9.9 (−4.6 to 24.7)                | −43.1 (−77.5 to −8.7)                | −33.2 (−67.8 to 1.5)                              | 0.061   |
| Ratio of total n-6 to n-3 fatty acids | 0.75 (1.12)      | −0.50 (−0.80 to −0.21)            | −1.74 (−2.03 to −1.44)               | −1.23 (−1.53 to −0.94)                             | <0.01   |

Boldface values indicate significance.

the potential beneficial interactions between RAAS therapy and n-3 PUFA.

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E.R.M. analyzed and interpreted data, conceptualized and designed the study, and drafted the manuscript. S.P.J. analyzed and interpreted data and drafted the manuscript.

C.A.A., E.G., K.H.-R., S.T., and M.R.B. drafted the manuscript. J.C. and L.J.A. conceptualized the study.

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Table 5—Reported side effects at the end of treatment periods

| Symptom                      | Baseline | Placebo | n-3 PUFA | Fish oil vs. placebo, P value |
|------------------------------|----------|---------|----------|------------------------------|
| Back pain                    | 8 (27)   | 7 (24)  | 10 (33)  | 0.34                         |
| Flu                          | 5 (17)   | 8 (28)  | 8 (27)   | 0.93                         |
| Infection                    | 2 (7)    | 2 (6)   | 0 (0)    | —                            |
| Pain                         | 9 (30)   | 8 (28)  | 9 (30)   | 0.85                         |
| Heartburn                    | 4 (13)   | 5 (17)  | 4 (14)   | 0.93                         |
| Reflux                       | 5 (17)   | 6 (21)  | 5 (17)   | 0.53                         |
| Stomach pain                 | 1 (3)    | 0 (0)   | 0 (0)    | —                            |
| Bloating                     | 2 (7)    | 4 (14)  | 3 (10)   | 0.28                         |
| Burping                      | 2 (7)    | 3 (10)  | 5 (17)   | 0.33                         |
| Diarrhea                     | 4 (13)   | 5 (17)  | 3 (10)   | 0.13                         |
| Loose stool                  | 4 (13)   | 7 (24)  | 4 (13)   | 0.23                         |
| Constipation                 | 8 (27)   | 8 (29)  | 6 (20)   | 0.22                         |
| Nausea                       | 1 (3)    | 3 (10)  | 3 (10)   | 0.98                         |
| Vomiting                     | 1 (3)    | 2 (7)   | 2 (7)    | 0.98                         |
| Skin rash                    | 0 (0)    | 1 (3)   | 0 (0)    | —                            |
| Taste change                 | 0 (0)    | 2 (7)   | 3 (10)   | 0.28                         |
| Fishy taste                  | 0 (0)    | 1 (4)   | 5 (17)   | 0.07                         |
| Fatigue                      | 3 (10)   | 7 (24)  | 6 (20)   | 0.55                         |
| Poor appetite                | 1 (3)    | 4 (14)  | 0 (0)    | —                            |

Data are N (%) unless otherwise indicated.

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