Dietary Enrichment with the Polyunsaturated Fatty Acid Eicosapentaenoic Acid Prevents Proteinuria and Prolongs Survival in NZB × NZW F_1 Mice

JAMES D. PRICKETT, DWIGHT R. ROBINSON, and ALFRED D. STEINBERG,
Arthritis Unit, Massachusetts General Hospital, Boston, Massachusetts 02114; Arthritis and Rheumatism Branch, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland 20014

ABSTRACT Prostaglandins and related compounds are active mediators of inflammation, but data concerning their role in the pathogenesis of the glomerulonephritis of New Zealand Black × New Zealand White (NZB × NZW) F_1 mice are conflicting. Dietary eicosapentaenoic acid (EPA, C20:5), a fatty acid analogue of arachidonic acid (C20:4), has been shown to impair platelet aggregation in humans, apparently through inhibition of the synthesis of prostaglandins and thromboxanes from arachidonic acid. We report here the effects of a diet high in EPA on the development of renal disease and survival in female NZB × NZW F_1 mice. Animals from 4–5 wk of age were fed diets containing 25% lipid, supplied either as beef tallow or menhaden oil, with fatty acid analysis of <0.05 and 14.4% EPA, respectively. In the first experiment, by 13.5 mo of age, mice on the beef tallow diet had all (9/9) developed proteinuria and the majority (6/9) had died, with renal histologic examination revealing severe glomerulonephritis. In contrast, none of 10 menhaden oil-fed animals had developed proteinuria, and all were alive at this time ($P < 0.005$ for both proteinuria and survival). In a second experiment using 50 mice in each dietary group, 56% of the beef tallow group vs. none of the menhaden oil group had developed proteinuria at 9 mo of age ($P < 0.005$). Native DNA binding at 6 mo of age was 23.9±14.7 vs. 10.1±9.7% in the beef and menhaden oil groups, respectively ($P < 0.01$). Weights were similar in all groups, and there was no evidence of essential fatty acid deficiency in any group. These results demonstrate that a diet high in EPA protects NZB × NZW F_1 mice from the development of glomerulonephritis.

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INTRODUCTION

Prostaglandins (PG)[1] and related compounds are involved in inflammatory reactions but their precise roles have not been well defined (1–3). The course of the glomerulonephritis in New Zealand Black × New Zealand White (NZB × NZW) F_1 mice, a model for human systemic lupus erythematosis (4, 5), may be improved by daily injections of large doses of PG (6). The effects of daily administration of large doses of PG to NZB × NZW F_1 mice may differ from the effects of endogenous PG, and therefore the possibility that inhibition of endogenous PG synthesis may retard development of glomerulonephritis in these animals has not been excluded. Evidence supporting this possibility is the demonstration that essential fatty acid (EFA) deficiency, a condition in which substrates for PG synthesis are reduced, delays the development of glomerulonephritis in NZB × NZW mice (7).

It has been previously reported that native Greenland Eskimos have prolonged bleeding times and abnormal platelet function, related to the lipid composition of their marine diet (8, 9). This alteration in platelet function has been attributed to the replacement of arachidonic acid in platelets by eicosapentaenoic acid (EPA), a constituent of lipids in marine animal tissues. It has been proposed that the mechanism of the anti-aggregating effects of EPA on platelets results from alterations in PG metabolism (10).

We have begun an investigation of the effects of an EPA-enriched diet on experimental inflammatory states. In this report we describe the ability of EPA to protect

[1] *Abbreviations used in this paper*: "beef," beef tallow supplemented diet; EFA, essential fatty acid; EPA, eicosapentaenoic acid; "fish," menhaden oil supplemented diet; NZB × NZW F_1 mice, New Zealand Black × New Zealand White mice; PG, prostaglandins.

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against renal disease and prolong survival in NZB × NZW F_1 mice.

METHODS

Diets. The basic diet consisted of a fat-free powder (ICN Nutritional Biochemicals, Cleveland, Ohio), which contains by weight 21% casein, 15.6% cellulose, 58.5% sucrose, and 4% balanced salt mixture, plus essential vitamins. This was mixed three parts to one by weight with either refined whole menhaden oil (Zapata Haynie Company, Reedville, Va.), a source rich in EPA, or melted beef tallow (ICN Nutritional). Fatty acid analyses of these lipids are given in Table I.

Animals. Female NZB × NZW F_1 mice were obtained from the Small Animals Section, Veterinary Resources Branch, of the National Institutes of Health, Bethesda, Maryland. An initial group (group 1) consisted of 10 animals on the EPA-enriched menhaden oil supplemented diet ("fish"), and 9 animals on the beef tallow diet ("beef"). These animals were entered at 5 wk of age. They were weighed collectively by dietary group at weekly intervals for the first 4.5 mo, and individually thereafter. Food intake was adjusted weekly to keep mean weights of each group comparable. Once it was clear that the animals would accept the diets, a second group of animals, group 2, was begun 4 mo later. These mice were 4 wk old at the onset of the study, and were divided into 50 animals on each diet. They were weighed individually on a weekly basis, with diets adjusted as above.

Proteinuria. Freshly voided urine samples were tested with standard dipsticks (Labstix, Ames Co., [Miles Laboratories, Inc.] Elkhart, Ind.) for all animals at monthly intervals, beginning at 4 mo of age. Animals were considered to have developed proteinuria when urine protein was ≥ 300 mg/dl.

Analysis of sera and diets. A subgroup of 27 fish-fed and 24 beef-fed animals in group 2 were bled via the retro-orbital venous sinus every 2 mo, beginning at 4 mo of age, for analysis of anti-double-stranded DNA antibodies by a modified Farr technique, as described (11), and for fatty acid analysis.

100 μl of sera from four animals in each dietary group, obtained at 8 mo of age, was extracted using an ethanol:ether procedure as described (12). 10 μl-samples of the melted beef tallow and menhaden oil were also subjected to analysis. Methyl esters of the extracted sera or oil samples were prepared using a 16-h incubation at 65°C with 0.5 cm^3 of 0.5 N methanolic HCl. Samples were evaporated, washed with 1.0 cm^3 methanol, taken to dryness, and resuspended in 200 μl chloroform. 2-μl aliquots were injected into a ⅛″ × 5′ stainless steel column packed with 10% Silar 10 C on Gas Chrom Q 100/200 mesh using a Perkin Elmer 900 gas chromatograph (Perkin Elmer Corp. Instrument Div. Norwalk, Conn.) This was programmed to begin at 150°, rising 16°/min to 225°C. Quantitative analyses agree within 14% using this system.

Statistical analysis. Proteinuria and survival were compared using a log rank analysis. Fatty acid levels and native DNA binding were compared using a nonpaired *t* test.

RESULTS

Proteinuria and survival. (Fig. 1) All of the beef-fed animals and none of the fish-fed animals in group 1 developed proteinuria by 13.5 mo of age ($P < 0.005$). The younger group 2 animals show a similar protection from proteinuria in the fish-fed animals ($P < 0.005$). The protective effect of the fish diet is complete; to date, none of the fish-fed animals in group 1 has had >30 mg/dl proteinuria. The same effect is seen in group 2, except for one fish-fed animal that had intermittent 100 mg/dl proteinuria for several months but is otherwise healthy. Histologic examinations of kidneys from all beef-fed animals that have died have shown severe glomerular and tubular disease with interstitial lymphoid infiltrates, graded 4+ according to the scale of Friend et al. (13). No fish-fed animals have been killed for histologic study.

In group 1, all fish-fed animals are alive at 13.5 mo, vs. only three of nine beef-fed animals ($P < 0.005$). In group 2, 3 of 50 beef-fed animals have died at 9 mo of age vs. none of the fish-fed animals.

Anti-double-stranded DNA antibodies. DNA binding at 6 mo of age in beef-fed animals was 23.9±14.7%, vs. 10.1±9.7% in fish-fed animals ($P < 0.01$).

Weights. Mean weights±SD for each diet in group 1 and group 2 are presented in Fig. 2. Animals in group 1 were slightly older and heavier at the initiation of the study. However, by 5 mo of age and thereafter, animals on both diets in group 1 and group 2 were of comparable weight. All animals were free of alopecia

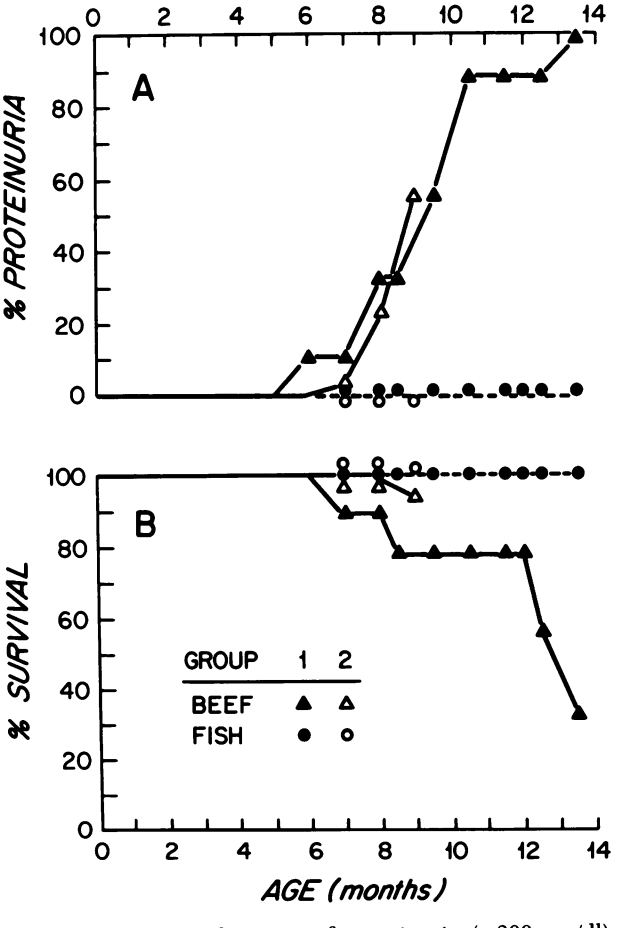

FIGURE 1 (A) Development of proteinuria (≥300 mg/dl). Group 1 beef vs. group 2 beef $P > 0.5$; group 1 beef vs. group 1 fish and group 2 beef vs. group 2 fish $P < 0.005$. Survival (B). For group 1, beef vs. fish $P < 0.005$. ▲△, beef; ●○, fish.

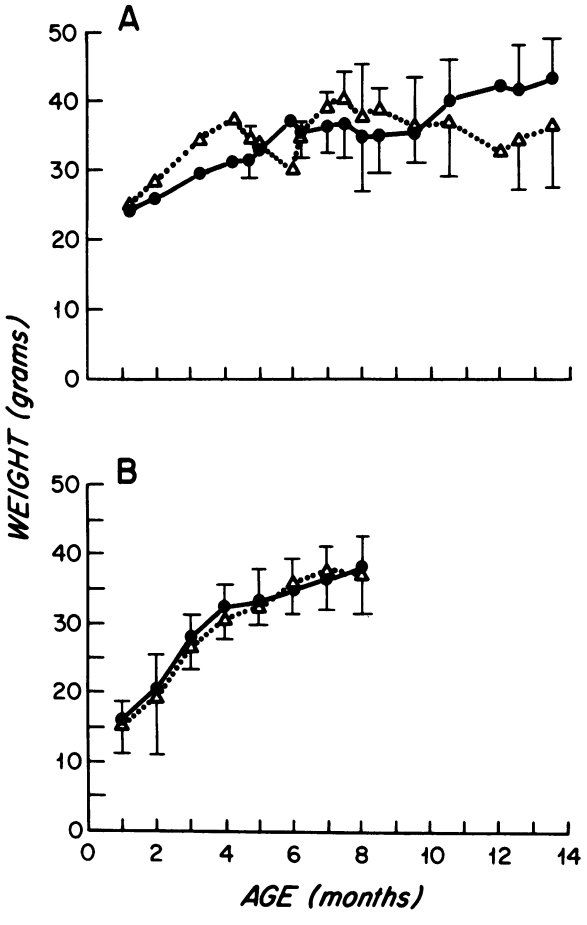

FIGURE 2 Mean weights for each diet (±SD when shown) for group 1 (A) and group 2 (B). △, beef; ●, fish.

or dermatitis, and before the development of renal disease were uniformly active and healthy in appearance.

Fatty acid analysis. Fatty acid analysis of the two dietary lipids and mouse sera are seen in Table I. Each dietary oil contains a comparable amount of linoleic acid. EPA constitutes 14.4% of the menhaden oil, but is undetectable in the beef tallow. Serum analysis reveals significantly lower levels of oleic (18:1), linoleic (18:2), and arachidonic (20:4) acids in fish-fed animals. Most striking, however, is the elevation of EPA in these animals.

DISCUSSION

A diet enriched in the polyunsaturated fatty acid, EPA, protects female NZB × NZW F_1 mice from the development of renal disease for at least 14 mo. In a control group of animals on a diet with the same total fat content, but lacking EPA, all developed proteinuria and two-thirds died over the same period ($P < 0.005$), similar to previous experiments with these mice on standard laboratory diets (4, 5). This was associated with a significant reduction in DNA binding activity in the fish-fed animals, at least through 6 mo of age. Although renal histopathology has not been obtained yet in the fish-fed animals, it is reasonable to assume a corresponding reduction in glomerular damage, inasmuch as previous studies have shown good correlations between the presence of proteinuria, measured either by dipstick or collections of 24-h urine samples, and the severity of renal pathology (4, 14, 15).

EFA deficiency, a state shown to modify certain animal models of inflammation (16, 17), cannot account for our results. Experimentally produced EFA deficiency in rodents produces alopecia, scaly dermatitis of the tail, and often severe growth retardation (16), none of which were manifested in our animals on either diet. Deficiency of EFA is characterized biochemically by serum polyunsaturated fatty acid ratios of C20:3/C20:4 > 0.4 (18). These ratios in the fish- and beef-fed animals were 0.05±0.006 and 0.08±0.04, respectively, demonstrating adequate dietary EFA.

Studies have shown that calorie restriction sufficient to reduce animal weight by 30%, but not variations in fat and protein composition of the diet, reduces renal disease and prolongs survival in NZB × NZW F_1 mice (13, 19). Previous studies using high dose PG (6, 14) or EFA-deficient diets (7) have not included weight data, or have allowed animal weights to vary by as much as 30% between groups, making it impossible to discount a protective effect of caloric restriction alone. Weight gain in our animals was closely controlled (Fig. 2), and thus variation in weights is not a factor in this study.

TABLE I
*Fatty Acid Analysis of Dietary Lipids and Mouse Sera**

Fatty acid	Menhaden oil‡§	Beef tallow‡	Sera, fish-fed animals ($n = 4$)	Sera, beef-fed animals ($n = 4$)
14:0	8.2	2.7	0.6±1.0	0.4±0.6
16:0	13.5	23.9	19.1±4.9	13.9±0.8
16:1	13.5	5.3	4.5±2.0	3.3±0.8
18:0	5.5	17.7	18.9±5.8	12.5±2.8
18:1 (oleic)	12.5	41.2	14.2±4.1	28.3±7.4‖
18:2 (linoleic)	5.2	6.1	3.2±0.7	14.6±4.2¶
20:3	1.4	<0.05	0.5±0.1	1.3±0.7
20:4 (arachidonic)	1.5	<0.05	9.9±2.5	14.9±2.4**
20:5 (EPA)	14.4	<0.05	18.4±8.4	<0.05¶

* As percent total fatty acid content, ±SD where indicated.
‡ Single determinations.
§ An additional 14% was identified as 18:3, 22:5, and 22:6.
‖ Fish vs. beef sera, $P < 0.02$.
¶ Fish vs. beef sera, $P < 0.01$.
** Fish vs. beef sera, $P < 0.05$.

In summary, enrichment of the diet of female NZB × NZW F_1 mice with the polyunsaturated fatty acid EPA protects against the development of renal disease and prolongs survival. This effect is not explained by difference in animal weights or by EFA deficiency, but appears to involve effects of enrichment of tissue lipids with EPA.

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