Original article
Clinical study of the effects on asthma-related QOL and asthma management of a medical food in adult asthma patients

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Abstract

Background:
Asthma can have a negative impact on quality of life although this is not well correlated with objective evaluations of pulmonary function. A medical food, EFF1009, containing the fatty acids gamma-linolenic acid (GLA) and eicosapentaenoic acid (EPA) decreases leukotriene B_4 synthesis in patients with asthma. Two previous clinical studies with EFF1009 provided preliminary evidence that the medical food improves asthma-related quality of life (ARQOL) and asthma management.

Objective:
To evaluate the impact on ARQOL of EFF1009 in adults with asthma.

Research design and methods:
The study was a randomized, prospective, double-blind, placebo-controlled, parallel group study in twenty-one ($N = 21$ evaluable) subjects with mild to moderate persistent asthma who consumed the medical food emulsion or placebo emulsion daily for 28 days. All participants continued their asthma medications throughout the study. ARQOL, including asthma signs and symptoms, and asthma control were measured using the Mini Asthma Quality of Life Questionnaire (MiniAQLQ) and the Asthma Control Questionnaire (ACQ), administered at baseline, Day 14 and Day 28. Safety and tolerability parameters, including adverse events, were monitored.

Results:
Baseline ARQOL scores, forced expiratory volume in one second (FEV_1) and other characteristics were balanced between both groups. Mean (standard error) total MiniAQLQ scores changed by 0.73 (0.38) and −0.22 (0.36) in the EFF1009 and placebo groups, respectively, ($p < 0.05$). The MiniAQLQ symptom domain score was improved in the EFF1009 group ($p < 0.05$). Total scores for the ACQ were not significantly improved in either group. Levels of the fatty acid EPA in plasma increased in the EFF1009 group but not the placebo group ($p < 0.03$). The medical food was well tolerated and no safety concerns were identified.

Conclusions:
The dietary addition of the medical food EFF1009 to asthma management regimens can improve patient perceived, ARQOL and can also improve asthma management as evidenced by reduced asthma symptoms. An additional study of the medical food, with larger subject population and longer treatment duration, is warranted to confirm these findings.

Introduction

Leukotrienes (LTs), one type of inflammatory eicosanoid derived from the essential fatty acid arachidonic acid (AA), contribute significantly to the pathophysiology of asthma and allergic rhinitis[1–4]. The biosynthesis of AA metabolites,

including the leukotrienes, can be measurably impacted by modifying the relative dietary intake of polyunsaturated fatty acids (PUFAs) such as gamma-linolenic acid (GLA) and eicosapentaenoic acid (EPA). It is well established that both EPA and the GLA metabolite dihomo-gamma-linolenic acid (DGLA) resident in immune cell membranes act as inhibitors of eicosanoid synthesis[5–8]. DGLA is a precursor of 15-hydroxyeicosatrienoic acid (15-HETrE), a metabolite that directly inhibits leukotriene biosynthesis[9–12]. When human diets are supplemented with GLA, its metabolite DGLA accumulates in immune cell membranes, and ex vivo synthesis of leukotriene B_4 is significantly decreased[13–15]. It is not known whether other leukotrienes are impacted by dietary GLA. In healthy individuals, the relative ratios of AA, DGLA and EPA in immune cell membranes, which are determined by dietary intakes[5,6] are sufficient to maintain leukotriene homeostasis. However, since 5-lipoxygenase (5-LO), the enzyme responsible for further conversion of AA to leukotrienes, is typically upregulated in asthmatic individuals[16–21], the relative proportions of precursor and inhibitor fatty acids in immune cell membranes are insufficient to maintain leukotriene homeostasis. As a result, individuals with asthma may benefit from a higher dietary intake of inhibitor fatty acids than can be obtained from a standard diet. Recognition of these special dietary needs led to the development of a medical food[22,23]. Furthermore, inhibition of the leukotriene pathway is expected to lead to a reduction of cysteinyl leukotrienes C_4, D_4, and E_4 (LTC$_4$, LTD$_4$, and LTE$_4$). LTC$_4$ and LTD$_4$ are potent bronchoconstrictors strongly implicated in asthma. Due to their rapid metabolism in vivo, the resulting metabolite LTE$_4$ is typically used as a surrogate biomarker for these important compounds.

The medical food EFF1009 is a flavored dietary emulsion product containing GLA and EPA from borage oil and fish oil, respectively. In previous studies, the medical food effectively reduced leukotriene B_4 synthetic capacity in both healthy subjects and patients with mild to moderate atopic asthma[23,24].

While a reduction in inflammatory mediators such as leukotrienes should theoretically improve asthma management, this theory should be investigated. Further, even objective assessments of lung function are often not well correlated with patient perceptions of their asthma status and asthma-related quality of life (ARQOL)[25]. Thus, it is important to include ARQOL measurements in asthma clinical trials. Validated questionnaires that measure ARQOL and asthma control have been developed and are now widely used[26–29]. Although the impact of the medical food EFF1009 on the management of the disease and ARQOL in patients with asthma has been evaluated in two previous studies[30], an additional, independent and more rigorous assessment of the impact on ARQOL was warranted.

In this study, safety was monitored, and effects on asthma-related quality of life and asthma control of the medical food product EFF1009 were evaluated using the MiniAQLQ[28] and the ACQ[29], respectively, in a double-blind, placebo-controlled design.

Patients and methods

Study design

This was a 28-day, randomized, double-blind, prospective, placebo-controlled, parallel group clinical study in subjects with mild to moderate persistent asthma performed at MetaClin Research, Inc. (Austin, TX). An external ethical review board (IntegReview) approved the protocol, and each subject provided written informed consent before entering the study. Subjects were recruited by MetaClin Research using local newspaper and radio advertisements. Eligibility criteria included non-smoking males and females aged 18–65 years with a body mass index (BMI) ≤ 32. All subjects had a history of asthma of at least one year, met National Asthma Education and Prevention Program (NAEPP) criteria[31] for mild or moderate asthma, and had a FEV$_1$ > 60% of predicted at screen. No subject had required more than 800 mcg/day of inhaled steroids or had a history of requiring systemic steroids within the year prior to study enrollment. Leukotriene synthesis inhibitors, leukotriene receptor antagonists, immunosuppressive drugs and dietary supplements containing GLA or EPA were disallowed during the study period. Subjects with a history of liver disease, hypersensitivity or allergy to fish, shellfish or soy, or females who were pregnant or nursing were excluded from the trial. Subjects were enrolled within two weeks of screening.

The efficacy endpoints were the change from baseline of MiniAQLQ, ACQ, and leukotrienes. The study was powered to an expected 24% decrease in ex vivo leukotriene B_4, since sufficient data were not available to base the power on MiniAQLQ scores. The fatty acids GLA and EPA were quantified in plasma as a measure of dosing compliance. An equal number of subjects were randomly assigned to the medical food emulsion (EFF1009) or placebo emulsion group. Each subject consumed the contents of a packet containing one serving of the emulsion once daily for 28 days. Efficacy endpoints, plasma fatty acids and safety parameters were measured at baseline and after 14 days and 28 days of daily emulsion ingestion.

Vital sign measurements, hematology and clinical chemistry tests were performed at baseline and at the end of the study period (analyses performed at Esoterix, Inc., Austin, TX). Baseline FEV$_1$ was measured for determination of eligibility at screening and to determine a complete ACQ score. Plasma fatty acids, stimulated whole blood leukotriene B_4 synthesis (analyses performed

at Pyxant Labs, Colorado Springs, CO), and urine levels of LTE$_4$, normalized for creatinine (analyses performed at Cayman Chemical, Analytical Testing Service, Ann Arbor, MI), were measured at baseline and after 14 days and 28 days of daily emulsion ingestion.

EFF1009 and placebo

The EFF1009 medical food was a flavored emulsion packaged as single serving tear-open pouches as described previously[30]. Each daily serving of EFF1009 delivered a minimum of 0.75 g GLA, 0.5 g EPA and 0.35 g docosahexaenoic acid (DHA). The placebo emulsion contained no GLA, EPA, or DHA, but was otherwise identical in composition and packaging to EFF1009.

Asthma-related QOL and asthma control assessments

ARQOL and asthma control were measured using the two self-administered validated instruments, the MiniAQLQ[28] and the ACQ[29], respectively.

The MiniAQLQ was developed specifically for use in clinical studies and surveys, has the same measurement properties as the longer AQLQ, and has been validated. The MiniAQLQ instrument is comprised of 15 questions in 4 domains: Symptoms (5 questions), Activities (4 questions), Emotions (3 questions) and Environment (3 questions). In all cases the questions refer to how the subject was feeling during the previous two weeks due to their asthma. For each question low scores indicate poor asthma-related quality of life compared to higher scores. The activity domain focuses on the extent of the perceived limitation while the other domains characterize frequency of a given symptom or other perception. The scale for each question is from 1 ('all of the time' or 'totally limited') to 7 ('none of the time' or 'not at all limited'). The total score is computed as the mean of responses to all 15 questions and each domain score is computed as the mean of the responses with the domain[28]. Changes in domain scores are only valid if there is a statistically significant change in the total score.

The Asthma Control Questionnaire (ACQ) is comprised of 1 question on airway caliber answered by the clinic staff and 6 questions that are answered by the study subjects. The subjects' questions focus on symptom frequency and severity, and the need for rescue medication (short-acting bronchodilator) during the previous week. The scale for each question is from 0, indicating good asthma control to 6, indicating poor asthma control. The total score is computed as the mean of responses to all of the questions. The ACQ does not have domains. The frequency of use of a short-acting bronchodilator (question number 6) was evaluated individually as well as being included in the total score since this treatment is allowed on an as needed basis and could potentially impact the study results. In this question, responses bracket the number of puffs used 'most days', e.g. a response of '0' means none, '1' means 1–2 puffs most days, '2' means 3–4 puffs most days, etc. The ACQ was developed with input from 92 asthma clinicians from 18 countries and has been validated[29].

Participants completed these questionnaires during their clinic visits at baseline (Day 0), Day 14, and Day 28. A fully completed questionnaire was required for inclusion in the analysis, except that study subjects were not required to answer the ACQ question on FEV$_1$. The validity and the measurement properties of the ACQ at the group level are not affected in the absence of FEV$_1$ measurement[32]. Screening FEV$_1$ was imputed into the Day 0 ACQ questionnaire only for the purpose of characterizing subjects' asthma as either 'well-controlled' or 'not well-controlled'[33,34] at baseline.

Biological fluid collection procedures

All subjects fasted for at least eight hours prior to collection of biological specimens. Plasma was prepared by centrifuging whole blood ($300 \times g$, 10 min, 4°C), separating the upper plasma layer, and centrifuging ($900 \times g$, 25 min, 4°C) to remove platelets. Plasma for fatty acid analysis was frozen immediately in a cryotube. Duplicate 1.5 mL aliquots of plasma for leukotriene analysis were mixed with previously prepared zymosan A (Sigma-Aldrich # Z4250) in HBSS buffer (0.04 g/mL), shaken for 30 minutes at 37°C, then centrifuged ($920 \times g$, 10 min, 4°C). The upper plasma layer was transferred to a cryotube. Urine was collected into a 10 mL tube and frozen. Plasma and urine samples were stored frozen (−80°C) until analysis.

Extraction, derivatization and quantitation of plasma fatty acids

Standard solutions of GLA (Sigma-Aldrich #L2378) and EPA (Cayman Chemical #90110) were prepared in both blank plasma and water in a range of concentrations (5–300 μg/mL) used to generate average response factors for these analytes and were injected at regular intervals in each analysis set. Both clinical plasma samples and prepared standards (0.5 mL) were extracted using procedures adapted from literature[35]. Briefly, lipids were extracted from plasma with CHCl$_3$-MeOH (2:1 v/v). Following separation by centrifugation (3000 rpm) from the salinified aqueous layer, the lipids were hydrolyzed by heating (1 hr, 80°C) in 3M KOH-MeOH (1:9 v/v). To isolate the fatty acids, the solution was acidified to pH = 2–2.5 with 2N H$_2$SO$_4$, extracted first in 1N H$_2$SO$_4$-hexane-iso-propanol (0.1:1:4), then with 2 mL of H$_2$O and 3 mL

hexane and centrifuged (3000 rpm, 5 min). The organic layer, containing fatty acids, was transferred to a clean tube and evaporated to dryness under N_2 (50°C). The fatty acids were derivatized to the corresponding *n*-butyl esters by the addition of 3 N HCl in *n*-BuOH (0.5 mL). The tubes were heated at 110°C for 30 minutes, then evaporated to dryness under N_2 (45°C). Finally, 1.0 mL of toluene was added and the solution sonicated for ~10 seconds prior to transfer to autosampler vials. Positive ion electron impact – gas chromatography/mass spectrometry (EI-GC/MS) was conducted on a ThermoFinnigan Model Trace DSQ/MSD instrument, equipped with a DB-5 MS column (J&W Scientific #123-5532). The column was heated from 150°C to 260°C (2°C/min, injector temp. 240°C, splitless mode, He carrier gas). The *n*-butyl esters were analyzed using selective ion-recording techniques for each compound (e.g. GLA m/z 334.2 ± 0.5). Quantitation of analytes was determined by comparison of peak heights, as determined by MS response, to the average response factors of the corresponding standards that were ≥ 1.5 times that of background GLA or EPA in plasma blanks. The limits of detection of GLA and EPA in plasma were approximately 100 ng/mL and 1 μg/mL, respectively.

Extraction and quantitation of leukotrienes

Quantitation of zymosan-stimulated whole blood plasma LTB_4, and its oxidation products 20-hydroxy-LTB_4 (20-OH-LTB_4) and 20-carboxy-LTB_4 (20-COOH-LTB_4) was performed using high performance liquid chromatography – electrospray ionization tandem mass spectrometry (LC/MS-MS). Standards (Cayman Chemical), ranging 1–80 ng/mL in 500 μL blank plasma, were used to generate linear calibration curves for each analyte, and quantitation of analytes was determined by comparison of MS response to the corresponding linear calibration formula. To both clinical and standard samples was added 20 ng/mL internal standard (LTB_4-d_4; Cayman Chemical), and 1.0 mL of MeOH-CH_3CN (1:1) extraction solvent. Following centrifugation (13,000 rpm) the upper liquid layer was transferred to an autosampler vial for analysis. The LC/MS-MS was a Sciex API 3000 Tandem Mass Spectrometer (Applied Biosystems) interfaced to an Agilent 1100 HPLC system. The MS was operated in the negative Turbo-Ionspray mode. The HPLC system was equipped with a Phenomenex Luna phenyl/hexyl column (150 × 2.0 mm; 3 μm). Gradient elution (flow rate 400 μL/min) was used, beginning with 70% mobile phase A (H_2O-MeOH, 95:5 v/v, with 10 mM NH_4OAc in both solvents) and 30% B (H_2O-MeOH, 5:95 v/v, 10 mM NH_4OAc in both solvents). Mobile phase B was increased linearly to 100% at 10 minutes, held for 3 minutes, and reduced to 30% at 13.1 minutes. This composition was held until 17 minutes. The estimated lower limit of quantitation (LLOQ) was 1 ng/mL plasma for LTB_4 and 20-OH-LTB_4. LLOQ for 20-COOH-LTB_4 was 10 ng/mL. Resulting levels of 20-COOH-LTB_4 were too low to be measured in these samples.

Urinary LTE_4 was quantified using the Leukotriene E_4 ACE^{TM} Competitive Enzyme Immunoassay Kit (Cayman Chemical, #520411), which is based on the competition between LTE_4 in the urine samples and LTE_4-acetylcholinesterase (AChE) conjugate for a limited amount of LTE_4 antiserum[36]. Urine samples were diluted two- or five-fold with EIA buffer for LTE_4 analysis. Creatinine was measured using the Creatinine Assay Kit (Cayman Chemical, # 500701).

Safety and tolerability assessment

The safety and tolerability of the medical food was monitored during this study by recording of adverse events and the analysis of vital sign measurements, hematology and clinical chemistry tests at baseline and at the end of the study period (analyses performed at Esoterix, Inc, Austin, TX). Baseline FEV_1 was measured for determination of eligibility at screening and to determine a complete ACQ score. Adverse events that occurred after administration of the first dose of test article were recorded in the appropriate Case Report Form at Day 14 and Day 28.

Statistical analyses

The MiniAQLQ and ACQ data from all subjects who completed both the baseline and at least one follow-up set of questionnaires were analyzed by comparing changes from baseline scores, including individual domain score analyses, by *t*-test (JMP Statistical Software, SAS Institute, Inc., Cary, NC). Leukotriene reduction was analyzed by comparing changes from baseline using ANCOVA adjusted by baseline leukotriene concentration.

Results

Subject characteristics

Baseline demographic and disease characteristics were similar between the two study groups (Table 1). There was no significant correlation between MiniAQLQ score at baseline and FEV_1 at screening (Figure 1), consistent with other reports[37,38]. Twenty-one of twenty-two enrolled subjects completed the study. One subject in the placebo group dropped out voluntarily due to the taste of the emulsion. Eleven (11) and 10 subjects completed the study in the EFF1009 and placebo groups, respectively, and were evaluable for efficacy.

Table 1. Demographic characteristics and baseline pulmonary function of subjects with asthma who were enrolled in the study.

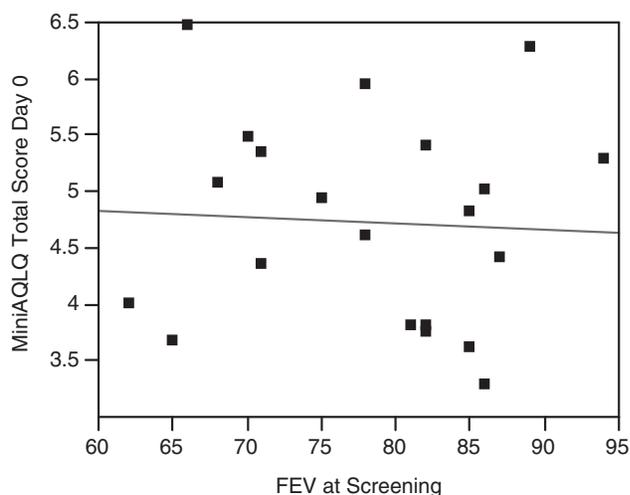| Asthma	Placebo $N = 11$	Active $N = 11$
Mean (SD) age, yrs	33 (13)	30 (10)
Mean (SD) body weight, Kg	66 (14)	75 (11)
Sex, no.		
Male	4	2
Female	7	9
Race, no.		
White	11	9
Black	0	1
Asian	0	1
Mean (SD) FEV_1, % predicted	80 (7.7)	77 (9.7)

Figure 1. Baseline MiniAQLQ and screening FEV_1 in adult subjects with mild to moderate persistent asthma. No significant correlation was found between the two measures.
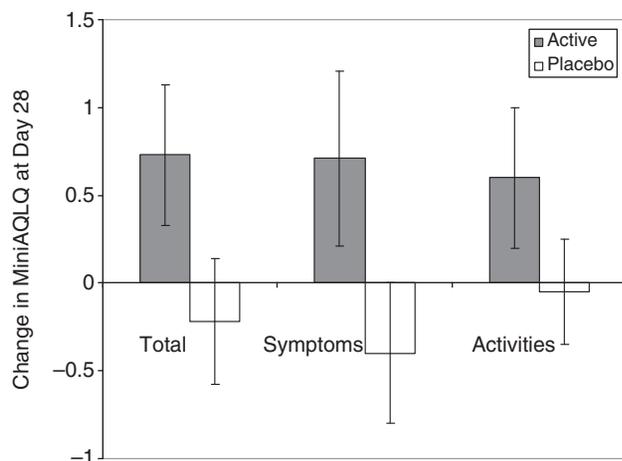

Figure 2. The change in scores from the MiniAQLQ (Day 28 minus Day 1) for adult subjects with mild to moderate persistent asthma who consumed EFF1009 or placebo daily for 28 days, mean and standard error. Changes in total score and symptoms domain score were significantly improved in the active group compared to placebo ($p < 0.05$). An increase in score of ≥ 0.5 is considered clinically significant.

Table 2. Study subjects who experienced adverse events (AE).

Treatment	Total Subjects N	Subjects Reporting Any AE		AEs Possibly or Probably Related to Test Article	
		N	%	N	%
	22	10	45	8	36
EFF1009	11	4	36	4	36
Placebo	11	6	54	4	36

Table 3. Adverse events (AE) deemed possibly or probably related to test article.

Event Description	EFF1009 $N = 11$	Placebo $N = 11$
Emesis	2	1
Nausea	3	
Dyspepsia		1
Abdominal cramping		1
Stomach cramping	1	
Stomach ache		1
Heartburn		1
Flatus or increased flatus	1	
Lightheadedness	1	
Headache		1
Total	8	6

Safety and tolerability

The medical food EFF1009 appeared to be well tolerated by most subjects in the study. There were no unexpected or unanticipated safety issues, and no serious adverse events occurred during the trial. Overall, adverse events were experienced by 54% of subjects who received placebo, and 36% of subjects who received EFF1009 (Table 2). Reported events were mild to moderate in severity and resolved without concomitant therapy. The incidence of adverse events that were deemed by the Investigator to be at least possibly related to the emulsion was comparable across both groups (36% and 36% of subjects who received EFF1009 and placebo, respectively). The majority of events were gastrointestinal in nature (88% EFF1009 vs. 83% placebo) and the incidence was comparable between the two groups (Table 3). The means of all laboratory analytes for both treatment groups were within normal limit ranges, and changes in vital sign measurements were unremarkable (Table 4). There were 10 abnormal test results reported for clinical laboratory analytes: 7 in the EFF1009 group (4 subjects) and 3 in the placebo group (3 subjects). The majority of abnormalities were not unusual, and the causal etiologies were often known. These data contribute to the safety profile for the medical food EFF1009, which includes safety monitoring data from two previous clinical studies and the determination by an

Table 4. Vital signs: mean (standard deviation).

Parameter	EFF1009		Placebo	
	Screen	Day 28	Screen	Day 28
Temperature	36.3 (0.7)	36.7 (0.6)	36.3 (0.6)	36.3 (0.8)
Pulse	72.7 (12.2)	71.0 (11.5)	63.6 (14.2)	66.5 (13.9)
Systolic blood pressure	111.5 (15.6)	112.9 (8.8)	118.3 (13.5)	117.0 (9.6)
Diastolic blood pressure	73.0 (12.7)	71.0 (8.2)	73.5 (10.7)	71.0 (8.0)

Table 5. Asthma-related quality of life and asthma management scores from the MiniAQLQ and ACQ questionnaires in subjects ($n = 21$) consuming EFF1009 or placebo daily for 28 days.

Measurement	Mean (SE)					
	Placebo Group ($N = 10$)			Active Group ($N = 11$)		
	Baseline	Day 28	Mean Change	Baseline	Day 28	Mean Change
MiniAQLQ Total Score	4.97 (0.30)	4.75 (0.27)	−0.22 (0.36)	4.50 (0.26)	5.22 (0.32)	0.73 (0.38)[a]
Symptoms Domain	4.92 (0.39)	4.52 (0.47)	−0.4 (0.41)	4.42 (0.25)	5.13 (0.40)	0.71 (0.46)[a]
Activity Limitation	5.9 (0.2)	5.8 (0.3)	−0.05 (0.3)	5.6 (0.3)	6.2 (0.2)	0.6 (0.4)
ACQ Total Score	1.46 (0.23)	1.61 (0.34)	0.15 (0.26)	1.61 (0.21)	1.39 (0.22)	−0.21 (0.23)

Note: in the MiniAQLQ an increased score is indicative of an improvement in asthma-related quality of life while in the ACQ a decreased score is indicative of improved asthma control.
[a]Significantly different from placebo, $p < 0.05$.

Expert Panel that the medical food is Generally Recognized as Safe (GRAS).

Efficacy

MiniAQLQ evaluation

At baseline, MiniAQLQ scores in both groups were less than optimal, 4.5 and 5.0 in the EFF1009 and placebo groups, respectively (Table 5). By Day 28 (Figure 2) a clinically and statistically significant improvement in the total MiniAQLQ score of 0.73 was reported by subjects in the EFF1009 group ($p < 0.05$), whereas there was no improvement in the placebo group (mean change of −0.22). A change of ≥0.5 in the total scale or domain scores is considered clinically significant[39-41]. The magnitude of the treatment effect compares favorably with other asthma management options[40]. Significant improvement ($p < 0.02$) was also evident by Day 14 in the EFF1009 group compared to placebo (data not shown).

When the MiniAQLQ domain scores were considered individually, significant improvements were observed for the symptoms domain in the EFF1009 group (Table 5). There was a clinically significant improvement in the activity domain in the EFF1009 group, but this did not reach statistical significance compared to placebo. The emotional function and environmental stimuli domains were not significantly impacted in either group (data not shown).

ACQ evaluation

In the ACQ, a score of 1.5 is considered a 'cut-point', separating those whose asthma may be 'well-controlled' from those whose asthma is 'not well-controlled'[33,34]. Sixty-four percent and 55% of subjects in the EFF1009 and placebo group, respectively, had baseline ACQ scores that were consistent with 'not well-controlled' asthma. There was no significant change in the ACQ total score in either group during the study (Table 5). One subject in the placebo group did not complete the ACQ questionnaire on Day 28 and was excluded from the analysis.

Nine (9) and 7 subjects in the EFF1009 and placebo groups, respectively, reported needing to use their rescue inhaler at least sometimes during the study. Of these, 78% percent of subjects in the EFF1009 group reported reduced rescue inhaler use frequency by Day 28 compared to baseline versus 14% of subjects in the placebo group. Mean rescue inhaler use among users in the EFF1009 group decreased from approximately 2–3 puffs per day at baseline to less than 1 puff per day by Day 28. Thus, the improvement in MiniAQLQ total score and symptom domain score cannot be attributed to an increase in rescue inhaler use among patients in the EFF1009 group.

Leukotrienes

Ex vivo LTB_4 from zymosan stimulated whole blood dropped an average of 20% in the EFF1009 group, but

Table 6. Changes in mean (standard error) urinary LTE_4 and ex vivo plasma LTB_4 in adult subjects with mild to moderate persistent asthma consuming EFF1009 or placebo for 28 days.

	LTB4 (ng/mL plasma)			LTE4 (pg/mg urine)*		
	Day 0	Day 14	Day 28	Day 0	Day 14	Day 28
EFF1009 ($N = 11$)	83.4 (10.8)	66.7 (9.6)	66.3 (12.1)	853 (226)	594 (165)	795 (151)**
Placebo ($N = 10$)	94.6 (14.9)	69.4 (8.4)	65.8 (8.3)	662 (117)	730 (145)	989 (199)

*Normalized for creatinine.
**Trend compared to placebo ($p < 0.08$).

Table 7. Changes in mean (standard error) plasma fatty acids in adult subjects with mild to moderate persistent asthma consuming EFF1009 or placebo emulsion for 28 days.

	GLA (µg/mL plasma)			EPA (µg/mL plasma)		
	Day 0	Day 14	Day 28	Day 0	Day 14	Day 28
EFF1009 ($N = 11$)	11.1 (1.9)	19.5 (2.1)	21.3 (3.8)	31.0 (3.3)	54.4 (5.7)*	62.5 (8.6)**
Placebo ($N = 10$)	12.3 (1.7)	16.6 (4.1)	20.3 (4.2)	37.4 (9.2)	42.7 (10.7)	40.0 (7.2)

*Change from Day 0 is significantly different from placebo, $p < 0.04$.
**Change from Day 0 is significantly different from placebo, $p < 0.006$.

this change was not significantly different from placebo (Table 6). Unanticipated and unexplained increases in plasma GLA in the placebo group described below may have contributed to this effect. Urinary LTE_4, normalized to creatinine, decreased in the EFF1009 group, as much as 30% at Day 14, and increased by almost 50% in the placebo group by Day 28. The changes in urinary LTE_4 in the EFF1009 group exhibited a statistical trend at Day 28 compared to placebo ($p < 0.08$).

Fatty acids

Increases in plasma levels of GLA and EPA in the EFF1009 group were as expected and indicated good compliance with the daily dosing regimen (Table 7). EPA is accumulated in plasma more readily than is GLA, and thus is the more reliable indicator of dosing compliance with the medical food. Plasma EPA increased ~100% by Day 28 in the EFF1009 group, versus ~7% in the placebo group. Elevations of GLA in the placebo group were unexpected. Site and data audits did not uncover any explanation for the GLA results in the placebo group. In the absence of specific dietary supplementation such elevations are biologically implausible, suggesting that some individuals in the placebo group may have been using disallowed dietary supplements containing GLA during the study.

Discussion

Improvement of ARQOL is an important goal of the asthma treatment regimen[37]. The ARQOL indicators deemed most important to physicians (sleep disturbance and activity limitation)[29] and to patients (symptoms control)[37] are amenable to patient self-assessment and were evaluated during this study. A previous open-label study[30] showed that these important indicators could be favorably impacted by one daily serving of EFF1009 used for four weeks along with asthma medications. However, due to inherent limitations of an open-label study an additional, clinic-based study was warranted. The current study corroborates those earlier findings relative to management of asthma symptoms and also demonstrates that ARQOL benefits are evident by 14 days, consistent with the observation that leukotriene biosynthetic capacity decreases after a minimum of two weeks of dietary supplementation with the medical food[23]. An additional study of the medical food, with a larger subject population and longer treatment duration, is warranted to confirm these findings. Further, information gained from the retrospective ACQ suggests that the medical food could potentially reduce the need for rescue inhaler medication, but this should be confirmed in a future study using a daily diary of inhaler use rather than via a question about the past week average use.

Statistically significant improvements in ARQOL were demonstrated according to the MiniAQLQ for subjects who consumed the medical food EFF1009 daily. A mean change of 0.73 shows that the medical food produced a clinically significant improvement in asthma-related quality of life as well. Importantly, the symptoms domain was also significantly improved, demonstrating that the medical food EFF1009 had a significant impact on asthma management.

Previous studies with the medical food have demonstrated that LTB_4 elaboration from zymosan stimulated whole blood is reduced 20–25%[23,24]. The same magnitude

of reduction was observed in the current study, however without reaching statistical significance. The unexpected reduction in LTB_4 in the placebo group is consistent with the rise in GLA, perhaps suggesting poor compliance with disallowed dietary supplements. Assuming that EFF1009 reduces the activity of 5-lipoxygenase, LTB_4 is a relevant biomarker for the entire leukotriene pathway, although it is generally accepted that the cysteinyl leukotrienes are more directly involved in the pathology of asthma than is LTB_4. This is the first study to evaluate the impact of the medical food EFF1009 on cysteinyl LTs. That impact was evaluated using spot samples of urine taken at each clinical visit for measurement of the metabolite LTE_4. While the results were encouraging, in any future study a more rigorous analysis of impact on cysteinyl LTs should be conducted, based on the collection of either first morning urine or 24-hour urine samples. Since a statistical trend of reduced LTE_4 was observed only in the EFF1009 group, this suggests the potential that the EPA and/or DHA component of the medical food, when ingested along with GLA, may specifically influence the regulation of the cysteinyl leukotrienes, possibly via the DHA metabolite, protectin $D1^{42}$.

Previous studies in patients with asthma who ingested either GLA or EPA, but not both, as a dietary supplement have yielded mixed, but primarily negative, results. For example, low dose GLA (0.3 to 0.5 g/day) produced no significant clinical benefit for atopic asthma compared to placebo in children after 16 weeks[43] or adults after 8 weeks[44]. High dose GLA alone (1.8–2.0 g/day) was reported to reduce ex vivo leukotriene B_4 production without a favorable impact on quality of life or asthma management[45,46]. Neither very low dose (0.1 g/day) nor very high dose (4 g/day) EPA in the form of purified ethyl esters ingested for eight weeks produced significant changes in pulmonary function in adult asthmatics[47]. Similarly, very high dose fish oil (2.7 g EPA/day plus 1.8 g DHA/day) or a diet rich in fish (averaging 3 g EPA/day) ingested for 6–10 weeks produced no significant change in symptoms, pulmonary function or medication requirements compared to placebo[48] or a control diet[49]. Methacholine-induced respiratory distress was increased by low dose EPA (ca. 0.7 g) but decreased by high dose (>3 g) EPA from fish oil in a study lasting one month[50]. Neither FEV_1 nor airway responsiveness to histamine were significantly improved in children with asthma ages 8 to 12 years after 3 or 6 months supplementation with 0.72 g EPA plus 0.48 g DHA compared to placebo, although both TNFα production from stimulated peripheral blood mononuclear cells and peripheral blood eosinophil numbers fell significantly from baseline in the active arm[51]. In a separate study lasting one year, relatively low dose EPA + DHA (1 g/day) significantly improved FEV_1 compared to placebo, but not until month 9 of the study[52]. High dose EPA (3.2 g EPA/day) had no significant impact on symptoms in

pollen sensitive, adult asthma patients followed through the respiratory allergy season[53]. EPA supplied at 3.2 g/day (+2.0 g/day DHA) has been reported to favorably impact exercise-induced asthma in elite athletes[54] as well as in nonathletes who participate in recreation[55]. Asthma symptoms and responsiveness to acetylcholine decreased significantly in children with asthma who were given fish oil for 10 months in an in-patient setting[56]. The late airway response to inhaled allergen was also attenuated by high dose 3.2 g/day EPA-rich fish oil after 10 weeks[57]. In contrast, airway responsiveness to histamine and to exercise was not significantly impacted by EPA at 3.2 g/day in patients with mild asthma although ex vivo LTB_4 levels were significantly reduced[58].

The GLA metabolite, DGLA, is a more potent inhibitor of the leukotriene pathway than is EPA[59] which has allowed for the use of relatively low doses of GLA (<1 g/day) to achieve significant reductions in leukotrienes[15,23,24,60]. However, a reduction in the capacity of the blood to synthesize LTB_4 when stimulated ex vivo has not been associated with a favorable effect on ARQOL or asthma management in most of the studies cited above, or for the current study's placebo patients, suggesting that this mechanism alone may not be sufficient for a meaningful impact on asthma. The authors think that the fish oil component of the medical food may be significantly contributing to the effectiveness of the EFF1009, as well as increasing its safety. In addition to preventing an increase in AA when GLA is fed[22], EPA-rich fish oil is known to down-regulate inflammatory cytokines, such as TNF-α and IL-1β, in asthmatics[55]. Thus, a combination of the fatty acids GLA and EPA could potentially reduce both leukotrienes and cytokines in asthma. In this study, an increase in plasma levels of both GLA and EPA (EFF1009 arm) but not GLA alone (placebo arm) was associated with improved asthma management and asthma related quality of life. Since the level of EPA in the medical food is low (500 mg EPA), and a low level of fish oil alone (700 mg EPA) was previously reported to exacerbate asthma[50], it is unlikely that the fish oil component of EFF1009 could be responsible for all of the benefit derived during this and previous studies with the product. Therefore, both fatty acids are required in combination to yield a benefit. As demonstrated in this clinical study, only in the EFF1009 group, where both EPA and GLA increased in plasma, was there an improvement in ARQOL, including the symptoms domain, and a strong statistical trend for reduced urinary LTE_4.

Although very high levels of fish oil appear to confer significant benefit in exercise induced asthma as described above, there are some difficulties associated with a dietary supplementation strategy using fish oil alone. First, low dose fish oil was reported to either exacerbate asthma[50] or to demonstrate a benefit only after 9 months of use[52]. In order to achieve positive results in a relatively short

period of time, very high levels of fish oil have been required, delivering >5 g EPA + DHA per day. Combined intakes of EPA + DHA are generally recognized as safe (GRAS) at 3 g per day or less due to concerns over prolonged bleeding times, increased LDL-cholesterol and impaired glycemic control at dietary intakes above 3 g per day[61,62]. Second, the sheer number of capsules required to deliver such high doses of the fatty acids would be expected to produce poor compliance with the regimen. Thus, for long-term management of asthma, fish oil alone may not be advisable.

The medical food, EFF1009, supplies 0.75 g GLA, 0.5 g EPA, and 0.3 g DHA per daily serving for adults, provided in a pleasantly flavored, emulsified food form that facilitates compliance and absorption[23]. The medical food has consistently produced a statistically significant improvement in patients with mild to moderate asthma within four weeks. In the current study, a significant improvement in ARQOL was reported within two weeks. Taken together, the previously published body of data along with results from this clinical study support the conclusion that a high dose of either GLA or EPA is neither required nor necessarily sufficient to produce a benefit, but rather, a low to moderate amount of both fatty acids combined appears to be optimal for benefit in the dietary management of asthma as well as being generally recognized as safe for long-term use.

Conclusions

This small, randomized, placebo-controlled study demonstrates that the daily consumption of a specially formulated medical food EFF1009 containing the fatty acids GLA and EPA is well tolerated and can improve the overall asthma-related quality of life and also improve the management of asthma symptoms. While this study corroborates two previous studies and adds to the body of data supporting safety and effectiveness of the medical food, a larger controlled study of longer treatment duration is warranted to confirm the specific findings.

Transparency

Declaration of funding
This work was funded by Efficas Inc., Boulder, CO, USA.

Declaration of financial/other relationships
J.L. has disclosed that she is employed by, and holds stock in, Efficas. D.S., G.B. and P.O.-C. have disclosed that they are former employees of Efficas. D.S. also has disclosed that he is a scientific consultant for, and holds stock in Efficas. E.D.P. has disclosed that he has no financial relationship with manufacturers that have an interest in the subject matter or materials discussed in the manuscript. J.J.P. has disclosed that she has

been a medical consultant to Efficas. P.O.-C. and G.B. have also disclosed that they are Efficas stockholders.

Some peer reviewers receive honoraria from CMRO for their review work. The peer reviewers of this paper have disclosed that they have no relevant financial relationships.

References

1. Busse WW. Role of leukotrienes in asthma and allergic rhinitis. Clin Exp Allergy 1996;26:868-79
2. Busse WW. Leukotrienes and inflammation. Am J Respir Critical Care Med 1998;157:S210-13
3. Peters-Golden M, Henderson WR. The role of leukotrienes in allergic rhinitis. Ann Allergy Asthma Immunol 2005;94:609-18
4. Wenzel SE. The role of leukotrienes in asthma. Prost Leuk Essent Fatty Acids 2003;69:145-55
5. Lands WEM, Libelt B, Morris A, et al. Maintenance of lower proportions of n-6 eicosanoid precursors in phospholipids of human plasma in response to added dietary n-3 fatty acids. Biochim Biophys Acta 1992;1180:147-62
6. Vas Dias FW. Eicosanoids – a brief overview. In: (R.J. Hamilton and R.D. Rice, eds.) Fish Oil: Technology, Nutrition and Marketing. P.J. Barnes & Associates, 1995: Chap. 7, 109
7. Kapoor R, Huang Y-S. Gamma linolenic acid: an antiinflammatory omega-6 fatty acid. Curr Pharm Biotech 2006;7:531-4
8. Zaloga GP. Dietary lipids: ancestral ligands and regulators of cell signalling pathways. Crit Care Med 1999;27:1646-8
9. Chilton-Lopez T, Surette ME, Swan DD, et al. Metabolism of gammalinolenic acid in human neutrophils. J Immunol 1996;156:2941-7
10. Chapkin RS, Miller CC, Somers SD, et al. Utilization of dihomo-gamma-linolenic acid (8,11,14-eicosatrienoic acid) by murine peritoneal macrophages. Biochim Biophys Acta 1988;959:322-31
11. Iverson L, Fogh K, Bojesen G, et al. Linoleic acid and dihomogammalinolenic acid inhibit leukotriene B$_4$ formation and stimulate the formation of their 15-lipoxygenase products by human neutrophils in vitro. Evidence of formation of antiinflammatory compounds. Agents Actions 1991;33:286-91
12. Iverson L, Fogh K, Kragballe K. Effect of dihomogammalinolenic acid and its 15-lipoxygenase metabolite on eicosanoid metabolism by human mononuclear leukocytes in vitro: selective inhibition of the 5-lipoxygenase pathway. Arch Dermatol Res 1992;284:222-6
13. Johnson MM, Swan DD, Surette ME, et al. Dietary supplementation with gamma-linolenic acid alters fatty acid content and eicosanoid production in healthy humans. J Nutr 1997;127:1435-44

14. Pullman-Mooar S, Laposata M, Lem D, et al. Alteration of the cellular fatty acid profile and the production of eicosanoids in human monocytes by gamma-linolenic acid. Arthritis Rheum 1990;33:1526-33

15. Ziboh VA, Fletcher MP. Dose-response effects of dietary gamma-linolenic acid-enriched oils on human polymorphonuclear-neutrophil biosynthesis of leukotriene B$_4$. Am J Clin Nutr 1992;55:39-45

16. Mita H, Yui Y, Taniguchi N, et al. Increased activity of 5-lipoxygenase in polymorphonuclear leukocytes from asthmatic patients. Life Sci 1985; 37:907-14

17. Sampson AP, Thomas RU, Costello JF, et al. Enhanced leukotriene synthesis in leukocytes of atopic and asthmatic subjects. Br J Clin Pharmacol 1992; 33:423-30

18. Pacheco Y, Hosni R, Chabannes B, et al. Leukotriene B$_4$ level in stimulated blood neutrophils and alveolar macrophages from healthy and asthmatic subjects. Effect of beta-2 agonist therapy. Eur J Clin Invest 1992; 22:732-9

19. Radeau T, Chavis C, Damon M, et al. Enhanced arachidonic acid metabolism and human neutrophil migration in asthma. Prost Leuk Essent Fatty Acids 1990;41:131-8

20. Shindo K, Koide K, Fukumura M. Enhancement of leukotriene B$_4$ release in stimulated asthmatic neutrophils by platelet activating factor. Thorax 1997; 52:1024-9

21. Cheria-Sammari S, Aloui R, Gormand F, et al. Leukotriene B4 production by blood neutrophils in allergic rhinitis – effects of cetirizine. Clin Exp Allergy 1995;25:729-36

22. Barham JB, Edens MB, Fonteh AN, et al. Addition of eicosapentaenoic acid to gamma-linolenic acid-supplemented diets prevents serum arachidonic acid accumulation in humans. J Nutr 2000;130:1925-31

23. Surette ME, Koumenis IL, Edens MB, et al. Inhibition of leukotriene synthesis, pharmacokinetics, and tolerability of a novel dietary fatty acid formulation in healthy adult subjects. Clin Ther 2003;25:948-71

24. Surette ME, Koumenis IL, Edens MB, et al. Inhibition of leukotriene biosynthesis by a novel dietary fatty acid formulation in patients with atopic asthma: a randomized, placebo-controlled, parallel-group, prospective trial. Clin Ther 2003;25:972-9

25. Food and Drug Administration. Guidance for Industry. Patient-reported outcome measures: use in medical product development to support labeling claims. U.S. Department of Health and Human Services. Rockville, MD: FDA/Center for Drug Evaluation and Researh, 2006. Available at www.fda.gov/CDER/GUIDANCE/5460dft.pdf [Last accessed 28 February 2008]

26. Juniper EF, Guyatt GH, Epstein RS, et al. Evaluation of impairment of health related quality of life in asthma: development of a questionnaire for use in clinical trials. Thorax 1992;47:76-83

27. Juniper EF, Buist AS, Cox FM, et al. Validation of a standardized version of the asthma quality of life questionnaire. Chest 1999;115:1265-70

28. Juniper EF, Guyatt GH, Cox FM, et al. Development and validation of the Mini Asthma Quality of Life Questionnaire. Eur Respir J 1999;14:32-8

29. Juniper EF, O'Byrne PM, Guyatt GH, et al. Development and validation of a questionnaire to measure asthma control. Eur Respir J 1999;14:902-7

30. Surette ME, Stull D, Lindemann J. The impact of a medical food containing gammalinolenic and eicosapentaenoic acids on asthma management and the quality of life in adult asthma patients. Curr Med Res Opin 2008;24:559-67

31. National Asthma Education and Prevention Program. Expert Panel Report: Guidelines for the Diagnosis and Management of Asthma-Update on Selected Topics 2002. U.S. Department of Health and Human Services, National Institutes of Health, National Heart, Lung and Blood Institute. NIH Publication No. 02-5074, June 2003

32. Juniper EF, O'Byrne PM, Roberts JN. Measuring asthma control in group studies: do we need airway calibre and rescue beta2-agonist use? Respir Med 2001;95:319-23

33. Juniper EF. Assessing asthma control. Curr Allergy Asthma Rep 2007; 7:390-4

34. Juniper EF, Bousquet J, Abetz L, et al. Identifying 'well-controlled' and 'not well-controlled' asthma using the Asthma Control Questionnaire. Respir Med 2006;100:616-21

35. Dole VP, Meinertz H. Microdetermination of long-chain fatty acids in plasma and tissues. J Biol Chem 1960;235:2595-9

36. Assay details. Available at: http://www.caymanchem.com/pdfs/520411.pdf [Last accessed 28 September 2009]

37. Moy ML, Israel E, Weiss ST, et al. Clinical predictors of health-related quality of life depend on asthma severity. Am J Resp Crit Care Med 2001;163:924-9

38. Juniper EF, Price DB, Stampone PA, et al. Clinically important improvements in asthma-specific quality of life, but no difference in conventional clinical indexes in patients changed from conventional beclomethasone dipropionate to approximately half the dose of extrafine beclomethasone dipropionate. Chest 2002;121:1824-32

39. Pinnock H, Juniper EF, Sheikh A. Concordance between supervised and postal administration of the Mini Asthma Quality of Life Questionnaire (MiniAQLQ) and Asthma Control Questionnaire (ACQ) was very high. J Clin Epidemiol 2005;58:809-14

40. Guyatt GH, Juniper EF, Walter SD, et al. Interpreting treatment effects in randomized trials. BMJ 1998;316:690-3

41. Juniper EF, Guyatt GH, Willan A, et al. Determining a minimal important change in a disease-specific Quality of Life Questionnaire. J Clin Epidemiol 1994;47:81-7

42. Levy BD, Kohli P, Gotlinger K, et al. Protectin D1 is generated in asthma and dampens airway inflammation and hyperresponsiveness. J Immunol 2007; 178:496-502

43. Hederos C-A, Berg A. Epogam evening primrose oil treatment in atopic dermatitis and asthma. Arch Dis Child 1996;75:494-7

44. Ebden P, Bevan C, Banks A, et al. A study of evening primrose seed oil in atopic asthma. Prost Leuk Essent Fatty Acids 1989;35:69-72

45. Stenius-Aarniala B, Aro A, Hakulinen A, et al. Evening primrose oil and fish oil are ineffective as supplementary treatment of bronchial asthma. Ann Allergy 1989;62:534-7

46. Ziboh VA, Naguwa S, Vang K, et al. Suppression of Leukotriene B$_4$ generation by ex vivo neutrophils isolated from asthma patients on dietary supplementation with gammalinolenic acid-containing borage oil: possible implication in asthma. Clin Dev Immunol 2004;11:13-21

47. Kirsch CM, Payan DG, Wong MYS, et al. Effect of eicosapentaenoic acid in asthma. Clin Allergy 1988;18:177-87

48. McDonald CF, Vecchio L, Pierce RJ, et al. Effect of fish-oil derived omega-3 fatty acid supplements on asthma control (Abstract). Aust NZ J Med 1990; 20:526

49. Picado C, Castillo JA, Schinca N, et al. Effects of a fish oil enriched diet on aspirin intolerant asthmatic patients: a pilot study. Thorax 1988;43:93-7

50. Broughton KS, Johnson CS, Pace BK, et al. Reduced asthma symptoms with n-3 fatty acid ingestion are related to 5-series leukotrienes production. Am J Clin Nutr 1997;65:1011-17

51. Hodge L, Salome CM, Hughes JM, et al. Effect of dietary intake of omega-3 and omega-6 fatty acids on severity of asthma in children. Eur Respir J 1998;11:361-5

52. Dry J, Vincent D. Effect of a fish oil diet on asthma: results of a 1-year double-blind study. Int Arch Allergy Appl Immunol 1991;95:156-7

53. Thien FCK, Mencia-Huerta J-M, Lee TH. Dietary fish oil effects on seasonal hay fever and asthma in pollen-sensitive subjects. Am Rev Respir Dis 1993; 14:1138-43

54. Mickleborough TD, Murray RL, Ionescu AA, et al. Fish oil supplementation reduces severity of exercise-induced bronchoconstriction in elite athletes. Am J Respir Crit Care Med 2003;168:1181-9

55. Mickleborough TD, Lindley MR, Ionescu AA, et al. Protective effect of fish oil supplementation on exercise-induced bronchoconstriction in asthma. Chest 2006;129:39-49

56. Nagakura T, Matsuda S, Shichijyo K, et al. Dietary supplementation with fish oil rich in ω-3 polyunsaturated fatty acids in children with bronchial asthma. Eur Respir J 2000;16:861-5

57. Arm JP, Horton CE, Spur BW, et al. The effects of dietary supplementation with fish oil lipids on the airways response to inhaled allergen in bronchial asthma. Am Rev Respir Dis 1989;139:1395-400

58. Arm JP, Horton CE, Mencia-Huerta J-M, et al. Effect of dietary supplementation with fish oil lipids on mild asthma. Thorax 1988;43:84-92

59. Guichardant M, Trailter H, Spielmann D, et al. Stearidonic acid, an inhibitor of the 5-lipoxygenase pathway. A comparison with timnodonic and dihomogam-malinolenic acid. Lipids 1993;28:321-4

60. Schubert R, Kitz R, Beermann C, et al. Influence of low-dose polyunsaturated fatty acids supplementation on the inflammatory response of healthy adults. Nutrition 2007;23:724-30

61. FDA. Substances affirmed as generally recognized as safe: Menhaden oil; Final rule (21 CFR, Part 184, Docket No. 86G-0289). Federal Register 1997; 62:30751-7

62. FDA. Agency Response Letter 2004; GRAS Notice No. GRN 000138 [Fish Oil]. U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN), Office of Food Additive Safety; College Park, MD. Available at http://www.cfsan.fda.gov/~rdb/opa-g138.html [Last accessed 28 February 2008]