Original Article

Low dose supplementation with two different marine oils does not reduce pro-inflammatory eicosanoids and cytokines *in vivo*

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In view of the reported potential anti-inflammatory activity of the New Zealand green lipped mussel (NZGLM), we aimed to compare the effect of low dose marine oil supplementation, from mussels and fish, in reducing blood markers of inflammation. Thirty apparently healthy males and females were recruited from the general public in Melbourne, Australia to participate in a double blind, randomised, parallel intervention study. Subjects were consuming approximately 73 mg of omega-3 long chain polyunsaturated fatty acids (n-3 LCPUFA) daily in their background diet prior to the commencement of the intervention. Subjects were randomly assigned to consume either 2 mL/day of the NZGLM oil preparation (mixed with olive oil and *dl*-α-tocopherol) or fish oil preparation (also mixed with olive oil and *dl*-α-tocopherol) for six weeks. Two mL of the oils contained 241 mg and 181 mg of n-3 LCPUFA, respectively. Neutrophil phospholipid fatty acids, serum thromboxane B_2 (TXB_2), stimulated monocyte production of prostaglandin E_2 (PGE_2), interleukin-1β (IL-1β) and tumor necrosis factor α (TNFα) were measured. During the intervention, the total intakes of n-3 LCPUFA from the background diet and the supplements were 199 mg/d and 173mg/day for the NZGLM and FO groups, respectively. Following six weeks of supplementation, both groups showed a small, but significant increase in neutrophil phospholipid proportion of eicosapentaenoic acid. The NZGLM group also showed a significant increase in docosahexaenoic acid levels. There were no significant changes with time or treatment for TXB_2, PGE_2, IL-1β or TNFα. This study showed that low dose supplementation with n-3 LCPUFA from two different marine oil preparations showed no difference in inflammatory markers in this group of healthy individuals. Further studies are warranted including dose response trials and studies in populations with inflammatory conditions.

Key Words: NZGLM; fatty acids; n-3 LCPUFA; eicosanoid; cytokine, gas chromatography

Introduction

Immuno-inflammatory conditions such as inflammatory bowel disease and arthritis are debilitating diseases affecting thousands of people around the world. Inflammation is generally caused by an increase in the production of pro-inflammatory eicosanoids and cytokines including prostaglandin E_2 (PGE_2), thromboxane B_2 (TXB_2), and leukotriene B_4 (LTB_4), interleukins 1, 2, 6 (IL), tumor necrosis factor alpha (TNFα) and interferon-γ.[1]

Traditional means of treating arthritis have included modulation of inflammatory mediator production with the use of non-steroidal anti-inflammatory drugs, however their use can result in ulceration of the gastric lining. Modulating the production of inflammatory mediators through dietary intervention with omega-3 long chain polyunsaturated fatty acids (n-3 LCPUFA) has been shown to reduce the symptoms of a number of inflammatory disorders without detrimental side effects.[2-4] Marine oils rich in n-3 LCPUFA, particularly fish oil, have been the focus of much clinical research. Hughes & Pinder[5] speculated that n-3 LCPUFA can reduce the production of pro-inflammatory markers through inhibition of human antigen presenting cell expression, intracellular adhesion and vascular cell adhesion molecules and reduced T-cell responses.[6,7] Meydani and colleagues[8] have shown that fish oil supplementation can reduce plasma levels of n-6 PUFA (arachidonic acid) and increase plasma levels of n-3 LCPUFA (eicosapentaenoic acid) as well as decreasing

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levels of IL-1β, IL-2, IL-6, PGE_2 and TNFα.

Over the past 20 or so years, research on the New Zealand Green lipped mussel (NZGLM) (freeze dried powder or oil extracted from the powder) has indicated that these preparations might have therapeutic value in the treatment of inflammatory conditions including arthritis and asthma.[9-11] The major fatty acids in the oil from the NZGLM include palmitic acid and the n-3 LCPUFA (docosahexaenoic acid; DHA and eicosapentaenoic acid; EPA).[12-14] Whitehouse *et al.*,[15] have reported that the NZGLM lipids (Lyprinol™) are more potent (on a mg/kg body weight dose rate) than fish oil in reducing induced inflammation in animals and that the bioactivity is related to the PUFAs. Furthermore, Dugas[16] demonstrated, in vitro using cultured human monocytes, that IL-4 stimulated monocyte production of LTB_4 in allergic patients was suppressed.

In view of the reported potential anti-inflammatory activity of the NZGLM, the aim of the present study was to determine the efficacy of the NZGLM lipids in reducing markers of inflammation (PGE_2, TXB_2, IL-1β, TNFα), in comparison with a typical fish oil rich in n-3 LCPUFA. The dose of marine oil supplement was approximately 200 mg/day and was chosen based on recommended intakes for the NZGLM product (Lyprinol™).

Methods

Subjects and study design

This was a randomised, double blind, parallel study in which 30 apparently healthy subjects (14 males and 16 females) were recruited. Subjects consumed one of two marine oils for six weeks, followed by a two-week washout period. The subjects underwent a strict screening process and were excluded if they exhibited symptoms of inflammation (sore joints, stiffness), had a past history of first generation inflammatory disease, high blood cholesterol (>5.5 mM), high blood pressure (>140/90) or a body mass index (BMI) greater than 30, were taking anti-inflammatory medication, statins, blood pressure lowering medication or dietary supplements (fish, evening primrose and flaxseed oil). Dietary intake was measured prior to and during the intervention period using weighed food records (WFR), which were assessed using the Diet Version 4 software (Xyris Software, Pty Ltd, Qld, Australia) with NUTTAB 95' database. Where foods were not present in the software program, composition tables were obtained from dietary panels on individual food products and from the relevant food companies. The study was designed to limit exogenous dietary n-3 LCPUFA (EPA, docosapentaenoic acid; DPA & DHA) and substitute with a marine oil source of n-3 LCPUFA. According to fatty acid dietary analysis of WFR using Foodworks® dietary software program (Xyris Software, Pty Ltd, Qld, Australia), total n-3 LCPUFA from subjects' background diet (in the weeks prior to baseline) in the FO and NZGLM groups were 63±54 mg/d and 83±18 mg/d, respectively (P>0.05). Subjects were asked to maintain their habitual intake of fish and green vegetables and consume no more than two serves of fatty fish (tuna, salmon, sardines) per week.

Fasting blood and anthropometric measurements were taken at baseline (day 0), halfway (day 21), end (day 42) and during the washout period at day 56 (post-supplementation). Blood was analysed for neutrophil phospholipid (PL) fatty acids, endotoxin-stimulated monocyte production of PGE_2, IL-1β and TNFα and serum levels of TXB_2. All subjects gave written informed consent before commencing the study and the study was approved by the Human Research Ethics Committee at RMIT University, Melbourne, Australia.

Marine oil preparations and compliance

Since the aim of the study was to compare the effect of two marine oils on inflammatory marker levels, the dose was based on oils as purchased rather than on a standardised n-3 PUFA content. The NZGLM oil was supplied by Pharmalink International (Queensland, Australia) and the fish oil was supplied by Felton Grimwade Bickford Pty Ltd (Oakleigh, Victoria, Australia). The study oil preparations were prepared by Felton Grimwade Bickford Pty Ltd which involved combining each marine oil (1 part) with olive oil (2 parts) and vitamin E, to mimic the commercial capsules sold as Lyprinol™, which contain 50 mg NZGLM lipids, 150 mg olive oil and 0.225 µg of *dl*-α-tocopherol. Lemon flavouring was added to the oils to mask the natural odour. Subjects were randomly assigned to take one of the two marine oils (requested dose was 2 mL of oil daily for six weeks, delivered using a dropper). Two mL of the NZGLM preparation contained 241 mg n-3 LCPUFA, supplying 97 mg EPA and 72 mg DHA with 8 mg *dl*-α-tocopherol/mL (equiv. to approx. 11 Lyprinol™ cap-sules), and 2 mL of the fish oil preparation contained 181 mg n-3 LCPUFA, supplying 87mg EPA and 50mg DHA, with 8 mg *dl*-α-tocopherol/mL. Prior to the study, each subject was given a previously weighed and labelled bottle of the allocated oil. Upon completion of the supplementation period (at day 42), subjects were asked to return the bottle of oil. The amount of oil consumed was calculated by subtracting the weight of the bottle at day 42 from the weight of the bottle at day 0. To maintain compliance throughout the study, subjects were each contacted weekly during the intervention to discuss any issues arising from the supplementation. Levels of fatty acids in neutrophils were determined at day 0, 42 and 56 using gas chromatography (GC), to determine if subjects were consuming the oils.

Anthropometry

Subjects' height and weight were recorded to calculate individuals BMI. Subjects had their percentage of body fat determined using a bioimpedance fat analyser (TBF-501 Tanita Corporation, Illinois, USA). Waist and hip measurements were taken using a metric tape measure to calculate the waist to hip ratio. Subjects' blood pressure and pulse were measured using a digital auto inflating blood pressure monitor (Lumiscope Co, Inc., NJ, USA). Subjects were seated for 15 minutes prior to these measurements to allow for their blood pressure to stabilise. To eliminate experimenter variability and diurnal variation, all measurements at each visit were conducted by KJM, at the same time and conducted at least twice to obtain a representative mean value.

Neutrophil phospholipid fatty acids

Whole blood was collected (20 mL) and added to tubes containing 4.5% EDTA (Sigma Chemical Co, St Louis, USA) and 6% dextran (Pharmacia, LKB, Uppsala, Sweden) in isotonic saline. Neutrophils were obtained through density centrifugation according to the method of Mantzioris *et al.*[17] The neutrophil pellet was resuspended in 1.5 mL of saline (0.9%) the lipids were extracted using chloroform: methanol (2:1, by vol., Merck, Germany) containing 0.005% butylated hydroxyanisole (Sigma Chemical Co, St Louis, USA). Samples were centrifuged and the bottom layer was reconstituted in chloroform: methanol (9:1, by vol., Merck, Germany). The total lipid extract was fractionated by thin layer chromatography retaining the PL fraction and trans-methylated according to the method of Murphy *et al.*[13] Fatty acid methyl esters were separated and quantified by capillary GC.[12] A standard mixture of fatty acid methyl esters (NuChek Prep Inc, Minnesota, USA) was used to identify fatty acids by retention times and to determine response factors. Data was reported as percent (%) of total neutrophil PL fatty acids.

Inflammatory markers

Thromboxane B_2

Following an overnight fast, 5 mL of venous blood was collected and allowed to clot in an incubated water bath for 30 minutes at 37°C. Cell free serum was separated from erythrocytes by centrifuging at 2000 rpm at 4°C, for 5 minutes and stored at -80°C until analysis.

Prostaglandin E_2, interleukin-1β *and tumor necrosis factor α*

Following an overnight fast, 5 mL of venous blood was collected and incubated with lipopolysaccharide (serotype 0111:B4; Sigma Chemical Co, St Louis, USA; final concentration: 0.2 mg/L) at 37°C buffered with 5% carbon dioxide for 24 hours, to stimulate PGE_2, IL-1β and TNFα production from monocytes. Cell-free plasma was obtained by centrifuging blood at 2000 rpm at 4°C for five minutes and stored at -80°C until analysis.

Immunoassays

PGE_2 and TXB_2 were measured by radioimmunoassay (RIA) with use of [^{3}H]PGE_2 and [^{3}H]TXB_2 (Amersham Australia Pty Ltd, Sydney, Australia), PGE_2 and TXB_2 (Cayman Chemical Co, Ann Arbor, MI, USA), and rabbit anti-PGE_2 (Sigma Chemical Co, St Louis, USA); rabbit anti-TXB_2 was prepared by inoculating rabbits with TXB_2-thyroglobulin conjugates according to the method of Mantzioris *et al.*[17] Cross-reactivities for the PGE_2 antiserum were <0.001% for TXB_2, 4.6% for 6-keto $PGF_{1\alpha}$, and 3.8% for $PGF_{2\alpha}$. Cross-reactivities for the TXB_2 antiserum were 0.06% for PGE_2, 0.05% for 6-keto $PGF_{1\alpha}$, and <0.05% for $PGF_{2\alpha}$. TNFα and IL-1β were measured by anti-PGE_2 (Sigma Chemical Co, St Louis, USA); rabbit anti-TXB_2 was prepared by inoculating rabbits with TXB_2-thyroglobulin conjugates according to the method enzyme-linked immunosorbent assay (ELISA) by using commercially developed matched-pair antibodies and recombinant TNFα and IL-1β standards (Endogen, Woburn, MA, USA). The range of detection was 0.07–5 µg/L for both TNFα and IL-1β. Samples for each assay were measured in triplicate and on the same day to reduce inter-assay variation.

Sample size and statistics

We estimated that a sample size of twelve subjects in each group would be a sufficient number to observe a significant change in IL-1β with a 0.05 level of significance and >90% power. A difference in IL-1β values of 1.5 ng/mL was shown to be statistically significant by Meydani *et al.*,[8] and represented a 43% change in the value of IL-1β over two months following fish oil supplementation which can be considered biologically relevant. Statistical analyses were carried out using SPSS (Statistical Package for Social Sciences version 8.0, 1997, Chicago, IL, USA). Descriptive statistics were performed providing mean and SD. Data were analysed using Repeated Measures ANOVA with post hoc analyses where significance was seen. Where total oil consumption and fatty acids (from the oil consumption) were compared, paired t-tests were conducted. Significance was P <0.05 unless otherwise stated. Data are reported as mean ± SD in the text and tables.

Results

Subject characteristics and compliance

The mean age of subjects in the NZGLM and fish oil groups was 43±10 yrs and 39±9.5 yrs, respectively. There were no significant changes in subject's weight, BMI or blood pressure dietary intake or other characteristics during the course of the study. These variables were not expected to change but were recorded at all timepoints to control for any confounding factors. The baseline characteristics of the subjects are shown in

Table 1. Characteristics of the subjects in the New Zealand Green Lipped mussel oil group (NZGLM) and fish oil group (FO) at day 0 (baseline), day 21 (3 weeks of supplementation), day 42 (following 6 weeks of supplementation) and day 56 (2 weeks washout, after supplementation had ceased).

	Subject characteristics[1]							
	NZGLM oil group (N = 12)				Fish oil group (N = 13)			
	Day 0	Day 21	Day 42	Day 56	Day 0	Day 21	Day 42	Day 56
Weight (kg)	75 ± 19	75 ± 19	75 ± 19	75 ± 19	74 ± 14	74 ± 14	74 ± 14	74 ± 14
Height (cm)	166 ± 11	166 ± 11	166 ± 11	166 ± 11	169 ± 7	169 ± 7	169 ± 7	169 ± 7
BMI	27 ± 4	27 ± 4	27 ± 5	28 ± 7	26 ± 3	26 ± 3	26 ± 3	26 ± 3
% Body fat	28 ± 7	28 ± 8	29 ± 7	28 ± 8	27 ± 7	28 ± 7	27 ± 7	28 ± 7
SBP (mmHg)	123 ± 14	123 ± 14	123 ± 15	125 ± 18	114 ± 10	118 ± 13	119 ± 11	114 ± 12
DBP (mmHg)	80 ± 11	82 ± 10	81 ± 11	78 ± 10	74 ± 10	74 ± 12	73 ± 11	73 ± 10
Pulse (bpm)	68 ± 7	69 ± 9	70 ± 8	71 ± 8	67 ± 7	69 ± 14	73 ± 12	69 ± 8

[1] Values expressed as Mean ± SD; NZGLM oil group (5 females, 7 males), FO group (6 females, 7 males).

Table 1. Two subjects from the NZGLM group withdrew from the study in the first week; one subject developed a rash and one subject felt nauseous after taking the oil. Two subjects from the fish oil group withdrew after two weeks due to reasons unrelated to the study. One subject was also excluded from all tests as the subject failed to consume the recommended dose of oil during the intervention period. Of the remaining group, 12 subjects from the NZGLM group and 13 subjects in the fish oil group completed the study.

The supply of surplus oil allowed the compliance with ingestion of oils to be estimated. Both groups consumed a mean of 71 mL of the marine oil, which was less than the anticipated (84 mL). The NZGLM group consumed on average 146 mg total n-3 LCPUFA (EPA, DPA & DHA), 81 mg EPA and 61 mg DHA per day for six weeks, while the fish oil group consumed on average 124 mg total n-3 LCPUFA, 73 mg EPA and 43 mg DHA per day for six weeks (Table 2).

The NZGLM group had a significantly higher intake of total MUFA (P<0.01), α-linolenic acid (18:3n-3; P <0.0001), EPA (P<0.05) and oleic acid (18:1n-9, P <0.01), compared with the fish oil group during the intervention. Dietary compliance was monitored using completed WFR during the eight week period. Dietary restrictions that were implemented prior to the study (restriction of fish intake) were adhered to during the intervention based on foods recorded by subjects in the WFR. During the intervention, the daily consumption of n-3 LCPUFA from exogenous sources (such as fish, eggs and lean meat) in the FO and NZGLM group was 49 ± 32 mg and 53 ± 38 mg, respectively. Thus, the total n-3 LCPUFA (treatment and background diet) was 199 mg and 173 mg n-3 LCPUFA for the NZGLM and FO group, respectively.

Neutrophil phospholipid fatty acids

There were small, but significant, increases in the proportion of EPA in the neutrophils in both groups following six weeks of supplementation (P=0.06 and P<0.05, respectively) and once supplementation was terminated, levels returned to baseline levels in both groups (P<0.05) (Table 3). In the NZGLM group only, the proportion of DHA increased significantly at the end of the supplementation period. Levels of 16:0 were significantly decreased at day 42 in both groups (P<0.01) and then returned to baseline levels two weeks after supplementation had ceased (P<0.01).

Thromboxane B_2 and prostaglandin E_2

Levels of TXB_2 and PGE_2 tended to decrease at day 42 of supplementation in both groups but did not reach statistical significance. This was most likely due to the wide range of values observed for both eicosanoids as indicated by the very large standard deviations as seen at baseline in Table 4.

Interleukin-1β and Tumor Necrosis Factor α

Similarly, IL-1β and TNFα decreased at day 42, but again were not statistically significant due to the wide range of values observed for both cytokines (Table 4).

Table 2. Amount of fatty acids consumed from the treatment oils, total for intervention (g) and daily consumption (mg) by the NZGLM and the fish oil group

	Fatty acids consumed[2]			
	Total (g) NZGLM group (N=12)	Mean consumed/ Day (mg)	Total (g) Fish oil group (N =13)	Mean consumed/ Day (mg)
Total oil (mL per 6 wks of study)	70.7 ± 8.1[1]	1.68	71.2 ± 7.1[1]	1.70
Total SFA	11.71	278.69	11.26	268.06
Oleic acid (18:1n-9)	32.35[3]	770.26	28.38	675.82
Total MUFA	36.71[3]	873.92	31.99	761.76
Arachidonic acid (20:4n-6)	0.36	8.47	0.18	4.39
Total n-6 PUFA	5.88	139.95	4.36	103.82
α-linolenic acid (18:3n-3)	1.49[3]	35.55	0.36	8.45
Octadecatetraenoic acid (18:4n-3)	0.74	17.52	0.68	16.27
Eicosapentaenoic acid (20:5n-3)	3.42[3]	81.45	3.08	73.31
Docosapentaenoic acid (22:5n-3)	0.18	4.35	0.34	7.98
Docosahexaenoic acid (22:6n-3)	2.54	60.47	1.79	42.70
Total n-3 PUFA	8.52	202.87	6.45	153.62
Total PUFA	14.40	342.81	10.81	257.44

[1] Values expressed as Mean ± SD; NZGLM group (5 females, 7 males), fish oil group (6 females, 7 males). [2]Values are mean of group.
[3] Significantly higher than the fish oil group based on paired-t-tests (P < 0.05).

Discussion

Studies in rats have suggested that the bioactivity of the oils of the NZGLM (*Perna canaliculus*) is greater than regular fish oils (on a mg/kg body weight dose rate basis) in reducing inflammation.[15] Thus the aim of the present study was to compare the effect of low dose administration of oils from the NZGLM and fish oil on the stimulated production of pro-inflammatory eicosanoids and cytokines. This is the first reported study to date to measure the effect of NZGLM lipids on blood markers of inflammation.

Following supplementation, there were no significant changes in inflammatory markers in either of the marine oil-fed groups or between the groups of apparently healthy volunteers. There was a very wide spread of values for most analytes which may have obscured changes with treatment or between treatments. Dietary nutrients (marine oils) and pharmacological drugs have been shown in animal and human studies to suppress an overactive immune response, however there is much variability in terms of response in humans as not all individuals react in

Table 3. Fatty acid composition of neutrophil phospholipid (% of total phospholipid fatty acids) for subjects from the NZGLM and fish oil group at day 0, day 42 and day 56 (2 weeks washout after supplementation had ceased)

	NZGLM group[1] (N = 12)			Fish oil group[1] (N = 13)		
Fatty acid	Day 0	Day 42	Day 56	Day 0	Day 42	Day 56
Palmitic acid (16:0)	13.5 ± 1.1	12.4 ± 0.8[2]	13.4 ± 1.0[3]	12.9 ± 1.0	12.3 ± 0.8[2]	13.2 ± 1.1[3]
Stearic acid (18:0)	15.6 ± 1.4	15.6 ± 1.2	14.8 ± 1.2[3]	15.3 ± 3.8	15.6 ± 1.6	14.9 ± 1.2[3]
Oleic acid (18:1n-9)	28.9 ± 2.2	29.4 ± 1.8	29.0 ± 1.8	29.2 ± 2.2	29.5 ± 1.6	29.5 ± 1.1
Linoleic acid (18:2n-6)	9.0 ± 1.0	8.9 ± 1.1	9.2 ± 1.2	8.4 ± 2.5	9.0 ± 1.1	9.0 ± 0.9
Arachidonic acid (20:4n-6)	13.1 ± 1.6	12.7 ± 1.5	12.8 ± 1.2	12.9 ± 0.8	13.0 ± 1.5	12.8 ± 1.0
α-linolenic acid (18:3n-3)	0.2 ± 0.2	0.2 ± 0.2	0.2 ± 0.2	0.2 ± 0.2	0.2 ± 0.2	0.2 ± 0.2
Eicosapentaenoic acid (20:5n-3)	0.5 ± 0.2	0.6 ± 0.2[2]	0.5 ± 0.1[3]	0.5 ± 0.2	0.6 ± 0.2[2]	0.9 ± 0.2[3]
Docosapentaenoic acid (22:5n-3)	0.3 ± 0.5	0.4 ± 0.3	0.4 ± 0.5	0.2 ± 0.2	0.3 ± 0.3[2]	0.2 ± 0.2
Docosahexaenoic acid (22:6n-3)	1.3 ± 0.3	1.6 ± 1.1[2]	1.4 ± 0.7[3]	1.3 ± 0.3	1.3 ± 0.3	1.1 ± 0.3[2,3]

[1] Values expressed as Mean ± SD; NZGLM group (5 females, 7 males), fish oil group (6 females, 7 males). [2] Significantly different to day 0 ($P < 0.05$) based on repeated measures ANOVA with simple contrasts. [3] Significantly different to day 42 (P=0.003) based on repeated measures ANOVA with simple contrasts.

Table 4. Levels of serum thromboxane B_2 (TXB_2) and lipopolysaccharide stimulated monocyte production of prostaglandin E_2 (PGE_2), interleukin-1β (IL-1β) and tumor necrosis factor α (TNFα) (ng/mL) for the New Zealand Green Lipped mussel oil group (NZGLM) and fish oil group (FO) at day 0 (baseline), day 21 (3 weeks of supplementation), day 42 (following 6 weeks of supplementation) and day 56 (2 weeks washout after supplementation had ceased)

	NZGLM oil group[1] (N = 12)				Fish oil group[1] (N = 13)			
	Day 0	Day 21	Day 42	Day 56	Day 0	Day 21	Day 42	Day 56
TXB_2	467 ± 186	477 ± 217	298 ± 197	307 ± 286	524 ± 317	420 ± 273	297 ± 184	480 ± 311
PGE_2	4.8 ± 2.1	3.4 ± 1.0	3.6 ± 2.3	4.1 ± 1.9	4.7 ± 1.6	3.5 ± 1.2	4.3 ± 2.2	4.0 ± 2.4
IL-1β	179 ± 75	150 ± 74	163 ± 63	158 ± 65	177 ± 77	151 ± 75	162 ± 101	150 ± 62
TNFα	38 ± 19	41 ± 22	33 ± 21	43 ± 17	46 ± 19	43 ± 24	38 ± 16	42 ± 17

[1] Values expressed as Mean ± SD; NZGLM oil group (5 females, 7 males), FO group (6 females, 7 males).

the same manner to any form of treatment.[18] Not all studies have shown positive health benefits especially in relation to fish oil supplementation and inflammation.

Endres *et al.*,[19] showed a reduction in IL-1 and TNFα production following six weeks of fish oil supplementation, however there were large standard deviations in the data, which suggests again different individual responses to treatments. Thus, it is likely that individual genotypic characteristics greatly influence an individual's anti-inflammatory response to fish oil, which could explain the large variability seen in our data.

There have been a number of *in vitro*, *ex vivo* and animal studies to indicate that the lipids of the NZGLM can reduce inflammation and production of proinflammatory eicosanoids.[15,16,20-26] Similarly, to date there are twelve published studies investigating the anti-inflammatory activity of the NZGLM preparations in humans; of these, only five have used Lyprinol™.[9-11,27-34] All of these studies with the exception of Caughey *et al.*,[30] indicated a reduction in either induced inflammation or symptoms of arthritis. Similarly, n-3 LCPUFA from fish oil has been shown to have positive health benefits on the risk factors of cardiovascular disease, diabetes, and ameliorate the symptoms associated with nephritis, respiratory distress as well as arthritis and acute and chronic inflammation.[3, 17, 35-37]

However, data from the present study do not support this research, which is most likely to be due to the low dose of n-3 LCPUFA given. The biochemical data from the current study does not support clinical data from previous research on the anti-inflammatory activity of the NZGLM in human clinical trials, most likely due to the fact that our subjects were free of any inflammatory condition. It is of interest that the intake of n-3 LCPUFA in this study of approximately 190 mg per day had a very small effect on the level of EPA and DHA in the neutrophils. Whether this was due entirely to the low dose (0.2 g/day) or to other factors (such as a high intake of LA) which can reduce the uptake of n-3 LCPUFA into neutrophils. In this study, olive oil was added to the marine oils. It was assumed that the low dose of olive would not influence the anti-inflammatory properties of the marine oils however there has been a recent report that extra-virgin olive oil contains a novel anti-inflammatory substance known as oleocanthal.[38] The oleocanthal content of different olive `oils has not yet been reported. In the present study, the same olive oil was used for both preparations.

Further studies are needed to investigate the reported bioactivity of the NZGLM using randomized controlled trials, with adequate controls and a larger cohort of subjects with differing severities of inflammatory conditions.

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Original Article

Low dose supplementation with two different marine oils does not reduce pro-inflammatory eicosanoids and cytokines *in vivo*

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低剂量补充两种海产油不会降低体内二十烷类物质和细胞因子等前炎症因子

文献报导了新西兰翡翠长嘴贻贝（NZGLM）的潜在抗炎活性，我们比较了低剂量补充海产贻贝油和海产鱼油对降低血液炎性指标的作用。方法：从澳大利亚墨尔本公众招募的三十名健康男女参加随机双盲平行干预实验。实验开始前，所有样本的每日饮食中含有约 73 mg 的 n-3 长链多不饱和脂肪酸（n-3 LCPUFA）。所有个体被随机指定连续六周每日摄入 2mg 与橄榄油、DL－α 生育酚混合的 NZGLM 油或鱼油制剂，2 ml 油中 n-3 LCPUFA 含量分别为 241 mg 和 181 mg。测嗜中性粒细胞磷脂脂肪酸、血清血栓素 B2（TXB2）、集落单核细胞产生的前列腺素 E2、白介素-1β（IL-1β）、癌症坏死因子α（TNFα）。结果：干预期间，NZGLM 油组和鱼油组每日从基础饮食和油制剂补充物中获得的 n-3 LCPUFA 总摄入量分别为 199 mg 和 173mg。油制剂补充干预结束后六周，NZGLM 油组和鱼油组嗜中性粒细胞磷脂中二十碳五烯酸的比例均有小的但显著的升高；NZGLM 油组二十二碳六烯酸水平也显著升高。TXB2、PGE2、IL-1β或 TNFα并不随处理的不同以及干预时间而表现出差异。结论：本研究表明，在健康个体中，低剂量补充来源于两种海产油制剂的 n-3 LCPUFA 并不会造成炎性指标的差异。但此结果需进行进一步的剂量反应实验和人群致炎条件实验确证。

关键词： 新西兰翡翠长嘴贻贝、脂肪酸、n-3 长链多不饱和脂肪酸、二十烷类物质、细胞因子、气相色谱。