Effect of dietary intake of omega-3 and omega-6 fatty acids on severity of asthma in children

L Hodge, C M Salome, J M Hughes, D Liu-Brennan, J Rimmer, M Allman, D Pang, C Armour, A J Woolcock

Institute of Respiratory Medicine

FISHERIES RESEARCH & DEVELOPMENT CORPORATION

Project No. : 95/121

EFFECT OF DIETARY INTAKE OF OMEGA-3 AND OMEGA-6 FATTY ACIDS ON SEVERITY OF ASTHMA IN CHILDREN

L Hodge, M.Sc. (Med.), Dip. Nutr. Diet.

C M Salome, B.Sc.

J M Hughes, PhD

D Liu-Brennan, B.Sc.

J Rimmer, MD, FRACP

M Allman, PhD

D Pang, B.Sc. Hons

C Armour, PhD

A J Woolcock, MD FRACP

Institute of Respiratory Medicine

P.O. Box M77

Missenden Road Post Office

CAMPERDOWN. N.S.W. 2050

Telephone : 9515 8383

Fax : 9550 6115

Fisheries Research and Development Corporation

Project No. : 95/121

ISBN No. : 0 646 32468 3

TABLE OF CONTENTS

ABBREVIATIONS

AA	Arachidonic Acid
ANOVA	Analysis of Variance
AHR	Airway hyperresponsiveness to histamine
DHA	Docosahexaenoic Acid
ELISA	Enzyme-linked immunosorbent assay
EPA	Eicosapentaenoic Acid
FEV_1	One second Forced Expiratory Volume
FVC	Forced Vital Capacity
LA	Linoleic Acid
αLNA	Alpha linolenic acid
PBMC	Peripheral Blood Mononuclear Cells
TNFα	Tumour Necrosis Factor alpha

95/131 Effect of dietary intake of omega-3 and omega-6 fatty acids on severity of asthma in children

PRINCIPAL INVESTIGATOR : L. Hodge
ADDRESS : Institute of Respiratory Medicine,
P.O. Box M77, Missenden Road Post Office,
Camperdown. N.S.W. 2050

OBJECTIVES :

1. To determine if low doses of fish oil, given as a dietary supplement to children, against a background diet which is low in the omega-6 fatty acid, linoleic acid, increases the level of the omega-3 fatty acid, eicosapentaenoic acid, in plasma and reduces the production of eosinophils and TNFα, both of which are known to be associated with inflammation.

2. To determine if this diet leads to an improvement in lung function, symptoms of asthma (cough, wheeze) and a reduction in medication use.

NON TECHNICAL SUMMARY :

We have previously shown that regular fish consumption and particularly consumption of oily fish is associated with reduced risk of children having asthma. Fish oil is high in a fatty acid of the omega-3 class known as eicosapentaenoic acid. This fatty acid is important in the inflammatory process and the products of its metabolism are less inflammatory than its omega-6 fatty acid counterpart - arachidonic acid, which is found in meat, eggs and dairy products. Linoleic acid is a precursor to arachidonic acid and is found in many vegetable oils and margarines. The use of vegetable oils and margarines has increased up to five-fold in the past 30 years and this period coincides with a doubling of the prevalence of childhood asthma.

Since inflammation in the airway walls is a fundamental abnormality in asthma, the association between oily fish consumption and reduced risk of asthma led to the hypothesis that the change in the ratio of omega-3 to omega-6 fatty acids in the diet may be one of the factors responsible for the increase in childhood asthma. It is also possible that a diet high in omega-6 fatty acids and low in omega-3 fatty acids can increase the severity of asthma or, alternatively, that a diet high in omega-3 fatty acids can reduce the severity of asthma.

In order to investigate the hypothesis that diets high in omega-3 fatty acids can reduce the severity of childhood asthma, thirty-nine asthmatic children aged 8-12 years participated in a double-blind, randomised, controlled trial for six months during which they received fish oil capsules plus canola oil and margarine (omega-3 group) or safflower oil capsules plus sunflower oil and margarine (omega-6 group). Plasma fatty acids, markers of inflammation (stimulated TNFα production and circulating eosinophil numbers) and lung function were measured at baseline and after three and six months of dietary modification. Day and night symptoms, peak flow rates and medication use were recorded for one week prior to laboratory visits. The major findings of this study were :

1. Plasma omega-3 fatty acids (eicosapentaenoic acid, docosahexaenoic acid) were not different at baseline between groups but were significantly greater in the omega-3 group than in the omega-6 group at three and six months ($p<0.00001$).

2. Of the omega-6 fatty acids, arachidonic acid was unchanged in both groups, but linoleic acid decreased in the omega-3 group and increased in the omega-6 group. These levels were significantly different between groups at three months ($p<0.01$) but not at six months.

3. In the omega-3 group TNFα production fell significantly compared with baseline at six months ($p=0.026$) but the magnitude of change between groups did not reach significance ($p=0.075$).

4. Circulating eosinophil numbers were significantly reduced in the omega-3 group compared with the omega-6 group. This reduction was not significant after log-transformation and the eosinophil numbers at 6 months in both groups were still above normal levels.

5. There were no significant changes in the clinical outcome measures of symptoms, lung function, peak flow rates and use of medication.

We conclude that a fish oil supplement along with the addition to the diet of oils and margarines high in omega-3 fatty acids increased the plasma levels of eicosapentaenoic acid and produced a downward trend in the production of inflammatory cells (eosinophils) and chemicals (TNFα) over 6 months, but had no effect on the clinical severity of asthma in the children studied. It is possible that clinical improvement may not be achieved until the levels of inflammatory cells and chemicals reach the normal range, suggesting that a longer period of supplementation may be required. Alternatively, fish oil may prevent the development of asthma and needs to be introduced earlier in life, before the disease becomes established. Further studies are required to investigate this latter explanation.

KEYWORDS : **asthma, fish oil, inflammation, omega-3 fatty acids, omega-6 fatty acids**

1. BACKGROUND

We have previously shown that regular fish consumption (1) and particularly consumption of oily fish (2) is associated with reduced risk of children having asthma. This association led to the hypothesis that the ratio of omega-3 to omega-6 fatty acids in the diet may be one of the factors which can influence clinical severity of asthma (3).

Previous studies have shown that dietary supplementation with fish oil, a rich source of the omega-3 fatty acid, eicosapentaenoic acid (EPA), and/or one of the vegetable sources of omega-3 fatty acids, alpha linolenic acid (αLNA), increases the levels of EPA in the phospholipids of cell membranes by up to 10 fold (4), and reduces the synthesis of the proinflammatory cytokines interleukin-1 and tumour necrosis factor alpha (TNFα) in human mononuclear cells (5). Although these changes have been associated with a reduction in the severity of late asthmatic responses to allergen (4), most of the clinical trials undertaken have shown no beneficial effect on the clinical severity of asthma. Generally, the studies have been short (8-10 weeks). Longer exposure periods may be required to reduce inflammation and thus induce clinical improvement, although a study of six months duration showed no effect on the development of seasonal hay fever and asthma (6). However, all of these studies were in adults whilst the epidemiological studies showing reduced risk have been in children.

The aim of the present study was to explore a possible mechanism for the findings of these epidemiological studies through a randomised controlled trial in which the diets of asthmatic children were supplemented with either omega-3 or omega-6 fatty acids. The effects on clinical, biochemical and inflammatory parameters were measured over six months.

2. NEED

The prevalence of asthma in children in Australia is extremely high and has increased dramatically over the last 20 years. There is circumstantial evidence to suggest that the increase in prevalence is associated with a change in the types of dietary fats used. There has been a shift away from animal derived fats such as lard, dripping and butter, towards margarines and oils, particularly those derived from sunflowers. Such changes in diet alter the types of fatty acids in cell membranes resulting in an increase in the quantity and activity of inflammatory mediators derived from these membranes. Fish oil has the potential to reverse these changes and reduce the morbidity and the incidence of asthma in children

We have shown that consumption of oily fish is associated with a reduced risk of asthma in children but it is not clear whether this effect is due to a therapeutic effect, reducing the severity of asthma in children who already have the disease, or a prophylactic effect, preventing the development of the disease in children. It is clear that both of these hypotheses need testing.

This project will test the hypothesis that a moderate fish oil supplement and modification of the background diet to include oils from the same class as the fish oils can reduce the severity of asthma in children. It will also look for evidence that minor modifications of the diet will lead to biochemical changes, that are likely to reduce the risk of asthma and provide a basis for future preventative interventions.

In the longer term, it will be essential to undertake studies to determine if fish oil supplements or increased consumption of fresh oily fish can prevent the development of asthma in "at risk" children. Previous studies of fish oil supplementation have used such high doses that they would be unlikely to be acceptable for use by pregnant women or young children. Future studies will be greatly facilitated if it is possible to recommend a diet or supplement which is both acceptable and effective.

3. OBJECTIVES

1. To determine if low doses of omega-3 fatty acids (1.2g/day), given as a dietary supplement, against a background diet which increases omega-3 and reduces omega-6 fatty acids, increases the level of EPA in plasma phospholipids, reduces the production of TNFα and numbers of circulating eosinophils.

2. To determine if treatment with supplementary omega-3 fatty acids and a diet which increases omega-3 and reduces omega-6 fatty acids over six months leads to an improvement in the lung function, airway hyperresponsiveness and symptoms of asthma (cough, wheeze) in children.

4. METHODS

Subjects

Forty-five asthmatic children, aged 8 - 12 years, with a history of episodic wheeze in the last 12 months and airway hyperresponsiveness to histamine (AHR) were recruited. Six subjects dropped out at baseline. The children were randomly allocated to one of two diet groups - twenty in the omega-3 group (11 female) and nineteen in the omega-6 group (11 female). Children with other significant diseases, taking regular oral corticosteroids or with known aspirin or dietary salicylate sensitivity were excluded. The study was approved by the Ethics Review Committee of the Central Sydney Area Health Service and by the Human Ethics Committee of the University of Sydney.

Study Design

There were two baseline visits separated by a two weeks, to establish the repeatability of the baseline measurements and to obtain diary card data of asthma severity. During the first baseline visit responses to allergen skin prick tests and the child's history of respiratory symptoms and medication use as well as parental smoking, race and social class were documented. The second baseline visit was followed by six months on a fat modified diet. During the diet period, subjects took supplementary capsules containing either fish oil (omega-3 group) or safflower/palm/olive oil (omega-6 group) and were asked to use exclusively the margarines and oils supplied. Subjects were reviewed at 12 weeks and finally at 24 weeks from commencement of the supplementation and dietary modification. At every visit lung function, AHR, height and weight were recorded and venous blood was collected for measurement of eosinophil levels, production of TNFα and the fatty acid composition of plasma. Severity of asthma was monitored via the completion of a parent supervised diary for one week prior to all but the first baseline visit. During the pre-visit week peak flow readings, medication requirements and symptom scores were recorded daily. At the same time a detailed dietary record was kept.(Appendix 1)

Diets

Omega-3 diet: Canola oil and canola-based margarines and salad dressings (Meadowlea Pty. Ltd., Sydney N.S.W.) were supplied to the family in unmarked containers to replace their usual oils and margarines. Canola oil is high in αlinolenic acid, an omega-3 fatty acid. Subjects were asked to use these oils and margarines exclusively and to have a meal containing fish at least once a month.

Omega-6 diet: Sunflower oil and sunflower oil based margarines and salad dressings (Meadowlea Pty. Ltd., Sydney N.S.W.) were supplied in unmarked containers to the families. Sunflower oil is high in linoleic acid, an omega-6 fatty acid. Subjects were asked not to eat fish and to use the supplied oils and margarines exclusively.

Supplementary capsules: All subjects were asked to take 4 supplementary capsules per day. The omega-3 group took MaxEPA (R.P. Scherer) containing 0.18g EPA and 0.12g docosahexaenoic acid (DHA), to give a total of 1.2 grams of omega-3 fatty acids per day. The omega-6 group took matched placebo capsules (R.P. Scherer), containing a combination of safflower (0.45g), palm (0.45g) and olive (0.1g) oils per capsule. No EPA or DHA was present in the placebo preparation.

Subjects and laboratory staff were blinded to the study groups. Compliance with taking the supplementary capsules was assessed by counting the number of unused capsules.

Diet diary

A detailed diary of all types and household measures of food and drinks consumed, including brand names, was kept for one week during the baseline and after three months and six months of dietary modification and supplementation (Appendix 1). Data from the diary was used to check compliance with the diet and that dietary intakes did not alter more than would be expected over the six months of study, taking into consideration the growth of the children.

Lung function and airway hyperresponsiveness

Lung function and AHR were measured at the beginning and end of baseline and after three months and six months of treatment. A Vitalograph wedge bellows spirometer (Vitalograph Ltd, Bucks, UK) was used to measure forced vital capacity (FVC) and one second forced expiratory volume (FEV_1). The highest of two values for FEV_1 repeatable to within 100ml was recorded and the percentage of predicted values (7) was calculated.

Airway hyperresponsiveness was measured using histamine inhalation tests performed according to the method of Yan *et al* (8). Briefly, histamine was administered to the subject via hand-held DeVilbiss No 45 plastic nebulisers in doubling doses, ranging from 0.03 to 7.8μ mol, until the FEV_1 fell by 20% or more. The dose of histamine causing the maximum fall in FEV_1 was used to calculate the dose response ratio which is the percentage fall in FEV_1 divided by the cumulative dose of histamine (9). A higher dose response ratio indicates more severe asthma. All short acting aerosol bronchodilators were withheld for six hours and long acting bronchodilators for 36 hours

Asthma severity

Asthma severity was measured for one week during the week prior to the second laboratory visit at baseline, and after three months and six months of the fat modified diet (Appendix 1). Asthma severity was measured using a composite severity score based on daily diary records of expiratory flow rate, day and night symptoms and medication use. Each component of the score contributes up to four points to a maximum composite score of 16. Expiratory flow rate was measured on waking, before bronchodilator, using an Assess peak flow meter (HealthScan Products, NJ, USA) or a Mini Wright peak flow meter (Clement Clarke International Ltd, Essex, UK) and scored according to the percent predicted value. Medication use was scored according to the frequency and type of medication. Symptom scores for wheeze, cough and shortness of breath were recorded for both day and night, with scores ranging from 0 (no symptoms) to 4 (symptoms which make normal activity, or sleeping, impossible).(Appendix 2)

Blood analysis

Venous blood (20ml) was collected at each laboratory visit. Full blood counts were performed on every occasion by automated full blood count analyser (Bayer Technicon H2). Peripheral blood mononuclear cells (PBMC) were purified from anti-coagulated peripheral blood by discontinuous density gradient centrifugation using mono-poly resolving medium (ICN Biomedicals) (10) and cultured in RPMI-1640 supplemented with Monomed A (CSL Ltd., Victoria, Australia) at $2x10^6$ cells/ml in a 5% CO_2 in air atmosphere at 37^oC. PBMC cultures were activated with lipopolysaccharide (10ng/ml), harvested and stored at -80^oC. Total PBMC synthesis of TNFα was assessed in the cell cultures after freeze-thawing (5) using a sandwich enzyme-linked immunosorbent assay (ELISA) and the human TNFα Duoset™ system (Genzyme Diagnostics, MA, USA).

For fatty acid analysis plasma lipids were extracted by the method of Bligh and Dyer (11). Phospholipids were separated from neutral lipids using a silica column. Tubes containing phospholipids were flushed with nitrogen and stored at $-80^{\circ c}$ until fatty acid determination. The phospholipids were transesterified using a one step methanolysis reaction (12) and fatty acids analysed using flame ionisation capillary gas chromatography (Hewlett-Packard Capillary Gas Chromatograph, North Ryde, NSW) using a fused carbon-silica column, coated with cyanopropylphenyl (J and W Scientific, Folsom, CA), hydrogen carrier gas and two step oven temperature program to allow optimal separation. Individual fatty acids (including LA, AA and EPA) were identified by comparison with a standard mixture (Nu Check Prep, Elysian, MN) to which EPA (Sigma, St Louis, MO) was added. The fatty acids were expressed as a percentage of total fatty acids.

Data Analysis

Data are reported as means $\pm$ 95% confidence intervals (95% ci). Since there were no significant differences between the first and second baseline values for any variable, mean baseline values were calculated and used for comparison. Differences between groups were determined by analysis of variance for repeated measures, for continuous variables, and by Chi-squared test for categorical variables. In addition, mean changes from baseline to three months and from baseline to six months were calculated and compared between groups by t-test. Values for dose response ratio were log transformed before analysis. Median values for eosinophil numbers and asthma severity scores were estimated and changes from baseline compared at 3 and 6 months by Mann-Whitney test.

5. RESULTS

Baseline characteristics of the two groups are shown in Table 1. At baseline there were no significant differences between the groups in lung function, as percent predicted, AHR, atopy, use of inhaled corticosteroids, use of bronchodilators, plasma fatty acid levels, eosinophil levels or mononuclear TNFα production. The omega-3 group was slightly older (mean age, $\pm$ 95% confidence intervals, 10.8 $\pm$ 0.6 years and 9.7 $\pm$ 0.4 years respectively $p<0.01$) and taller (mean height, $\pm$ 95% confidence intervals, 146 $\pm$ 4 cm and 139 $\pm$ 4 cm respectively $p=0.02$) than the omega-6 group.

There was no significant difference between groups in the quantity of oil and margarine consumed throughout the study. The omega-3 group ate significantly more fresh fish than the omega-6 group (mean intake $\pm$ 95% confidence intervals, 370 $\pm$ 148g/mth and 109 $\pm$ 70g/mth respectively, $p=0.0045$). The mean number of capsules taken per day was 3 out of a recommended 4 in both groups, but no child averaged less than 2 capsules per day. One child in the omega-3 group and two children in the omega-6 group experienced some discomfort after taking the capsules which was not related to their asthma and medication use did not change significantly throughout the study (Tables 16A & B).

Objective 1. ***To determine if low doses of omega-3 fatty acids (1.2g/day), given as a dietary supplement, against a background diet which increases omega-3 and reduces omega-6 fatty acids :***

*a. **Raises the level of EPA in plasma phospholipids;***
*b. **reduces the production of TNFα and,***
*c. **reduces the numbers of circulating eosinophils.***

Summary : ***a. The level of EPA in the omega-3 group was five times higher at three months and 3 times higher at 6 months compared with the baseline values, whilst the levels in the omega-6 group remained unchanged.***

b. TNFα production was significantly reduced in the omega-3 group compared with baseline, whilst the levels were unchanged in the omega-6 group. However, the difference between groups for TNFα production at six months did not reach significance, possibly due to the large variability in readings.

c. Numbers of circulating eosinophils were reduced in the omega-3 group and not in the omega-6 group, but the difference was not significant after log transformation.

Fatty acids

Plasma phospholipid omega-3 fatty acids, as a percent of total fatty acids, increased in the omega-3 group and was virtually unchanged in the omega-6 group. The mean change ($\pm$ 95% ci) in total omega-3 fatty acids at 3 months was 3.18 $\pm$ 0.88% vs -0.21 $\pm$ 0.24% p<0.0000001 and the mean change at 6 months was 2.19 $\pm$ 0.67% vs 0.05 $\pm$ 0.41% p<0.00001(Table 11, Figure 1).

Plasma phospholipid EPA also increased in the omega-3 group compared with the omega-6 group. The mean change in plasma EPA at 3 months was 1.98 ± 0.53% (omega-3) vs -0.11 ± 0.09% (omega-6) p<0.0000001 and the mean change at 6 months was 1.75 ± 0.45% (omega-3) vs 0.19 ± 0.44% (omega-6) p=0.0024 (Table 12, figure 2).

The percentage of plasma LA decreased in the omega-3 group and increased in the omega-6 group. The mean change between groups was significantly different at three months (omega-3 group: -2.04 ± 1.92% vs omega-6 group: 1.69 ± 1.85% p=0.0099) but not at six months (omega-3 group: -0.83 ± 1.28% vs omega-6 group: 1.17 ± 1.81% p=0.0809) (Table 13, figure 3).

Changes in plasma AA were small and not significantly different between groups at either 3 or 6 months (Table 14).

TNFα

In the omega-3 group TNFα production from stimulated PBMCs fell significantly over the six month period from 1300 ± 316 pg/ml to 896 ± 211pg/ml (p = 0.026), but the magnitude of the changes did not differ significantly between groups (mean change at six months in the omega-3 group was -416 ± 331 pg/ml and in the omega-6 group was 44 ± 359 pg/ml; p = 0.075) (Table 15, figure 6). There were no significant differences between groups in TNFα production over time, (p = 0.22 using repeated measures analysis of variance (ANOVA)).

Peripheral Blood eosinophils

In the omega-3 group, eosinophil numbers fell throughout the study from a median value of 0.91×10^9/L at baseline, to 0.74×10^9/L at 3 months and 0.65×10^9/L at 6 months. In the omega-6 group eosinophil numbers rose from a median value of 0.62×10^9/L at baseline, to 0.70×10^9/L at 3 months and 0.81×10^9/L at 6 months. However, the magnitude of these changes did not differ significantly between groups (p=0.11, Mann-Whitney test) (Table 10, figure 7).

Objective 2. ***To determine if treatment with supplementary omega-3 fatty acids and a diet which increases omega-3 and reduces omega-6 fatty acids over six months leads to an improvement in the lung function, airway hyperresponsiveness and symptoms of asthma (cough, wheeze) in children.***

Summary : ***There was no change in either group for any of these parameters.***

Lung function and asthma severity

There was no significant change in spirometric function, dose response ratio to histamine or asthma severity scores at either 3 months or 6 months in either group. Mean FEV_1 at six months in the omega-3 group was 83 ± 5% predicted and in the omega-6 group was 84 ± 5% predicted (Table 2) and mean FVC at six months in the omega-3 group was 85 ± 5% predicted compared with 87 ± 3% predicted in the omega-6 group (Table 3). Median asthma severity score (including peak flow, day and night-time symptom and medication scores - Tables 5, 6, 7 & 8) was 7 in the omega-3 group and 8 in the omega-6 group (Table 9, figure 4)). The mean change in dose response ratio at six months in the omega-3 group was -0.4 ± 0.6 doubling doses and in the omega-6 group was 0.5 ± 0.9 doubling doses (p=0.10) (Table 4, figure 5).

6. DISCUSSION

This study has shown that, in asthmatic children, a modest fish oil supplement of 1.2g/day of omega-3 fatty acids and relatively minor changes to the diet caused a fivefold increase in plasma EPA. Although there were no significant clinical effects, there was a trend towards reduced TNFα production and reduced numbers of circulating eosinophils.

The changes in omega-3 fatty acids in plasma phospholipids achieved in this study are similar to studies which used much larger supplements of fish oil (10-15 g/day) (13) suggesting that significant omega-3 incorporation into phospholipids can be attained with relatively low doses of fish oil accompanied by a background diet low in omega-6 fatty acids. Changes of this magnitude have been associated with significant reductions in neutrophil chemotaxis (14). The observed changes in plasma EPA levels confirm that compliance was good over the whole period of the trial.

Arachidonic acid is a relatively abundent fatty acid found in foods, particularly meat, and it has been suggested that high levels of this fatty acid contribute to the severity of asthma (15). Linoleic acid is the metabolic precursor of AA and so it could be expected that an increase in LA levels would result in an increase in AA or, alternatively, that a reduction in LA would result in a reduction in AA. However, in this study, the levels of AA did not change in either group. The absence of any change in the omega-6 group suggests that, under normal circumstances, the capacity of the liver to form AA from LA is limited. Supplementation of LA in rats has no effect on either the LA, EPA or AA (16).

Previous studies have shown that an increase in EPA in the diet results in a reduction in AA in the plasma and cell phospholipids (13)(17) but, in these studies, the intake of LA was not modified. High dietary levels of EPA, with modification of other dietary fatty acids is likely to increase competition between AA and EPA for sites on the phospholipids, resulting in decrease in AA levels. In our study, the reduction of LA in the diet may have freed some sites on the phospholipids for occupation by EPA without the need for displacing the AA.

The net effect of reducing the LA intake and increasing the EPA is a change in the ratio of EPA to AA, without affecting the levels of AA. It is therefore possible that a deficiency of EPA is the important factor in modulation of the inflammatory response, rather than an excess of AA as was previously thought (15).

At 6 months the difference in linoleic acid between the groups was no longer significant. This could indicate that compliance was reduced in the seond half of the study. The diet diaries showed that both groups continued to use the oils and margarines supplied but it is possible that they did not take their capsules with the same regularity. Alternatively, a homeostatic mechanism may be responsible for maintenance of the balance of fatty acids which can be overridden initially with a change in the diet but cannot be sustained to the same degree over extended periods. The decline in omega-3 fatty acid levels at six months in the omega-3 group could also be explained in a similar way.

TNFα production is implicated in the pathogenesis of asthma since it is increased in asthmatics (18) and increases airway responsiveness both in vitro (19) and in vivo (20). The effect of fatty acid intake on TNFα production has been well established and it has been shown that significant reductions in stimulated TNFα production can be achieved with smaller numbers of subjects and shorter duration of the study period than the present study (21). However, the subjects of these studies were healthy male adults. It is possible that any changes in an asthmatic subject would be slower due to the presence of active inflammation. The fact that there was a downward trend in TNFα production which was consistent at both three months and six months suggests that a study of longer duration might have produced more significant effects. Circulating eosinophil numbers also tended to decrease throughout the study and this could indicate a down-regulation of the inflammatory process. The effect of fatty acid intake on circulating eosinophil numbers has not been examined in any other study.

Changing the fatty acid intake had no effect on any clinical measure of asthma severity in these asthmatic children. This finding is in accordance with those of previous studies in adult asthmatics (17), and suggests that, in children with existing asthma, modification of fat intake is unlikely to have any short term therapeutic benefit. In the absence of any therapeutic effect, a number of plausible mechanisms may explain the observed reduction in the risk of having asthma in children who eat oily fish. If the diet measured by a food frequency questionnaire is typical of lifetime dietary habits of children and parents, it is possible that a diet rich in omega-3 fatty acids during early life, or even prenatally, may prevent the development of asthma in susceptible children. Alternatively, modest differences in dietary fats over a longer period of time may modify cytokine production and inflammatory processes, potentially reducing symptoms in the long term. In the present study TNFα production continued to fall at six months, even though the changes in omega-3 fatty acids in the plasma phospholipids were maximal at three months. Finally, it is possible that fish oil does not reduce the risk of asthma and that some other chemical in oily fish is responsible.

We conclude that a fish oil supplement along with the addition to the diet of canola oil and canola oil margarine over six months increased the plasma levels of omega-3 fatty acids and reduced the levels of LA, but had no effect on the clinical severity of asthma in these children. However, there was a downward trend in both eosinophil numbers and TNFα production in the omega-3 group. This suggests that increases in dietary omega-3 fatty acids over a longer period of time, say years, may be required to reduce the severity of existing asthma. It is yet to be determined if increasing dietary omega-3 fatty acid intake in early life can prevent the development of asthma.

7. IMPLICATIONS AND RECOMMENDATIONS

1. We have shown that fish oil supplements and a diet which reduces linoleic acid can produce biochemical changes indicative of a reduced inflammatory response. The significance of these changes is uncertain because no clinical improvements were observed. There are several possible explanations :

a. A longer period of supplementation may be required. In those children with established asthma the levels of inflammatory mediators may need to be closer to normal levels before any clinical benefit is observed.

b. The correct balance of dietary fatty acids needs to be introduced either prior to or in the early stages of asthma. Once asthma is well established there are changes to the airway wall which perpetuate the disease and dietary manipulation may not be enough to reverse these changes.

c. The correct balance of dietary fatty acids needs to be achieved before birth through the maternal diet to prevent the development of asthma. It is known that long chain fatty acids are essential for normal growth and development of the foetus. It is also known that children with a head circumference greater than 37cm at birth are three times more likely to develop asthma (22) and that fatty acids measured in umbilical vein blood immediately after delivery of the placenta are correlated with head circumference (23)

d. Some other component in fresh oily fish is responsible for the observed reduced risk of asthma in the children surveyed.

2. The following are recommendations for further studies to investigate these possibilities.

   a. A study similar to the present study but extended to one year or more

   b. A prospective longitudinal study of children who, from their family history, have an increased risk of developing asthma, which encourages the inclusion of fresh, oily fish in infancy and early childhood and is accompanied by the avoidance of oils and margarines high in omega-6 fatty acids.

   c. A study of women at high risk of having children who will develop asthma which increases the ratio of omega-3 to omega-6 fatty acids in the diet during pregnancy through reducing the intake of oils and margarines high in omega-6 and increasing the consumption of foods high in omega-3 fatty acids such as fresh, oily fish.

   d. A study which compares fish oil supplementation with fresh, oily fish consumption in subjects with asthma.

8. INTELLECTUAL PROPERTY

No commercially vaulable intellectual property arises from this project.

9. ACKNOWLEDGEMENTS

The authors wish to thank R.P.Scherer Ltd for providing the MaxEPA capsules and Meadowlea Pty Ltd for providing the oil, margarine and mayonnaise for the study. We would also like to thank the children and their families for their willing participation in this study.

10. REFERENCES

1. Peat JK, Salome CM, Woolcock AJ. Factors associated with bronchial hyperresponsiveness in Australian adults and children. Eur Respir J 1992;5:921-29.

2. Hodge L, Salome CM, Peat JK, Haby MM, Xuan W, Woolcock AJ. Consumption of oily fish and childhood asthma risk. Medical Journal of Australia 1996;164:137-140.

3. Hodge L, Peat JK, Salome CM. Increased consumption of polyunsaturated oils may be a cause of increased prevalence of childhood asthma. Aust NZ J Med 1994;24:727.

4. Arm JP, Horton CE, Spur BW, Mencia-Huerta J-M, Lee TH. The effects of dietary supplementation with fish oil lipids on the airways response to inhaled allergen in bronchial asthma. Am Rev Respir Dis 1989;139:1395-1400.

5. Endres S, Ghorbani R, Kelley VE, et al. The effect of dietary supplementation with n-3 polyunsaturated fatty acids on the synthesis of interleukin-1 and tumor necrosis factor by mononuclear cells. N Engl J Med 1989;320:265-271.

6. Thien FCK, Menci-Huerta JM, Lee TH. Dietary fish oil effects on seasonal hay fever and asthma in pollen-sensitive subjects. Am Rev Respir Dis 1993;147:1138-1143.

7. Polgar G, Promadhat V. Pulmonary function testing in children: techniques and standards. Philadelphia: Saunders, 1971.

8. Yan K, C S, Woolcock A. A rapid method for the measurement of bronchial responsiveness. Thorax 1983;38:760-765.

9. Peat JK, Salome CM, Berry G, Woolcock AJ. Relation of dose-response slope to respiratory symptoms in a population of Australian schoolchildren. American Review of Respiratory Disease 1991;144:663-667.

10. Ferrante A, Thong YH. Optimal conditions for simultaneous purification of mononuclear and polymorphonuclear leucocytes from human peripheral blood by the hypaque-ficoll method. Journal of Immunological Methods 1980;36:109-117.

11. Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry 1959;37:911-917.

12. Lepage R. Diet esterification of all classes of lipids in a one-step reaction. Journal of Lipid Research 1986;27:114-120.

13. Gibney MJ, Hunter B. The effects of short- and long-term supplementation with fish oil on the incorporation of n-3 polyunsaturated fatty acids into the cells of the immune system in healthy volunteers. European Journal of Clinical Nutrition 1993;1993:255-259.

14. Dyerberg J, Schmidt EB. Modulation of atherthrombogenesis by n-3 polyunsaturated fatty acids. In: Sinclair A, Gibson R, eds. Third International Congress on Essential Fatty Acids and Eicosanoids. Adelaide: AOCS Press, 1992:293-296.

15. Lindahl O, Lindwall L, Spangberg A, Stenram A, Ockerman PA. Vegan regimen with reduced medication in the treatment of bronchial asthma. Journal of Asthma 1985;22(1):45-55.

16. Mann NJ, Warrick GE, O'Dea K, Knapp HR, Sinclair AJ. The effect of linoleic, arachidonic and eicosapentaenoic acid supplementation on prostacyclin production in rats. Lipids 1994;29:157-162.

17. Kirsch CM, Payan DG, Wong MYS, et al. Effect of eicosapentanoic acid in asthma. Clin Allergy 1988;18:177-187.

18. Broide DH, Lotz M, Cuomo AJ, Coburn DA, Federman EC, Wasserman SI. Cytokines in symptomatic asthma airways. Journal of Allergy and Clinical Immunology 1992;89:958-967.

19. Anticevich SZ, Hughes JM, Black JL, Armour CL. Induction of human airway hyperresponsiveness by tumour necrosis factor-alpha. European Journal of Pharmacology 1995;285:221-225.

20. Thomas PS, Yates DH, Barnes PJ. Tumor necrosis factor-alpha increases airway responsiveness and sputum neutrophilia in normal human subjects. American Journal of Respiratory and Critical Care Medicine 1995;152(1):76-80.

21. Caughey GE, Mantzioris E, Gibson RA, Cleland LG, James M. The effect on human tumour necrosis factor alpha and interleukin 1-beta production of diets enriched in omega-3 fatty acids from vegetable oil or fish oil. American Journal of Clinical Nutrition 1996;63:116-122.

22. Beasley R, Fergusson D, Crane J, Horwood J. Perinatal factors and atopic disease in childhood. Thoracic Society of Australia and New Zealand Annual Scientific Meeting. Wellington, New Zealand, 1997:104.

23. Leaf AA, Leighfield MJ, Costeloe KL, Crawford MA. Long chain polyunsaturated fatty acids and fetal growth. Early Human Development 1992;30:183-191.

FIGURES

FIGURE 1

CHANGE IN TOTAL OMEGA-3 FATTY ACIDS AS A PERCENTAGE OF TOTAL FATTY ACIDS

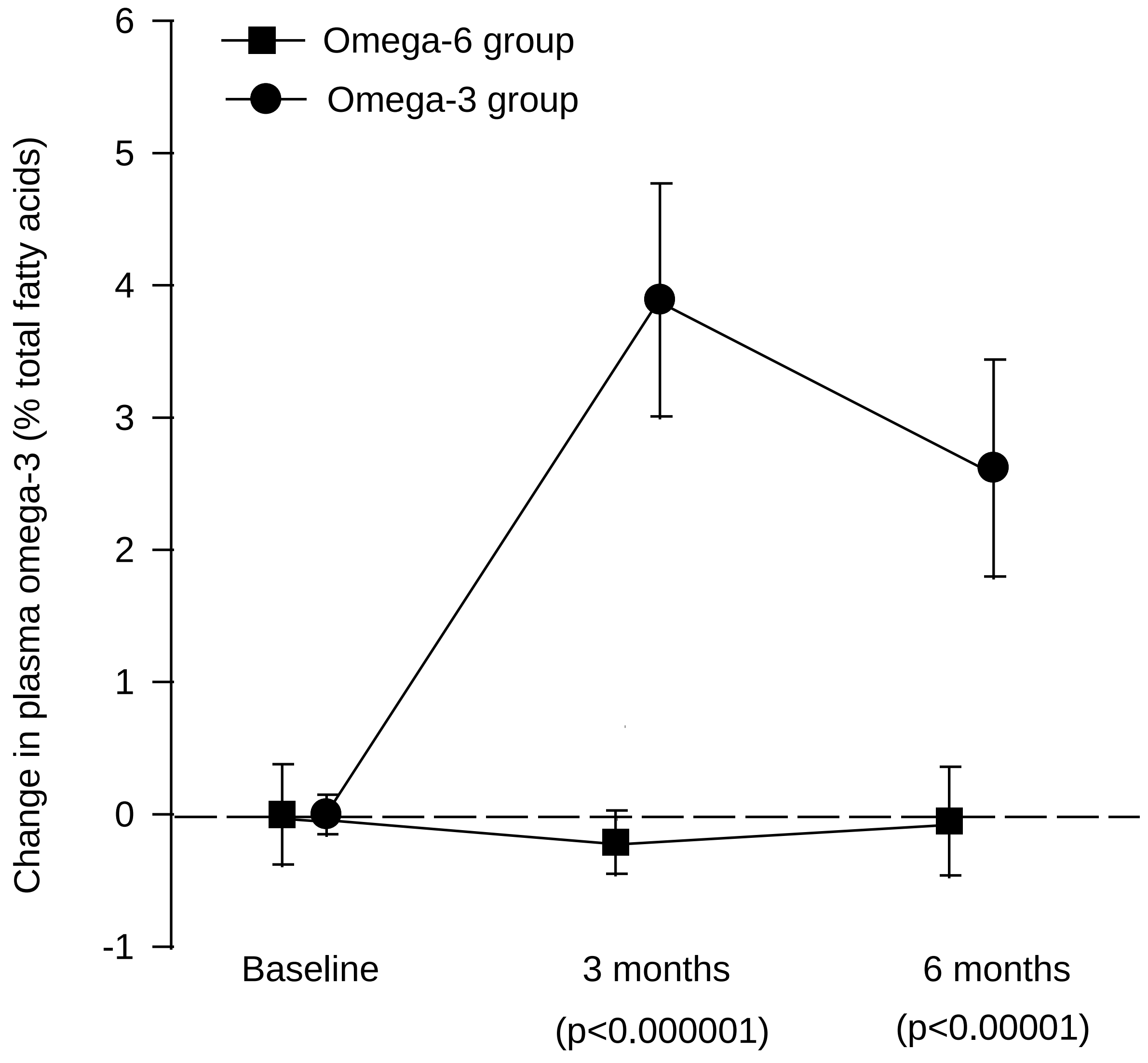


FIGURE 2

CHANGE IN PLASMA EICOSAPENTAENOIC ACID (EPA) AS A PERCENTAGE OF TOTAL FATTY ACIDS

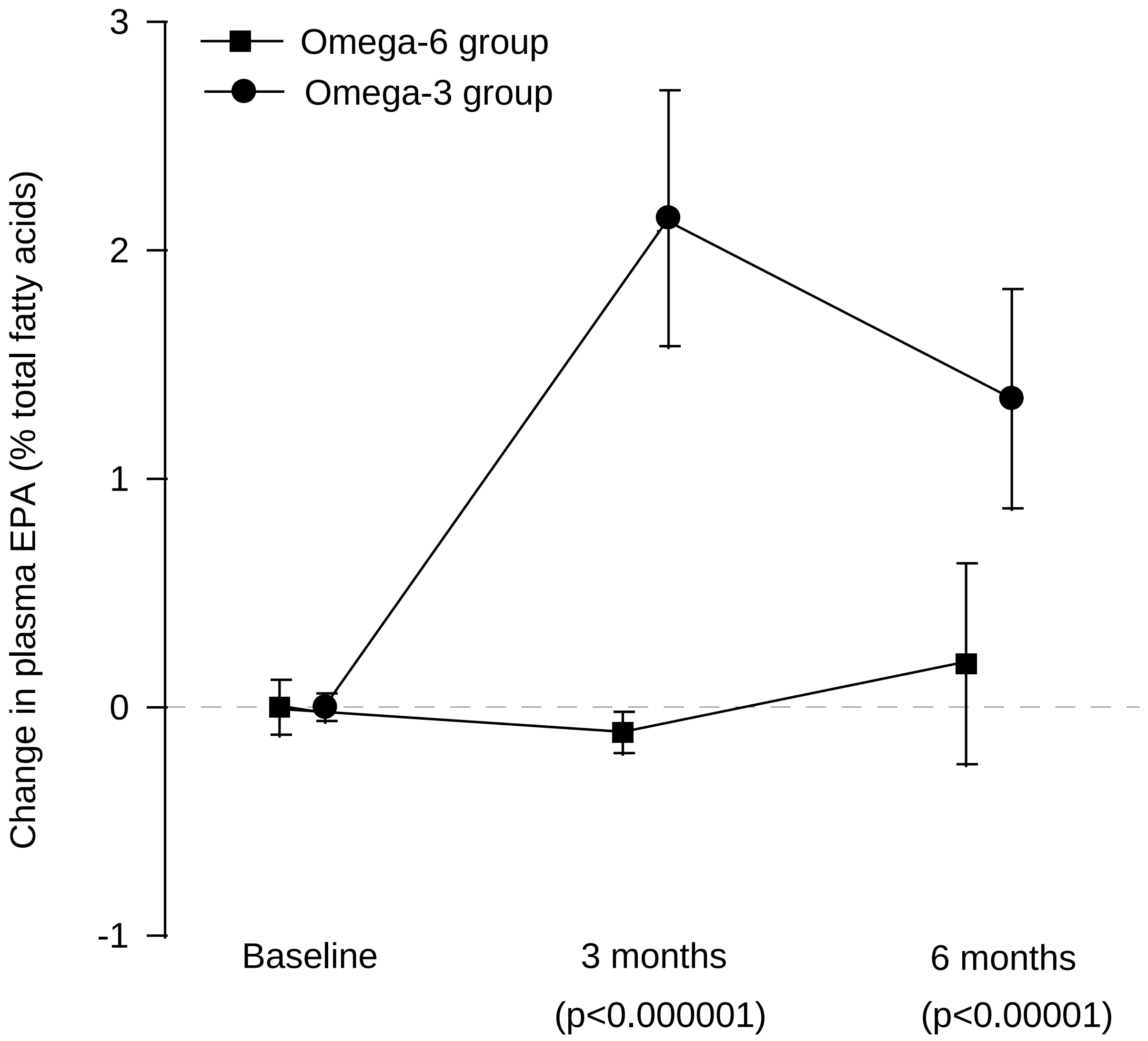


FIGURE 3

PLASMA LINOLEIC ACID AS A PERCENTAGE OF TOTAL FATTY ACIDS

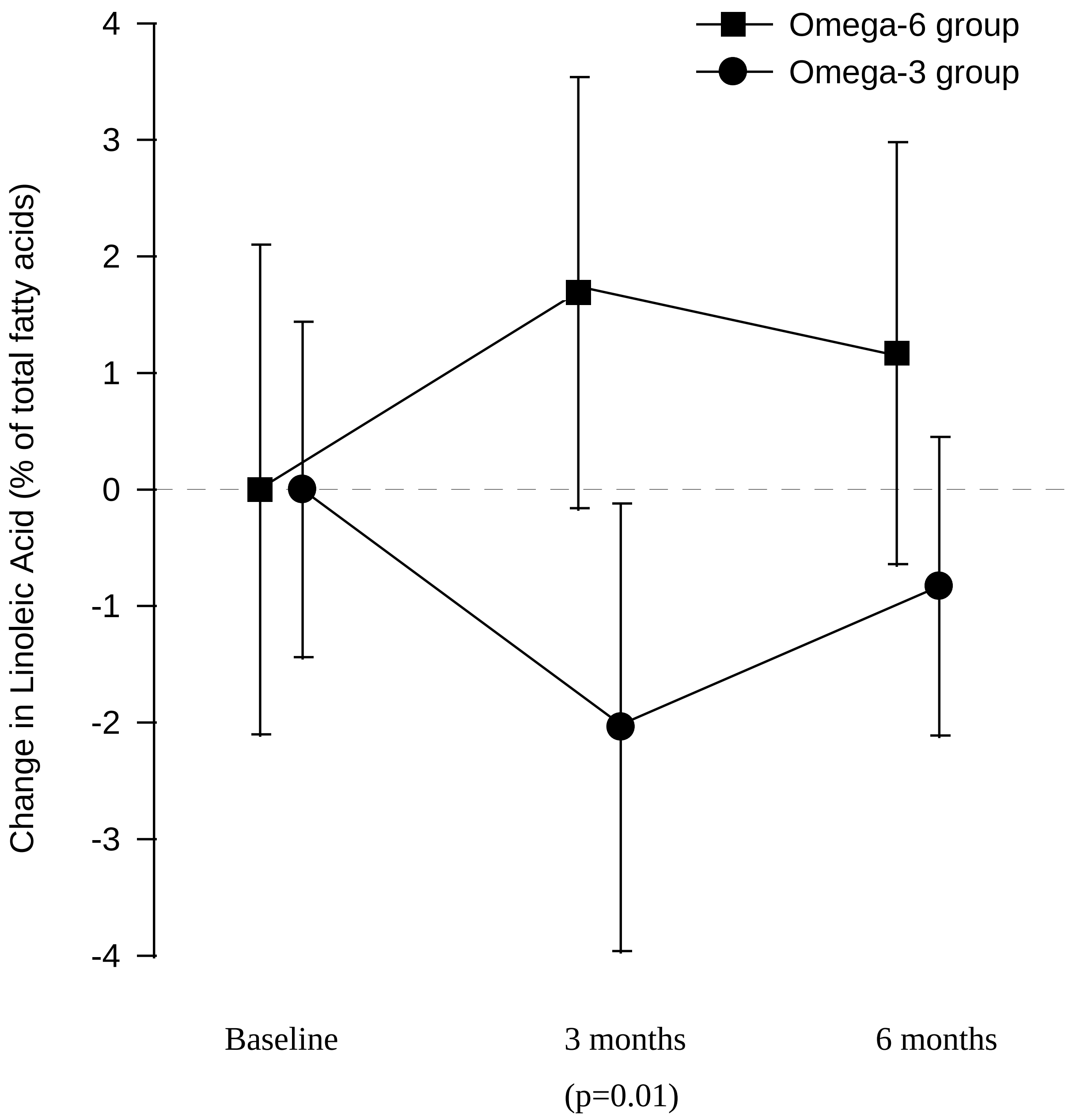


FIGURE 4

CHANGE IN SYMPTOM SCORES

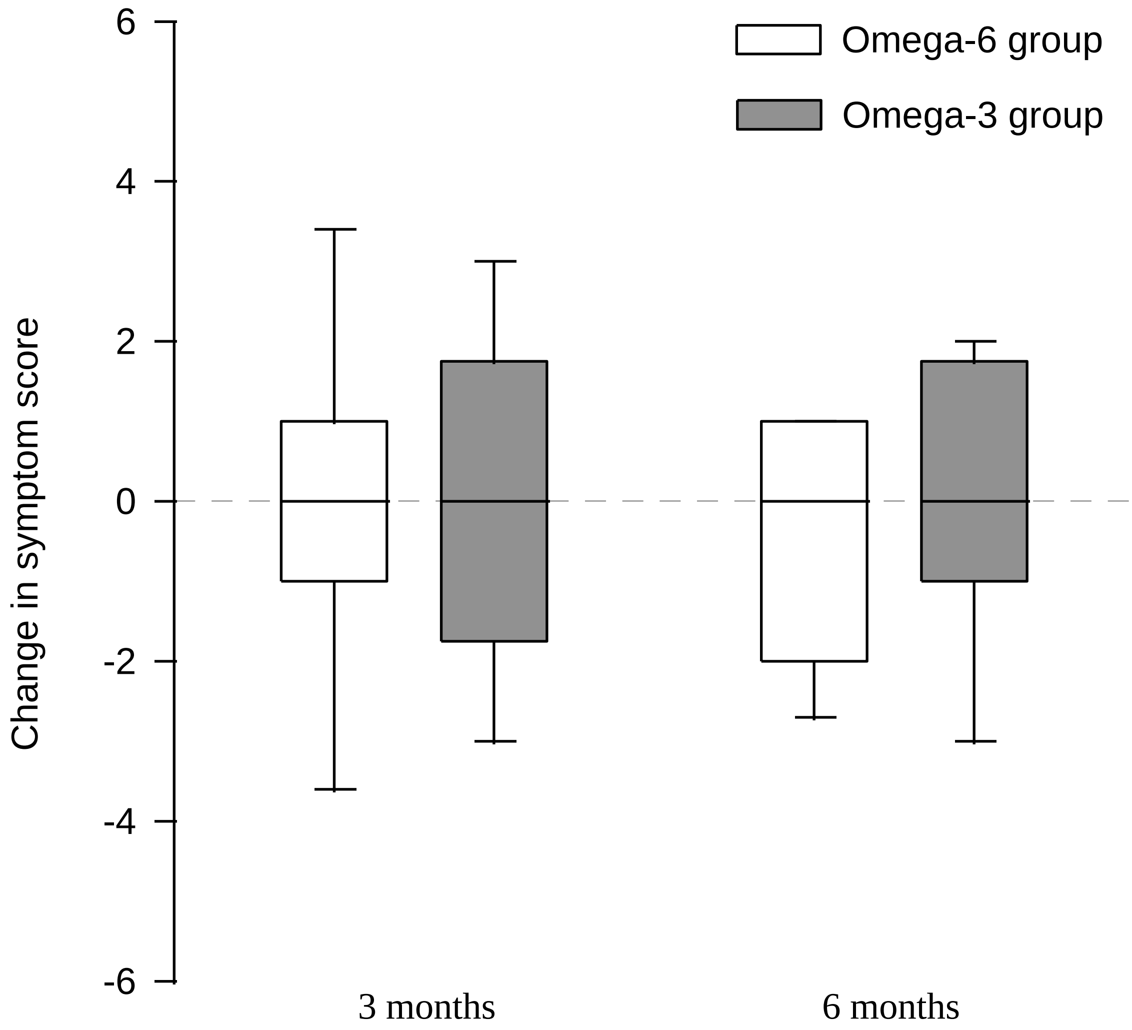


FIGURE 5

CHANGE IN DOSE RESPONSE RATIO

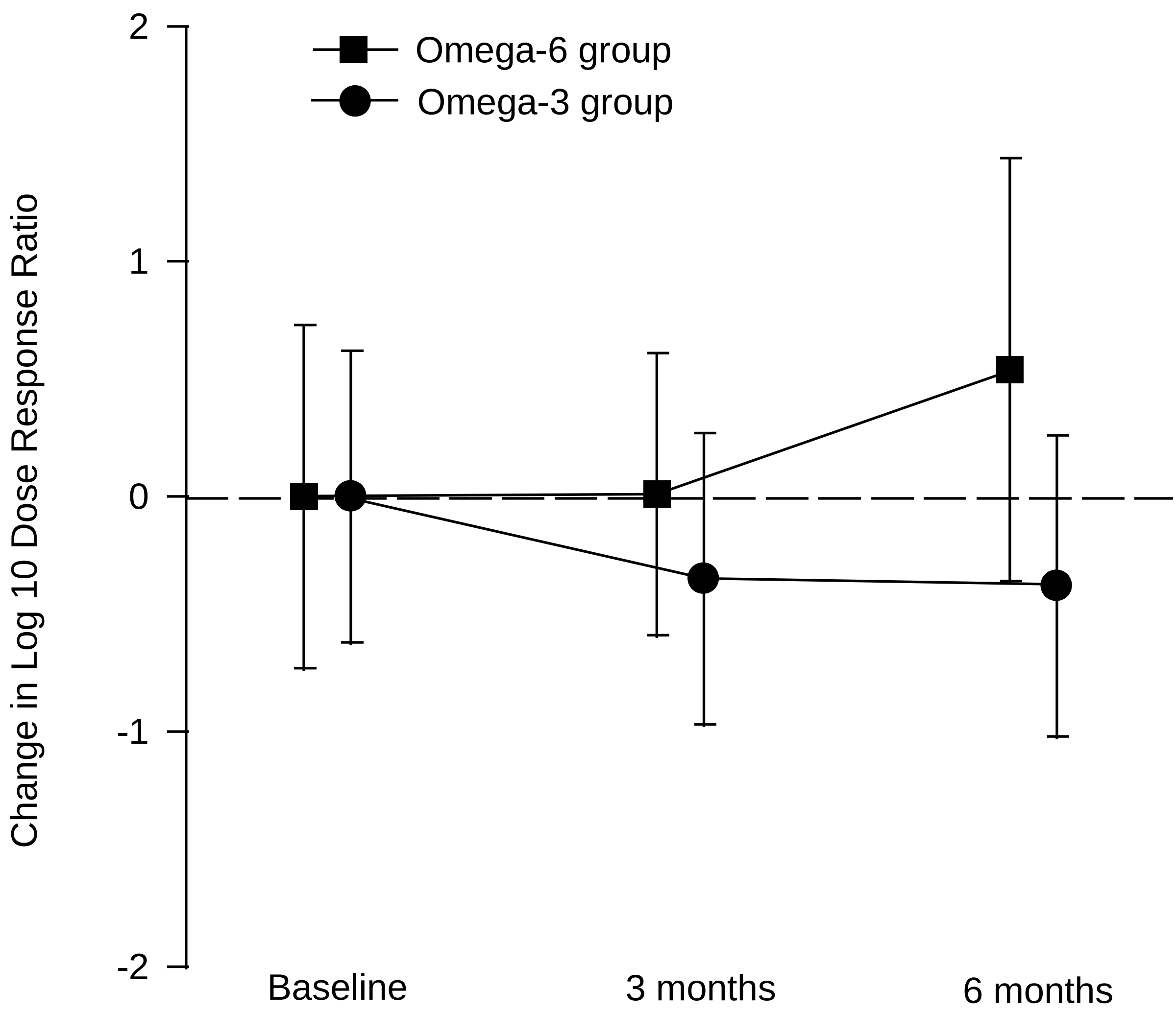


FIGURE 6

CHANGE IN TNF α

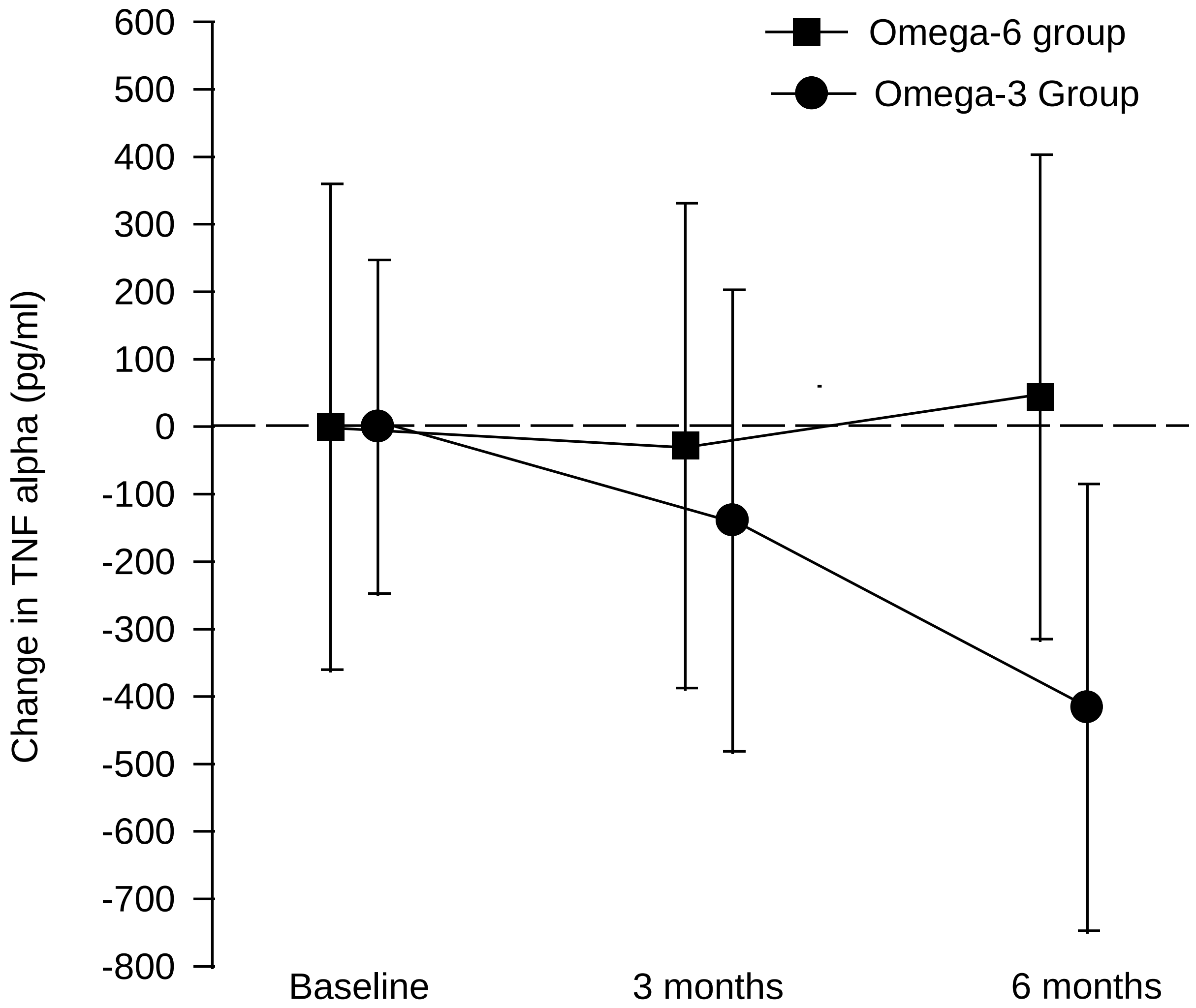
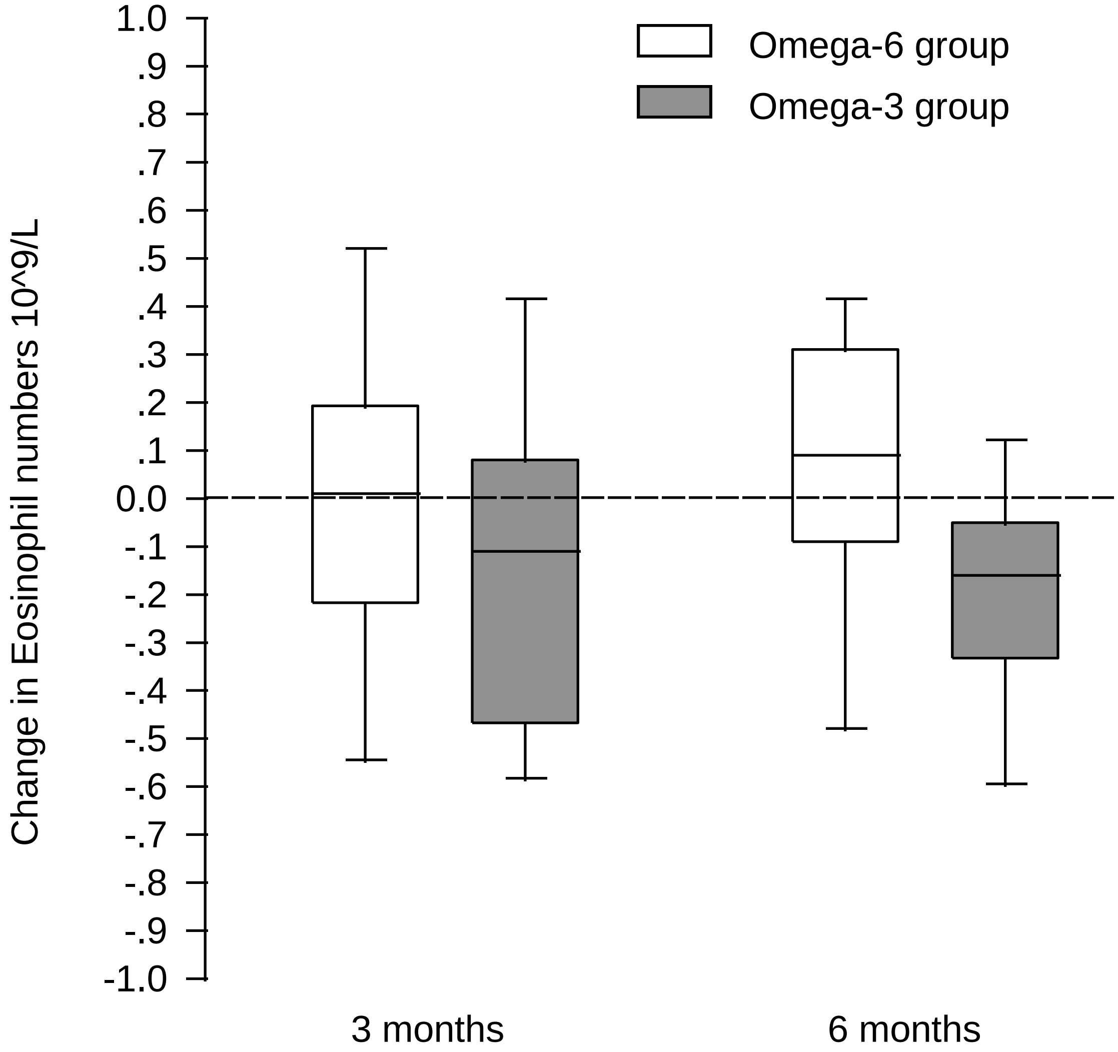


FIGURE 7

CHANGE IN EOSINOPHIL NUMBERS



TABLES

TABLE 1

BASELINE DATA

Baseline details of the two subject groups. Values are group means ± 95% confidence intervals, except for the asthma severity score and eosinophil numbers which are given as medians and interquartile ranges.

	Omega-6 group	**Omega-3 group**
Number of subjects	19	20
Females:Males	11:8	12:8
Age (years) *	9.7 ± 0.4	10.8 ± 0.6
Height (cm) *	139 ± 4	146 ± 4
Number atopic	17	19
Median asthma score	8 (7, 9.5)	8 (5.5, 9.5)
FEV1 (% predicted)	86 ± 7	81 ± 6
FVC (% predicted)	87 ± 6	86± 6
Dose response ratio	30.8 (ci: 16.7, 56.7)	37.3 (ci: 18.8, 74.3)
Medication		
Inhaled steroids	13	13
Beta agonists	15	20
Cromoglycate	6	4
Plasma omega-3 (% total fatty acids)	2.26 ± 0.22	2.14 ± 0.37
Plasma EPA (% total fatty acids-TFA)	0.54 ± 0.09	0.45 ± 0.11
Plasma Linoleic Acid (%TFA)	34.43 ± 1.59	35.82 ± 1.38
Plasma Arachidonic Acid (%TFA)	4.56 ± 0.36	4.56 ± 0.39
TNFα production (pg/ml)	1284 ± 230	1300 ± 316
Eosinophils (x 10^9/L)	0.62 (0.55, 0.93)	0.91 (0.53, 1.18)

* $p < 0.05$

TABLE 2

ONE SECOND FORCED EXPIRATORY VOLUME AS A PERCENTAGE OF PREDICTED VALUES

GROUP A

SUBJECT	RUN-IN 1	RUN-IN 2	MEAN OF RUN-IN VALUES	DIFF BETW RUN-IN VALUES	3 MONTHS	6 MONTHS	DIFFERENCE FROM MEAN OF RUN-IN AT 3 MONTHS	DIFFERENCE FROM MEAN OF RUN-IN AT 6 MONTHS
9	56	61	59	5	70	84	12	26
10	45	95	70	50	107	62	37	-8
12	83	89	86	6	98	*	12	*
13	98	93	96	-5	97	71	2	-25
14	92	87	90	-5	82	74	-8	-16
15	107	93	100	-14	94	102	-6	2
16	83	101	92	18	90	84	-2	-8
17	100	115	108	15	100	81	-8	-27
18	94	96	95	2	86	86	-9	-9
19	60	67	64	7	88	68	25	5
23	107	100	104	-7	84	73	-20	-31
25	61	50	56	-11	*	95	*	40
27	104	101	103	-3	91	99	-12	-4
30	82	76	79	-6	64	91	-15	12
31	95	98	97	3	101	82	5	-15
35	77	81	79	4	75	77	-4	-2
36	94	94	94	0	100	94	6	0
37	90	97	94	7	100	98	7	5
38	67	84	76	17	89	85	14	10
count	19	19	19	19	18	18	18	18
mean	84	88	86	4	90	84	2	-2
std. dev.	18	16	16	14	12	11	14	18
ci-width	8	7	7	6	5	5	7	8
ci-high	92	95	93	11	95	89	9	6
ci-low	76	81	79	-2	84	78	-5	-11

GROUP B

SUBJECT	RUN-IN 1	RUN-IN 2	MEAN OF RUN-IN VALUES	DIFF BETW RUN-IN VALUES	3 MONTHS	6 MONTHS	DIFFERENCE FROM MEAN OF RUN-IN AT 3 MONTHS	DIFFERENCE FROM MEAN OF RUN-IN AT 6 MONTHS
1	55	64	60	9	95	65	36	6
2	81	75	78	-6	89	80	11	2
3	79	75	77	-4	79	80	2	3
4	94	97	96	3	94	94	-2	-2
5	98	90	94	-8	80	102	-14	8
6	77	82	80	5	79	70	-1	-10
7	76	80	78	4	87	85	9	7
8	85	78	82	-7	73	76	-9	-6
11	100	102	101	2	105	103	4	2
20	87	91	89	4	81	79	-8	-10
21	94	91	93	-3	82	91	-11	-2
22	89	100	95	11	88	87	-7	-8
24	58	53	56	-5	65	70	10	15
26	72	63	68	-9	63	71	-5	4
28	45	77	61	32	98	95	37	34
29	87	84	86	-3	67	67	-19	-19
32	78	67	73	-11	76	80	4	8
33	103	92	98	-11	100	97	3	-1
34	85	84	85	-1	86	81	2	-4
39	75	81	78	6	81	96	3	18
count	20	20	20	20	20	20	20	20
mean	81	81	81	0	83	83	2	2
std. dev.	15	13	13	10	12	12	14	11
ci-width	7	6	6	4	5	5	6	5
ci-high	88	87	87	5	88	89	8	7
ci-low	74	76	75	-4	78	78	-4	-3

TABLE 3

FORCED VITAL CAPACITY AS A PERCENTAGE OF PREDICTED VALUES

GROUP A

SUBJECT	RUN-IN 1	RUN-IN 2	MEAN RUN-IN	DIFF BETW RUN-IN VALUES	3 MONTHS	6 MONTHS	DIFFERENCE FROM MEAN OF RUN-IN AT 3 MONTHS	DIFFERENCE FROM MEAN OF RUN-IN AT 6 MONTHS
9	62	67	65	5	78	90	14	26
10	49	96	73	47	107	83	35	11
12	92	96	94	4	104	*	10	*
13	97	93	95	-4	97	83	2	-12
14	86	78	82	-8	85	71	3	-11
15	110	85	98	-25	95	98	-3	1
16	70	103	87	33	92	86	6	-1
17	108	106	107	-2	100	92	-7	-15
18	97	93	95	-4	82	88	-13	-7
19	83	79	81	-4	93	86	12	5
23	109	109	109	0	94	90	-15	-19
25	56	56	56	0	*	96	*	40
27	94	95	95	1	86	92	-9	-3
30	84	76	80	-8	74	83	-6	3
31	102	103	103	1	102	86	-1	-17
35	87	92	90	5	87	86	-3	-4
36	95	95	95	0	103	86	8	-9
37	76	88	82	12	90	86	8	4
38	73	80	77	7	84	80	8	4
count	19	19	19	19	18	18	18	18
mean	86	89	87	3	92	87	3	0
std. dev.	18	14	14	15	9	6	12	15
ci-width	8	6	6	7	4	3	5	7
ci-high	94	95	94	10	96	90	8	7
ci-low	78	83	81	-4	88	84	-3	-7

GROUP B

SUBJECT	RUN-IN 1	RUN-IN 2	MEAN RUN-IN	DIFF BETW RUN-IN VALUES	3 MONTHS	6 MONTHS	DIFFERENCE FROM MEAN OF RUN-IN AT 3 MONTHS	DIFFERENCE FROM MEAN OF RUN-IN AT 6 MONTHS
1	57	78	68	21	91	59	24	-9
2	100	91	96	-9	101	96	6	1
3	89	91	90	2	87	95	-3	5
4	95	96	96	1	90	95	-6	-1
5	100	88	94	-12	86	98	-8	4
6	96	106	101	10	103	103	2	2
7	81	82	82	1	82	87	1	6
8	80	77	79	-3	64	78	-15	-1
11	100	99	100	-1	99	101	-1	2
20	79	86	83	7	72	67	-11	-16
21	94	84	89	-10	88	86	-1	-3
22	103	111	107	8	98	71	-9	-36
24	64	62	63	-2	77	78	14	15
26	88	84	86	-4	76	79	-10	-7
28	64	71	68	7	85	85	18	18
29	93	93	93	0	81	86	-12	-7
32	73	67	70	-6	73	77	3	7
33	96	88	92	-8	101	90	9	-2
34	92	91	92	-1	85	84	-7	-8
39	67	74	71	7	76	88	6	18
count	20	20	20	20	20	20	20	20
mean	86	86	86	0	86	85	0	-1
std. dev.	14	12	13	8	11	11	10	12
ci-width	6	5	6	3	5	5	4	5
ci-high	92	91	91	4	91	90	4	5
ci-low	79	81	80	-3	81	80	-4	-6

TABLE 4

DOSE RESPONSE RATIO AS A MEASURE OF AIRWAY HYPERRESPONSIVENESS

GROUP A

SUBJECT	RUN-IN 1 (DRR)	RUN-IN 2 (DRR)	MEAN RUN-IN (DRR)	DIFF BETW RUN-INS (doub. dos.)	3 MONTHS (DRR)	6 MONTHS (DRR)	DIFFERENCE FROM MEAN OF RUN-IN AT 3 MONTHS (doubling dose)	DIFFERENCE FROM MEAN OF RUN-IN AT 6 MONTHS (doubling dose)
9	*	*	*	*	*	250.0	*	*
10	*	19.5	19.5	*	4.6	266.7	-2.1	3.8
12	32.7	111.1	60.2	1.8	38.8	*	-0.6	*
13	16.1	158.3	50.5	3.3	40.8	19.4	-0.3	-1.4
14	26.5	6.6	13.3	-2.0	45.6	12.5	1.8	-0.1
15	91.7	120.8	105.2	0.4	191.7	20.4	0.9	-2.4
16	120.8	29.6	59.8	-2.0	70.8	4.1	0.2	-3.9
17	5.6	10.6	7.7	0.9	9.0	53.1	0.2	2.8
18	31.6	95.8	55.1	1.6	51.0	95.8	-0.1	0.8
19	137.5	666.7	302.8	2.3	46.9	383.3	-2.7	0.3
23	9.4	40.8	19.6	2.1	39.8	104.2	1.0	2.4
25	15.0	57.1	29.3	1.9	*	31.6	*	0.1
27	1.0	2.4	1.6	1.3	4.4	6.4	1.5	2.0
30	145.8	55.1	89.6	-1.4	116.7	87.5	0.4	0.0
31	154.2	104.2	126.7	-0.6	129.2	333.3	0.0	1.4
35	49.0	49.0	49.0	0.0	187.5	44.9	1.9	-0.1
36	55.1	67.3	60.9	0.3	42.9	133.3	-0.5	1.1
37	3.6	7.2	5.1	1.0	4.9	6.9	-0.1	0.4
38	6.9	5.4	6.1	-0.4	2.4	21.4	-1.3	1.8
count	17.0	18.0	18.0	17.0	17.0	18.0	17.0	17.0
mean	25.5	37.6	30.8	0.6	31.2	47.4	0.0	0.5
std. dev.	4.3	4.2	3.8	1.5	4.0	4.1	1.3	1.9
ci-width	*	*	*	0.7	*	*	0.6	0.9
ci-high	51.0	72.7	56.7	1.3	33.1	49.3	0.6	1.4
ci-low	12.8	19.4	16.7	-0.1	29.2	45.4	-0.6	-0.4

GROUP B

SUBJECT	RUN-IN 1 (DRR)	RUN-IN 2 (DRR)	MEAN RUN-IN (DRR)	DIFF BETW RUN-INS (doub. dos.)	3 MONTHS (DRR)	6 MONTHS (DRR)	DIFFERENCE FROM MEAN OF RUN-IN AT 3 MONTHS (doubling dose)	DIFFERENCE FROM MEAN OF RUN-IN AT 6 MONTHS (doubling dose)
1	583.3	533.3	557.8	-0.1	67.3	61.2	-3.1	-3.2
2	141.7	100.0	119.0	-0.5	208.3	42.9	0.8	-1.5
3	400.0	258.3	321.5	-0.6	204.2	266.7	-0.7	-0.3
4	7.1	7.4	7.3	0.1	4.6	21.4	-0.7	1.6
5	53.1	51.0	52.0	-0.1	18.9	24.5	-1.5	-1.1
6	466.7	69.8	180.4	-2.8	104.2	183.3	-0.8	0.0
7	2.7	4.6	3.5	0.8	3.7	5.6	0.1	0.7
8	10.6	19.4	14.3	0.9	4.6	21.7	-1.6	0.6
11	95.8	20.4	44.2	-2.2	12.0	16.1	-1.9	-1.5
20	24.5	8.2	14.2	-1.6	13.3	14.4	-0.1	0.0
21	13.3	13.3	13.3	0.0	16.1	24.5	0.3	0.9
22	17.2	2.2	6.1	-3.0	11.0	3.6	0.8	-0.8
24	59.2	27.6	40.4	-1.1	120.8	44.9	1.6	0.2
26	466.7	250.0	341.6	-0.9	53.1	112.5	-2.7	-1.6
28	*	25.7	25.7	*	23.9	11.0	-0.1	-1.2
29	100.0	83.3	91.3	-0.3	87.5	225.0	-0.1	1.3
32	21.4	17.2	19.2	-0.3	21.4	24.5	0.2	0.4
33	91.7	583.3	231.2	2.7	145.8	158.3	-0.7	-0.5
34	12.2	23.5	16.9	0.9	145.8	65.3	3.1	2.0
39	5.1	1.8	3.0	-1.5	2.7	0.3	-0.2	-3.6
count	19.0	20.0	20.0	19.0	20.0	20.0	20.0	20.0
mean	45.4	31.6	37.3	-0.5	29.2	28.6	-0.4	-0.4
std. dev.	5.2	5.3	4.8	1.4	4.2	5.1	1.4	1.5
ci-width	*	*	*	0.6	*	*	0.6	0.6
ci-high	95.0	65.7	74.3	0.1	54.6	58.4	0.3	0.3
ci-low	21.7	15.2	18.8	-1.1	15.7	14.0	-1.0	-1.0

TABLE 5

PEAK FLOW AND PEAK FLOW SCORES

GROUP A

SUBJECT	RUN-IN % predicted	RUN-IN score	3 MONTHS % predicted	3 MONTHS score	6 MONTHS % predicted	6 MONTHS score	DIFFERENCE FROM RUN-IN at 3 months	DIFFERENCE FROM RUN-IN at 6 months
9	83	2	68.0	4	70.4	3	2	1
10	86	1	85.7	1	81.9	2	0	1
12	81	2	*	*	*	*	*	*
13	66	4	46.5	4	57.0	4	0	0
14	77	3	72.6	3	72.0	3	0	0
15	81	2	69.5	4	82.7	2	2	0
16	75	3	73.0	3	62.3	4	0	1
17	74	3	56.2	4	77.4	3	1	0
18	79	2	*	*	62.0	4	-2	2
19	49	4	50.2	4	37.1	4	0	0
23	51	4	49.8	4	68.7	4	0	0
25	61	4	56.3	4	59.1	4	0	0
27	66	4	73.1	3	57.4	4	-1	0
30	89	1	47.2	4	63.0	4	3	3
31	106	0	103.6	0	93.0	0	0	0
35	64	4	59.7	4	61.4	4	0	0
36	92	1	89.4	1	78.7	2	0	1
37	87	1	91.0	1	95.8	0	0	-1
38	73	3	102.5	0	94.5	0	-3	-3
count	19	19	17	17	18	18	18	18
mean	75.8	2.5	70.3	2.8	70.8	2.8	0.1	0.3
median		3.0		4.0		3.5	0.0	0.0

GROUP B

SUBJECT	RUN-IN % predicted	RUN-IN score	3 MONTHS % predicted	3 MONTHS score	6 MONTHS % predicted	6 MONTHS score	DIFFERENCE FROM RUN-IN at 3 months	DIFFERENCE FROM RUN-IN at 6 months
2	63	4	48.8	4	62.5	4	0	0
3	49	4	44.1	4	63.0	4	0	0
4	81	2	45.3	4	73.3	3	2	1
5	62	4	70.7	3	125.0	0	-1	-4
6	76	3	76.1	3	56.7	4	0	1
7	71	3	77.0	3	62.2	4	0	1
8	82	2	69.9	4	74.1	3	2	1
11	83	2	135.9	0	140.7	0	-2	-2
20	75	3	74.8	3	74.3	3	0	0
21	64	4	81.4	2	74.5	3	-2	-1
22	76	3	84.3	2	74.1	3	-1	0
24	74	3	72.0	3	76.8	3	0	0
26	58	4	45.1	4	39.2	4	0	0
28	59	4	66.5	4	67.0	4	0	0
29	52	4	39.9	4	41.7	4	0	0
32	87	1	84.0	2	79.9	2	1	1
33	60	4	90.5	1	103.7	0	-3	-4
34	77	3	83.0	2	85.7	1	-1	-2
39	84	2	65.8	4	68.5	4	2	2
count	19	19	19	19	19	19	19	19
mean	70	3	71.3	2.9	75.9	2.8	-0.2	-0.3
median		3.0		3.0		3.0	0.0	0.0

TABLE 6

DAYTIME SYMPTOM SCORES

GROUP A

SUBJECT	RUN-IN	3 MONTHS	6 MONTHS	DIFFERENCE FROM RUN-IN at 3 months	DIFFERENCE FROM RUN-IN at 6 months
9	2	2	2	0	0
10	1	1	2	0	1
12	1	*	*	*	*
13	1	1	2	0	1
14	2	0	0	-2	-2
15	2	3	2	1	0
16	2	1	1	-1	-1
17	2	2	2	0	0
18	1	*	0	*	-1
19	3	3	3	0	0
23	2	3	3	1	1
25	1	1	2	0	1
27	3	0	1	-3	-2
30	2	3	2	1	0
31	3	3	3	0	0
35	2	1	1	-1	-1
36	0	0	0	0	0
37	1	0	0	-1	-1
38	2	2	2	0	0
count	19	17	18	17	18
mean	1.7	1.5	1.6	-0.3	-0.2
median	2.0	1.0	2.0	0.0	0.0

GROUP B

SUBJECT	RUN-IN	3 MONTHS	6 MONTHS	DIFFERENCE FROM RUN-IN at 3 months	DIFFERENCE FROM RUN-IN at 6 months
2	3	1	3	-2	0
3	2	2	2	0	0
4	2	3	2	1	0
5	1	3	2	2	1
6	1	0	3	-1	2
7	3	2	2	-1	-1
8	0	0	0	0	0
11	1	0	0	-1	-1
20	1	1	2	0	1
21	2	1	1	-1	-1
22	2	2	3	0	1
24	1	1	0	0	-1
26	2	3	3	1	1
28	1	0	3	-1	2
29	3	3	3	0	0
32	0	0	3	0	3
33	1	3	3	2	2
34	0	2	0	2	0
39	0	1	0	1	0
count	19	19	19	19	19
mean	1	1.5	1.8	0	0
median	1.0	1.0	2.0	0.0	0.0

TABLE 7

NIGHT-TIME SYMPTOM SCORES

GROUP A

SUBJECT	RUN-IN	3 MONTHS	6 MONTHS	DIFFERENCE FROM RUN-IN at 3 months	DIFFERENCE FROM RUN-IN at 6 months
9	2	1	1	-1	-1
10	2	0	1	-2	-1
12	3	*	*	*	*
13	2	1	3	-1	1
14	2	0	0	-2	-2
15	3	4	4	1	1
16	2	2	2	0	0
17	3	3	3	0	0
18	3	*	0	*	-3
19	2	3	2	1	0
23	4	4	1	0	-3
25	2	1	0	-1	-2
27	2	1	1	-1	-1
30	3	3	1	0	-2
31	3	3	3	0	0
35	3	3	2	0	-1
36	0	0	0	0	0
37	1	0	0	-1	-1
38	0	2	2	2	2
count	19	17	18	17	18
mean	2.2	1.8	1.4	-0.3	-0.7
median	2.0	2.0	1.0	0.0	-1.0

GROUP B

SUBJECT	RUN-IN	3 MONTHS	6 MONTHS	DIFFERENCE FROM RUN-IN at 3 months	DIFFERENCE FROM RUN-IN at 6 months
2	0	0	0	0	0
3	3	3	2	0	-1
4	3	3	2	0	-1
5	2	3	2	1	0
6	2	2	2	0	0
7	2	2	2	0	0
8	0	0	0	0	0
11	0	0	0	0	0
20	2	3	1	1	-1
21	3	3	2	0	-1
22	2	0	2	-2	0
24	3	2	3	-1	0
26	3	3	3	0	0
28	3	0	0	-3	-3
29	2	3	3	1	1
32	2	0	0	-2	-2
33	0	0	3	0	3
34	3	3	3	0	0
39	0	0	0	0	0
count	19	19	19	19	19
mean	2	1.6	1.6	0	0
median	2.0	2.0	2.0	0.0	0.0

TABLE 8

MEDICATION SCORES

GROUP A

SUBJECT	RUN-IN	3 MONTHS	6 MONTHS	DIFFERENCE FROM RUN-IN at 3 months	DIFFERENCE FROM RUN-IN at 6 months
9	2	2	2	0	0
10	2	2	2	0	0
12	1	*	*	*	*
13	2	2	2	0	0
14	2	2	1	0	-1
15	1	1	1	0	0
16	1	1	1	0	0
17	2	2	2	0	0
18	1	*	1	*	0
19	1	1	1	0	0
23	2	2	2	0	0
25	2	2	2	0	0
27	2	2	2	0	0
30	1	1	1	0	0
31	2	2	2	0	0
35	2	2	2	0	0
36	2	2	2	0	0
37	0	2	2	2	2
38	2	2	2	0	0
count	19	17	18	17	18
mean	1.6	1.8	1.7	0.1	0.1
median	2.0	2.0	2.0	0.0	0.0

GROUP B

SUBJECT	RUN-IN	3 MONTHS	6 MONTHS	DIFFERENCE FROM RUN-IN at 3 months	DIFFERENCE FROM RUN-IN at 6 months
2	0	0	2	0	2
3	1	1	1	0	0
4	1	1	0	0	-1
5	2	2	2	0	0
6	1	2	2	1	1
7	0	0	2	0	2
8	2	2	0	0	-2
11	0	0	0	0	0
20	0	2	0	2	0
21	2	2	0	0	-2
22	2	2	2	0	0
24	1	1	2	0	1
26	2	2	2	0	0
28	2	2	2	0	0
29	2	2	2	0	0
32	2	2	2	0	0
33	0	2	0	2	0
34	2	2	2	0	0
39	0	0	0	0	0
count	19	19	19	19	19
mean	1.2	1.4	1.2	0.3	0.1
median	1.0	2.0	2.0	0.0	0.0

TABLE 9

TOTAL SYMPTOM AND MEDICATION SCORES

GROUP A

SUBJECT	RUN-IN	3 MONTHS	6 MONTHS	DIFFERENCE FROM RUN-IN at 3 months	DIFFERENCE FROM RUN-IN at 6 months
9	8	9	8	1	0
10	6	4	7	-2	1
12	7	*	*	*	*
13	9	8	11	-1	2
14	9	5	4	-4	-5
15	8	12	9	4	1
16	8	7	8	-1	0
17	10	11	10	1	0
18	7	*	5	*	-2
19	10	11	10	1	0
23	12	13	10	1	-2
25	9	8	8	-1	-1
27	11	6	8	-5	-3
30	7	11	8	4	1
31	8	8	8	0	0
35	11	10	9	-1	-2
36	3	3	4	0	1
37	3	3	2	0	-1
38	7	6	6	-1	-1
count	19	17	18	17	18
mean	8.1	7.9	7.5	-0.2	-0.6
median	8.0	8.0	8.0	0.0	0.0

GROUP B

SUBJECT	RUN-IN	3 MONTHS	6 MONTHS	DIFFERENCE FROM RUN-IN at 3 months	DIFFERENCE FROM RUN-IN at 6 months
2	7	5	9	-2	2
3	10	10	9	0	-1
4	8	11	7	3	-1
5	9	11	6	2	-3
6	7	7	11	0	4
7	8	7	10	-1	2
8	4	6	3	2	-1
11	3	0	0	-3	-3
20	6	9	6	3	0
21	11	8	6	-3	-5
22	9	6	10	-3	1
24	8	7	8	-1	0
26	11	12	12	1	1
28	10	6	9	-4	-1
29	11	12	12	1	1
32	5	4	7	-1	2
33	5	6	6	1	1
34	8	9	6	1	-2
39	2	5	4	3	2
count	19	19	19	19	19
mean	7	7	7	0	0
median	8.0	7.0	7.0	0.0	0.0

TABLE 10

EOSINOPHIL COUNTS

GROUP A

SUBJECT	MEAN RUN-IN (10^9/L)	3 MONTHS (10^9/L)	6 MONTHS (10^9/L)	DIFFERENCE FROM RUN-IN AT 3 mths	6 mths
9	1.65	1.37	1.56	-0.28	-0.09
10	0.62	0.63	0.81	0.01	0.19
12	0.62	0.70	*	0.08	*
13	0.48	0.76	0.81	0.28	0.33
14	1.25	0.51	0.82	-0.74	-0.43
15	0.72	1.40	1.31	0.68	0.59
16	0.59	1.32	0.67	0.73	0.08
17	0.56	0.41	0.56	-0.15	0.00
18	0.74	0.83	0.91	0.09	0.17
19	0.84	1.01	0.81	0.17	-0.03
23	0.56	0.77	0.92	0.21	0.36
25	0.42	0.62	0.37	0.20	-0.05
27	0.37	0.36	0.47	-0.01	0.10
30	0.83	0.68	0.33	-0.15	-0.50
31	1.31	0.79	1.75	-0.52	0.44
35	0.52	0.64	0.73	0.12	0.21
36	1.12	0.56	0.56	-0.56	-0.56
37	0.53	0.48	0.22	-0.05	-0.31
38	1.02	0.78	1.33	-0.24	0.31
count	19	19	18	19	18
mean	0.78	0.77	0.83	-0.01	0.05
median	0.62	0.70	0.81	0.01	0.09

GROUP B

SUBJECT	MEAN RUN-IN (10^9/L)	3 MONTHS (10^9/L)	6 MONTHS (10^9/L)	DIFFERENCE FROM RUN-IN AT 3 mths	6 mths
1	2.24	1.63	2.00	-0.61	-0.24
2	1.02	0.54	0.67	-0.48	-0.35
3	1.11	0.57	0.50	-0.54	-0.61
4	0.34	0.46	0.32	0.12	-0.02
5	*	0.65	0.36	*	*
6	1.34	1.25	0.85	-0.09	-0.49
7	1.11	0.47	0.54	-0.64	-0.57
8	0.49	0.34	0.39	-0.15	-0.10
11	0.74	0.95	0.46	0.21	-0.28
20	1.43	1.32	0.62	-0.11	-0.81
21	1.33	1.29	1.22	-0.04	-0.11
22	0.39	0.85	0.34	0.46	-0.05
24	0.78	1.66	0.61	0.88	-0.17
26	1.25	0.77	1.09	-0.48	-0.16
28	0.96	0.72	0.98	-0.24	0.02
29	0.50	0.85	0.99	0.35	0.49
32	0.91	0.48	0.83	-0.43	-0.08
33	0.88	0.76	0.83	-0.12	-0.05
34	0.56	0.51	0.75	-0.05	0.19
39	0.27	0.17	0.09	-0.10	-0.18
count	19	20	20	19	19
mean	0.93	0.81	0.72	-0.11	-0.19
median	0.91	0.74	0.65	-0.11	-0.16

TABLE 11

TOTAL OMEGA-3 FATTY ACIDS AS A PERCENTAGE TOTAL PLASMA FATTY ACIDS

GROUP A

SUBJECT	RUN-IN 1	RUN-IN 2	3 MONTHS	6 MONTHS	DIFFERENCE BETWEEN RUN-INS	MEAN RUN-IN	DIFFERENCE FROM MEAN RUN-IN AT 3 MONTHS	DIFFERENCE FROM MEAN RUN-IN AT 6 MONTHS
9	1.6	1.5	1.4	1.5	-0.1	1.55	-0.15	-0.05
10	2.1	5.0	2.5	6.2	2.9	3.55	-1.05	2.65
12	3.1	2.7	2.2	*	-0.4	2.90	-0.70	*
13	2.6	1.9	2.8	1.4	-0.7	2.25	0.55	-0.85
14	2.3	2.7	1.9	1.5	0.4	2.50	-0.60	-1.00
15	2.5	2.7	1.8	2.2	0.2	2.60	-0.80	-0.40
16	1.9	2.2	1.9	2.1	0.3	2.05	-0.15	0.05
17	1.8	3.1	1.8	1.9	1.3	2.45	-0.65	-0.55
18	2.1	2.1	2.9	2.3	0.0	2.10	0.80	0.20
23	2.7	2.3	2.4	2.3	-0.4	2.50	-0.10	-0.20
19	1.7	2.1	1.5	1.6	0.4	1.90	-0.40	-0.30
25	*	*	*	*	*	*	*	*
27	1.9	2.1	2.2	2.4	0.2	2.00	0.20	0.40
30	2.4	2.2	1.9	2.0	-0.2	2.30	-0.40	-0.30
31	2.1	2.3	2.2	2.7	0.2	2.20	0.00	0.50
35	2.0	1.9	1.9	1.8	-0.1	1.95	-0.05	-0.15
36	1.5	1.5	1.7	1.4	0.0	1.50	0.20	-0.10
37	1.9	1.9	2.3	3.3	0.0	1.90	0.40	1.40
38	2.8	2.0	1.5	1.9	-0.8	2.40	-0.90	-0.50
count	18	18	18	17	18	18	18	17
mean	2.2	2.3	2.0	2.3	0.2	2.26	-0.21	0.05
std dev	0.4	0.8	0.4	1.1	0.8	0.48	0.52	0.87
ci width	0.2	0.4	0.2	0.5	0.4	0.22	0.24	0.41
ci high	2.4	2.7	2.2	2.8	0.6	2.48	0.03	0.46
ci low	2.0	2.0	1.8	1.7	-0.2	2.03	-0.45	-0.37

GROUP B

SUBJECT	RUN-IN 1	RUN-IN 2	3 MONTHS	6 MONTHS	DIFFERENCE BETWEEN RUN-INS	MEAN RUN-IN	DIFFERENCE FROM MEAN RUN-IN AT 3 MONTHS	DIFFERENCE FROM MEAN RUN-IN AT 6 MONTHS
1	1.5	1.4	2.0	1.5	-0.1	1.45	0.55	0.05
2	1.6	1.9	5.1	3.1	0.3	1.75	3.35	1.35
3	2.1	1.8	6.7	3.7	-0.3	1.95	4.75	1.75
4	1.5	1.6	5.7	2.9	0.1	1.55	4.15	1.35
5	1.7	1.9	5.3	2.0	0.2	1.80	3.50	0.20
6	1.6	1.6	3.1	5.4	0.0	1.60	1.50	3.80
7	1.9	1.8	5.5	4.3	-0.1	1.85	3.65	2.45
8	1.5	1.8	9.0	5.4	0.3	1.65	7.35	3.75
11	1.9	1.7	1.8	5.3	-0.2	1.80	0.00	3.50
21	*	2.2	5.0	3.8	*	2.20	2.80	1.60
20	2.7	2.3	9.8	4.8	-0.4	2.50	7.30	2.30
22	1.9	2.0	3.9	5.2	0.1	1.95	1.95	3.25
24	3.7	4.1	7.2	3.7	0.4	3.90	3.30	-0.20
26	1.7	1.8	5.7	5.7	0.1	1.75	3.95	3.95
28	*	4.9	7.8	7.1	*	4.90	2.90	2.20
29	2.2	*	3.9	2.8	*	2.20	1.70	0.60
32	1.7	1.6	5.3	3.3	-0.1	1.65	3.65	1.65
33	1.9	2.8	5.3	5.1	0.9	2.35	2.95	2.75
34	1.6	1.8	6.2	7.0	0.2	1.70	4.50	5.30
39	2.3	2.1	1.9	*	-0.2	2.20	-0.30	*
count	18	19	20	19	17	20	20	19
mean	1.94	2.16	5.31	4.32	0.07	2.14	3.18	2.19
std dev	0.54	0.89	2.18	1.54	0.31	0.84	2.01	1.49
ci width	0.25	0.40	0.96	0.69	0.15	0.37	0.88	0.67
ci high	2.20	2.56	6.27	5.02	0.22	2.50	4.06	2.86
ci low	1.69	1.76	4.35	3.63	-0.08	1.77	2.29	1.52

TABLE 12

EICOSAPENTAENOIC ACID AS A PERCENTAGE OF TOTAL PLASMA FATTY ACIDS

GROUP A

SUBJECT	RUN-IN 1	RUN-IN 2	3 MONTHS	6 MONTHS	DIFFERENCE BETWEEN RUN-INS	MEAN RUN-IN	DIFFERENCE FROM MEAN RUN-IN AT 3 MONTHS	DIFFERENCE FROM MEAN RUN-IN AT 6 MONTHS
9	0.3	0.2	0.3	0.3	-0.1	0.25	0.05	0.05
10	0.5	1.1	0.5	4.4	0.6	0.80	-0.30	3.6
12	1.1	0.7	0.7	*	-0.4	0.90	-0.20	*
13	0.5	0.6	0.3	0.4	0.1	0.55	-0.25	-0.15
14	0.5	0.7	0.4	0.4	0.2	0.60	-0.20	-0.2
15	0.8	0.9	0.5	0.7	0.1	0.85	-0.35	-0.15
16	0.4	0.5	0.4	0.4	0.1	0.45	-0.05	-0.05
17	0.4	1.2	0.3	0.4	0.8	0.80	-0.50	-0.4
18	0.5	0.6	0.4	0.3	0.1	0.55	-0.15	-0.25
23	0.5	0.5	0.5	0.5	0.0	0.50	0.00	0
19	0.3	0.5	0.3	0.3	0.2	0.40	-0.10	-0.1
25	*	*	*	*	*	*	*	*
27	0.3	0.3	0.5	0.7	0.0	0.30	0.20	0.4
30	0.4	0.4	0.3	0.2	0.0	0.40	-0.10	-0.2
31	0.4	0.5	0.8	0.6	0.1	0.45	0.35	0.15
35	0.6	0.5	0.4	0.5	-0.1	0.55	-0.15	-0.05
36	0.3	0.4	0.3	0.3	0.1	0.35	-0.05	-0.05
37	0.4	0.5	0.5	1.2	0.1	0.45	0.05	0.75
38	0.6	0.6	0.3	0.4	0.0	0.60	-0.30	-0.2
count	18	18	18	17	18	18	18	17
mean	0.5	0.6	0.4	0.7	0.1	0.54	-0.11	0.19
std dev	0.2	0.3	0.1	1.0	0.3	0.19	0.20	0.92
cl width	0.1	0.1	0.1	0.5	0.1	0.09	0.09	0.44
cl high	0.6	0.7	0.5	1.2	0.2	0.63	-0.02	0.62
cl low	0.4	0.5	0.4	0.2	0.0	0.45	-0.21	-0.25

GROUP B

SUBJECT	RUN-IN 1	RUN-IN 2	3 MONTHS	6 MONTHS	DIFFERENCE BETWEEN RUN-INS	MEAN RUN-IN	DIFFERENCE FROM MEAN RUN-IN AT 3 MONTHS	DIFFERENCE FROM MEAN RUN-IN AT 6 MONTHS
1	0.3	0.4	0.4	0.4	0.1	0.35	0.05	0.05
2	0.4	0.4	2.5	1.8	0.0	0.40	2.10	1.4
3	0.4	0.3	3.0	1.4	-0.1	0.35	2.65	1.05
4	0.3	0.3	3.0	1.2	0.0	0.30	2.70	0.9
5	0.4	0.4	2.5	0.6	0.0	0.40	2.10	0.2
6	0.5	0.6	1.4	2.7	0.1	0.55	0.85	2.15
7	0.5	0.5	3.3	2.0	0.0	0.50	2.80	1.5
8	0.2	0.2	4.2	2.8	0.0	0.20	4.00	2.6
11	0.5	0.4	0.4	2.3	-0.1	0.45	-0.05	1.85
21	*	0.4	1.9	1.2	*	0.40	1.50	0.8
20	0.8	0.6	5.2	1.5	-0.2	0.70	4.50	0.8
22	0.5	0.5	1.9	2.7	0.0	0.50	1.40	2.2
24	1.0	1.3	3.8	1.0	0.3	1.15	2.65	-0.15
26	0.4	0.4	2.6	0.5	0.0	0.40	2.20	0.1
28	*	2.2	4.6	3.5	*	2.20	2.40	1.3
29	0.3	*	1.5	0.7	*	0.30	1.20	0.4
32	0.4	0.4	2.6	1.1	0.0	0.40	2.20	0.7
33	0.4	0.5	2.6	2.1	0.1	0.45	2.15	1.65
34	0.4	0.3	2.5	3.7	-0.1	0.35	2.15	3.35
39	0.5	0.4	0.4	*	-0.1	0.45	-0.05	*
count	18	19	20	19	17	20	20	19
mean	0.46	0.55	2.52	1.75	0.00	0.54	1.98	1.20
std dev	0.19	0.46	1.33	1.00	0.11	0.44	1.20	0.94
cl width	0.09	0.21	0.58	0.45	0.05	0.19	0.53	0.42
cl high	0.54	0.76	3.10	2.20	0.05	0.73	2.50	1.63
cl low	0.37	0.35	1.93	1.30	-0.05	0.35	1.45	0.78

TABLE 13

LINOLEIC ACID AS A PERCENTAGE OF TOTAL PLASMA FATTY ACIDS

GROUP A

SUBJECT	RUN-IN 1	RUN-IN-2	3 MONTHS	6 MONTHS	DIFFERENCE BETWEEN RUN-INS	MEAN RUN-IN	DIFFERENCE FROM MEAN RUN-IN AT 3 MONTHS	DIFFERENCE FROM MEAN RUN-IN AT 6 MONTHS
9	38.6	34.2	33.0	36.4	-4.4	36.40	-3.40	0.00
10	30.0	33.6	34.7	31.2	3.6	31.80	2.90	-0.60
12	35.1	32.7	35.2	*	-2.4	33.90	1.30	*
13	25.7	33.4	31.9	31.2	7.7	29.55	2.35	1.65
14	30.7	37.2	35.9	34.9	6.5	33.95	1.95	0.95
15	28.2	30.2	41.3	39.2	2.0	29.20	12.10	10.00
16	39.4	36.9	40.5	33.2	-2.5	38.15	2.35	-4.95
17	34.5	35.3	35.6	31.2	0.8	34.90	0.70	-3.70
18	43.7	40.2	37.8	43.2	-3.5	41.95	-4.15	1.25
23	34.0	37.7	43.7	40.3	3.7	35.85	7.85	4.45
19	34.4	38.2	36.5	38.0	3.8	36.30	0.20	1.70
25	*	*	*	*	*	*	*	*
27	36.9	33.4	35.8	32.4	-3.5	35.15	0.65	-2.75
30	39.9	30.3	37.5	40.2	-9.6	35.10	2.40	5.10
31	25.6	30.7	33.2	32.9	5.1	28.15	5.05	4.75
35	37.6	34.2	33.3	38.0	-3.4	35.90	-2.60	2.10
36	37.1	39.0	35.0	34.4	1.9	38.05	-3.05	-3.65
37	33.8	32.4	33.0	32.9	-1.4	33.10	-0.10	-0.20
38	33.3	31.2	36.1	36.0	-2.1	32.25	3.85	3.75
count	18	18	18	17	18	18	18	17
mean	34.4	34.5	36.1	35.6	0.1	34.43	1.69	1.17
std dev	4.9	3.1	3.1	3.7	4.5	3.44	4.01	3.80
ci width	2.3	1.4	1.4	1.8	2.1	1.59	1.85	1.81
ci high	36.6	35.9	37.6	37.4	2.2	36.01	3.54	2.97
ci low	32.1	33.1	34.7	33.9	-1.9	32.84	-0.17	-0.64

GROUP B

SUBJECT	RUN-IN 1	RUN-IN-2	3 MONTHS	6 MONTHS	DIFFERENCE BETWEEN RUN-INS	MEAN RUN-IN	DIFFERENCE FROM MEAN RUN-IN AT 3 MONTHS	DIFFERENCE FROM MEAN RUN-IN AT 6 MONTHS
1	36.5	36.0	33.8	31.5	-0.5	36.25	-2.45	-4.75
2	38.6	30.6	33.9	37.2	-8.0	34.60	-0.70	2.60
3	44.5	42.7	39.1	42.5	-1.8	43.60	-4.50	-1.10
4	28.9	33.3	36.7	30.8	4.4	31.10	5.60	-0.30
5	36.3	35.3	33.9	34.3	-1.0	35.80	-1.90	-1.50
6	37.1	38.7	33.5	29.4	1.6	37.90	-4.40	-8.50
7	41.2	39.5	40.5	38.0	-1.7	40.35	0.15	-2.35
8	32.8	32.3	17.2	35.0	-0.5	32.55	-15.35	2.45
11	32.8	32.8	31.5	32.6	0.0	32.80	-1.30	-0.20
21	*	32.5	25.9	29.5	*	32.50	-6.60	-3.00
20	32.8	38.3	28.9	32.2	5.5	35.55	-6.65	-3.35
22	40.0	36.9	39.9	40.2	-3.1	38.45	1.45	1.75
24	39.1	35.1	36.4	36.1	-4.0	37.10	-0.70	-1.00
26	36.6	36.0	32.5	35.0	-0.6	36.30	-3.80	-1.30
28	*	40.2	38.9	41.4	*	40.20	-1.30	1.20
29	33.9	*	36.4	35.7	*	33.90	2.50	1.80
32	35.6	34.6	31.8	33.2	-1.0	35.10	-3.30	-1.90
33	34.5	33.1	34.5	36.7	-1.4	33.80	0.70	2.90
34	33.0	32.0	34.4	33.2	-1.0	32.50	1.90	0.70
39	37.4	34.6	35.9	*	-2.8	36.00	-0.10	*
count	18	19	20	19	17	20	20	19
mean	36.20	35.50	33.78	34.97	-0.94	35.82	-2.04	-0.83
std dev	3.69	3.21	5.31	3.76	3.03	3.14	4.37	2.85
ci width	1.71	1.44	2.33	1.69	1.44	1.38	1.92	1.28
ci high	37.91	36.94	36.11	36.67	0.50	37.19	-0.12	0.45
ci low	34.49	34.06	31.45	33.28	-2.37	34.44	-3.95	-2.12

TABLE 14

ARACHIDONIC ACID AS A PERCENTAGE OF TOTAL FATTY ACIDS

GROUP A

SUBJECT	RUN-IN 1	RUN-IN 2	3 MONTHS	6 MONTHS	DIFFERENCE BETWEEN RUN-INS	MEAN OF RUN-INS	DIFFERENCE FROM MEAN RUN-IN AT 3 MONTHS	DIFFERENCE FROM MEAN RUN-IN AT 6 MONTHS
9	5.3	4.0	4.4	4.4	-1.3	4.65	-0.25	-0.25
10	3.8	3.9	4.1	4.1	0.1	3.85	0.25	0.25
12	5.7	5.0	5.1	*	-0.7	5.35	-0.25	*
13	2.7	4.0	3.1	3.1	1.3	3.35	-0.25	-0.25
14	4.0	5.4	4.5	4.5	1.4	4.70	-0.20	-0.20
15	3.6	4.5	4.6	4.6	0.9	4.05	0.55	0.55
16	3.9	5.0	4.9	4.9	1.1	4.45	0.45	0.45
17	4.2	5.0	4.3	4.3	0.8	4.60	-0.30	-0.30
18	6.2	6.1	4.5	4.5	-0.1	6.15	-1.65	-1.65
23	5.2	5.4	5.8	5.8	0.2	5.30	0.50	0.50
19	4.3	5.5	4.8	4.8	1.2	4.90	-0.10	-0.10
25	*	*	*	*	*	*	*	*
27	4.2	4.7	3.5	3.5	0.5	4.45	-0.95	-0.95
30	3.6	2.9	3.6	3.6	-0.7	3.25	0.35	0.35
31	3.6	4.0	4.4	4.4	0.4	3.80	0.60	0.60
35	4.9	4.2	3.5	3.5	-0.7	4.55	-1.05	-1.05
36	4.3	4.3	3.2	3.2	0.0	4.30	-1.10	-1.10
37	4.4	4.5	4.2	4.2	0.1	4.45	-0.25	-0.25
38	6.3	5.7	4.7	4.7	-0.6	6.00	-1.30	-1.30
count	18	18	18	17	18	18	18	17
mean	4.5	4.7	4.3	4.2	0.2	4.56	-0.28	-0.28
std dev	1.0	0.8	0.7	0.7	0.8	0.79	0.68	0.71
ci width	0.4	0.4	0.3	0.3	0.4	0.36	0.32	0.34
ci high	4.9	5.0	4.6	4.6	0.6	4.93	0.04	0.06
ci low	4.0	4.3	4.0	3.9	-0.2	4.20	-0.59	-0.61

GROUP B

SUBJECT	RUN-IN 1	RUN-IN 2	3 MONTHS	6 MONTHS	DIFFERENCE BETWEEN RUN-INS	MEAN OF RUN-INS	DIFFERENCE FROM MEAN RUN-IN AT 3 MONTHS	DIFFERENCE FROM MEAN RUN-IN AT 6 MONTHS
1	4.3	3.9	4.2	4.2	-0.4	4.10	0.10	0.10
2	3.7	3.0	4.1	4.1	-0.7	3.35	0.75	0.75
3	6.2	5.1	4.9	4.9	-1.1	5.65	-0.75	-0.75
4	3.0	3.5	4.4	4.4	0.5	3.25	1.15	1.15
5	4.3	4.3	4.1	4.1	0.0	4.30	-0.20	-0.20
6	5.2	5.5	4.7	4.7	0.3	5.35	-0.65	-0.65
7	4.7	2.4	5.5	5.5	-2.3	3.55	1.95	1.95
8	2.8	2.6	2.0	2.0	-0.2	2.70	-0.70	-0.70
11	5.9	5.0	4.2	4.2	-0.9	5.45	-1.25	-1.25
21	*	5.2	3.5	3.5	*	5.20	-1.70	-1.70
20	6.5	5.2	3.8	3.8	-1.3	5.85	-2.05	-2.05
22	5.9	5.6	4.7	4.7	-0.3	5.75	-1.05	-1.05
24	5.1	4.9	5.4	5.4	-0.2	5.00	0.40	0.40
26	4.9	4.3	4.8	4.8	-0.6	4.60	0.20	0.20
28	*	5.1	5.2	5.2	*	5.10	0.10	0.10
29	4.7	*	4.6	4.6	*	4.70	-0.10	-0.10
32	4.4	3.7	4.8	4.8	-0.7	4.05	0.75	0.75
33	5.2	4.6	5.4	5.4	-0.6	4.90	0.50	0.50
34	4.4	4.0	3.9	3.9	-0.4	4.20	-0.30	-0.30
39	4.6	3.8	4.3	*	-0.8	4.20	0.10	*
count	18	19	20	19	17	20	20	19
mean	4.77	4.30	4.43	4.43	-0.57	4.56	-0.14	-0.15
std dev	1.00	0.96	0.80	0.82	0.64	0.89	0.97	0.99
ci width	0.46	0.43	0.35	0.37	0.31	0.39	0.42	0.45
ci high	5.23	4.73	4.77	4.80	-0.26	4.95	0.29	0.30
ci low	4.30	3.87	4.08	4.06	-0.88	4.17	-0.56	-0.60

TABLE 15

LIPOPOLYSACCHARIDE STIMULATED TNF α PRODUCTION FROM PERIPHERAL BLOOD MONONUCLEAR CELLS

GROUP A

SUBJECT	RUN-IN 1	RUN-IN 2	MEAN RUN-IN	DIFFERENCE BETWEEN RUN-INS	3 MONTHS	6 MONTHS	DIFFERENCE FROM MEAN OF RUN-IN AT 3 MONTHS	DIFFERENCE FROM MEAN OF RUN-IN AT 6 MONTHS	PERCENTAGE DIFFERENCE AT 3 MONTHS	PERCENTAGE DIFFERENCE AT 6 MONTHS
9	2080	1470	1775	-610	3160	948	1385	-827	78	-47
10	492	1122	807	630	1318	984	511	177	63	22
12	1658	2800	2229	1142	640	•	-1589	•	-71	•
13	690	786	738	96	570	1466	-168	728	-23	99
14	1766	1480	1623	-286	1034	1238	-589	-385	-36	-24
15	512	828	670	316	634	2440	-36	1770	-5	264
16	1810	604	1207	-1206	816	1070	-391	-137	-32	-11
17	1092	1124	1108	32	1214	452	106	-656	10	-59
18	512	618	565	106	954	1726	389	1161	69	205
19	1326	1046	1186	-280	1114	389	-72	-797	-6	-67
23	1002	504	753	-498	848	874	95	121	13	16
25	986	360	673	-626	2240	348	1567	-325	233	-48
27	1062	1070	1066	8	2160	1762	1094	696	103	65
30	3660	1510	1510	-2150	180	458	-1330	-1052	-88	-70
31	1462	1582	1522	120	1058	1172	-464	-350	-30	-23
35	1448	2280	1864	832	1818	1904	-46	40	-2	2
36	650	1814	1232	1164	846	1294	-386	62	-31	5
37	2120	1720	1920	-400	1380	1386	-540	-534	-28	-28
38	2320	1574	1947	-746	1878	3040	-69	1093	-4	56
count	19	19	19	19	19	18	19	18	19	18
mean	1403	1279	1284	-124	1256	1275	-28	44	11	20
stdev	793	625	511	801	717	718	799	776	73	91
cl-width	357	281	230	360	322	331	359	359	33	42
cl-high	1759	1560	1514	236	1578	1607	331	402	44	62
cl-low	1046	997	1054	-484	933	944	-387	-315	-22	-22

GROUP B

SUBJECT	RUN-IN 1	RUN-IN 2	MEAN RUN-IN	DIFFERENCE BETWEEN RUN-INS	3 MONTHS	6 MONTHS	DIFFERENCE FROM MEAN OF RUN-IN AT 3 MONTHS	DIFFERENCE FROM MEAN OF RUN-IN AT 6 MONTHS	PERCENTAGE DIFFERENCE AT 3 MONTHS	PERCENTAGE DIFFERENCE AT 6 MONTHS
1	474	380	427	-94	1170	1302	743	875	174	205
2	468	806	637	338	954	1220	317	583	50	92
3	•	•	•	•	•	•	•	•	•	•
4	1622	974	1298	-648	740	543	-558	-755	-43	-58
5	2020	1038	1529	-982	2660	688	1131	-841	74	-55
6	1046	1414	1230	368	260	488	-970	-742	-79	-60
7	•	3100	3100	•	2920	1378	-180	-1722	-6	-56
8	•	1860	1860	•	728	1724	-1132	-136	-61	-7
11	1726	1478	1602	-248	702	754	-900	-848	-56	-53
20	1118	1084	1101	-34	748	•	-353	•	-32	•
21	716	•	716	•	520	240	-196	-476	-27	-66
22	850	1194	1022	344	1390	806	368	-216	36	-21
24	598	326	462	-272	452	476	-10	14	-2	3
26	1200	970	1085	-230	1468	684	383	-401	35	-37
28	•	2280	2280	•	872	486	-1408	-1794	-62	-79
29	796	•	796	•	•	554	•	-242	•	-30
32	2500	1576	2038	-924	2880	1580	842	-458	41	-22
33	1484	910	1197	-574	788	970	-409	-227	-34	-19
34	1006	1040	1023	34	984	1338	-39	315	-4	31
39										
count	15	16	18	13	17	17	17	17	17	17
mean	1175	1277	1300	-225	1190	896	-139	-416	0	-14
stdev	592	694	684	454	837	444	719	697	63	70
cl-width	299	340	316	247	398	211	342	331	30	33
cl-high	1474	1617	1616	22	1588	1107	203	-85	30	19
cl-low	875	937	984	-472	793	685	-481	-747	-30	-47

TABLE 16A

FINAL QUESTIONNAIRE - GROUP A

1 = yes 2 = no FRESH FISH : 0 = none 1 = oily fish 2 = non-oily fish 3 = both

MEDICATION : -1 = decrease; 0 = no change; +1 = increase
PERCEPTION : -2 = much worse +2 = much better

SUBJECT	KNOWLEDGE OF GROUP	VITAMIN SUPPLEMENTS	MARGARINE g/day	OIL mls/day	CAPSULES No./day	SIDE EFFECTS	TYPE OF FRESH FISH	QUANTITY g/month	PERCEPTION (of symptoms)	MEDICATION INCREASE/ DECREASE
9	2	1	15.4	7.4	3.9	2	3	125	1	1
10	2	1	17	1.5	3.9	2	2	173	-1	1
12	•	•	•	•	•	•	•	•	•	•
13	2	1	14.3	11.9	2.7	1	2	200	-1	1
14	2	1	7.4	6.7	3.8	2	0	0	0	1
15	2	1	11.9	5.36	3.1	2	3	600	1	0
16	2	2	11.9	0	2.7	2	2	33	0	0
17	2	2	7.4	1.98	3.6	2	0	0	2	0
18	2	2	6.7	16.4	4.0	2	1	33	0	0
19	2	2	15	13	3.3	2	0	0	1	0
23	2	2	11.9	10.7	4.1	1	2	100	0	-1
25	2	2	19	7	3.9	2	0	0	0	0
27	2	1	14.9	12.6	2.9	2	0	0	1	0
30	2	1	1.5	2.6	2.1	2	3	75	-1	0
31	2	2	5.5	4.4	3.2	2	2	75	-1	1
35	2	2	12.6	6	2.7	2	3	300	1	0
36	2	2	13.4	11.9	3.4	2	2	33	1	0
37	2	2	19.3	4.5	2.4	2	1	8	2	1
38	2	1	5.8	12.3	3.1	2	3	200	1	0
count	18	18	18	18	18.0	18	18	18	18	18
mean/yes	0 yes	8 yes	11.7	7.6	3.3	2 yes	5 = 0	109	0 = -2	1 = -1
stdev			5.0	4.7	0.6		2 = 1	151	4 = -1	11 = 0
ci-width			2.3	2.2	0.3		6 = 2	70	5 = 0	6 = 1
ci-high			14.0	9.8	3.5		5 = 3	178	7 = 1	
ci-low			9.4	5.4	3.0			39	2 = 2	

TABLE 16B

FINAL QUESTIONNAIRE - GROUP B

1 = yes 2 = no FRESH FISH : 0 = none 1 = oily fish 2 = non-oily fish 3 = both

MEDICATION : -1 = decrease; 0 = no change; +1 = increase
PERCEPTION : -2 = much worse +2 = much better

SUBJECT	KNOWLEDGE OF GROUP	VITAMIN SUPPLEMENTS	MARGARINE g/day	OIL mls/day	CAPSULES No./day	SIDE EFFECTS	TYPE OF FRESH FISH	QUANTITY g/month	PERCEPTION (of symptoms)	MEDICATION INCREASE/ DECREASE
1	2	1	11	6	2.7	2	1	150	2	-1
2	2	1	16	12	2.2	2	2	130	2	0
3	2	1	13	1	2.4	2	3	300	2	0
4	2	2	9	16	3.8	2	2	975	2	-1
5	2	2	9	1.5	2.7	2	2	75	0	0
6	2	2	5	24	2.7	2	3	175	1	0
7	2	2	9	3	3.3	2	3	150	-1	1
8	2	1	4	16	3.8	2	0	50	1	-1
11	2	2	13	1	3.4	2	3	500	1	-1
20	2	2	15	5	3.8	2	1	200	2	0
21	2	2	15	5	3.8	2	1	100	2	0
22	2	2	19	4	2.7	2	3	200	1	0
24	2	1	12	18	2.7	2	1	1000	1	-1
26	2	2	9.7	3.3	3.9	1	3	200	0	1
28	2	2	10.8	7.5	3.2	2	3	1000	0	1
29	1	2	7.5	8.6	2.5	2	3	450	0	0
32	2	2	14.1	14.1	4.0	2	2	450	1	0
33	1	2	15.9	13.2	3.4	2	2	400	0	0
34	1	2	7.1	2.4	3.2	2	0	0	0	0
39	2	2	21	6	2.4	2	3	900	0	0
count	20	20	20	20	20.0	20	20	20	20	20
mean/yes	3 yes	5 yes	11.81	8.38	3.1	1 yes	2 = 0	370	0 = -2	5 = -1
stdev			4.45	6.58	0.6		4 = 1	337	1 = -1	12 = 0
ci-width			1.95	2.88	0.3		5 = 2	148	7 = 0	3 = 1
ci-high			13.76	11.26	3.4		9 = 3	518	6 = 1	
ci-low			9.85	5.50	2.9			223	6 = 2	

APPENDIX 1

A. Food Diary

There is always a great temptation to change the way that you eat when you know that someone will be looking at it, but please *resist this temptation!* We need to know how your child eats normally so that we can explain any differences in his/her blood analysis. Although a dietitian will be analysing your child's diet, she will not be making any comments on the foods eaten unless you specifically request this information. Our interest in this study is the type of fats your child consumes and their relationship to the chemicals his/her body is producing, not whether it is a good or bad diet. So please, **be totally honest with us and encourage your child to eat as normally as possible** while you are keeping the diary.

Each day you should record all foods and drinks consumed including snacks like fruit, bread, lollies, chocolates, biscuits, cakes, etc. It is important to describe each food or drink by **type and brand** where appropriate. We also need to know the **quantity**. In some foods the number and approximate size will be enough, for example "1 medium". For other foods, household measures will be more appropriate. These measures should be the standard ones used in recipes as follows :

1 cup = 250 mls measuring cup

1 teaspoon = 5 mls

1 tablespoon = 20 mls

The **method of cooking** is also important. Foods should be described wherever possible or appropriate as boiled, steamed, microwaved, fried, baked (no fat or oil), or roasted (with fat or oil)

<u>**Oils and margarines**</u> should be recorded by the number of grams, teaspoons or tablespoons. It is only necessary to record the brand and

type when the margarine and oils used are not those supplied by us (e.g. Meadowlea polyunsaturated margarine).

Drinks, rice, pasta, breakfast cereals, icecream and some vegetables and fruits can be recorded by the cup such as "1/2 cup milk" or "1 cup boiled rice" or "1/4 cup (1 scoop) icecream" or "1/4 cup of steamed peas" or "1/2 cup grapes" . Smaller quantities can be recorded by the tablespoon or teaspoon. Vegetables such as potatoes can be approximated by number and size for example "1 medium roasted potato". Many fruits can be measured in a similar way for example, "1 large apple", or "3 small apricots".

Meats can be described by type, approximate size, method of cooking and whether the fat was eaten or cut off. For example "3 thin beef sausages, grilled " or "2 small forequarter lamb chops, barbecued, fat eaten" or "3 large slices of lean roast lamb leg"

Home made stews and casseroles should be described by their ingredients and the approximate portion consumed. For example 1/4 homemade stew with 1 tablespoon oil, 1 medium potato, 3 medium carrots, 1 large onion, 1 kg chuck steak.

When recording **prepackaged foods** it is important to note down the size of the packet and the brand, for example "1 x 25g packet of plain Smiths crisps" or "1 x 250 ml 100% juice Apple Popper". Sometimes only part of the packet is consumed so the portion, say "1/2 packet Maggi Instant Noodles" could be recorded or even the number, for example "5 Birdseye 15's Fish Fingers" or "8 squares Cadbury plain milk chocolate" or "1 1/2 cups Kelloggs Cornflakes".

B. Asthma Diary

On each diary page is a section to record information about your child's asthma. This should be done first thing in the morning and before bed at night.

Morning Section

Night-time symptoms

Tick the box which best describes your child's asthma during the previous night. If your child takes bronchodilator sprays, such as Ventolin, Bricanyl, Alupent, Respolin or Serevent, or tablets, medicines or sprinkles, such as Nuelin or Theodur, before bed, please tick the box marked Bronchodilator before bed, either 'YES' or 'NO'.

Peak flow readings

Measure peak flows first thing in the morning, before your child has had any bronchodilator sprays. Record three tries.

Evening Section

Daytime symptoms

Tick the box which best describes your child's asthma during the day.

Medication use

Record the amount of each medication taken in the last 24 hours, ie. since the record you made in yesterday's diary.

Example

DATE : 20-3-95 **DAY :** Monday

EXAMPLE	BREAKFAST	MORNING SNACKS	LUNCH	AFTERNOON SNACKS	DINNER	EVENING SNACKS
FOOD & DRINK (brand/type and quantity)	2 Weetbix 1 teaspoon sugar ½ cup milk (whole) ½ cup orange juice (fresh squeezed)	1 medium apple 1 × 250 ml apple Popper	2 slices Fielders white sandwich loaf + 2 teaspoons Flora polyunsaturated margarine 1 slice Kraft Cheddar cheese 1 Redskin	1 × 25g Uncle Toby's Choc Chip Muesli Bar 2 milk Arrowroot biscuits 1 cup milk (whole)	1 medium potato (chips, home-cooked) (in Vegetable oil) ¼ cup fresh peas (boiled) ¼ cup fresh carrots (boiled) 1 grilled chump chop (medium) 2 scoops Peters Natural Vanilla Icecream	–

MORNING SECTION

NIGHT-TIME SYMPTOMS (last night) Please tick :	
Slept through the night; woke at usual time, not tight on waking	☐
Slept through the night, woke at usual time, tight on waking	☑
Woke with asthma at night, symptoms relieved by bronchodilator	☐
Woke with asthma at night, symptoms not relieved by bronchodilator	☐
Bronchodilator before bed	Yes ☑ No ☐

PEAK FLOWS Record 3 tries
1. 200
2. 220
3. 215

EVENING SECTION :

RECORD DAYTIME SYMPTOMS Please tick :	
No symptoms	☐
Occasional symptoms, normal activity possible, no extra treatment needed	☐
Symptoms, normal activity possible after extra treatment taken	☑
Symptoms which interfered with normal activity despite extra treatment	☐
Symptoms which made normal activity impossible	☐

ASTHMA MEDICATIONS

Medication and Strength	Amount taken since last night
Ventolin	4 puffs
Intal 5mg	4 puffs
Becotide 50	8 puffs

APPENDIX 2

Symptom score (Freq and severity)	Medication	AM pre BD PEF (% recent best)
0 No symptoms all week	**0** BD/SCG* (on 0-3 days)	**0** > 92.5%
1 1 on 3 days or less	**1** BD/SCG* (on > 4 days)	**1** 85% - 92.5%
2 1 on ≥ 4 days or 2 on ≤ 2 days	**2** Steroid aerosol (<1000μg / day)	**2** 77.5% - 84.9%
3 2 on ≥ 3days or 3 on ≤ 2 days	**3** Steroid aerosol (>1000μg / day)	**3** 70% - 77.4%
4 3 on ≥ 3days or 4 on any day	**4** Oral steroid (1 or more days)	**4** < 70%

Symptom score (day)

0 = no symptoms
1 = occasional symptoms which do not interfere with normal activity and do not require extra medication.
2 = symptoms which do not interfere with normal activity, provided extra medication is taken.
3 = symptoms which interfere with normal activity despite extra medication.
4 = symptoms which make normal activity impossible.

Symptom score (night):

0 = no BD treatment before bed, sleep through the night, wake at usual time, not tight on waking
1 = no BD treatment before bed, sleep throught the night, wake at usual time, tight on waking
2 = sleep through the night, provided BD treatment taken at bed-time
3 = wake at night - return to sleep after BD treatment
4 = wake at night, can't get back to sleep even after BD treatment