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Effect of dietary fish oil supplementation on the antigen-induced late-phase response in the skin

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*We have studied the effect of dietary fish oil supplementation (3.2 gm/day of eicosapentaenoic acid and 2.2 gm/day of docosahexaenoic acid) for 10 weeks on the antigen-induced, cutaneous late-phase response (LPR) in 16 atopic individuals. All subjects developed an LPR to intradermal allergen injection. The mean $\pm$ SEM area of induration at 6 hours was 1840 ± 472 mm². A biopsy was performed at the LPR site at 6 hours. Subjects were then randomized to receive fish oil or placebo olive oil in a double-blind, parallel fashion, for 10 weeks. After the diet, there were no significant differences between fish oil and placebo treatment in the size of the immediate wheal-and-flare response or the extent of induration at 4 and 6 hours, as compared with prediet values in each group. Histologically, at 6 hours, there was a significant infiltrate into the allergen site compared with that of the control diluent site of total inflammatory cells ($p < 0.002$) as well as of eosinophils ($p < 0.001$). However, there were no significant differences when pre- and postdiet values were compared. Hence, we conclude that dietary fish oil supplementation does not inhibit the cutaneous LPR clinically or histologically. (*J ALLERGY CLIN IMMUNOL* 1992;89:829-35.)*

Key words: Allergens, late-phase response, eicosapentaenoic acid, fish oils, eosinophils

On exposure to relevant allergen, atopic individuals mount a well-characterized, IgE-mediated immediate reaction. In addition, an LPR occurs some hours later that is more complex than the early reaction and in-

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Supported in part by a grant from the BUPA Medical Foundation. Received for publication Oct. 16, 1991.

Revised Nov. 27, 1991.

Accepted for publication Dec. 4, 1991.

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1/1/35446

Abbreviations used

EPA:	Eicosapentaenoic acid
DCHA:	Docosahexaenoic acid
LPR:	Late-phase response
BAL:	Bronchoalveolar lavage
BALF:	Bronchoalveolar lavage fluid
SQ:	Standardized quality
SPT:	Skin prick test
LTC ₄ , LTB ₄ :	Leukotrienes C ₄ and B ₄

volves recruitment of inflammatory cells from the circulation. This LPR has been described with allergen reactions in skin, nose, and lung¹⁻³ and may be a more relevant model of chronic allergic inflammation.

Fish oil is abundant in EPA and DCHA. Dietary supplementation with these fatty acids leads to the incorporation of EPA into the membrane phospholipids of cells and tissues. The presence of EPA in cells and tissues has been demonstrated to modulate inflammation by depressing inflammatory cellular function, by reducing mediator generation, and by producing alternative mediators with attenuated activity.^{4,5} We have recently demonstrated that dietary supplementation with EPA for 10 weeks in subjects with allergic asthma significantly attenuates the late-phase bronchoconstriction to inhaled allergen challenge.⁶ Animal models of the LPR suggest that the histologic appearances are similar in the skin and the lung.⁷ Therefore, the skin is believed to provide a useful model for studying the inflammatory changes in asthmatic airways. The purpose of this study was to investigate the target-organ specificity of dietary EPA supplementation on the attenuation of the bronchial LPR by observing its effect on the cutaneous LPR.

MATERIAL AND METHODS

Subjects

Eighteen (11 male and seven female subjects, aged 21 to 56 years) atopic individuals were recruited from the Allergy Clinic of Guy's Hospital. Subjects with any medical disorder other than allergic asthma or rhinitis were excluded, as were individuals who had ever received injection immunotherapy or who were currently taking oral medication. Subjects with grass-pollen sensitivity were not studied during the pollen season. The studies were approved by the Ethical Committee of Guy's Hospital, and the study was performed with each volunteer's informed consent.

Skin testing

Lyophilized extracts of *Dermatophagoides pteronyssinus* (house dust mite), *Phleum pratense* (timothy grass), or *Felis domesticus* (cat) were kindly supplied by ALK Laboratories, Copenhagen, Denmark (courtesy of Dr. Henning Lowenstein). The lyophilized extracts (1 million SQ units) were reconstituted in 1 ml of ALK diluent (isotonic solution with 0.3 mg/ml of human serum albumin and 0.5% phenol) and then diluted to 12,000, 1200, or 400 SQ U/ml with the same diluent. One batch of allergen was used throughout the study. On the subject's arrival in the clinic, SPTs were carried out with concentrated (1 million SQ U/ml) grass pollen, house dust mite, cat-antigen solutions, and diluent control. The mean diameter of the wheal and flare was recorded at 15 and 20 minutes after each test. Antigen was then injected intradermally into the nondominant supinated forearm. The concentration of antigen used for the intradermal injection was determined by the size of the reaction to the SPT. If the SPT was <8 mm in diameter, 0.02 ml of the 12,000 SQ U/ml solution was administered (240 SQ units); if the SPT wheal was between 8 and 10 mm, 0.02 ml of the 1200 SQ U/ml solution (24 SQ units) was administered; and if the SPT wheal was >10 mm, 0.02 ml of

the 400 SQ U/ml of solution (8 SQ units) was injected. The control solution was injected at a site approximately 7 cm below the antigen injection.

Clinical measurement of skin response

Measurements of the wheal-and-flare responses were made at 15 and 30 minutes after the skin test, and the size of the late response was measured at 4 and 6 hours after injection. The outlines of the skin responses were delineated with a marking pen and traced onto plastic transparencies. The areas contained within were later calculated with the MacDraft program on an Apple Macintosh computer (Apple Computer, Inc., Cupertino, Calif.). After 6 hours, biopsies were performed at the allergen and control sites.

Skin biopsies

The biopsy site was first anesthetized with 1% lidocaine without epinephrine. The biopsies were performed by use of a 4 mm punch in all subjects at 6 hours after the intradermal injection at both antigen and control diluent sites. After biopsy, the wound was closed with one suture to facilitate healing. The biopsy specimens were placed in 10% formal saline for 24 hours and embedded in paraffin.

Histology

Paraffin-embedded biopsy specimens were sectioned and stained with hematoxylin and eosin. Sections were coded and read blind by an investigator who had no involvement with the clinical portion of the study. Cell counts were performed of extravascular eosinophils and the total inflammatory cell infiltrate, based on standard histologic appearances. The number of cells in each biopsy specimen were counted and averaged over five random high-power fields with a graticule eyepiece with a viewing area of 0.273 mm².

Study design

At the initial study day, baseline skin responses to allergen were measured and biopsies were performed. Subjects were then randomized to receive 18 capsules a day of Max-EPA (Seven Seas, Ltd., Marfleet, Hull, U. K.) (3.2 gm of EPA and 2.2 gm of DCHA) or identical placebo capsules containing olive oil for 10 weeks in a double-blind fashion. Their usual diets were unchanged. At the end of 10 weeks, the same concentration of allergen was injected intradermally into the same forearm and responses were measured as before. A biopsy was performed at the allergen injection site at 6 hours, and the specimen was processed in an identical fashion.

Compliance assessment

Compliance was assessed by EPA incorporation into leukocyte membrane phospholipid. Neutrophils were purified from 20 ml of anticoagulated blood to >95% by means of dextran sedimentation (Pharmacia, Buckingham, England) and centrifugation on a cushion of Lymphoprep (Nyegaard, Ltd., Birmingham, England). A portion of 1×10^7 cells was resuspended in 500 μ l of Hanks' balanced salt solution

TABLE I. Neutrophil and plasma phospholipid analysis

	18:2 n - 6 Linoleic	20:4 n - 6 Arachidonic	20:5 n - 3 EPA	22:6 n - 3 DCHA
Plasma fatty acid content (% $\pm$ SEM of total)*				
Placebo (n = 8)				
Before diet	30.50% $\pm$ 1.77%	6.59% $\pm$ 0.27%	0.77% $\pm$ 0.10%	1.70% $\pm$ 0.07%
After diet	29.88% $\pm$ 1.42%	6.50% $\pm$ 0.25%	1.01% $\pm$ 0.14%	1.81% $\pm$ 0.16%
Max-EPA (n = 7)				
Before diet	30.39% $\pm$ 1.67%	5.41% $\pm$ 0.64%	0.67% $\pm$ 0.11%	1.61% $\pm$ 0.27%
After diet	28.22% $\pm$ 1.42%	5.16% $\pm$ 0.45%	6.13% $\pm$ 1.17%	4.36% $\pm$ 0.34%
Neutrophil extract fatty acid content (% $\pm$ SEM of total)*				
Placebo (n = 8)				
Before diet	10.57% $\pm$ 0.65%	12.81% $\pm$ 0.62%	0.79% $\pm$ 0.50%	1.12% $\pm$ 0.12%
After diet	9.23% $\pm$ 0.35%	12.66% $\pm$ 0.70%	0.25% $\pm$ 0.04%	1.09% $\pm$ 0.10%
Max-EPA (n = 7)				
Before diet	11.14% $\pm$ 0.42%	11.89% $\pm$ 0.47%	0.30% $\pm$ 0.08%	0.86% $\pm$ 0.10%
After diet	10.55% $\pm$ 0.58%	10.41% $\pm$ 0.72%	3.09% $\pm$ 0.34%	2.04% $\pm$ 0.26%

*Mean (SEM) fatty acid composition of plasma and neutrophil extracts expressed as a percentage of total fatty acid content before and after dietary supplementation with placebo and Max-EPA.

without calcium or magnesium and stored under argon at -70°C before extraction of phospholipids. Neutrophil phospholipids were extracted according to the method of Bligh and Dyer.³⁸ Samples were first extracted in chloroform (BDH Chemicals, Ltd., Dorset, England). After being dried under a steady stream of nitrogen, samples were resuspended in boron trifluoride (Sigma Chemical Co., Poole, Dorset, England) and heated to 100°C under nitrogen for 90 minutes to esterify the fatty acids. Samples were then extracted into hexane (BDH Chemicals, Ltd.) and stored under nitrogen before analysis for fatty acid composition by gas chromatography.³⁹ Gas chromatographic analysis was performed by Dr. J. M. Mencia-Huerta at the Institut Henri Beaufour, Paris, France.

Statistics

The skin clinical responses were analyzed with the Mann-Whitney U test. The cellular infiltrate in the skin biopsy specimens was analyzed similarly.

RESULTS

Clinical

Sixteen subjects completed the study, eight of whom were randomized to receive placebo and eight received the active fish oil capsules. One 50-year-old male subject randomized to receive fish oil developed symptomatic diabetic mellitus while he was receiving the capsules and was withdrawn from the study. Another subject was lost to follow-up. The results of compliance assessment are presented in Table I. One subject receiving fish oil had high plasma levels of N-3 fatty acids but not in neutrophil membrane, suggesting lack of long-term compliance, and his data were withdrawn from analysis. After allergen injection,

all subjects developed an immediate wheal-and-flare reaction as well as an LPR that occurred 4 to 6 hours later. There were no statistically significant differences between the sizes of the early phase reaction wheal and flare or the LPR at 4 and 6 hours after fish oil or placebo therapy as compared with prediet values (Fig. 1).

Biopsies

The numbers of eosinophils and total inflammatory cells infiltrating the skin biopsy sites are illustrated in Figs. 2 and 3. There was a significant total inflammatory cell ($p < 0.002$) as well as eosinophil ($p < 0.001$) infiltrate in the allergen-challenge biopsy sites compared with that in the control-diluent injection biopsy prediet site. After 10 weeks of fish oil or placebo olive oil capsules, there were no statistically significant differences in the numbers of eosinophils or total inflammatory cells infiltrating the allergen-challenge biopsy sites compared to prediet values.

DISCUSSION

The late cutaneous response to aeroallergens was first noted more than 100 years ago by Blackley.⁸ However, it has only been in recent years that research has focused on this LPR because it is believed that the inflammatory changes observed more accurately reflect clinical disease than the immediate reaction.

The LPR has been extensively studied in the skin and has also been demonstrated in both the upper and lower airways. The LPRs demonstrate a number of similarities. In both the skin⁹ and lung,¹⁰ an LPR can be induced if a sufficient dose of allergen is admin-

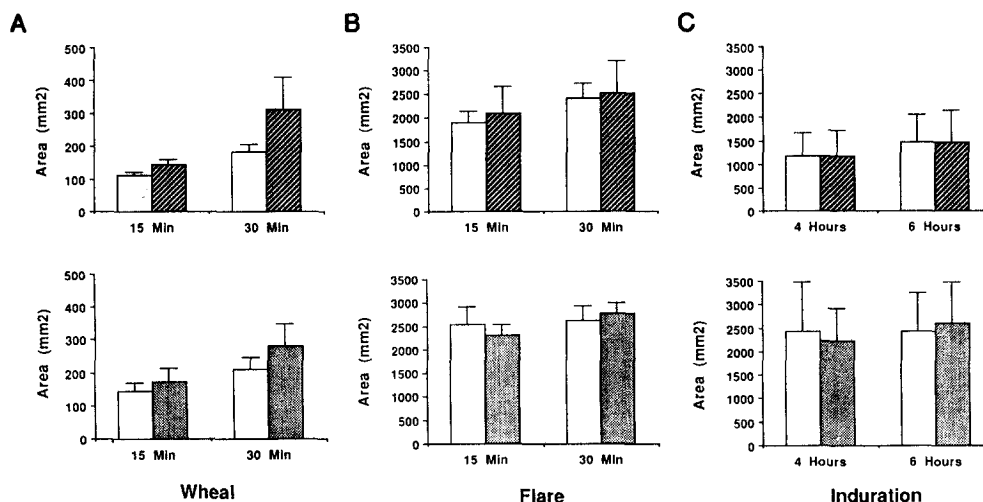


FIG. 1. Bar graphs. Mean areas $\pm$ SEM of **A**, immediate wheal and **B**, reaction at 15 and 30 minutes, and **C**, mean areas of induration at 4 and 6 hours in patients before diet as well as after fish oil and after placebo therapy. No significant differences between groups or before and after diet.

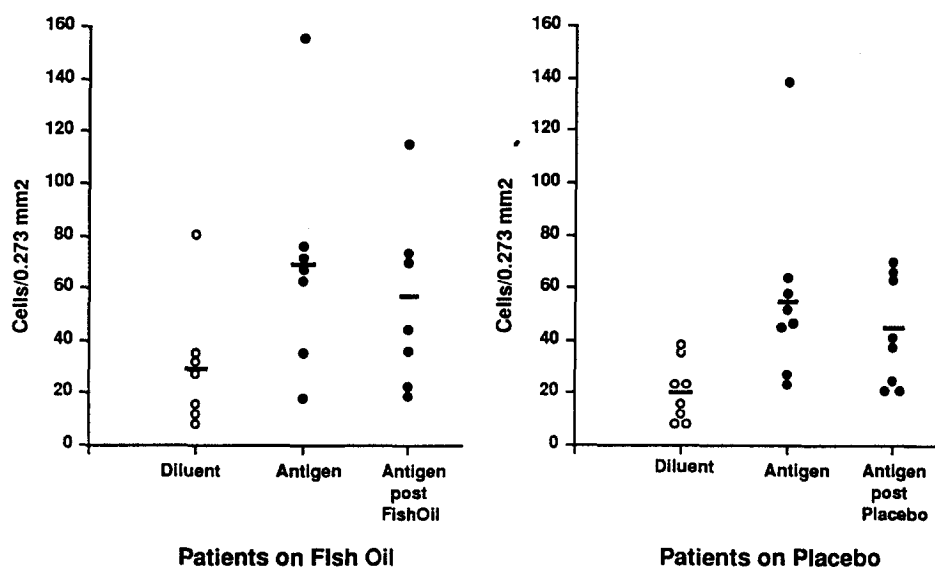


FIG. 2. Counts of total inflammatory cellular infiltrate in allergen biopsy site as compared with that of control diluent biopsy values before and after diets. Significant difference ($p < 0.002$) between allergen and control biopsy sites before diet, but none in the allergen site after diet as compared with that of before diet.

istered. The skin LPR has been demonstrated to be IgE mediated,^{1, 11} and similarly, a high level of circulating IgE antibody is an important determinant of the asthmatic LPR.^{3, 12} Both the cutaneous and pulmonary responses are inhibited by corticosteroids.^{13, 14}

In the skin, investigators have used a blister chamber to study preformed and newly generated mediator

release after allergen challenge and during the LPR. LTC₄ is found in skin-chamber fluids from the first hour and in peaks 4 to 6 hours after antigen challenge, corresponding to a visible LPR. In the airways, LTC₄ is found as early as 5 minutes after endobronchial allergen challenge.¹⁵ However, the levels found bear no relation to the development of LPR.¹⁶ After inhalational challenge, a small, but significant, increase

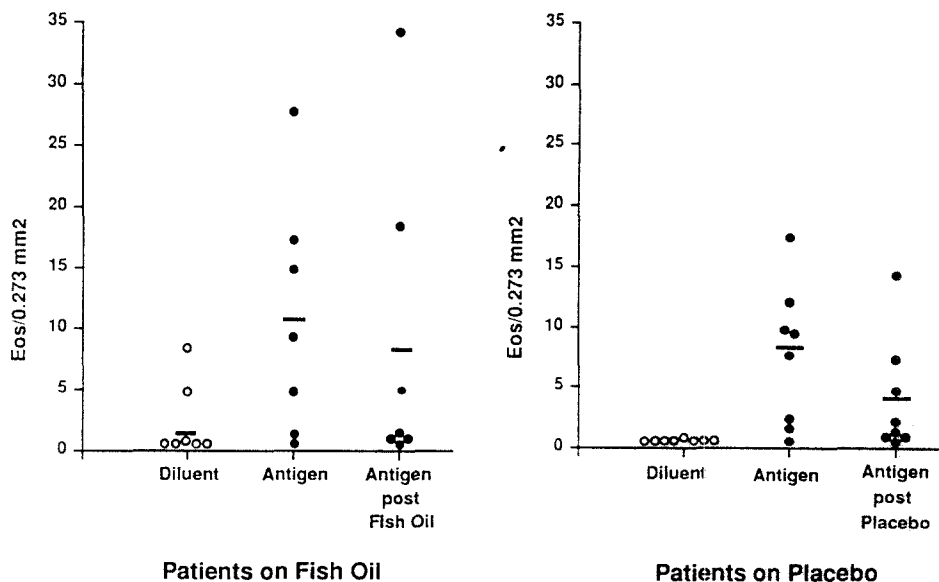


FIG. 3. Eosinophil infiltrate into allergen biopsy sites was significant compared with that of diluent before diet biopsy site ($p < 0.001$), but no significant difference between allergen sites before and after diet.

in LTC₄ levels has been found in BALF at 6 hours in association with LPR. There was no consistent change in levels of LTB₄.

A characteristic cellular infiltrate is associated with the LPR after cutaneous allergen challenge. In skin biopsy specimens at 6 hours, neutrophils and activated eosinophils are prominent cells, as are CD3⁺ T-lymphocytes, mainly of the CD4⁺ helper/inducer phenotype.¹⁷ By 24 and 48 hours, eosinophils and CD4⁺ T-lymphocytes remain the persistent cells. In the lung, BAL 6 hours after inhalational allergen challenge similarly revealed significant increases in the lymphocytes, neutrophils, and eosinophils associated with development of LPR. There was also a significant decrease in the percentage of mast cells recovered from BALF.¹⁸

EPA, 3.2 gm/day dietary supplementation for 10 weeks in healthy human subjects³ and individuals with asthma,^{6, 40} reduces neutrophil chemotaxis to multiple stimuli, as well as adherence to endothelial cells and generation of LTB₄. EPA has also been demonstrated to significantly attenuate the LPR after allergen bronchial challenge in atopic subjects with asthma.⁶ However, this study has demonstrated that this attenuation does not extend to the skin LPR, despite the same dose and duration of EPA supplementation. This finding may be due to differences in the nature of the LPR between the skin and lung, despite the similarities noted above. Other investigators have also found differences between the skin and lung LPR in terms of modulation by drug therapy. For example, both the

xanthine bronchodilators, enprophylline and theophylline, have been reported to inhibit the bronchial LPR,¹⁹ but they did not have an effect on the cutaneous LPR.²⁰ Similarly, the selective H₁ antagonist, cetirizine, has been reported to attenuate the bronchial LPR clinically²¹ as well as inhibit eosinophil influx in BALF.²² However, some investigators have found that it did not suppress the cutaneous LPR clinically,²³ although other investigators have noted a suppressive effect on eosinophil recruitment and infiltration.^{24, 25} Direct comparison is difficult since the doses of cetirizine used in these studies were different and since additional studies would be needed to ascertain whether there is a genuine difference between the cutaneous and bronchial responses.

The occurrence and intensity of skin and lung LPRs to ragweed or grass-pollen antigen have been studied in the same subjects. In that study,²⁶ all seven subjects developed LPRs in the skin and lung with similar timing; however, there was no correlation between the intensity of the two types of LPRs in individual subjects. Antigen-specific IgE correlated with the intensity of the cutaneous LPR, whereas serum neutrophil chemotactic activity tended to correlate with the intensity and timing of the bronchial response. A previous study had demonstrated a correlation between the bronchial LPR and serum neutrophil chemotactic activity²⁷ but no correlation with total or specific IgE. Both these studies suggest that bronchial LPR may be more complex than the cutaneous response.

In recent years, there has been much interest in the

heterogeneity of mast cells. In rodents, distinct differences exist in morphologic and functional characteristics of mast cells derived from various tissues that allow these mast cells to be subdivided into connective tissue and mucosal types. Although no such relationship exists in human mast cells, there are, nevertheless, differences between mast cells from the skin and those from the lung. For example, lung mast cells stain mainly with alcian blue, whereas skin mast cells stain with safranin O. Although both types of cells are activated by IgE-dependent mechanisms, skin mast cells are also activated by the nonspecific mast cell-degranulating agent, compound 48/80, and by the neuropeptide, substance P, whereas lung mast cells are not. Sodium cromoglycate reduces histamine release from lung mast cells by a mechanism that exhibits rapid tachyphylaxis. In contrast, histamine release from skin mast cells is unaffected by sodium cromoglycate. Moreover, lung mast cells produce more LTC₄ than skin mast cells on activation.²⁸ This mast cell heterogeneity may partially explain some of the differences between skin and lung responses after allergen challenge. Mast cells have also been recently demonstrated to produce cytokines, such as interleukins-4 and 5 and granulocyte-macrophage colony-stimulating factor, which can activate and recruit eosinophils and other inflammatory cells.²⁹ These cytokines provide additional evidence for a putative link between mast cells and the LPR. Although there have been no studies of possible differences between the cytokine profiles of skin and lung mast cells, such differences could further explain the differences in LPRs between the two tissues.

Much data concerning EPA modulation of the inflammatory response have been work with neutrophils and mononuclear cells in which they are known to inhibit cellular function^{5, 30} as well as mediator generation^{4, 31, 32} and cytokine production.^{33, 34} However, in the allergic inflammatory response, and especially the LPR, it appears that eosinophils and mast cells play far more prominent roles than in other inflammatory conditions. Although there is some evidence to suggest that dietary EPA supplementation can inhibit eosinophil recruitment in nasal antigen challenge,³⁵ there is no data available on its effects with respect to other eosinophil functions and mediator generation. There is also no data on EPA effects on human mast cells, but it has been demonstrated to up regulate PAF generation from cultured murine mast cells without having any effect on histamine release.³⁶ Hence, EPA may not significantly suppress the function of, and mediator generation by, these cells important in the LPR. Furthermore, although dietary supplementation with fish oil led to a significant at-

tenuation of the bronchial LPR, there were no changes in the resting nonspecific airway responsiveness.^{6, 40} Although the exact mechanism of bronchial hyperresponsiveness remains unclear, airway reactivity has been correlated with the increased numbers of eosinophils and mast cells in the BALF.³⁷ This finding suggests that, even with clinically measurable attenuation of the bronchial LPR by EPA, there may still be basal activation and release of mediators by mast cells and eosinophils, leading to bronchial hyperresponsiveness. Inasmuch as the attenuation of the bronchial LPR by EPA may be due to inhibition of neutrophil recruitment and function, we can speculate that this may be unimportant for the pathogenesis of basal airway reactivity.

We have studied the effect of dietary EPA supplementation on the development of the antigen-induced LPR in the skin. In contrast to the bronchial LPR, there is no significant attenuation of the cutaneous response, clinically or histopathologically. This may be due to differences in the pathogenesis of the LPR between these two tissues as have been discussed. Furthermore, this could also suggest that eosinophil function and mast cell mediator release, which are important factors in the pathogenesis of the LPR, are not inhibited by EPA.

We thank Seven Seas Ltd., Marfleet, Hull, U. K., for supplying the Max-EPA and matched placebo capsules, and Prof. D. A. Levison, Department of Histopathology, Guy's Hospital, for his advice and the use of his department's facilities.

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