# The Eicosapentaenoic to Docosahexaenoic Acid Ratio of Diets Affects the Pathogenesis of Arthritis in Lew/SSN Rats[1]

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ABSTRACT Dietary-induced changes in tissue levels of polyunsaturated fatty acids modify inflammatory reactions through changes in the synthesis of lipid and peptide mediators of inflammation. Four semipurified 20% fat diets, based on beef tallow (BT), safflower oil (SFO), docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) were provided. The DHA and EPA ratios of the (n-3) fatty acid-based diets were 1.1 and 3.4, respectively. The effect of prefeeding diets differing in EPA to DHA ratios prior to the induction of streptococcal cell wall (SCW) arthritis in female Lew/SSN rats was examined. Weanling rats were fed diets for 5 wk before arthritis induction and 5 wk post-arthritis induction. Footpad thickness, hock circumference, plasma and macrophage fatty acids and histological assessment were compared. There were no differences in food intake and final body weights among the groups. Footpad inflammation, reported as percentage change (adjusted for growth) was greatest for rats fed the BT-based diet, intermediate in those fed the SFO-based diet and least for the rats fed the EPA- and DHA-based diets ($P < 0.05$). Macrophage phospholipids revealed cellular incorporation of EPA and DHA from the fish-oil based diets which modified lipid and peptide mediators of inflammation. Histological sections of rat hocks ranked by severity of arthritis-related changes suggested that the SFO- and EPA-based diets were more successful in ameliorating the destructive arthritic phase in hock joints than the BT- and DHA-based diets ($P = 0.09$) in this model of arthritis. The course of SCW-induced arthritis can be altered by diet-induced changes in macrophage fatty acid composition. The EPA-based diet is more effective in suppression of inflammation than the DHA-based diet. J. Nutr. 130: 559–565, 2000.

KEY WORDS: • *EPA* • *DHA* • *Lew* • *SSN rats* • *arthritis* • *dietary fat*

Polyarthritis induced in an appropriately susceptible strain of rat by intraperitoneal injection (ip)[4] of a sterile aqueous suspension of cell wall fragments in a volume of PBS provides a model for chronic proliferative synovitis with a defined humoral and cellular immunogenic response to the arthrogen (Trentham 1987). This immunopotentiating activity results from type-A bacterial species. The gram-positive bacterial cell walls consist of peptidoglycan linked covalently to a complex carbohydrate heteropolymer referred to as a peptidoglycan polysaccharide complex (PG-PS) which is poorly degraded, persisting in tissue for long periods of time.

Cromartie et al. (1977) initially developed this model of streptococcal cell wall (SCW) polymers and described it as the development of acute erythema with swelling in peripheral joints, 24-h post-systemic injection. This reaction peaked in severity at 3 d, then subsided. Fourteen to 28 d later, a chronic phase of swelling developed that persisted for months and ultimately destroyed the more severely involved joints.

Diet-induced changes in tissue fatty acid composition may modify inflammatory reactions. For example, in vitro studies have shown that eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) inhibit a number of lymphocyte functions (Calder, 1997). In vivo studies in laboratory animals utilizing diets rich in (n-3) polyunsaturated fatty acids (PUFA) (linseed, canola, fish oil) result in suppressed lymphocyte proliferation and activity (Calder et al. 1995). Direct comparisons of the effects of diets rich in (n-3) fatty acids indicate that fish oil is more suppressive than linseed or canola oil and (n-6) PUFA-rich diets appear to be less suppressive than those containing (n-3) PUFA (Mantzioris et al. 1994). Meta-analysis of clinical studies in rheumatoid arthritis (RA) (James and Cleland 1997) has shown that fish oil has moderate therapeutic effects when given in large quantities. Thus optimization of (n-3) PUFA treatment is essential for the improvement of the efficacy of this treatment: for improvement in compliance and to make it more practical.

There is little information concerning which (n-3) PUFA (EPA or DHA) is immunosuppressive, or how much (n-3) PUFA in relation to (n-6) PUFA is necessary to bring about

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[4] Abbreviations used: AA, arachidonic acid; BT, beef tallow; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; i.p., intraperitoneal injection; LA, linoleic acid; LNA, $\alpha$-linolenic acid; MUFA, monounsaturated fatty acids; ND, no disease; PG PS, peptidoglycan polysaccharide; PUFA, polyunsaturated fatty acids; RA, rheumatoid arthritis; SCW, streptococcal cell wall; SFO, safflower oil; SPF, specific pathogen-free.


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**TABLE 1**

*Fatty acid composition of the lipid of the experimental diets*[1]

| Fatty acid | Beef tallow | Safflower oil | DHA | EPA |
|---|---|---|---|---|
| | *g/100 g fatty acids* | | | |
| 14:0 | 2.5 | 0.2 | 2.0 | 1.4 |
| 16:0 | 25.0 | 8.1 | 18.3 | 15.3 |
| 16:1(n-7) | 1.5 | 0.2 | 2.3 | 3.3 |
| 18:0 | 17.7 | 2.9 | 13.4 | 9.9 |
| 18:1(n-9) | 41.3 | 16.0 | 29.9 | 27.4 |
| 18:1(n-7) | 1.8 | 0.8 | 1.6 | 0.0 |
| 18:2(n-6) | 9.2 | 70.8 | 7.5 | 8.6 |
| 18.3(n-3) | 0.4 | 0.6 | 0.5 | 0.6 |
| 20:1(n-9) | 0.4 | 0.4 | 0.4 | 0.4 |
| 20:3(n-6) | 0.0 | 0.0 | 0.1 | 0.2 |
| 20:4(n-6) | 0.0 | 0.0 | 0.6 | 0.8 |
| 20:5(n-3) | 0.0 | 0.0 | 11.0 | 22.2 |
| 22:5(n-3) | 0.0 | 0.0 | 2.1 | 1.9 |
| 22:6(n-3) | 0.0 | 0.0 | 10.0 | 6.6 |
| Total SFA | 45.3 | 11.3 | 33.8 | 26.6 |
| Total MUFA | 44.9 | 17.5 | 34.3 | 31.1 |
| Total (n-6) PUFA | 9.4 | 70.8 | 8.4 | 10.0 |
| Total (n-3) PUFA | 0.4 | 0.6 | 23.6 | 32.3 |
| EPA/DHA | | | 1.1 | 3.4 |

[1] SFA, saturated fatty acids; PUFA, polyunsaturated fatty acid; MUFA, monounsaturated fatty acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

these effects (Jeffery et al. 1997). Thus, in this study, the effect of two diets with different ratios of EPA and DHA were compared and contrasted with beef tallow (BT)- and safflower oil (SFO)-based diets. We investigated the effect of prefeeding these diets to weanling rats (prior to the induction of arthritis) on plasma total fatty acids, plasma phospholipids and macrophage phospholipids. We also investigated the effects of the induced synovitis on hock circumference and footpad thickness as evidence of inflammation and histological changes in hock sections as evidence of arthritic activity in joints.

## METHODS

***Animals.*** Weanling female (SPF) Lew/SSN rats (albino, a,h,c: RT1[l]) aged 3 wk, weighing between 30 to 50 g were obtained from the Animal Resources Center (Perth, WA, Australia). The rats were housed at the Faculty of Medicine and Health Sciences, University of Newcastle, in polypropylene cages with stainless steel wire tops for a period of 5 wk prior to induction of arthritis and then a further 5-wk post-induction (to observe the acute, chronic and remittive phases of the induced arthritis). The rats were housed individually in an open system with recycled paper-based bedding (Fibercycle, Mudgeerba, Australia) which was changed twice weekly. Free access to autoclaved water was provided via elongated waterspouts, and experimental diets were placed in special containers on the cage floor. Relative humidity ranged between 45 and 55% (humidity was measured but not regulated in the Medical Science Building animal facility). The environmental conditions included 23–25°C, (regulated at 23°C prior to arthritis induction and 25°C post-induction) 12:12 h artificial photoperiod and housing, handling, pain management and sample collection procedures conformed to the policies and recommendations of the University of Newcastle's Animal Care and Ethics Committee.

***Diets.*** The four experimental diets were derived from a semipurified 20% corn oil diet (without the corn oil) (Cat. No. 960245; ICN Nutritional Biochemical Cleveland, OH). Each diet contained 200 $g \cdot kg^{-1}$ lipid mixture to provide diets predominantly either BT or SFO or DHA and EPA, BT and SFO were used as control diets. (**Table 1**). Proportions of the basal diet components and oil combinations used in the ICN semipurified diet are listed in **Table 2**. Formulation of these diets followed the protocol set out by the Ad Hoc Committee (1977) and Reeve et al. (1993). All diets were standardized to contain 600 $mg \cdot kg^{-1}$ (racemic mix of RRR and SRR isomers) $\alpha$-tocopherol acetate (ICN). DHA and EPA oils were provided by Lube AS (Hadsund, Denmark). The vitamin E was necessary to compensate for the vitamin E in the fish oil, to ensure that the vitamin E concentration was the same in all diets. Diets were prepared weekly, flushed with nitrogen, stored at −20°C and provided fresh to the rats each day. There were five to seven rats allocated to each diet, with two rats in the BT and DHA groups without arthritis. Four rats were used as controls or as no disease (ND) rats for growth comparison purposes. Oral buprenorphine was used for pain management. Sublingual tablets (0.3 mg) dissolved in jelly were provided 12 hourly.

***Induction and monitoring of arthritis.*** Rats ($n$ = 5–7) weighing 150 g and prefed experimental diets were injected intraperitoneally with sterile peptidoglycan group A polysaccharide (PG-APS) polymers produced from *Streptococcus pyrogenes* (Lot No. 12202) from Lee Laboratories (Grayson, GA) at a dose rate of 15 $\mu g$ rhamnose $\cdot$ g $body^{-1}$. All rats displayed a marked acute onset of arthritis followed by remission, prior to the development of the chronic phase of this model of arthritis. Rat hock circumferences and footpad thicknesses were measured prior to arthritis induction and at 4-d intervals until the termination of the experiment. Footpad thicknesses (as a measure of edema and arthritis activity) were measured using a specially adapted dial calliper, and hock circumferences were measured using a modified rule tool calibrated to gradations of 0.01 mm. An average of three measures was taken at each stage. Rats were checked daily using an adaptation of the Morton and Griffiths Adverse Effects Scoring

**TABLE 2**

*Proportions of the basal diet components and oil combinations used in the ICN semi-purified diets*

| Component[1] | |
|---|---|
| | *g/kg diet* |
| High nitrogen casein | 200.0 |
| DL-Methionine | 3.0 |
| Sucrose | 355.8 |
| Corn starch | 150.0 |
| Alpacel (nonnutritive bulk) | 50.0 |
| DL-$\alpha$-tocopherol acetate powder (250 IU/g) | 1.2 |
| AIN-76 Mineral mix (AIN-93M-MX)[2] | 40.0 |
| ICN Vitamin mix (AIN-93-VX)[3] | 12.0 |
| Total lipid[4] | 200.0 |
| *Oil Combinations* | |
| BT diet | |
| Beef tallow | 180.0 |
| Safflower oil | 20.0 |
| SFO diet, Safflower oil | 200.0 |
| DHA diet | |
| Beef tallow | 126.0 |
| Safflower oil | 20.0 |
| DHA oil | 54.0 |
| EPA diet | |
| Beef tallow | 110.0 |
| Safflower oil | 20.0 |
| EPA oil | 70.0 |

[1] Ingredients of semipurified diet purchased as high corn oil diet (20% corn oil) without the added corn oil. (ICN Nutritional Biochemical, Cleveland, OH. Catalogue No. 960246.) Lipid content was added to compensated for the absence of corn oil.

[2] American Institute of Nutrition. Report of the American Institute of Nutrition Ad Hoc Committee on Standards for Nutritional Studies. J. Nutr. 1997; 107:1340–1348.

[3] Reeve et al. (1993).

[4] Fatty acids and the proportions of each used to constitute the experimental diets are listed in Table 1.

## TABLE 3

*Adverse Effects Scoring Chart for arthritic laboratory animals in distress*[1]

| Score | Appearance | Body weight | Clinical signs | Response to stimuli | Behavior |
|---|---|---|---|---|---|
| 0 | Normal | Maintained body temperature/ respiration/heart rate | Normal | Normal | Normal |
| 1 | Lack of grooming | 5% weight loss | Minor changes in respiration | Slightly changed response | Minor changes |
| 2 | Starry coat | Food intake decreased 5–10% weight loss | Increased body temperature/heart rate/respiration | Moderate change in response | Not normal |
| 3 | Coat very starry/abnormal postures | Food intake nil 20% weight loss | 50% increase in respiration/heart rate | Violent reaction/weak reaction/comatosed | Self mutilating/ vocalizing |

| Scoring[2] | |
|---|---|
| Normal | (0–4) |
| Monitor carefully | (5–9) |
| Pain relief must be considered | (10–14) |
| Ample evidence of severe pain and distress termination must be considered | (15–20) |

[1] Adapted from Morton and Griffiths (1985).
[2] Method of scoring, the scores for each variable are added together to provide a total score of distress.

Chart (Morton and Griffiths 1985) (**Table 3**). If the severity of the arthritis indicated the need for greater pain management, a subcutaneous injection of buprenorphine (0.01 mg) was given. Rats were routinely monitored until the termination of the experiment.

***Isolation and purification of peritoneal macrophages.*** Three days before collection of elicited macrophages, anesthetized rats were given an intraperitoneal injection of 50 g/L cornstarch solution (20 mL · kg body weight$^{-1}$). Cells were collected by peritoneal lavage with 20 mL of warm PBS (Sigma Chemical Co., St. Louis, MO). The total cell population from one animal was pelleted by centrifugation at 400 × *g* for 10 min, resuspended in Dulbecco's modification of Eagle's medium medium containing 5% heat-inactivated fetal calf serum, plated in four 100 x 15 mm sterile polystyrene culture dishes, incubated in 5% $CO_2$ atmosphere at 37°C for 3 h to allow macrophage adherence. Nonadherent cells and medium were then removed by aspiration and culture dishes rinsed with a volume of warm PBS. This removed up to 50% of the total cell number but provided a macrophage population that was >90% pure as indicated by morphology and staining with $\alpha$ naphthyl acetate esterase (Sigma Chemical Co.). Cell viability as determined by phase contrast microscopy and trypan blue exclusion was >95%.

***Histology.*** At the conclusion of the experiment, rats were killed by $CO_2$ inhalation, skinned hock joints were removed, fixed in 10% formalin, progressively decalcified with final fixing in amyl acetate. Paraffin sections were then prepared and stained with hematoxylin and eosin stain. The sections were then assessed independently by a veterinary pathologist (Veterinary Pathology Services, Cooparoo, Australia) who was unaware of the diets and diet group of the rats. This laboratory was certified by the American College of Pathologists. The rat hocks were graded by severity of histological change overall and by seven aspects of joint change as indicated in **Table 4**.

***Lipid extraction and analysis.*** Blood was collected from anesthetized rats by cardiac puncture, plasma was separated by centrifugation at 1300 × *g* and stored at −20°C until analyzed. Freshly adhered macrophages were harvested using a plastic scraper and lysed by sonication for 2 min over ice with a cell disrupter in chloroform: methanol (2:1, v/v) with 0.002% of butylated hydroxytoluene and stored at −20°C until analyzed. Plasma samples were transesterified for plasma fatty acid analysis using the method by Lepage and Roy (1986). Lipids from plasma samples were extracted using the method by Folch et al. (1957) and phospholipid fractions separated by TLC (Christie 1982). Heptadecanoic acid was added to the lipid extracts as an internal standard and separation was carried out on silica gel plates by using hexane/diethyl ether/acetic acid (85:15:1) as the solvent system. After plates were dried and phospholipids identified, the phospholipids were scraped from the plates and redissolved in hexane. Macrophage and plasma phospholipids were then methylated using (14 g/100 g) $BF_3$-methanol reagent and heated for 1 h at 100°C

## TABLE 4

*Dietary treatment effects on the severity of histological changes in hocks of Les/SSN rats fed following streptococcal cell wall (SCW) arthritis induction*[1]

| Variable[3] | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|---|
| | *n* | Rankings[2] | | | | | | | |
| Beef tallow | 5 | 2.6 ± 0.4 | 2.6 ± 0.6 | 1.8 ± 0.5 | 2.0 ± 0.6 | 1.4 ± 0.4 | 1.4 ± 0.4 | 2.6 ± 0.2 | 2.6 ± 0.4 |
| Safflower oil | 7 | 2.1 ± 0.3 | 1.4 ± 0.3 | 1.0 ± 0.1 | 1.6 ± 0.4 | 1.4 ± 0.4 | 1.3 ± 0.3 | 1.0 ± 0.1 | 2.3 ± 0.3 |
| DHA | 5 | 2.4 ± 0.2 | 2.3 ± 0.3 | 1.0 ± 0.4 | 2.9 ± 0.3 | 1.0 ± 0.1 | 1.9 ± 0.3 | 2.6 ± 0.3 | 2.6 ± 0.2 |
| EPA | 7 | 2.4 ± 0.2 | 1.8 ± 0.4 | 1.0 ± 0.1 | 2.7 ± 0.4 | 1.4 ± 0.4 | 1.0 ± 0.2 | 2.4 ± 0.1 | 2.4 ± 0.1 |

[1] Data are means ± SEM of ranks.
[2] Rankings: 1 = normal; 2 = minimal change; 3 = mild change; 4 = moderate change; 5 = severe change.
[3] Variables: (1) synovial hyperplasia; (2) periarticular soft tissue inflammation (nonsuppurative); (3) periarticular soft tissue inflammation (suppurative), (4) periarticular fibroplasia, (5) pannus formation, (6) erosion, destruction of synovium, cartilage, bone, (7) osteoclastic activity, osteoporosis, (8) overall grade of arthritic changes.

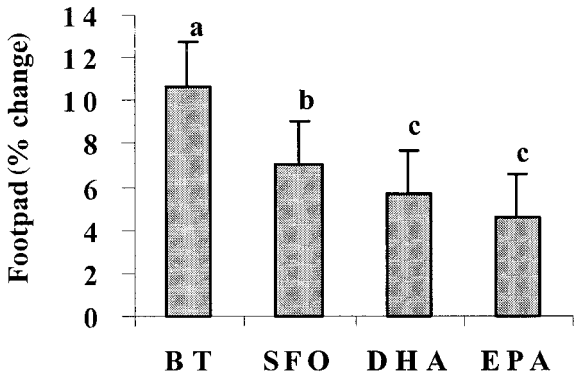


**FIGURE 1** Percentage change in footpad thicknesses as a result of inflammation induced by Streptoccocal cell wall (SCW) model of arthritis in female Lew/SSN rats fed beef tallow (BT), safflower oil (SFO), DHA or EPA diets for 10 wk. Data are mean ± SEM, $n = 5–7$. Values that do not share a letter are significantly different, $P < 0.05$.

using a method of Metcalfe and Schmitz (1961). Analysis of methyl esters was performed using capillary gas chromatography (Hewlett-Packard HP6890, NSW, Australia). The column was a 30 m x 0.25 mm (DB-225) fused carbon-silica column coated with cyanopropylphenyl (J&W Scientific, Folsom, CA), and a method modified by Garg and Blake (1997) was used to analyze the fatty acids. Methyl esters were separated using ultra high purity hydrogen as the carrier gas, injector and detector temperatures were maintained at 250°C, column temperature was programmed for 170 to 220°C at a rate of 10°C per min up to 190°C and then a rate of 3°C per min up to 220°C. The total run time was 30 min, split ratio was 10:1 and a 5 μL injection volume was used. The chromatograph utilized a flame ionizer detector and auto-sampler. Sample fatty acid methyl ester (FAME) peaks were identified by retention time comparison of authentic standard mixtures of FAME (Nu-Chek-Prep Inc., Elysian, MN), using Chemstations version A.04.02 software for gas chromatographic analysis.

***Statistical analysis.*** Data are means ± SEM of five to seven rats fed each diet. Differences among blood lipids were determined using Kruskal Wallis test and footpad, and hock differences were determined using random intercept, normal model for each rat, with fixed time and group effects. For all comparisons, $P < 0.05$ was considered significant.

Usually, a one-way ANOVA test would be used to analyze continuous variables such as blood lipids. However, ANOVA assumes that the outcome is normally distributed. In this case, small numbers and a question over the distribution of the outcomes mean that the nonparametric analog of one-way ANOVA must be used to analyze blood lipid levels. The analyses of footpad and hock differences over time, the repeated nature of the measurements must be taken into account. Because there is no reason to believe that a rat footpad or hock measurement changes over time except for treatment differences, an ANOVA model with a random intercept for each rat is an appropriate method of analysis in the case of these measurements (Laird and Ware 1982).

## RESULTS

***Rat growth.*** The diets used in this study were isocaloric, and there was no difference in food intake among rats fed the different diets (data not shown). Final body weights, and thus the total growth, were not different among rats fed the different diets. Rats displayed a marked acute onset of arthritis followed by remission, prior to the development of the chronic phase of this model of arthritis. All rats given an ip injection of PG-PS developed arthritis. Body weights and food intake decreased after the induction of arthritis in all groups. ND rats maintained a steady rate of growth and rats were more active and had a lower mean body weight ($P < 0.05$) than the arthritic rats.

***Footpad and hock swelling.*** Footpad inflammation of rats fed the four diets varied in intensity (**Fig. 1**). There was a significantly greater percentage change in footpad thickness (adjusted for growth) in the BT diet-fed rats than in the SFO, DHA and EPA diet-fed rats. Inflammation in DHA and EPA diet-fed rats was significantly less than in those fed SFO diet ($P < 0.05$), (Fig. 1). The percentage change in hock circumferences (adjusted for growth) for the SFO, DHA and EPA diet-fed rats did not differ significantly from that of the BT diet-fed rats (data not shown).

***Histology.*** The histological sections of hock joints were assessed for severity of change overall and a number of particular changes were related to the effects of inflammation and arthritis (Table 4). The overall severity of change suggested a trend ($P = 0.09$) that rats fed the BT and DHA diets were the most severely affected and those consuming EPA and SFO-based diets were less affected.

***Modification of plasma and cellular lipids.*** EPA [20:5(n-3)] was 1.5 g/100 g fatty acid in macrophage phospholipids of rats fed the EPA diet, a level greater than in the other three groups ($P < 0.05$), (**Table 5**). DHA in macrophage phospholipids of the EPA and DHA diet-fed rats was significantly greater than in the BT and SFO diet-fed rats (Table 5). The incorporation of EPA, docosapentaenoic acid (DPA) [22:5(n-3)] and DHA in plasma phospholipids of the EPA and DHA diet-fed rats was significantly different from those of the BT and SFO diet-fed rats (**Table 6**). The levels of palmitic acid (16:0) and 18:1(n-9) were lower in the plasma of rats fed the SFO diet than in rats fed the other diets (**Table 7**). However, the 18:1(n-9) concentration in macrophage phospholipids did not differ among groups (Table 5). Rats fed the SFO diet had plasma concentrations of linoleic acid (LA) [18:2(n-6)] that

## TABLE 5

*Effect of diets containing beef tallow (BT), safflower oil (SFO), docosahexaenoic acid (DHA) or eicosapentaenoic acid (EPA) on the fatty acid composition of macrophage phospholipids in arthritic Lew/SSN rats*[1]

| | Diet | | | |
|---|---|---|---|---|
| Fatty acid | BT | SFO | DHA | EPA |
| *n* | 5 | 7 | 5 | 7 |
| | *g/100 g total fatty acids* | | | |
| 16:0 | 17.8 ± 1.2 | 21.2 ± 1.8 | 15.0 ± 0.5 | 15.3 ± 0.8 |
| 18:0 | 14.6 ± 1.5[a] | 14.4 ± 0.9[a] | 12.9 ± 0.7[b] | 12.6 ± 0.7[b] |
| 18:1(n-9) | 30.8 ± 1.6 | 28.6 ± 2.0 | 27.1 ± 1.3 | 26.3 ± 2.1 |
| 18:2(n-6) | 5.3 ± 0.2[a] | 5.5 ± 0.6[a] | 4.3 ± 0.3[b] | 3.9 ± 0.3[b] |
| 18:3(n-6) | 0.2 ± 0.1 | 0.6 ± 0.1 | 0.4 ± 0.1 | 0.6 ± 0.2 |
| 18:3(n-3) | 1.3 ± 0.4[d] | 3.3 ± 0.5[c] | 4.8 ± 0.6[b] | 6.1 ± 0.6[a] |
| 20:3(n-6) | 1.6 ± 0.6[b] | 2.1 ± 0.7[a] | 0.1 ± 0.1[c] | 1.9 ± 0.8[b] |
| 20:4(n-6) | 1.8 ± 1.0[a] | 1.4 ± 0.2[a] | 0.9 ± 0.2[b] | 0.9 ± 0.1[b] |
| 20:5(n-3) | 0.5 ± 0.2[b] | 0.9 ± 0.3[b] | 0.7 ± 0.1[b] | 1.5 ± 0.2[a] |
| 22:5(n-3) | ND[2] | 2.4 ± 1.5[b] | 10.2 ± 1.4[a] | 8.3 ± 2.4[a] |
| 22:6(n-3) | ND | 0.5 ± 0.2[b] | 1.5 ± 0.1[a] | 1.7 ± 0.2[a] |
| 24:1(n-9) | ND | 0.4 ± 0.4[b] | 3.1 ± 0.1[a] | 2.4 ± 0.4[a] |
| Total (n-6) | 9.1 ± 0.5[b] | 9.7 ± 0.4[b] | 5.8 ± 0.1[a] | 7.5 ± 0.3[c] |
| Total (n-3) | 1.9 ± 0.1[b] | 7.2 ± 0.6[a] | 17.4 ± 0.5[c] | 17.7 ± 0.9[c] |
| EPA/DHA | ND | 1.8 | 0.5 | 0.9 |

[1] Values are means ± SEM. Values in a row with different superscripts differ, $P < 0.05$.
[2] Not detected.

**TABLE 6**

*Effect of diets containing beef tallow (BT), safflower oil (SFO), docosahexaenoic acid (DHA) or eicosapentaenoic acid (EPA) on the fatty acid composition of plasma phospholipids in arthritic Lew/SSN rats[1]*

| Fatty acid | BT | SFO | DHA | EPA |
|---|---|---|---|---|
| *n* | 5 | 7 | 5 | 7 |
| | *g/100 g total fatty acids* | | | |
| 16:0 | 14.4 ± 0.3[a] | 14.9 ± 0.4[a] | 16.6 ± 0.6[b] | 17.6 ± 0.6[b] |
| 18:0 | 35.0 ± 0.6 | 33.7 ± 1.9 | 31.6 ± 0.6 | 33.7 ± 1.1 |
| 18:1(n-9) | 6.8 ± 0.1[a] | 4.9 ± 1.7[b] | 6.8 ± 0.1[a] | 6.0 ± 0.5[a] |
| 18:2(n-6) | 6.4 ± 0.2[a] | 16.6 ± 1.5[b] | 9.6 ± 0.3[c] | 7.6 ± 0.3[d] |
| 18:3(n-6) | 0.1 ± 0.1 | 0.1 ± 0.1 | 0.1 ± 0.1 | 0.1 ± 0.1 |
| 18:3(n-3) | 0.2 ± 0.1 | 0.1 ± 0.1 | 0.2 ± 0.1 | 0.2 ± 0.1 |
| 20:3(n-6) | 0.6 ± 0.1[a] | 0.3 ± 0.1[b] | 0.7 ± 0.1[a] | 0.8 ± 0.1[a] |
| 20:4(n-6) | 24.9 ± 0.9[a] | 23.0 ± 1.1[a] | 8.8 ± 0.5[b] | 10.2 ± 1.1[b] |
| 20:5(n-3) | 0.3 ± 0.1[a] | ND[2] | 6.8 ± 0.2[b] | 5.8 ± 0.4[b] |
| 22:5(n-3) | 0.6 ± 0.1[a] | 0.6 ± 0.2[a] | 1.2 ± 0.1[b] | 2.1 ± 0.1[c] |
| 22:6(n-3) | 6.6 ± 0.4[a] | 2.3 ± 0.5[b] | 13.3 ± 0.3[c] | 11.1 ± 0.7[d] |
| 24:1(n-9) | 1.5 ± 0.1[a] | 1.1 ± 0.1[b] | 1.5 ± 0.1[a] | 1.6 ± 0.0[a] |
| Total (n-6) | 32.2 ± 0.3[a] | 40.1 ± 0.6[b] | 19.4 ± 0.2[c] | 18.8 ± 0.3[c] |
| Total (n-3) | 7.8 ± 0.2[a] | 3.1 ± 0.2[b] | 21.7 ± 0.2[c] | 19.2 ± 0.3[d] |
| EPA/DHA | 0.1 | ND | 0.5 | 1.0 |

[1] Values are means ± SEM. Values in a row with different superscripts differ, $P < 0.05$.
[2] Not detected.

were more than twice that of other groups ($P < 0.05$) (Table 7), but this difference was not evident in macrophage phospholipids where levels were slightly ($P < 0.05$) greater in rats fed SFO and BT diets than in rats fed EPA and DHA diets (Table 5). Plasma concentrations of arachidonic acid (AA) [20:4(n-6)] in rats fed BT and SFO diets were more than double those of the EPA and DHA diet-fed rats (Table 7) and these proportional differences were similar in macrophage phospholipids (Table 5).

## DISCUSSION

Clinical studies have demonstrated dietary fish oil containing EPA and DHA has moderate beneficial effects in rheumatoid arthritis patients (Cleland et al. 1988, Kremer et al. 1995). The purpose of this study was to examine the effect of varying the dietary ratios of EPA/DHA on the pathogenesis of the SCW-induced arthritis in female Lew/SSN rats. The characteristics of the diets used in this study were selected so that they would be relevant to humans with RA. All rat diets contained a mix of fatty acids, the lipid content of which was 38% of energy, which is in agreement with a previous human study (Volker et al. 1998). EPA and DHA content of the diets provided 13 and 10% of dietary energy, respectively, which is comparable with human supplementation regimens. We compared differing ratios of EPA and DHA to determine the optimal ratio for supplementation. A BT-based diet was selected as the control diet and the SFO-based diet was used to mirror the (n-6) fatty acid content of the Western diet.

Cromartie et al. (1977) initially described this animal model of arthritis. It is characterized by acute inflammation in peripheral joints in 100% of susceptible strains within 24 h. Acute inflammation is caused by SCW fragment deposition in the synovium. This is a T cell-independent process, associated with complement activation, which subsides in 3–5 d. Histologically, the rapid onset early phase of the disease is characterized by synovial abnormalities consistent with microvascular injury accompanied by perivascular infiltrate of polymorphonuclear and mononuclear cells. The interstitial spaces exhibit edema and fibrin deposits. The secondary, chronic synovitis is T cell-dependent and includes pannus formation, angiogenesis, erosions and joint destruction. The chronic phase is characterized by synovial lining cell layer proliferation, fibroblast-like cells in the sublining stroma and destructive invasion of periarticular bone. In RA, the site of synovitis is in the pannus, which is responsible for the characteristic erosions (Chu et al. 1992, Yanni et al. 1994).

The extent of inflammation observed in the four diet groups, measured from arthritis induction to peak of acute arthritic flare, varied. Footpad thicknesses (a measure of inflammation and edema) were greater in the rats fed the BT diet than in the EPA, DHA, and SFO diet-fed rats (Fig. 1). All rats displayed a marked acute onset of arthritis followed by remission, prior to the development of the chronic phase of this model of arthritis. The immunomodulatory effects of these diets are likely to be related to the changed availability of AA. Because dietary AA was restricted, so too was the production of AA-derived mediators of inflammation (Li et al. 1994).

The histological grading of hock change overall in the dietary groups did not mirror the results of the changes in footpad thicknesses and hock circumferences (Table 4). The severity of arthritic change was greatest in rats fed BT and DHA diets and least for the EPA and SFO diet-fed rats. The severity of change in the rats fed the BT diet was expected because SFA do not influence lipid mediators of inflammation (Grimble 1994). However, the severity of arthritic change in rats fed the DHA diet suggested that it may have been an efficient immunosuppressant, so that once the rat immune systems were suppressed, the DHA diet did not modulate inflammatory mediators. The degree of overall change in the rats fed EPA diets provided further evidence of the immuno-

**TABLE 7**

*Effect of diets containing beef tallow (BT), safflower oil (SFO), docosahexaenoic acid (DHA) or eicosapentaenoic acid (EPA) on the fatty acid composition of plasma fatty acids in arthritic Lew/SSN rats[1]*

| Fatty acid | BT | SFO | DHA | EPA |
|---|---|---|---|---|
| *n* | 5 | 7 | 5 | 7 |
| | *g/100 g total fatty acids* | | | |
| 16:0 | 17.8 ± 0.7[a] | 14.4 ± 0.9[b] | 16.9 ± 0.1[a] | 17.4 ± 0.9[a] |
| 18:0 | 15.9 ± 0.6 | 16.2 ± 1.2 | 15.8 ± 0.3 | 13.5 ± 1.6 |
| 18:1(n-9) | 19.4 ± 1.4[a] | 6.2 ± 1.2[b] | 12.5 ± 0.6[c] | 13.1 ± 0.5[c] |
| 18:2(n-6) | 7.8 ± 0.2[a] | 27.4 ± 4.2[b] | 9.6 ± 0.2[c] | 8.7 ± 0.1[c] |
| 18:3(n-6) | 0.3 ± 0.1[a] | 0.6 ± 0.1[b] | 0.1 ± 0.1[c] | 0.2 ± 0.1[c] |
| 18:3(n-3) | 0.2 ± 0.1 | 0.2 ± 0.1 | 0.3 ± 0.1 | 0.3 ± 0.1 |
| 20:3(n-6) | 0.4 ± 0.1 | 0.3 ± 0.1 | 0.4 ± 0.1 | 0.4 ± 0.1 |
| 20:4(n-6) | 29.0 ± 1.4[a] | 30.3 ± 4.4[a] | 11.0 ± 0.8[b] | 11.2 ± 0.5[b] |
| 20:5(n-3) | 0.6 ± 0.4[a] | ND[2] | 15.9 ± 0.3[b] | 17.3 ± 0.3[c] |
| 22:5(n-3) | 0.3 ± 0.1[a] | 0.1 ± 0.1[b] | 1.4 ± 0.1[c] | 2.2 ± 0.1[d] |
| 22:6(n-3) | 3.8 ± 0.1[a] | 1.2 ± 0.3[b] | 11.4 ± 0.1[c] | 9.8 ± 0.2[d] |
| 24:1(n-9) | 0.9 ± 0.1 | 0.7 ± 0.1 | 0.8 ± 0.1 | 0.7 ± 0.1 |
| Total (n-6) | 37.6 ± 0.4[a] | 58.7 ± 2.2[b] | 21.2 ± 0.2[c] | 20.6 ± 0.1[c] |
| Total (n-3) | 5.1 ± 0.1[a] | 1.6 ± 0.1[b] | 19.1 ± 0.1[c] | 29.7 ± 0.1[d] |
| EPA/DHA | 0.2 | ND | 1.4 | 1.8 |

[1] Values are means ± SEM. Values in a row with different superscripts differ, $P < 0.05$.
[2] Not detected.

suppressive effect of the EPA diet (Whelan 1996). The ranking of the SFO diet-fed rats with the EPA diet-fed rats was surprising. This could have resulted from the high level of dietary LA which was responsible for a negative feedback inhibition of LA conversion to AA and thus the suppression of the production of AA-derived mediators of inflammation (Whelan 1996). This effect could be related to the ratio of SFA to MUFA in the diet because this ratio can influence the immunomodulatory effects of dietary lipids (Jeffery et al. 1997). These results could also indicate that the SFO-based diet did not suppress the immune system activity of the rats prior to induction of arthritis.

Fatty acid composition of rat plasma and macrophages generally reflected those of the diets (Tables 5, 6 and 7). The SFO diet had a high proportion of LA but macrophage phospholipid levels in rats fed that diet did not reflect the same proportions (Table 5). There is a linear relationship between the proportion of DHA in the diet and the proportion of LA in plasma (Petersen et al. 1998) This reflects the regulation of AA production via a feedback inhibition (Garg and Li 1994, Whelan 1996). The high proportion of LA in the SFO diet would suppress endogenous AA production through an inhibitory feedback mechanism (Garg et al. 1988). The proportion of AA was high in the plasma of SFO- and BT-fed rats and low in the plasma of rats fed EPA and DHA diets (Table 7).

The plasma of EPA diet-fed rats contained a high proportion of EPA and DHA, whereas the plasma of DHA diet-fed rats contained a higher proportion of DHA. In the plasma of EPA and DHA diet-fed rats, the LA levels were low and also were low in macrophage phospholipids (Tables 5 and 7). AA plasma levels in EPA and DHA diet-fed rats were also low and remained low in macrophage phospholipids, which reflected the ability of EPA and DHA to competitively inhibit AA production (Calder 1998, Garg et al. 1988, Whelan 1996). The (n-6) to (n-3) balance of the EPA and DHA diets was manipulated to provide a comparison of the immunomodulating effects of these diets.

In several animal models of autoimmune disease (Byleveld et al. 1998, Leslie et al. 1985, Prickett et al. 1984), an effect of dietary fish oil on the severity of inflammation has been observed. Prickett et al. (1984) and Leslie et al. (1985) observed the effect of fish oil-based diets in collagen-induced arthritis in Sprague-Dawley rats and B10.RIII mice, respectively. Prickett and coworkers (1984) observed that a 10% fish oil diet enhanced the susceptibility of rats to induction of arthritis; however, the severity of arthritis was not affected by diet. Leslie and colleagues (1985) reported that there was a reduced incidence and severity of arthritis in a 5% fish oil diet compared with a corn oil diet. Feeding diets to mice for 26 d prior to induction of arthritis delayed the onset of arthritis, with reduced incidence and severity compared with corn oil-fed mice. Female mice fed a fish oil diet had a reduced incidence of arthritis compared with males fed the same diet. Byleveld and colleagues (1998) utilized a 20% fish oil diet in SPF male Swiss mice as a model of respiratory infection. They observed that diets rich in (n-3) fatty acids, which reduced the risk of cardiovascular disease and inflammatory diseases, are unlikely to compromise the outcomes of infection or clearance of infection. RA clinical studies in humans (Cleland et al. 1988, Kremer et al. 1990, 1995) have utilized pharmacological doses of EPA and DHA (1.0 to 7.1 g/d), in as much as 16 g d of fat without any increases in immunomodulatory effects.

The current study indicates that changing the lipid characteristics of the diet has an immunomodulatory effect in this animal model of arthritis. Fish oil may influence the pathogenesis of SCW-induced arthritis and that (n-3) fatty acids found in fish oil may elicit an effect. However, the total PUFA content of the diet and the balance of (n-6) to (n-3) fatty acids are important in determining the overall effect. The ratio of EPA/DHA can be fine-tuned to optimize this immunoregulatory effect. Further research is necessary to determine the exact effects in more lengthy trials.

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