Suppression of autoimmune disease by dietary n-3 fatty acids

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Abstract Previous studies have demonstrated that dietary fish oil preparations have anti-inflammatory effects in humans and in experimental animals, but the individual components of fish oils that are responsible for their anti-inflammatory effects have not been documented. We therefore investigated in (NZB × NZW)F1 mice, a model for human systemic lupus erythematosus, the effects of diets containing ethyl esters of two purified n-3 fatty acids, eicosapentaenoic acid (EPA-E) and docosahexaenoic acid (DHA-E), a refined fish oil triglyceride (FO) which contained 55% n-3 fatty acids, and beef tallow (BT) which contains no n-3 fatty acids. The diets were initiated prior to the development of overt renal disease at age 22 weeks, and continued for 14 weeks. The extent of the renal disease was quantified by light microscopy and by proteinuria. Diets containing either 10 wt% FO, 10% EPA-E, or 6% or 10% DHA-E alleviated the severity of the renal disease, compared to the BT diet, whereas diets containing either 3% or 6% EPA-E or 3% DHA-E were less effective. Two diets containing approximately 3:1 mixtures of EPA-E and DHA-E alleviated the renal disease to a greater extent than expected for either of these fatty acids given singly. We believe that these experiments provide the first demonstration of anti-inflammatory effects of individual dietary n-3 fatty acids. The results also indicate that the anti-inflammatory effects of fish oils depend on synergistic effects of at least two n-3 fatty acids.—**Robinson, D. R., L-L. Xu, S. Tateno, M. Guo, and R. B. Colvin.** Suppression of autoimmune disease by dietary n-3 fatty acids. *J. Lipid Res.* 1993. **34:** 1435-1444.

Supplementary key words (NZB × NZW)F1 mice • eicosapentaenoic acid ethyl ester • docosahexaenoic acid ethyl ester • fish oil • autoimmune glomerulonephritis

Dietary marine lipids have been shown to alleviate the severity of autoimmune disease in inbred strains of mice that are models for the human autoimmune disease, systemic lupus erythematosus (SLE) (1-4). Several clinical trials have demonstrated statistically significant, but only modest, anti-inflammatory effects of dietary supplements of fish oils in human autoimmune disease, including rheumatoid arthritis and SLE (see ref. 5). In order to improve the therapeutic effects of n-3 fatty acids on human autoimmune diseases, it would be useful to better understand the mechanisms of the protective effects of n-3 fatty acids on experimental models of autoimmune disease.

The inbred autoimmune murine strain, (NZB × NZW)F_1, (NZB/W) has several features which make it a useful model for these purposes. This strain reproducibly develops a spontaneous glomerulonephritis similar to the renal disease in human SLE (6, 7). Dietary marine lipids can dramatically reduce the severity of the murine renal pathology. This protective effect is dose-dependent, and can be quantitated histologically, which allows comparisons of different dietary lipid preparations (1-4, 8). Although the effects of dietary n-3 fatty acids on the glomerular lesion in NZB/W and other autoimmune murine strains has been extensively studied, the mechanisms of the protective effects of n-3 fatty acids remain unknown. The protective effects of marine lipids are generally assumed to be related to their content of n-3 fatty acids, but this assumption has not been proven, either for autoimmune disease or for effects of marine lipids on several other pathologic states, such as atherosclerosis (1-4, 8, 9). If the n-3 fatty acids are responsible for the ability of fish oils to suppress autoimmune disease, the relative effectiveness of different n-3 fatty acids would be of both theoretical and practical significance.

In order to help clarify these issues, we have carried out a study of the effects of diets containing various quantities of purified n-3 fatty acids in the form of their ethyl esters, and have compared the effects of these diets to a diet containing a fish oil triglyceride preparation and to a diet containing beef tallow, which lacks n-3 fatty acids (5). The results of these studies demonstrate that either of two n-3 fatty acids, eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA), can individually alleviate the severity of autoimmune glomerulonephritis. We also

Abbreviations: NZB/W, New Zealand Black-White F1 hybrid mice (NZB × NZW)F1; EPA and EPA-E, eicosapentaenoic acid and its ethyl ester; DHA and DHA-E, docosapentaenoic acid and its ethyl ester; AA, arachidonic acid; BT, beef tallow; FO, fish oil; SLE, systemic lupus erythematosus.

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found that DHA was effective at lower doses than EPA, and that these two fatty acids act synergistically to alleviate autoimmune glomerulonephritis.

METHODS

Experimental procedures are described in detail in the accompanying manuscript (5). Briefly, female NZB/NZW mice were fed with Purina lab chow until age 22 weeks, at which time no significant renal disease was present, based on the absence of proteinuria. At age 22 weeks the mice were randomly divided into groups and were placed on one of several experimental diets. The experiments were continued for a further 14 weeks, at which time the control mice fed with beef tallow (BT) had developed definite histologic changes of glomerulonephritis that could be compared to the mice that were fed the marine lipid diets. Proteinuria was measured by the dipstick method (Albustix®, Ames, Elkhart, IN) at weekly intervals. Each animal was considered to have developed proteinunia when the urinary protein concentration reached 100 mg/dl (2+) or greater on two consecutive measurements.

The formulation of the experimental diets is described in detail elsewhere (5). Briefly, the diets consisted of a nutritionally balanced fat-free powder to which was added 10 wt% of either one of the n-3 fatty acid preparations or BT. In addition, 2 wt% safflower oil was added to all diets to provide adequate quantities of n-6 essential fatty acid. In diets containing less than 10% marine lipids, BT was added to bring the lipid content up to 10%, exclusive of the safflower oil. Therefore, all experimental diets contained 12 wt% of total lipid. The purpose of these experiments was to compare the effects of different n-3 fatty acid preparations with a diet containing negligible quantities of n-3 fatty acids on the severity of murine autoimmune glomerulonephritis, as reflected by proteinuria and, more accurately, by histologic changes in the kidney. The fatty acid compositions of the lipid preparations used in these diets are presented in detail in the accompanying manuscript (5). Briefly, in the BT diet approximately 95% of the fatty acids were either saturated or monoenoic, in nearly equal proportions, with 4.7% n-6 PUFA, as linoleic acid, and only 0.7% n-3 PUFA. The fish oil (FO) diet contained 60% PUFA, including 55% n-3 PUFA, with 31% EPA and 13% DHA, and 3.5% n-6 PUFA. The EPA ethyl ester (EPA-E) diet contained 99% PUFA, including 94% EPA, 4.8% arachidonic acid (AA), and 2.3% 18:4n-3. The DHA ethyl ester (DHA-E) diet contained 98% PUFA, including 90% DHA, 3.6% 22:5n-3, 2.8% 22:5n-6, and 0.9% EPA. The safflower oil diet contained 77% n-6 PUFA, nearly all 18:2n-6, 13% monoenoic, and 10% saturated fatty acids.

Groups of 11 to 15 mice were studied in each dietary group. Not all groups were studied contemporaneously. In order to assure that groups studied at one time were comparable to groups studied at other times, three separate groups of mice fed with 10% BT and three groups fed with 10% FO were studied over the duration of these experiments separate experiments. These three BT and three FO groups were analyzed separately, but no significant differences in histologic changes in the kidneys between the three BT groups or between the three FO groups were found. Therefore, the BT and 10% FO were pooled and each reported as single groups. These two dietary groups served as a basis for comparisons with groups fed the n-3 fatty acid ethyl ester preparations, and the 5% FO group. After the completion of these experiments, a final experiment was carried out in order to confirm the previous observation of an apparent synergistic effect of a diet containing a combination of EPA-E and DHA-E on the development of the renal disease. This experiment consisted of three groups: BT, 10% FO, and a diet combining EPA-E with DHA-E, and the results were analyzed separately from the previous experiments.

At the time the rats were killed, the kidneys were removed rapidly and fixed in 10% formalin. The tissues were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Light microscopy was carried out independently by two observers (RBC and MG) without knowledge of the dietary histories. Any discrepancies between the evaluations of the two observers were subsequently resolved by mutual agreement after jointly reviewing the sections in question. Renal biopsy changes were analyzed based on a modification of published criteria, with specific histologic features graded on a scale of 0, 1, 2, or 3; corresponding to normal, mild, moderate, or severe, respectively, as previously described by others (10). The chronicity index was the sum of the scores for glomerular sclerosis, interstitial fibrosis, tubular atrophy, and interstitial mononuclear cell infiltrate (each score on a scale of 0-3). No histologic changes of vascular disease were found in these studies. The overall severity of the glomerular pathology was defined as the average of the scores for the four glomerular histological features that were graded: cellularity, capillary wall thickening, mesangial material, and sclerosis, as described previously (4). A full longitudinal section through each kidney was reviewed, and at least 100 glomeruli were analyzed. In addition, the number of histologically normal glomeruli were tabulated for each dietary group as another indication of the degree of severity of the renal disease. Glomeruli were considered to be normal when the scores were 0 or 1 for glomerular hypercellularity, and 0 for all other histologic parameters. The P values for comparisons of histopathologic changes among different dietary groups were calculated with t-tests or Fisher Exact tests. The P values for group comparisons of proteinuria were calculated with Mann-Whitney U tests.

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RESULTS

We used two different parameters to measure the severity of the glomerulonephritis that developed in NZB/W mice. The first parameter was proteinuria, which was absent in all mice at the time when they were assigned to the experimental diets at age 22 weeks. Since proteinuria developed in a majority of mice fed the BT diet over the subsequent 13 weeks, reduction in the numbers of mice that developed proteinuria was considered as an index of alleviation of glomerulonephritis by several of the marine lipid diets. Proteinuria is a manifestation of renal injury that was followed throughout the experiment, reflecting the rate of progression of the renal disease. The second parameter that reflected the severity of the renal disease was the histopathology of the kidney, using histologic criteria generally accepted for assessing the severity of glomerulonephritis in systemic lupus erythematosus (10). In comparing the different diets, we will use primarily the histologic observations, since histologic changes have been shown to correlate better with survival than proteinuria (11).

Urinary protein measurements were made at weekly intervals for all mice during the period that experimental diets were fed. The results are presented in Figs. 1–4. By the end of the 13-week experimental period, 77% of the BT group had developed proteinuria, compared to 11% of the 10% FO group, a difference that was highly statistically significant (P = 0.0001, **Fig. 1**). An intermediate number of mice, 38%, developed proteinuria on the 5% FO diet, and this differed significantly from the BT group (P = 0.038), but did not differ significantly from the 10% FO group. In **Fig. 2** it is demonstrated that EPA suppressed the development of proteinuria to a similar extent as did the 10% FO diet (P = 0.0003 and 0.002 vs. BT, for the 10% and the 6% EPA diets, respectively). However, the 3% EPA diet did not differ from BT. The results shown in **Fig. 3** demonstrate that all of the DHA diets suppress proteinuria (P = 0.0001, 0.0005, and 0.035, for the 10%, 6%, and 3% DHA diets, respectively). Therefore, both of the individual n–3 fatty acids suppress the renal disease in these mice, as assessed by proteinuria, and the DHA is more effective than EPA. Finally, in a separate experiment, we compared a diet containing approximately the same quantities of EPA and DHA as the 10% FO diet, 3.3% EPA-E and 1.3% DHA-E, to two new groups of mice fed with either the BT or the 10% FO diet, over the same period of time. The results are shown in **Fig. 4,** and demonstrate that the diet containing EPA-E and DHA-E reduces proteinuria similarly to the 10% FO diet (P = 0.002 and 0.0002 for the EPA-E + DHA-E diet and the 10% FO diets, respectively, vs. BT). An additional group fed with a similar diet containing 2.8% EPA-E + 0.8% DHA-E gave essentially the same results as the previous EPA + DHA diet (P = 0.023 vs. BT, results not shown).

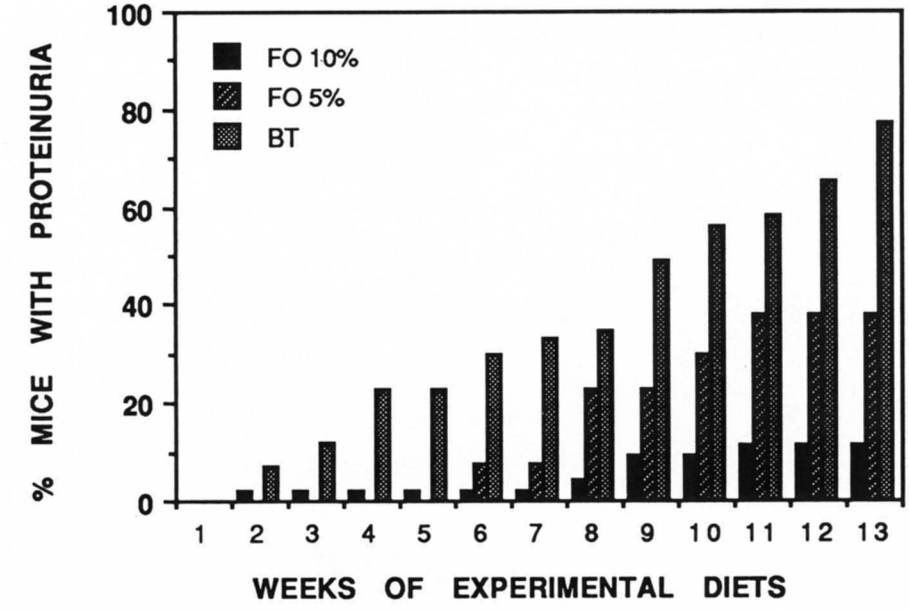

Fig. 1. Reduction of proteinuria in NWB/W mice by fish oil (FO) diets. Mice were fed with experimental diets beginning at age 22 weeks. The diets consisted of a fat-free balanced diet to which 12 wt% lipid was added. Of the lipid, 10 wt% was either beef tallow (BT) or marine lipid, and diets with <10% marine lipid contained sufficient BT to bring the total of the BT plus marine lipid up to 10%. All diets contained 2 wt% safflower oil to provide sufficient essential fatty acid. In the results shown here, the percentage of mice developing proteinuria at 13 weeks after initiation of the diets was significantly less that of the BT group for both the 10% FO and the 5% FO groups; P = 0.0001 and 0.038, respectively, vs. BT. Proteinuria is defined as protein equal to or greater than 100 mg/dl.

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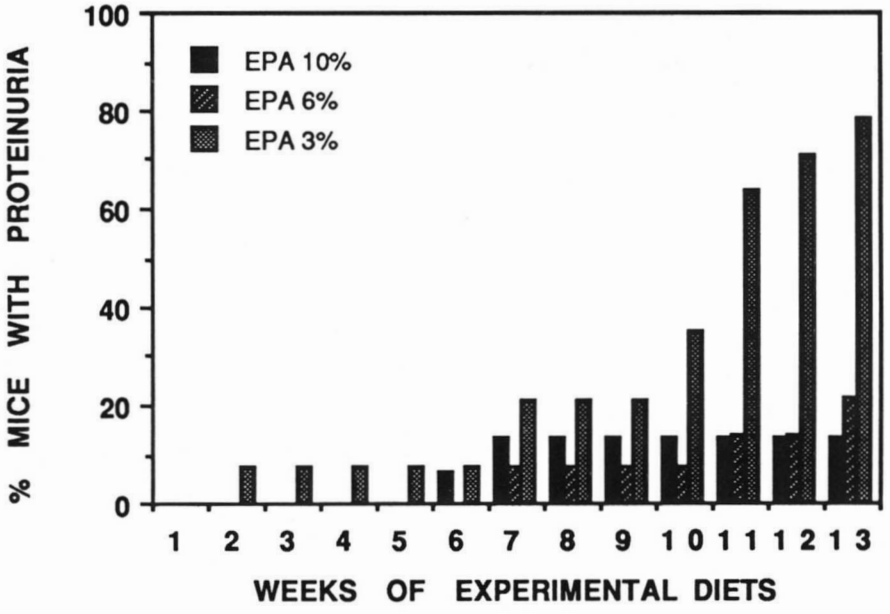

Fig. 2. Reduction of proteinuria by dietary eicosapentaenoic acid (EPA). Each diet contained EPA ethyl ester (EPA-E), wt% as indicated. The proteinuria was significantly reduced by the 10% and 6% EPA-E diets; $P = 0.0003$ and 0.002, respectively, vs. BT (Fig. 1).

The results of the histologic analyses for all of the mice studied are given in Tables 1 and 2. Photomicrographs of representative sections illustrating the glomerular pathologic changes seen in the mice from the experiments reported in **Table 1** are shown in **Fig. 5.** In the experiments shown in Table 1 there was no significant scarring or deposition of extracellular mesangial material in any of the dietary groups. Hypercellularity and capillary wall thickening were significantly less severe, and the percentage of mice with normal glomeruli was greater in animals fed the 10% FO than for those fed the 10% BT diet. This protection is lost by reducing the FO to 5%, since the 5% FO group does not differ significantly from the BT group (Table 1).

The EPA-E alleviates the severity of the renal disease to a similar degree as the 10% FO, but only at the highest

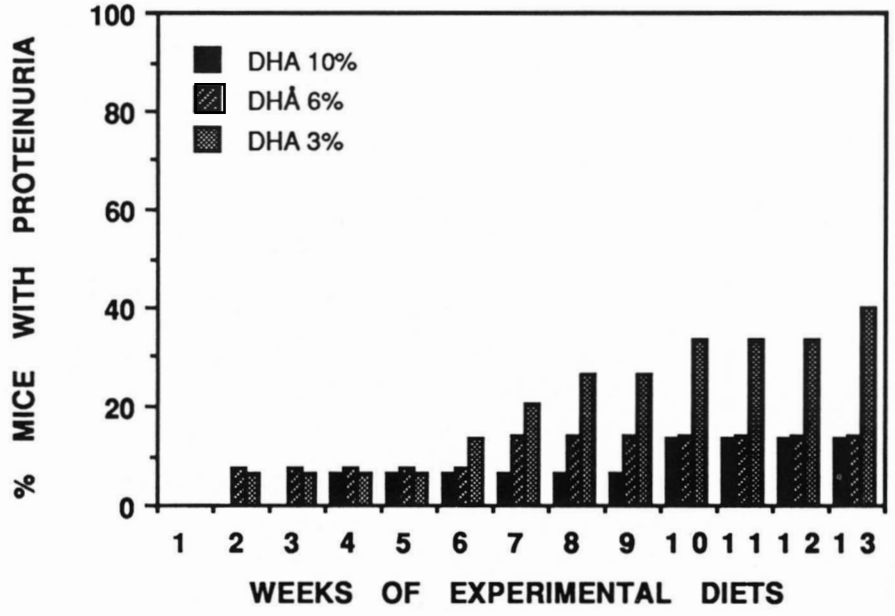

Fig. 3. Reduction of proteinuria by dietary docosahexaenoic acid (DHA). Each diet contained DHA ethyl ester (DHA-E), wt% as indicated. Proteinuria was significantly reduced by each of these diets; $P = 0.0001$, 0.0005, 0.035, for 10%, 6%, and 3%, respectively, vs. BT.

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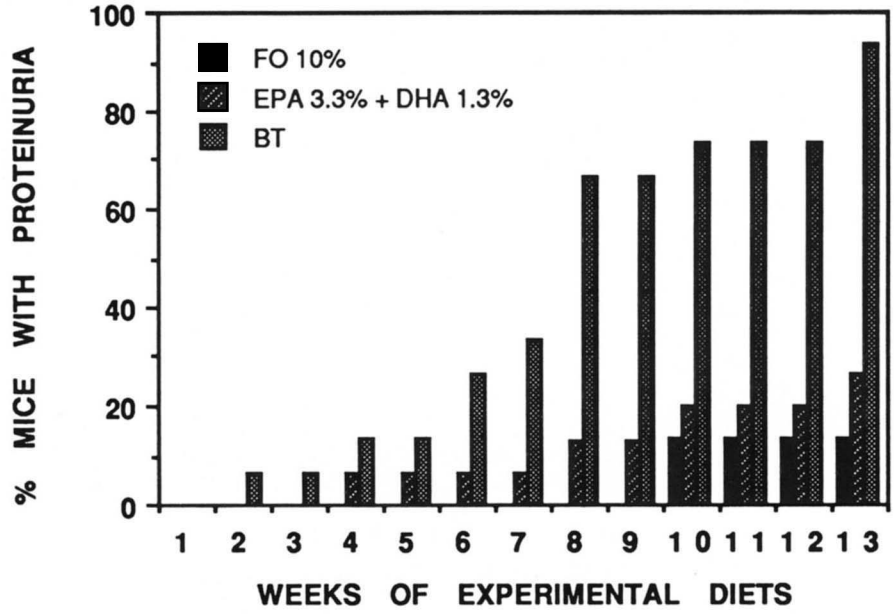

Fig. 4. Reduction of proteinuria by a diet containing a mixture of 3.3% EPA and 1.3% DHA; quantities similar to those in the 10% FO diet. Proteinuria was reduced significantly for both the FO and the EPA + DHA diets; P = 0.0002 and 0.0019, respectively, vs. BT.

dose of EPA-E of 10%. The DHA-E also alleviates the severity of the renal disease at least as effectively as the 10% FO, at doses of both 6% and 10%, while the 3% DHA-E does not differ significantly from the BT diet. Since the 3% EPA-E diet contains nearly the same quantity of EPA as the 10% FO diet, and the 6% EPA contains a slightly higher quantity of total n-3 fatty acids than the 10% FO diet, the lack of efficacy of these two EPA-E diets suggested the possibility that a combination of more than one n-3 fatty acid may be required for optimal alleviation of this murine autoimmune disease.

We therefore determined the effects of diets that combined both EPA-E and DHA-E in two groups of mice. The first group was fed a diet containing both 2.8% EPA-E and 0.8% DHA-E, to give an n-3 fatty acid content only slightly greater than the 3% EPA-E and the 3% DHA-E diets, and similar to the content of EPA and DHA in the 10% FO diet. With this diet the degree of cellularity and the percentage of mice with normal glomeruli did not differ from the BT controls, but the degree of

TABLE 1. Effects of n-3 fatty acids on renal pathology in NZB/W mice

Dietary Group	No. of Mice	Glomerular Cellularity[a]	Glomerular Capillary Thickening[a]	% Mice with Normal Glomeruli[b]
BT	35	2.1 ± 0.2	1.8 ± 0.2	17
FO 10%	44	1.0 ± 0.2[c]	0.5 ± 0.1[c]	70[e]
FO 5%	13	2.2 ± 0.2[d]	1.5 ± 0.3[d]	23[d]
EPA-E 3%	13	2.7 ± 0.2[d]	2.0 ± 0.3[d]	8[d]
6%	12	2.1 ± 0.2[e]	1.4 ± 0.3[d]	8[d]
10%	15	0.7 ± 0.3[c]	0.3 ± 0.2[c]	73[f]
DHA-E 3%	14	1.7 ± 0.3	1.1 ± 0.3[e]	36
6%	14	0.7 ± 0.2[c]	0.1 ± 0.1[c]	93[c,e]
10%	14	0.5 ± 0.2[c]	0.1 ± 0.1[c]	93[c,e]
EPA-E 2.8% + DHA-E 0.8%	11	2.0 ± 0.3[e]	0.7 ± 0.2[f]	27

[a]Severity of glomerular pathology measured scores ranging from 0 (normal) to 3 (most severe). Mean scores ± SEM are listed. In the experiments reported in this table, glomerular mesangial material, sclerosis, and tubulo-interstitial changes were insignificant in all groups.

[b]Glomeruli were considered normal glomeruli when the mean cellularity scores were either 0 or 1, and capillary thickening scores were 0, for each individual mouse kidney section. The number of mice with normal glomeruli within a dietary group was divided by the total number of mice in that group (× 100) to give the % of mice with normal glomeruli.

[c]$P < 0.001$ vs. BT.

[d]$P < 0.001$ vs. FO 10%; n.s. vs. BT.

[e]$P < 0.05$ vs. FO 10%; n.s. vs. BT.

[f]$P < 0.02$ vs. BT.

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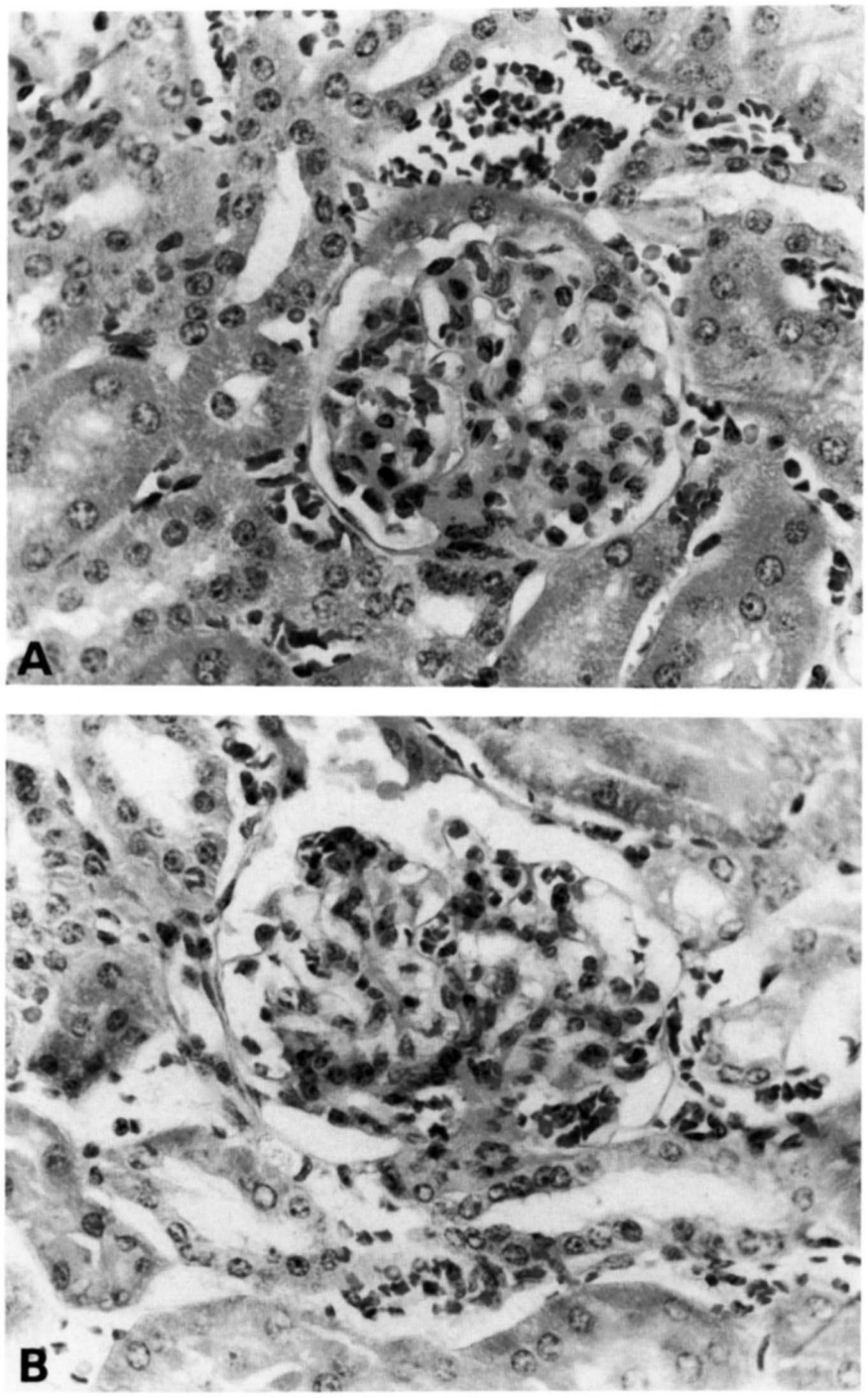

Fig. 5. Representative sections from NZB/NZW mice kidneys illustrating glomerular lesions of different histologic grades. The sections are chosen to illustrate the grading system for cellularity and capillary thickening. A, Normal glomerulus (cellularity 0, capillary thickening 0). B, Almost normal glomerulus (cellularity 1, capillary thickening 0). C, Moderate glomerular lesions (cellularity 2, capillary thickening 2). D, Severe glomerular lesions (cellularity 3, capillary thickening 3). All sections stained with hematoxylin and eosin, magnification × 500.

capillary wall thickening was less severe and similar to the 10% FO diet.

To confirm the observation that EPA-E and DHA-E may act synergistically to alleviate autoimmune renal disease, an additional experiment was performed in which the diet contained similar quantities of EPA and DHA as were present in the 10% FO diet (**Table 2**). The data in Table 2 demonstrate that the 10% FO diet results in significant alleviation of all the pathological changes examined, both in the glomeruli and in the tubulo-

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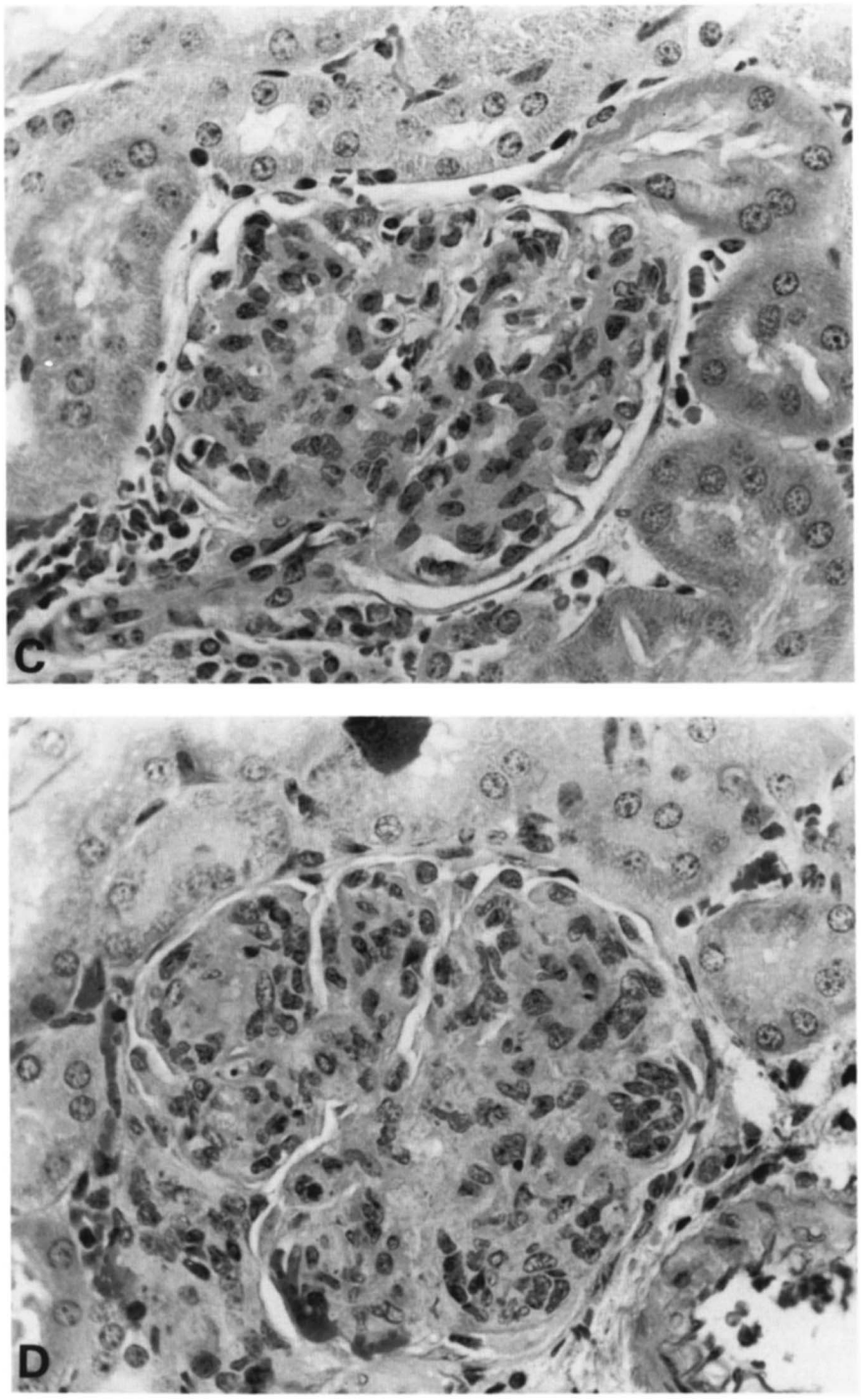

interstitial regions of the kidneys. Nearly identical results were found for mice given the combined n-3 fatty acid diet as for mice given the 10% FO diet. The effectiveness of both of these n-3 fatty acid diets in alleviating the renal disease is summarized by the values for the overall severity of the glomerular disease, and by the chronicity index. In this experiment, which was performed approximately 1 year after the completion of the previous experiments, the FO and BT groups had more advanced lesions than those described in Table 1, despite the same ex-

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TABLE 2. Effects of a diet combining EPA-E and DHA-E on renal pathology in NZB/W mice[a]

Dietary Group	No. of Mice	Glomerular Pathology: Cellularity	Capillary Thickening	Mesangial Material	Sclerosis	Overall Severity[b]
BT	13	3.2 ± 0.2	2.5 ± 0.3	2.5 ± 0.4	1.8 ± 0.2	2.5 ± 0.2
FO 10%	15	1.8 ± 0.4[d]	1.1 ± 0.3[d]	1.5 ± 0.2[c]	0.5 ± 0.3[c]	1.2 ± 0.3[d]
EPA-E 3.3% + DHA-E 1.3%	14	2.1 ± 0.3[d]	0.9 ± 0.3[d]	1.7 ± 0.2	0.2 ± 0.1[d]	1.2 ± 0.2[d]

		Tubulo-Interstitial Pathology: Atrophy	Fibrosis	Lymphoid Infiltrates	Chronicity Index[c]
BT	13	1.6 ± 0.3	1.2 ± 0.2	1.3 ± 0.2	5.9 ± 0.9
FO 10%	15	0.5 ± 0.2[c]	0.5 ± 0.3[c]	0.5 ± 0.2[c]	2.0 ± 0.9[d]
EPA-E 3.3% + DHA-E 1.3%	14	0.3 ± 0.2[d]	0.1 ± 0.1[d]	0.3 ± 0.2[d]	0.9 ± 0.5[d]

[a]See footnote to Table 1 and Methods for pathology scoring system.
[b]Overall severity is the mean of the four glomerular pathologic changes: cellularity, capillary thickening, mesangial material, and sclerosis.
[c]Chronicity index is the sum of the scores for glomerular sclerosis plus the three tubulo-interstitial changes.
[d]$P < 0.01$ vs. BT group.
[c]$P < 0.05$ vs. BT group.

perimental protocol in the two experiments, but the intergroup comparisons are still valid. These differences were manifested by the presence of significant degrees of glomerular mesangial material, glomerular sclerosis, and tubulo-interstitial pathology, all of which were present to a negligible degree in the experiments reported in Table 1.

DISCUSSION

Previous reports have documented that fish oil diets alleviate the glomerulonephritis in murine strains that develop autoimmune disease. The results reported in the present study further characterize the beneficial effects of marine lipids on murine autoimmune disease. The severity of this autoimmune disease is conveniently quantitated by urinary protein measurements and by renal histology, allowing comparison of different groups of mice that were fed different lipid preparations. Although the protective effects of fish oils on murine autoimmune disease could logically be attributed to the n-3 fatty acids that are abundant in fish oils, the experiments reported here validate that prediction unequivocally. The ability of EPA to inhibit the formation of eicosanoids derived from arachidonic acid as well as the fact that eicosanoids derived from EPA often differ from their arachidonic acid-derived analogues in biological activity, have been invoked to account for the anti-inflammatory activities of marine lipids (9). The results reported here clearly show that the ability of fish oil preparations to alleviate the severity of autoimmune glomerulonephritis, an effect that may be considered anti-inflammatory, cannot be accounted for by EPA alone.

The experiments reported here document that both EPA and DHA, given separately in sufficient quantities, were capable of reducing the severity of autoimmune glomerulonephritis. However, in order to alleviate the renal disease to the same degree as the 10% FO diet, larger quantities of either EPA or DHA were required when either was given alone than the quantities that were present in the FO diet. For example, neither the 3% nor the 6% EPA-E diet significantly lessens the degree of glomerular pathology from that in the BT controls, although reduction of proteinuria is seen with the 6% EPA (Table 1, Fig. 2). Since the 10% FO diet contains approximately 3% EPA, it is clear that the protective effects of the FO diet cannot be attributed to its EPA content alone. Similarly, the DHA alone in the 10% FO diet cannot account for the FO effects. A trivial explanation for these observations is the possibility that the ethyl esters of these fatty acids might be poorly absorbed and/or incorporated into tissue lipids. Evidence against this possibility is presented in the accompanying paper, and rests primarily on the observation that the FO and the ethyl ester diets containing similar quantities of n-3 fatty acids lead to similar contents of n-3 fatty acids in tissue phospholipids (5). Furthermore, the combined ethyl ester diet containing a total of 4.6% n-3 fatty acids (Table 2), also contained essentially the same quantities of EPA and DHA that were present in the 10% FO diet. Since this combined n-3 fatty acid diet was as effective as the 10% FO diet in alleviating autoimmune renal disease, it is unlikely that the n-3 fatty acid ethyl esters are poorly absorbed or poorly utilized. Finally, others have recently reported that n-3 fatty acids are well absorbed from the gastrointestinal tract (12-15). These considerations suggest that while the n-3 fatty acids in fish oil are responsible for the protective effects of fish oils on autoimmune disease, no single n-3

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fatty acid in fish oils can account for their beneficial effects. This conclusion suggests that there may be synergistic effects of different n-3 fatty acids, and the two experiments reported here with diets combining EPA and DHA ethyl esters provide direct evidence supporting that hypothesis. The mechanism of the synergistic effects of the two n-3 fatty acids is unknown. It does not appear to be related to elevation of the total n-3 fatty acid levels in phospholipids, since the total n-3 fatty acid levels are no greater with the mixed n-3 fatty acid diet than with diets containing either EPA-E or DHA-E alone, in similar quantities (5).

Comparisons of the FO diet, in which the n-3 fatty acids are preferentially incorporated into the *sn*-2 position of triglycerides, with n-3 fatty acid ethyl esters requires the assumption that the n-3 fatty acids would be incorporated into tissue phospholipids and exert similar biological effects regardless of whether they were ingested as triglycerides or as ethyl esters. This assumption may or may not be valid, since the PUFA administered as triglycerides may, in part, be absorbed as monoglycerides, which may be distributed to tissues by different pathways than the PUFA, which are administered as ethyl esters, and absorbed as free fatty acids (14, 15). However, we know of no evidence that either the tissue incorporation or the biological effects of orally administered PUFA differ with ethyl ester or triglyceride forms.

In some of these experiments, there is a lack of correlation between the elevation of phospholipid n-3 fatty acid levels and the protective effects of the different diets. This is most obvious in comparing the 5% and 10% FO diets, which are associated with essentially identical phospholipid n-3 fatty acid levels (5). However, there is no significant alleviation of renal pathology, and only a mild degree of improvement in proteinuria associated with the 5% FO diet, whereas the 10% FO diet was highly effective in reducing both the severity of renal pathology and proteinuria. Since this autoimmune renal disease evolves over the 13-week period of administration of the experimental diets, it is possible that the 10% FO diet is more effective than the 5% FO diet because it changes the fatty acid composition of phospholipids more rapidly than the 5% FO diet, but our fatty acid analyses were carried out only after 13 weeks of the experimental diets. Previous studies have demonstrated that the efficacy of marine lipid diets was reduced when these diets are begun at later rather than earlier ages, indicating that the efficacy of the n-3 fatty acid diets is dependent on the stage of the autoimmune disease at the time of administration of the diets (8).

Several clinical trials have been reported in which patients with rheumatoid arthritis were given dietary supplements of marine lipid preparations containing up to approximately 6 gm of n-3 fatty acids daily (see ref. 5). Assuming a daily caloric intake of 2000 calories for patients with rheumatoid arthritis, 6 gm of n-3 fatty acids daily would provides only 2.7% of energy as n-3 fatty acids. This may be compared to the requirement for at least 9.8% energy for major beneficial effects on murine autoimmune disease, calculated above. Although it is not possible to extrapolate from the treatment of murine to human autoimmune disease, it seems reasonable to postulate that more significant anti-inflammatory effects for rheumatoid arthritis could be achieved than the modest effects which have been seen if larger quantities of n-3 fatty acids were ingested than the quantities that were used in previous clinical trials.

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