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A fish oil diet reduces the severity of collagen induced arthritis after onset of the disease

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SUMMARY

We have previously reported that compared to a corn oil diet a fish oil diet (5% by weight) fed to B10R.III mice before the induction of collagen induced arthritis markedly reduced disease severity. In this study we determine whether a fish oil diet could reduce the severity of collagen induced arthritis if begun after the arthritis was clinically apparent. Mice were initially fed either a fish oil or corn oil diet and immunized with bovine type II collagen 4 weeks later. At the onset of collagen-induced arthritis, half of the corn oil fed mice were switched to fish oil and arthritis assessed on a weekly basis. Four weeks after the diet change until killing 5 weeks later, the mice switched to fish oil developed much less severe arthritis than the corn oil fed controls. Thus the severity index of corn oil fed mice ranged between 9·4 and 7·1; the severity index of fish oil fed mice was between 6·8 and 4·3 while the mice switched to fish oil ranged between 7·2 and 5·6. Analysis of peritoneal macrophages 13 weeks after immunization showed that macrophages from fish oil fed mice incorporated eicosapentaenoic acid into phospholipids and produced less arachidonate products than corn oil fed mice. There was no difference between macrophages obtained from mice switched from corn oil to fish oil and those maintained on fish oil with respect to fatty acid composition of membrane phospholipids or prostaglandin profile. These results suggest that arthritis severity may be modulated after the onset of CIA by altering the PG profile of macrophages present at inflammatory sites.

Keywords fish oil collagen arthritis mice

INTRODUCTION

Collagen-induced arthritis (CIA) is an experimental model in which rats (Trentham, Townes & Kang, 1977), mice (Courtenay *et al.*, 1980) and non-human primates (Cathcart *et al.*, 1986) develop arthritis when injected with type II collagen (CII). Although immunity to CII plays a significant role in the pathogenesis of this disease the presence of anti-CII antibodies alone is not sufficient to produce the signs and symptoms of CIA (Wooley *et al.*, 1981). Moreover incidence and/or severity of CIA can be influenced by other factors such as heredity (Wooley *et al.*, 1981), gender (Leslie *et al.*, 1985; 1987; Holmdahl *et al.*, 1985) and diet (Prickett, Trentham & Robinson, 1984). In a previous report by Leslie *et al.* (1985) we found that mice maintained before disease induction on a diet in which the only source of fat was fish oil, rather than corn oil, developed significantly less severe disease. Fish oil diets are unique in that they contain eicosapentaenoic acid (EPA), an unusual prostaglandin (PG) precursor, which is entirely absent from vegetable oil and non-marine animal fats. Since EPA is rapidly incorporated into macrophage phospholipids which then alter the PG profile of this cell, the decreased severity of CIA may reflect an altered ratio of EPA to arachidonate (AA) metabolite(s) at sites of articular swelling. The aim of this experiment was to determine if the clinical and metabolic effects of an EPA enriched diet extend beyond the induction of CIA to alleviate arthritis severity after the disease is established.

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MATERIALS AND METHODS

Diet

The two diets used in this experiment were similar except for the source of fat (5% by weight). In the control diet the fat was corn oil (Mazola, Best Foods, Union, NJ) and in the experimental diet it was fish oil (Maxepa; R. P. Scherer, NA, Troy, MI). The other components of this diet by weight were: vitamin-free

casein, 18%; corn starch, 33·55%; sucrose, 33·55%; cellulose, 5%; DL-methionine 0·3%; choline chloride, 0·1%; salt mix AIN 76, 3·5%; vitamin mix, 1%. The composition of the vitamin mix per kg was as follows: Vitamin A acetate, 150,000 IU; vitamin D, 15,000 IU; vitamin E, 3,000 IU; menadione sodium bisulphite, 200 mg; biotin, 20 mg; folacin, 200 mg; inositol, 2,380 mg; niacin, 3,000 mg; calcium pantothenate, 1,600 mg; riboflavin, 700 mg; thiamin, 600 mg; vitamin B6, 7800 mg; vitamin B12, 1 mg.

Mice

Colonies of B10.RIII mice were established from breeding pairs obtained from Dr Chella S. David of Mayo Medical School, Rochester, MN. Eight-week-old mice separated by sex were housed four to a cage and given fresh food and water *ad libitum* every day. Forty-six mice fed a corn oil diet and 25 mice fed a fish oil diet were weighed at weekly intervals until the termination of the experiment 17 weeks later. After 4 weeks on these diets all mice were injected with CII and examined every 4 or 5 days for the onset of arthritis. When arthritis was first observed, 31 days after immunization half of the corn oil fed mice were distributed between two groups. The first group continued to be fed the corn oil diet while the second group was switched to a fish oil diet. The three groups of mice (corn oil fed, fish oil fed and switched group) were monitored for arthritis for a further 9 weeks. Mice were then killed and peritoneal macrophages isolated by adherence. Macrophages were either analysed for fatty acids present in their phospholipid pool or incubated for 24 h and the incubation medium analysed for PG and thromboxane (TX). Thirty-two days after immunization, blood samples were taken by retroorbital sinus puncture. Eighty days after immunization, when the severity of the arthritis was declining, a second blood sample was taken. The serum was stored at −20°C for determination of anti-collagen antibodies.

Determination of anti-collagen antibodies

Antibody levels to native type II collagen were measured on serially diluted sera by a passive haemagglutination assay, using tanned type O Rh-negative red blood cells to which native bovine type II collagen had been covalently attached (Cathcart *et al.*, 1986). In mouse serum, this assay provides an excellent quantitative correlation ($P < 0.001$) with an enzyme-linked IgG-specific immunosorbent assay.

CII injections

Native CII was isolated from fetal bovine nasal septa or articular cartilage as described by Leslie *et al.* (1985). Amino acid composition was consistent with that described for this type of collagen and yielded a single band on SDS gels. CII (100 μg in 50 μl of 0·5 M acetic acid) was emulsified in 50 μl of complete Freunds adjuvant and injected intradermally into the base of the tail.

Arthritis assessment

The severity of CIA was recorded for 13 weeks after immunization by a single observer who was blinded to the experimental protocol. Arthritis was assessed clinically every week by scoring each paw on a 0–4 scale: 0, none; 1, probable swelling; 2, definite but minimal swelling; 3, moderate and 4, severe. The severity index based on equal numbers of males and females in each group, was the total severity score at each time of observation divided by the number of mice with disease. The highest score (maximum 16) attained by each mouse within the entire period of observation was used to calculate mean peak severity.

Macrophage preparation

Six millilitres of buffer (HBSS) was injected intraperitoneally and the abdominal fluid withdrawn after massage. Cells from this fluid were gently pelleted, washed twice with HBSS and resuspended in 1 ml RPMI 1640 medium containing 10% FCS. After adjusting the cell concentration to $1{\cdot}5 \times 10^6$ cells per ml, 1 ml samples were placed on plastic titre wells, incubated for 2 h and the non-adherent cells removed by washing. By counting the non-adherent cells the number of adherent cells were obtained by difference. These adherent cells were then used for phospholipid extraction or were further incubated for PG and TX analysis of the medium.

Fatty acid analysis

Total lipids were extracted from macrophages by the method of Folch, Lees & Sloane-Stanley (1957). Briefly, the cells were pelleted and 12 ml of chloroform/methanol (2:1) added. After sonicating, 2·5 ml of 0·88% KCl was added, and the mixture centrifuged (10,000 ***g*** for 10 min). The lower phase was washed and neutral lipids, free fatty acids and glycolipids were removed by passage through a silicic acid column and the phospholipid fraction eluted with methanol (Vance & Sweeley, 1967). After alkaline methanolysis (Kishimoto & Hoshi, 1972) the fatty acid methyl esters (FAME) were extracted, washed, concentrated and analysed by gas chromatography. Dietary fatty acids were similarly converted to their methyl esters.

Gas chromatography

The FAME fraction was dissolved in 20 μl of hexane, and 4 μl injected. The FAME was eluted from the column (OV 225) with a linear thermal gradient of 170–220°C over 25 min, and held at the final temperature for a further 25 min. To separate docosapentaenoic (DPA) from docosahexaenoic acid (DHA), the FAME fraction was also chromatographed over a second column (OV 1) with a linear thermal gradient of 170–220°C. The detector was of the flame ionization type.

PG determinations

PGE_2 and PGI_2 (assayed as 6 keto $PGF_{1\alpha}$) and TXA_2 (assayed as TXB_2) were quantified by RIA using antibodies described by Leslie *et al.* (1984; 1985).

RESULTS

Fatty acid composition of the diet

As previously documented (Leslie *et al.* 1985) the fish oil contained only 2% of the essential omega 6 fatty acid, linoleic acid (LA) compared to the 65% found in corn oil (Fig. 1). The three omega 3 fatty acids found in fish oil EPA, DPA and DHA were entirely absent from corn oil.

Fatty acid composition of macrophage phospholipids

The fish oil fed group incorporated EPA and its metabolite DPA, whereas EPA and DPA were entirely absent from the

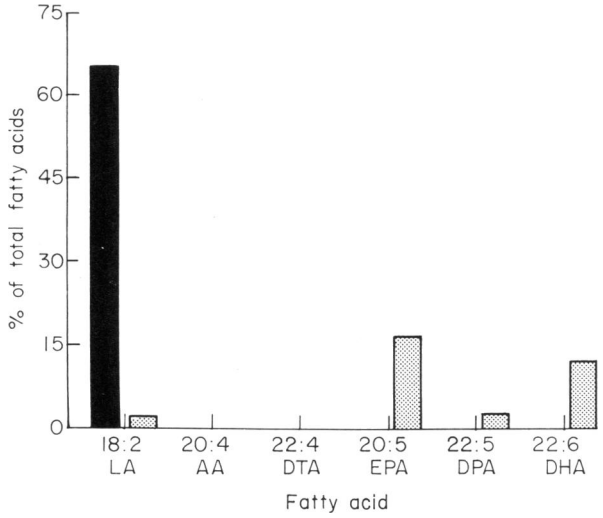

Fig. 1. The fatty acid composition of fish oil (▨) and corn oil (■). The fatty acids present in the corn oil and fish oil diets were analysed as described in Materials and Methods. LA, 18:2 omega 6 fatty acid, linoleate; AA, 20:4 omega 6 fatty acid, arachidonate; DTA, 22:4 omega 6 fatty acid, docosatetraenoate; EPA, 20:5 omega 3 fatty acid, eicosapentaenoate; DPA, 22:5 omega 3 fatty acid, docosopentaenoate; DHA 22:6 omega 3 fatty acid, docosahexaenoate. EPA, DPA and DHA were only present in fish oil while the omega 6 essential fatty acid LA, was significantly less than that present in corn oil.

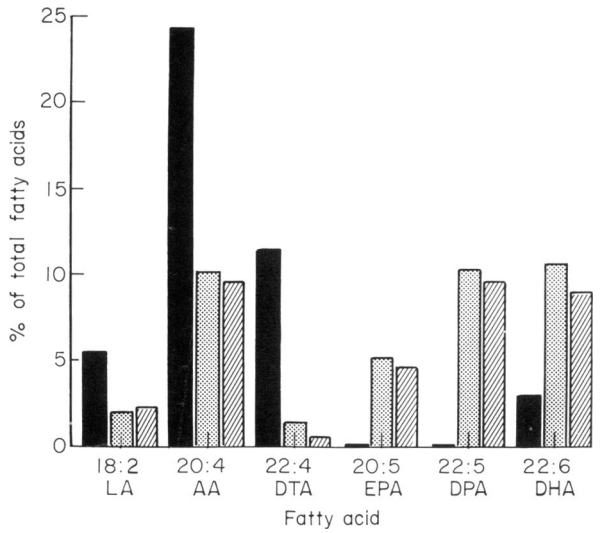

Fig. 2. The fatty acid composition of macrophage phospholipids isolated from mice fed a fish oil diet (□), a corn oil diet (■) or switched from a corn to fish oil diet (■). Macrophages were isolated from mice on the three different dietary protocols 9 weeks after immunization. Phospholipids were extracted and after acid methanolysis fatty acids were determined as described in Materials and Methods. The group of mice which had been switched to fish oil showed the characteristic fatty acid profile of the fish oil fed group. Fatty acid symbols are described in Fig. 1.

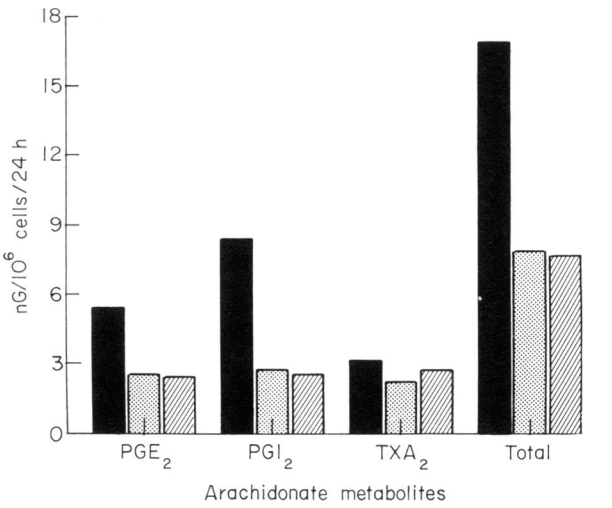

Fig. 3. The prostaglandin profile of macrophages isolated from mice fed a fish oil diet (▨), a corn oil diet (■) or switched from a corn to a fish oil diet (■). Thirteen weeks after immunization macrophages isolated from mice on the three different dietary protocols were incubated for 24 h and PG and TX in the medium assayed by RIA. The group of mice switched to fish oil showed a macrophage PG profile characteristic of the fish oil fed rather than corn oil fed group.

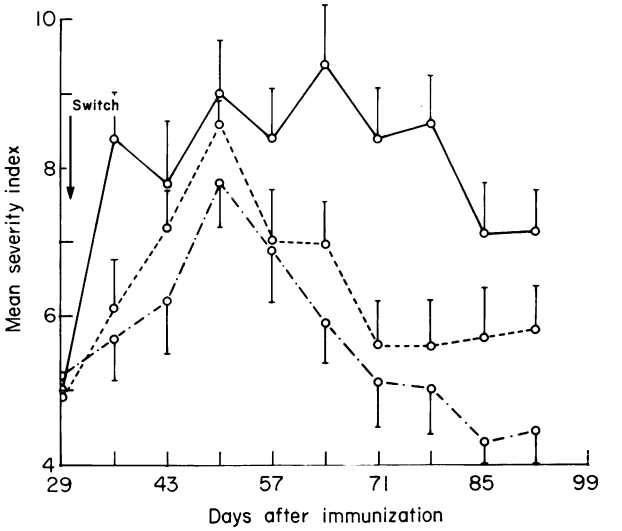

Fig. 4. The severity of arthritis in mice fed a fish oil diet (O- · -O), a corn oil diet(O——O) or switched from a corn to a fish oil diet (O- - -O). Eight-week-old B10.RIII mice were fed either a fish or corn oil diet and after 4 weeks all mice were immunized with CII. At the onset of arthritis 31 days later, half of the corn oil fed mice were switched to fish oil. Arthritis was assessed and the mean severity index +s.e.m. calculated weekly (Materials and Methods) for 9 weeks for all three dietary groups. At each time of assessment the corn oil fed group had more severe arthritis than the fish oil fed group or the group switched to fish oil after the onset of arthritis. By the Sign test this was highly significant ($P < 0.001$).

macrophages of the corn oil fed group. Compared to the fish oil fed group the corn oil fed group had a greater percentage of AA and LA. The group of mice which had been switched to fish oil after 31 days on the corn oil diet showed the characteristic fatty acid profile of the fish oil fed group (Fig. 2).

Table 1. Effect of diet on antibody responses to immunization with Type II collagen

Diet	Arthritis	Antibody titre after immunization	
		32 days	80 days
Fish	Present	11·5±1·2	13·0±1·7
Switched	Present	13·0±0·7	13·9±1·3
Corn	Present	12·7±0·9	13·2±1·1
Fish	Absent	9·1±2·2	12·3±1·8
Switched	Absent	10·8±1·7	11·7±2·4
Corn	Absent	10·1±1·6	11·1±1·6

B10.RIII mice fed a fish or corn oil diet or switched from a corn to fish oil diet 31 days after immunization were bled retroorbitally 32 days (1 day after switch) and 80 days after immunization with type II collagen. Anti-collagen antibody titres, expressed as the mean $-\log_2$ (dilution) ±s.d., were determined by passive haemaglutination. Fish oil fed mice with CIA had significantly less ($P<0·001$) antibody than the other two groups 32 days after immunization. At 80 days there were no differences between the groups. In all groups mice without arthritis had consistently less antibody than mice with arthritis.

Table 2. The effect of diet on growth of B10.RIII mice

Dietary group	% weight gain ± s.d.
Chow	27·5±3·4
Fish	27·5±8·8
Switch	26·2±8·2
Corn	32·0±8·4

Eight week B10.RIII mice were fed for 17 weeks standard rodent chow or a diet in which the only source of fat was fish or corn oil. The switched group was fed the corn oil diet for 8 weeks and the fish oil diet for the remaining 9 weeks. There was no significant difference in weight gain between the four groups.

PG determinations

In the corn oil fed mice, significantly more PG was found in the medium, relative to the fish oil fed mice. The group of mice switched to fish oil showed a macrophage PG profile characteristic of the fish oil fed rather than the corn oil fed group (Fig. 3).

Arthritis incidence and severity

Arthritis was first observed 31 days after immunization when 36% of the fish oil fed mice and 48% of the corn oil fed mice had arthritis with severity indices of 5·2 and 5·0 respectively. After division of the corn oil fed group each had the same % mice with disease (48%) with the same severity index (5·0). By the end of the experiment 9 weeks later, 76% of the fish oil fed mice, 77% of the corn oil fed mice and 80% of the switched mice had developed the disease. As previously reported (Leslie, Gonnerman & Cathcart, 1987) the incidence of disease among males 82%) was greater than among females (74%). Based on equal numbers of males and females in the dietary groups, the corn oil fed group had higher severity indices than the fish oil fed group at each time of arthritis assessment (Fig. 4). By the Sign test for nonparametric data (Rosner, 1982) this was highly significant ($P<0·001$). The mice switched to a fish oil diet 31 days after immunization had a mean severity index only slightly less than that of the corn oil group for 3 weeks after the switch. Four weeks after the switch and for the remainder of the experiment the severity index fell to resemble that of the fish oil group. Again by the Sign test for nonparametric data this decreased severity compared with the corn oil fed control is highly significant ($P<0·001$). Throughout the observation period, female mice had significantly less severe disease than males from the same dietary group (data not shown). The mean peak severities of the fish oil fed group (8·6±1·1 males and 7·3±0·9 females) and the switch group (10·1±0·8 males and 7·3±0·8 females) were significantly less at the $P<0·1$ level than that of the corn oil fed group (12·2±1·3 males and 9·1±0·9 females).

Diet and anti-collagen antibodies

Between the two bleeding times the antibody titre approximately doubled. In every group, mice that failed to get arthritis by the end of the experiment had significantly lower titres than mice with arthritis. Of mice with arthritis the fish oil fed group had significantly less antibody 32 days after immunization while at 80 days there were no differences between the groups (Table 1).

Mice weights

Mice fed fish oil or switched to fish oil gained weight at the same rate as mice fed rodent chow. Although there was a tendency for mice on the corn oil diet to gain more weight than the other groups this was not significant (Table 2).

DISCUSSION

A fish oil diet was first applied to CIA by Prickett *et al.* (1984) who found that the incidence of arthritis in female Sprague-Dawley rats was increased by feeding a fish oil compared to a beef tallow diet. A divergent clinical response to fish oil diets in different species may be due to genetic factors (Griffiths & DeWitt, 1984) since when a fish oil diet was fed to B10.RIII mice before immunization with CII we found arthritis severity to be less than in corn oil-fed mice (Leslie *et al.*, 1985). We now show that a fish oil diet is still able to reduce arthritis severity even when begun after the onset of arthritis. Although recent reports by Kremer *et al.* (1985) and Sperling *et al.* (1986) suggest that an EPA-enriched diet can benefit patients with rheumatoid arthritis, this is the first time a salutatory effect has been documented in an animal with progressive arthritis.

Stuart *et al.* (1982) suggested that high titres of anticollagen antibodies have a role in CIA in that, as we found, lower titres are associated with mice that never develop the disease. We also noted that 32 days after collagen immunization antibody production was much less in the fish oil fed mice, the same group that developed less severe disease relative to the corn oil fed mice. However the mice switched from corn oil to a fish oil diet also developed less severe arthritis without any apparent effect on antibody titres. If a fish oil diet modulates CIA by retarding anticollagen antibody production, either the antibodies assayed here do not reflect the actual level of antibody subclass to the arthrogenic epitope(s) and/or other factors are influencing disease severity.

Metabolic alterations that accompany a fish oil diet include an increase in generation of immunoreactive LTB_5 over LTB_4 in response to an inflammatory stimulus (Lee *et al.*, 1985), a decrease in the two series of PG produced by kidney and liver slices (Kelley *et al.*, 1985), an increase in the synthesis of three series of PG in the kidney and decreased surface Ia expression by resident peritoneal macrophages (Kelley *et al.*, 1985). Incubated macrophages from mice continuously fed or switched to a fish oil diet also showed a reduced accumulation in the amount of two series of PG in the medium which reflected the decreased AA and/or incorporation of EPA in the phospholipid pool. We also noted that with mice fed a fish oil diet the increase in EPA:AA ratio in macrophage phospholipids occurred rapidly and had plateaued by 5 weeks on the diet (Leslie *et al.*, 1985). Thus the clinical improvement which was most evident 4 weeks after the switch to fish oil, fits into the expected time frame for maximum change in macrophage fatty acid profile and eicosanoid production typical of a fish oil diet.

It is well documented that PGs play a role in immunity and inflammation (Goodwin & Webb, 1980). When the PG profile of inflammatory cells is altered by a fish oil diet these cells show functional changes that tend to reduce inflammation (Leitch *et al.*, 1984; Lee *et al.*, 1985; Sperling *et al.*, 1985). Since there appears to be a correlation between immune cell function and macrophage eiscosanoid profiles, it is not unexpected that fish oil diets decrease disease severity in CIA even during the inflammatory response. The finding that dietary manipulation does not have a consistent effect on the incidence of CIA also supports our view that fish oil does not directly affect the mechanism by which collagen induces arthritis (Leslie *et al.* 1987) but rather suppresses inflammation in target organs.

Until recently, diet alone as a therapeutic measure has not proved beneficial when examined in a controlled fashion (Ziff, 1983). However, our findings in murine CIA taken together with those of Robinson *et al.* (1986) in murine lupus, demonstrate the potential for fish oil diets to retard inflammatory processes. Although further work is needed to determine the active components of these diets and the specific cellular and biochemical events through which they mediate their clinical effects, EPA enriched diets hold promise as non-invasive, low cost anti-rheumatic disease agents.

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REFERENCES

CATHCART, E.S., HAYES, K.C., GONNERMAN, W.A., LAZZARI, A.A. & FRANZBLAU, C. (1986) Experimental arthritis in nonhuman primate (I) induction by bovine type II collagen. *Lab. Invest.* **54,** 26.

COURTENAY, J.S., DALLMAN, M.J., DAYAN, A.D., MARTIN, A. & MOSEDALE, B. (1980) Immunization against heterologous type II collagen induces arthritis in mice. *Nature* **283,** 666.

FOLCH, J., LEES, M. & SLOANE-STANLEY, G.M. (1957) A simple method for the isolation and purification of total lipids from animal tissues. *J. biol. Chem.* **266,** 497.

GOODWIN, J.S. & WEBB, D.R. (1980) Regulation of the immune response by prostaglandins. *Clinical Immunol. Immunopathol.* **15,** 106.

GRIFFITHS, M.M. & DEWITT, C.W. (1984) Genetic control of collagen-induced arthritis in rats: the immune response to type II collagen among susceptible and resistant strains and evidence for multiple gene control. *J. Immunol.* **132,** 2830.

HOLMDAHL, R., JANSSON, L., LARSSON, E., RUBIN, K. & KARESKOG, L. (1986) Homologous type II collagen induces chronic and progressive arthritis in mice. *Arthr. Rheum.* **29,** 106.

KELLEY, V.C., FERRETTI, A., IZUI, S. & STROM, T.B. (1985) A fish oil diet rich in eicosapentaenoic acid reduces cyclooxygenase metabolites and suppresses lupus in MRL-1pr mice. *J. Immunol.* **134,** 1914.

KISHIMOTO, Y. & HOSHI, M. (1972) Isolation, purification and assay of fatty acids and steroids from the nervous system. *Methods Enzymol.* **3,** 75.

KREMER, J.M., BIGAUOETTE, J., MICHALEK, A.V., TIMCHALK, M.A., LININGER, L., RYNES, R.I., HUYCK, C., ZIEMINSKI, J. & BARTHOLOMEW, L.E. (1985) Effects of manipulation of dietary fatty acids on clinical manifestations of rheumatoid arthritis. *Lancet* **i,** 184.

LEE, T.H., HOOVER, R.L., WILLIAMS, J.D., SPERLING, R.I., ROBINSON, D.R., COREY, E.J., LEWIS, R.A. & AUSTEN, K.F. (1985) Effect of dietary enrichment with eicosapentaenoic and docosahexaenoic acids on *in vitro* neutrophil and monocyte leukotriene generation and neutrophil function. *N. Engl. J. Med.* **312,** 1217.

LEITCH, A.G., LEE, T.H., RINGEL, E.W., PRICKETT, J.D., ROBINSON, D.R., PYNE, S.G., COREY, E.J., DRAZEN, J.M., AUSTEN, K.F. & LEWIS, R.A. (1984) Immunologically induced generation of tetraene and pentaene leukotrienes in the peritoneal cavities of menhaden-fed rats. *J. Immunol.* **132,** 2559.

LESLIE, C.A., PAVLAKIS, A., WHEELER, J. & KRANE, R. (1984) Arachidonate cascade products in rabbit bladder: neurophysiological significance? *J. Urol.* **132,** 376.

LESLIE, C.A., GONNERMAN, W.A., ULLMAN, M.D., HAYES, K.C., FRANZBLAU, C. & CATHCART, E.S. (1985) Dietary fish oil modulates macrophage fatty acids and decreases arthritis susceptibility in mice. *J. exp. Med.* **163,** 1336.

LESLIE, C.A., GONNERMAN, W.A. & CATHCART, E.S. (1987) Gender differences in eicosanoid production from macrophages of arthritis susceptible mice. *J. Immunol.* **138,** 413.

PRICKETT, J.D., TRENTHAM, D.E. & ROBINSON, D.R. (1984) Dietary fish oil augments the induction of arthritis in rats immunized with type II collagen. *J. Immunol.* **132,** 725.

ROBINSON, D.R., PRICKETT, J.D., MAKOUL, R.T., STEINBERG, A.D. & COLVIN, R.B. (1986) Dietary fish oil reduces progression of establshed renal disease in (NZB × NZW) F_1 mice and delays renal disease in BXSB and MRL/1 strains. *Arthritis Rheum.* **29,** 539.

ROSNER, B. (1982) *Fundamentals of Biostatistics*, p. 264. PWS Publishers, Boston.

SPERLING, R.I., WEINBLATT, M., ROBIN, J.L., COBLYN, J., FRASER, P. LEWIS, R.A. & AUSTEN, K.F. (1986) Effects of dietary fish oil in in vitro leukocyte function, leukotriene generation and activity o disease in rheumatoid arthritis. *Arthr. Rheum.* **29,** S18.

STUART, J.M., CREMER, M.A., TOWNES, A.S. & KANG, A.H. (1982) Type II collagen-induced arthritis in rats: passive transfer with serum and evidence that IgG anticollagen antibodies can cause arthritis. *J. exp Med.* **155,** 1.

TRENTHAM, D.E., TOWNES, A.S. & KANG, A.H. (1977) An experimenta model of arthritis. *J. exp. Med.* **146,** 857.

VANCE, D.E. & SWEELEY, C.C. (1967) Quantitative determination of th neutral glycosyl ceramides in human blood. *J. Lipid Res.* **8,** 621.

WOOLEY, P.H., LUTHRA, H.S., STUART, J.M. & DAVID, C.S. (1981) Type II collagen-induced arthritis in mice (I) major histocompatibility complex (I-Region) linkage and antibody correlates. *J. exp. Med.* **154** 688.

WOOLEY, P.H., DILLON, A.M., LUTHRA, H.S., STUART, J.M. & DAVID C.S. (1983) Genetic control of type II collagen-induced arthritis in mice: factors influencing disease susceptibility and evidence fo multiple MHC-associated gene control. *Transplant. Proc.* **25,** 180.

ZIFF, M. (1983) Diet in the treatment of rheumatoid arthritis. *Arth Rheum.* **26,** 457.