Treatment Of Children's Asthma With A Lipid Extract Of The New Zealand Green Lipped Mussel (Perna Canaliculus) (Lyprinol®) - A Double Blind, Randomised Controlled Trial In Children With Moderate To Severe Chronic Obstructive Asthma.

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Citation

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Abstract

The efficacy and safety of an oral standardised lipid extract of New Zealand green lipped mussel (Perna canaliculus) marketed as Lyprinol
® was assessed as maintenance therapy for children with moderate asthma. A total of 71 children aged 6 to 13 years were enrolled in a 16-week, single centre, double-masked, placebo-controlled, parallel-group trial and randomly assigned to receive either Lyprinol or placebo (2 capsules twice daily). Patients were maintained on as-needed beta-agonist therapy and inhaled corticosteroid (ICS) throughout the study. Results. Lyprinol improved the percentage of children reporting little or no trouble with their asthma at three months of treatment (97% vs. 76% p=0.057). Both groups were able to tolerate a dose reduction of ICS. There were fewer mild and moderate asthma exacerbations overall in the Lyprinol group. Lyprinol was well tolerated. It appears from this study that Lyprinol is a safe nutritional supplement for children with moderate asthma and that larger prospective controlled studies should explore its potential use as a nutraceutical in asthma as an addition to conventional treatment.

INTRODUCTION

Inflammatory cellular infiltrates, including lymphocytes, eosinophils, and mast cells, are found in the airways of patients with asthma, even during periods of clinical stability[1]. These cells produce multiple inflammatory mediators, such as histamine, cytokines, and the cysteinyl leukotrienes (leukotrienes C4, D4, and E4). Compelling evidence exists for the role of the cysteinyl leukotrienes in the pathophysiology of asthma[1,2,3,4]. These mediators are potent bronchoconstricting agents and induce other features typical of asthma, including airway-wall edema and mucus hypersecretion[5].

Interest in the possible health benefits of dietary marine n-3 fatty acids (fish oil) developed following observations that those populations with a high dietary intake of fish also have a low incidence of asthma[6]. The potential anti-inflammatory effect of fish oil may relate to its constituent eicosapentaenoic acid (EPA), being a competitive substrate with arachidonic acid in generating metabolites. EPA is a substrate for the generation of less active prostanoids and leukotrienes than arachidonic acid, thereby potentially acting to reduce airway inflammation and bronchoconstriction[7].

The influence of a diet rich in EPA and DHA in the role of airway inflammation in asthma has been the subject of interest of several small trials[8,9,10].

To date, the evidence for beneficial effects of a dietary intake which is high in marine n-3 fatty acids is controversial. Whilst some support for this hypothesis has occurred in epidemiological studies, the data from intervention studies has been conflicting. Black[11] hypothesized that dietary fatty acid intake may influence the development of allergic sensitization by increasing the formation of prostaglandin E2; which in turn promotes T helper lymphocyte Th2 responses and stimulates the

formulation of immunoglobulin E. As dietary fat intake patterns have changed over the past few decades, with an increase in saturated and omega-6 fat consumption, and a reduction in polyunsaturated omega-3 fat intake, this may be an important factor in the increasing prevalence of asthma and possibly other allergic diseases. Although used for asthma, the efficacy of dietary marine fatty acids (fish oil) is controversial and a review[12] of the clinical trials of asthma and fish oils by Monteleone et al. concluded that there was no evidence of clinical improvement in people with asthma using fish oil supplementation, despite some changes seen in inflammatory cell functions. In a review Woods et al.[13] analyzed nine trials of fish oil supplementation in asthma without finding any consistent effect on all analyzable outcomes. More recently an experimental clinical study in mild asthma suggests bronchial allergic inflammation is related to a diet deficient in polyunsaturated fatty acids[14].

Oily fish and shellfish are rich in the omega-3 fatty acids EPA and DHA. Lyprinol® is a natural, standardised lipid extract of New Zealand green lipped mussel (Perna canaliculus) containing a unique combination of free fatty acids, sterol esters, polar lipids and carotenoids. The lipid extract can be characterized as a mixture belonging to a lipid group called sterol esters (SE). The SE from Lyprinol capsules contains a characteristic fatty acid and sterol composition[15]. The fatty acids in the SE fraction are mainly myristic acid, palmitic acid, palmitoleic acid, stearic acid, oleic acid, linoleic acid, EPA and DHA. The sterols found in this fraction include cholesterol, cholesta-3,5-diene, 26,27-dinoergostadienol, cholesta-5,22-dien-3-ol, ergosta-5,22-dien-3-ol. Animal studies have suggested that the potency of the anti-inflammatory effect of Lyprinol is greater than fish oils[16]. There is some experimental evidence of anti-inflammatory activity of Lyprinol in a rat model[17]. Few controlled trials have examined Lyprinol in asthma. Emelyanov et al. [18] investigated the effects of Lyprinol in 46 adult patients with atopic asthma. These patients were steroid-naïve and had relatively mild asthma. These authors found a significant decrease in daytime wheeze and an increase in morning peak expiratory flow in the treated group. While they found no significant change in FEV1 they did demonstrate a reduction in hydrogen peroxide in expired breath condensate which was used as a marker of airway inflammation.

In the study reported here we sought to investigate the use of Lyprinol in children with moderate chronic persistent asthma taking regular inhaled corticosteroid (ICS) using clinical endpoints relevant to paediatric asthma management.

PATIENTS AND METHODS

PATIENT POPULATION

The trial was conducted in Auckland New Zealand. Children aged 6 to 13 years of age were enrolled if they met the following criteria: documented history of asthma with diagnosis consistent with standard guidelines accepted by National Asthma Council of Australia, asthma treated only with beta-agonist and inhaled corticosteroid (receiving a daily dose of inhaled corticosteroids 50-500 micrograms beclomethasone / budesonide or 50-250 micrograms fluticasone). Participants had to be able to swallow capsules and were having a minimum of symptoms at the date of enrollment. Children were recruited from the greater Auckland area from advertising in general and health oriented magazines in the Auckland area and through pharmacies, asthma society, General Practice and Paediatrician contacts.

Children were excluded if they required regular oral corticosteroids or had required oral, depot or parenteral corticosteroid therapy in the 30 days before the first screening visit. Similarly subjects using oral leukotriene antagonists, long acting bronchodilators, or Lyprinol, other fish oil supplement or other investigational medicine were excluded. Other exclusion criteria were: hospitalisation on more than two occasions for asthma in the preceding 12 months, receiving antibiotic therapy for chest infection during the run-in period of 4 weeks, those with clinical or laboratory evidence of a serious, uncontrolled systemic disease or any disease that is likely to interfere with the objectives of the study. Other disorders excluded were disease of the ears, nose, throat or sinuses likely to require surgical intervention during the study period, congenital heart disease, hepatic disease or chronic respiratory disease other than asthma. Demographic information was collected including family history pertaining to allergy and asthma, environmental history including existence of pets, crowding, type of dwelling and education level of biological parents if known. Some of this information was used to categorise children using a deprivation index based mainly on location of dwelling[19]

Subjects were excluded if they were considered unable or unlikely to comply with study requirements or those with a known allergy to Lyprinol or any fish, seafood or products

of marine food. All parents of patients enrolled in the trial or legal guardians of patients gave written informed consent and the trial was approved by the Northern Regional Ethics Committee (NTY/05/06/041) in late 2005 and reports renewed annually during the course of the study.

STUDY DESIGN

The study was a prospective double blind parallel randomized parallel-group controlled trial. Primary outcome measures were inhaled corticosteroid dose reduction with secondary outcome measures of asthma exacerbation rate, asthma symptom score, Juniper quality of life score [http://www.qoltech.co.uk], use of short acting beta agonist medication and lung function performance including end-tidal breath nitric oxide level.

Children meeting the inclusion and exclusion criteria were screened by the clinical study team (one paediatrician, one general practitioner and 2-5 asthma nurses). The study was designed by the principal investigators without influence from study sponsors with a final protocol (LYPNZ01) documented in August 2005. The study was managed by Asthma New Zealand/The Lung Association and study visits were conducted at their main office and clinical rooms at 581 Mt Eden Rd Mt Eden, Auckland, New Zealand. The study sponsor was Pharmalink International Limited. The study period commenced in May 2006 and the last child enrolled completed the trial in 22 nd April 2009. Data were held on written clinical record files and abstracted by study co-ordinator to an Excel™ spreadsheet. The diagnosis of asthma was reviewed by the clinician study team for accuracy during the screening phase of each child entering the protocol and at the end of study data collection by two clinicians independent of the study team. The clinical study team was blinded with respect to placebo or Lyprinol capsules until after the dataset was closed and audited.

Study medication was either Lyprinol brand capsules or matching placebo provided by the Lyprinol manufacturer.

Lyprinol® contains 50 mg of a unique combination of omega-3 polyunsaturated fatty acids (mostly eicopentaenoic acid and docosahexaenoic acid) the product of a patented extraction process. The unique combination of fatty acids produced by the extraction process is named PCSO-524. This product is then dissolved in 100 mg olive oil. It is a commercial product sold under the brand name Lyprinol® and Omega XL®. The placebo was given in identical capsules, manufactured for Pharmalink International Limited, containing 150 mg olive oil.

Both the Lyprinol® and placebo capsules had been manufactured to have identical taste and smell. Preparation and packaging of of study medication was performed centrally and randomisation was performed in blocks of twenty with all study medication being shipped to the study centre at the start and half way through the trial. There was monthly dispensing to each patient during the trial period. Patients were asked to return unused medication at each monthly visit during the treatment trial. Unused study medication was accounted for and destroyed. Study medication was pre-labelled with random codes and the identities of these codes were not made available to study staff. Permitted medications were ICS (fluticasone, beclomethasone or budesonide) in defined doses and the short acting beta agonists (salbutamol or terbutaline). No other asthma medication was permitted, although antihistamines, or nasal sprays were permitted for co-existing allergic conditions.

Patients were seen for two run-in visits at fortnightly intervals when suitability for entry to the trial was assessed and asthma stabilized. During the screening period, patients recorded asthma symptoms, beta-agonist use, and peak expiratory flow rates (PEFRs) on diary cards. Other assessments included the medical history, interviews about subjective symptoms, a physical examination, measurement of vital signs, an estimation of end-tidal Nitric Oxide, and pulmonary function testing. Once allocated to treatment at baseline (visit 3) they were seen monthly for 4 months while on study medication (visits 4 through 7). The dose of study medication for both groups was 2 capsules taken orally twice daily. The final follow-up visit was one month after study medication was completed. A total of 8 study related visits was usual. Children were weighed and height recorded on study entry and at visit 6 to ensure spirometry recordings and dosing were consistent and accounted for growth during the study period. Study flow is shown in Table 1.

Figure 1

Table 1. Study procedure

Visit No.	Week No.	Study Procedures
1	-4	Review and discuss trial information, provide copies of Patient Information and Informed Consent forms to take home Physical examination, medication review, spirometry, peak flow, Nitric Oxide (NO) measurement, eligibility review, Juniper Quality of Life (QOL), Height / Weight Provide diary card for recording symptoms, peak flow and medication taken.
2	-2	Physical examination, medication review, spirometry, peak flow, Nitric Oxide (NO) measurement, QOL. Review diary card, discuss patient information consent
3	0	Obtain Written Informed Consent and confirm eligibility. Physical examination, medication review, spirometry, Nitric Oxide (NO) measurement, QOL. Confirm baseline dose of Inhaled corticosteroids Randomise and dispense study medication Provide diary card and contact details for Investigators
4	4	Physical examination, medication review, spirometry, peak flow, Nitric Oxide (NO) measurement, QOL. Reduce Inhaled corticosteroid by 25% if asthma is stable Review diary card for adverse events, medications, peak flow monitoring, symptoms Collect returned study medication and dispense next months medication.
5	8	Physical examination, medication review, spirometry, peak flow, Nitric Oxide (NO) measurement, QOL. Reduce Inhaled corticosteroid by 25% if asthma is stable Review diary card for adverse events, medications, peak flow monitoring, symptoms Collect returned study medication and dispense next months medication.
6	12	Physical examination, medication review, spirometry, peak flow, Nitric Oxide (NO) measurement, QOL. Height / Weight Reduce Inhaled corticosteroid by 25% if asthma is stable Review diary card for adverse events, medications, peak flow monitoring, symptoms Collect returned study medication and dispense next months medication.
7	16	Physical examination, medication review, spirometry, peak flow, Nitric Oxide (NO) measurement, QOL. Review diary card for adverse events, medications, peak flow monitoring, symptoms Collect returned study medication and dispense next months medication.
8	20	Follow-up visit Physical examination, medication review, spirometry, peak flow, Nitric Oxide (NO) measurement, QOL Specifically ask for any adverse events and changes to medications

Primary endpoint measures were reduction from baseline of inhaled corticosteroid dose in micrograms of fluticasone. When fluticasone was not the inhaled corticosteroid used the dose was converted to fluticasone equivalent dose in the ratio of 1: 2: 2 (fluticasone: beclomethasone: budesonide).

Doses were those actually taken recorded from compliance checks. At visits 4-6 inclusive inhaled corticosteroid dose was reduced by 25% provided symptom diary cards, spirometry and NiOx measurements, together with clinician assessment, indicated that the child's asthma was stable. If no change or an increase in inhaled corticosteroid dose was clinically appropriate at the assessment visit this was performed and the process repeated at the subsequent study visit. Children would be withdrawn from the trial if their assessed requirement for inhaled corticosteroid exceeded a daily dose of 250 micrograms fluticasone (or equivalent dose of beclomethasone or budesonide).

At each study visit the Nitric oxide measurements were performed using Aerocrine AB NIOX Nitric Oxide Monitoring System (Aerocrine AB, Sundbybergsvägen 9 SE-171 73 Solna, Sweden). The machine was kept in a room controlled temperature of 18 degrees Celsius and the machine and NIOX calibration gas 200ppb NO cylinder were all maintained and serviced as per manufacturer's specifications. Spirometry was performed according to Thoracic Society Australia and New Zealand guidelines[20] using a Micro Medical Micro Lab spirometer (Cardinal Health, 7000 Cardinal Place, Dublin, OH 43017) which was serviced and calibration certificated throughout the trial. NO estimations were performed first and parents were advised to omit foods rich in nitrates in the child's diet for the day of testing. These foods included cured meats/sausage, beetroot, radish, lettuce, spinach and rhubarb.

FEV 1 was measured by spirometry, and the percent of predicted FEV 1 was calculated at screening, at the enrollment visit (baseline), and at each office visit (weeks 4, 8, 12, 16 and 20). For each measurement, the highest of three forced expiratory maneuvers was used for analysis. The diary symptom score sheet is shown in figure 2. During the treatment period, patients recorded on diary cards daytime asthma symptoms scores, night-time awakenings, beta-agonist use (salbutamol or terbutaline micrograms used). Spacer devices were used throughout with detailed instructions and coaching of subjects to provide consistency of dosing.

The Juniper pediatric quality of life questionnaire (self administered New Zealand version) is a validated inventory to assess asthma symptoms relevant to children used with permission for this study[21,22,23,24].

SAFETY ASSESSMENTS

Adverse experiences (spontaneously provided or elicited through interviews), the results of respiratory function testing, and findings on physical examination were used to evaluate the safety of the treatment. NiOx testing was included in the protocol and children were not weaned from ICS if a NiOx reading had increased by 20 bpb. Asthma exacerbations were considered adverse events and their severity was defined as:

Mild where there was increased use of SABA over baseline only e.g colds etc where SABA use increased for a few days (typically 3-5 days), Moderate when a nebulizer , oral steroid was prescribed or a doctor seen with worsening asthma, or had school absence because of symptoms or signs. If a child had a moderate exacerbation in the month any mild exacerbations/ increased SABA use was not recorded (i.e only the more severe exacerbation is recorded). Severe exacerbations were defined as those children with deteriorating asthma who were admitted to hospital.

STATISTICAL ANALYSIS

A preplanned analysis was determined with the difference of any changes in these measures of outcome between the two groups over time being the primary focus of the trial.

Statistical tests in the analysis of longitudinal data were performed using SAS version 9.1[25].

Generalized linear mixed models were used to investigate changes over time in the two groups in the major outcomes. These models allow for the correlations between repeated measures. These analyses model the correlation between measurements on the same participant. An appropriate link function was used for normally distributed, binomial and ordinal data. Confidence limits or probability levels were used. Pre-investigation power studies suggested a sample size of 120 patients (60 in each group) was needed. It was considered that a 25% reduction in the use of inhaled corticosteroids over the course of the study would be a clinically important change.

Descriptive statistics were used to evaluate the percent change from baseline at end point for each efficacy assessment.

RESULTS

Of 87 patients screened for the trial, 73 were randomized to treatment (Figure 3), and 71 completed the 7-month protocol. Figure 1 shows disposition of patients through the trial.

Figure 2

Figure 1: Disposition and follow up of subjects during trial

74 children consented and randomized
73 children initiated on study medication (ITT)
71 children entered and completed trial with complete data records (per protocol)

Fourteen children were excluded on screening (16.1%) predominantly for inability to swallow capsules, despite coaching. The severity asthma of five children on screening was deemed to be too severe to participate in the trial. Two children were randomized to study medication but did not complete the protocol both leaving the country and not responding to followup (one, randomized to placebo, after visit 5, the other, randomized to Lyprinol, after visit 7). The parents of one child withdrew consent just after randomization but before taking any study medication.

DEMOGRAPHIC AND BASELINE CHARACTERISTICS

Neither sex nor age differed significantly between treatment groups (Table 2). Mean age at asthma diagnosis was similar although more NZ European children were represented in the control group. Baseline asthma characteristics were similar between groups, with mean percentage predicted FEV1 estimations being 94.2 for the Lyprinol group and 90.7 for the control group. Inhaled fluticasone dose at baseline was comparable (177 mcg / day for Lyprinol and 173 mcg/ day for placebo group)

Figure 3

Table 2: Baseline characteristics at randomization

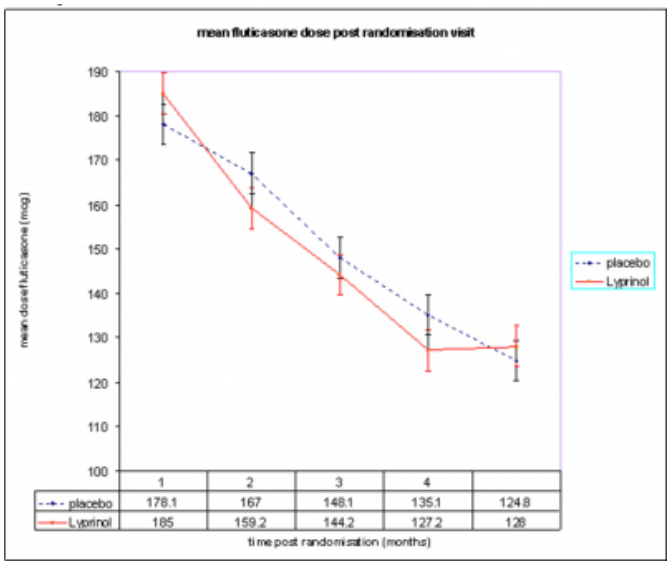
	Lyprinol n= 36	**Placebo** n= 35
Male (%)	21(58%)	19(54%)
Age at diagnosis Mean (std. dev)	3.8 yrs (2.4)	3.6 yrs (2.0)
Inhaled corticosteroid dose at study entry (fluticasone equivalent mcg/ day) Mean (SEM)	177 (56)	173 (59)
NZ European	13(36%)	22(63%)
Maori or Pacific Islander	11(31%)	7(20%)
% predicted FEV1 Mean (SEM)	94.2 (9.4)	90.7(9.4)

PRIMARY OUTCOME - REDUCTION OF INHALED CORTICOSTEROIDS

A general linear mixed model for repeated measures was used to investigate whether the changes in Inhaled corticosteroids over the 4 months of treatment differed between the Lyprinol and placebo groups. An unstructured covariance was used. The dose of inhaled corticosteroids at last visit before capsules were issued was included as a covariate in the model together with the treatment group, visit and the interaction between visit and treatment group which was effect of interest. Both groups were able to decrease inhaled steroids over time (mean reduction fluticasone 42.8 mcg/day for control group and 57.8 mcg/day for Lyprinol group). This difference was not significant (p=0.27). Figure 2 shows mean doses of ICS for the Lyprinol and placebo group for the four months on study medication.

Figure 4

Figure 2. Estimated mean dose of inhaled corticosteroid (mcg fluticasone equivalent) taken by time after randomisation



SECONDARY OUTCOMES ASTHMA SYMPTOMS AND RESCUE BETA-AGONIST USE AND EXACERBATIONS.

1. USE OF SHORT ACTING BETA AGONIST (SABA)

As there was no use of rescue inhaler in fifty percent of the children during the trial this outcome was treated as a binary variable (used SABA or not), and a generalized linear mixed model with a logit link was used to investigate changes over the length of the trial. The use of SABA recorded at the last visit before capsules were issued was included as a covariate. There was less use of rescue beta-agonist use in the Lyprinol group in visits 4, 6 and 7 but confidence intervals overlap (Table 3). Overall there was no evidence of a difference in the change of use of SABA over time between the Lyprinol and placebo groups (p=0.67)

Figure 5

Table 3– Percentage of children using beta agonist at each study visit referenced to baseline

Visit	% using beta-agonist rescue	95% confidence intervals	% using beta-agonist rescue	95% confidence intervals
	Placebo		Lyprinol	
4	64.1	46.2,78.7	51.2	34.7,67.5
5	44.9	28.9,62.0	49	31.9,66.5
6	52.3	35.3,68.9	43.2	27.4,60.5
7	53.8	36.8,70.0	42.6	27.1,59.7

2. QUALITY OF LIFE SCORE

The Juniper score was summed over the full 23 items. 15 % of the scores were 161 indicating that all items were scored at the maximal score of 7 ("none of the time/not bothered by asthma") It was decided to look at two groups of scores, those of 138 or more (an average of 6 or more) and those with less than 138 . 63% scored at least 138. The quality of life score data was highly skewed towards the maximal score in both groups and it was considered reasonable to create a cut point to produce a binary variable.

A generalized linear mixed model with a logit link was used to investigate changes in quality of life over the length of the trial. The Juniper score recorded at the at last visit before capsules were issued was included as a covariate. There was evidence that the pattern of change was different for the two groups over time (p=0.057) with similar levels of QOL initially with the Lyprinol group reporting fewer problems at visit 6. (see Table 4.)

Figure 6

Table 4 – Quality of Life scores (Juniper scale) referenced to baseline

Visit	Placebo		Lyprinol	
	% with none or hardly any problems	95% confidence intervals	% with none or hardly any problems	95% confidence intervals
4	86.1	60.1,96.2	76.8	50.5,91.5
5	76.1	46.3,92.2	66.5	37.9,86.6
6	75.8	45.7,92.1	96.8*	87.1,99.3
7	90.2	67.9,97.6	85.2	62.4,95.3

* p=0.057

3. SYMPTOM DIARY SCORES

Most participants had few symptoms as measured on the symptom score.

No difference was found in the pattern of breathlessness between the Lyprinol and placebo group (p = 0.43), the pattern of cough, wheeze or tight chest during the day (p = 0.98) or the pattern of sleep loss (p = 0.52)

4. ASTHMA EXACERBATIONS

Asthma exacerbations, as expected in a study of moderately severe asthma during the study were common. Most of these were mild. There were a total of 64 mild and moderate exacerbations recorded with 28 children recorded with no exacerbations during the study period. There were 48 mild exacerbations recorded (26 in the placebo group, 22 in the Lyprinol group). There were 16 moderate exacerbations (10 in the placebo group and 6 in the Lyprinol group). There was 1 severe exacerbation in a child randomized to placebo. These results are summarized in tables 5 and 6.

The numbers were small and the zero cells mean that the logistic regressions are not appropriate so the raw percentages are presented in the tables. Overall there was an annualized rate of moderate exacerbation (moderate exacerbations per patient per year) of 0.86 in the placebo group compared to 0.5 in the Lyprinol group.

Figure 7

Table 5 Moderate exacerbations. Absent from school because of asthma or received a SABA nebulisation at medical centre or clinic

visit	Placebo	Lyprinol
	Number (Percentage) with moderate exacerbation of asthma in preceding month	
4	2(5.7%)	1(5.3%)
5	1(2.9%)	3(8.8%)
6	4(11.4%)	2 (5.5%)
7	3(8.6%)	0

Figure 8

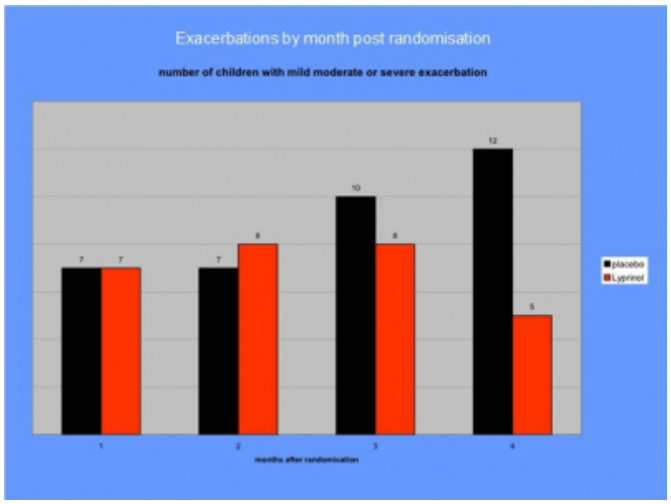
Table 6. Number of asthma exacerbations during month before study visit by severity

		Placebo		
Study Visit	none	mild	moderate	Total children with exacerbation data
4	28	5	2	35
5	28	6	1	35
6	25	6	4	35
7	23	9	3	35
Total	104	26	10	140

		Lyprinol		
Study Visit	none	mild	moderate	Total
4	29	6	1	36
5	26	5	3	34
6	28	6	2	36
7	31	5	0	36
Total	114	22	6	142

Figure 9

Figure 3



SAFETY

Adverse events possibly related to study medication were reported for 2 children. These consisted of generalized itching for one (Lyprinol) and the exacerbation of pre-existing eczema of another (placebo). Upper respiratory tract infection was the most common adverse event in both groups. No deaths occurred during four months of treatment with only one admission to hospital with asthma in a child (randomized to placebo) several weeks before her final study visit. No adverse events led to withdrawal of children from the study. Two children had diarrhea (one Lyprinol; one placebo) during the study period and two children had urticarial rash (both placebo).

DISCUSSION AND CONCLUSIONS

Inhaled corticosteroids (ICS) are the mainstay of treatment in chronic persistent childhood asthma. Unnecessarily large ICS doses have been shown to cause harm in a number of ways including growth retardation and adrenal suppression in children[26]. Long acting beta agonists, with their own risks, have been well demonstrated to be steroid-sparing [27] but few studies have looked at nutritional supplements in this regard.

This study was designed to explore the hypothesis that the use of the marine oil dietary supplement Lyprinol might reduce the need for inhaled corticosteroids in the pediatric patient with moderate asthma. While the primary outcome measure failed to reach statistical significance to demonstrate an effect of at least 25% ICS dose reduction, there was a trend to decreased ICS dosing in the Lyprinol group at visits 5, 6 and 7, with a reversal of this trend one month after ceasing study medication (visit 8). With regard to secondary endpoints there was a trend to improvement in the Juniper quality of life score. We were interested to see a reduction in the annualized exacerbation rate for both mild and moderate exacerbations in this trial and although numbers are small this could be consistent with some beneficial effect of the dietary supplement.

There are limitations of the current study. Preliminary power calculations indicated that 60 children in each arm of the study would be sufficient to demonstrate an effect and we could not recruit sufficient numbers in the time period available. Recruitment was slower and more difficult than anticipated. Although pediatric guidelines exist in New Zealand for the management of asthma a number of children with moderate asthma have been escalated to higher levels of treatment with long acting beta agonists and LABA/ICS combination inhalers, and were not eligible for entry to this study. Study recruitment targeted through pharmacies was very limited and may have reflected a perception that the study was promoting less use of standard ICS therapies. The study recruitment was thus hampered and with budgetary and time constraints the study ceased recruiting in November 2008 with approximately 40% fewer participants than

planned. There may have been uncontrolled factors operating in this study, for example there was no food diary or control for consumption of oily fish in children; but these children ate what most of their New Zealander peers were eating. Also our small dataset was unable to support a subgroup analysis to investigate whether Lyprinol might have a differential effect on children with more severe asthma.

We observed an ability of the study protocol to safely reduce ICS doses by a mean of approximately 25% using clinical and respiratory function monitoring including end-tidal nitric oxide monitoring. This is consistent with a study by Smith et al.[28] who followed 110 asthmatic adults and children for a year demonstrating the ability to safely ween patients from their initial doses of ICS.

Therefore we cannot firmly conclude that the supplementation of 4 capsules of Lyprinol each day enhances ICS dose reduction in this group of children with moderate asthma, despite the trend we report.

With regard to secondary outcome measures there was a difference bordering on significance noted in the quality of life scores at visit 6 with more reporting none, or hardly any asthma problems in the Lyprinol group (97% vs 76%). This indicates that Lyprinol may improve some aspects of quality of life in children with moderate asthma. We used a binary outcome measure for this score because the data was skewed towards the maximum score for the total quality of life. We were unable to transform it sufficiently to satisfy the conditions of normality for use as a continuous variable. The generalised linear mixed models for repeated measures did not have options for ordinal variables therefore a cut point was used to produce a binary outcome.

Exacerbations of asthma in the two groups present interesting data. Despite the statistical analysis problems presented in small numbers in subgroups, there is some consistency in the direction of effect which lends some support to the usefulness of this dietary supplement. If the asthma exacerbation rate can be annualized for both groups there appears to be an exacerbation rate reduction of 18% for mild exacerbations and 42% for moderate exacerbations. This would be the equivalent of a number needed to treat (NNT) of 5.6 and 2.4 respectively. The confidence intervals around these rates are large in view of the small numbers in the study who had moderate exacerbations (see table 6).

Much of the interest in n-3 fatty acid supplementation for asthma began in the era when the neutrophil was already considered to have an important role in the pathogenesis of asthma. It appears that the most profound anti-inflammatory effects of n-3 fatty acids are on neutrophil function and mediator generation. This may explain why there may be more demonstrable clinical benefit in diseases where there is neutrophilic inflammation such as rheumatoid arthritis, psoriasis, cystic fibrosis and inflammatory bowel disease. Since, in asthma, eosinophils and mast cells are thought to be more important effector cells, N-3 fatty acids that do not have a significant anti-inflammatory effect on eosinophils and mast cells in vitro have not been seriously considered as beneficial. Neutrophils may however play more of a role in some forms of chronic, severe asthma and asthma associated with sudden severe attacks[29].

Recent evidence is suggesting different T-cell subpopulations may be responsible for the wide spectrum of clinical phenotypes seen in asthma[30]. It may be that nutritional supplementation with Lyprinol may benefit some clinical phenotypes of asthma more than others and further studies in asthma patients with "noneosinophilic" asthma may be a future step.

In this study we were not able to use lung function outcomes as measures of effect in that the protocol was deliberately set to reduce ICS in a planned step-wise fashion. These measures were useful in monitoring safety in the trial. We were impressed by the rarity of adverse reactions seen in this study of highly allergic children ingesting a regular quantity of marine oil. Rashes and itch were noted but were relatively rare and the supplement appears safe and easy to use although larger studies in pediatric asthma populations will be needed to confirm this. The main cause for study exclusion was inability to swallow capsules but even the youngest children in the study could do this consistently and coaching improved this.

Some thought was given to the amount of extra oil the dose of 4 capsules per day would add to the diet of a younger child and consideration is given to using a smaller dose to children. While this is the standard adult Lyprinol dose, there is no reason to believe this dose needs to be altered for children. The dose of four capsules per day contributes 0.2 gram of the mussel oil and 0.6 gram of olive oil. In the context of dietary fat, this is a very low amount (approximately 1/3 of the amount of the fat in a typical

serving of salad dressing). One study of marine omega-3 oils in children was noted where about 0.5 grams per day were ingested without untoward effects[31].

The present trial shows that Lyprinol was well tolerated by a number of children with moderate asthma over the age range 6-13 years. Side effects to Lyprinol appear to be uncommon in number and minor in nature in this group of children. The side effect of slight itching was noted in this trial (3/35 placebo, 1/36 in Lyprinol group).

This study builds on the previous study of Lyprinol in asthma showing that it is a safe nutritional supplement for children with moderate asthma. It appears that there is a trend to inhaled corticosteroid sparing and a trend to reduction in mild and moderate exacerbations together with an improvement shown in quality of life as assessed by the Juniper pediatric asthma inventory.

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