CHEST Online Supplement

The Effect of Omega-3 Fatty Acids on Bronchial Hyperresponsiveness, Sputum Eosinophilia, and Mast Cell Mediators in Asthma

John D. Brannan, PhD; Johan Bood, PhD; Ahmad Alkhabaz, MD; David Balgoma, PhD; Joceline Otis, BSc; Ingrid Delin, BSc; Barbro Dahlén, MD, PhD; Craig E. Wheelock, PhD; Parameswaran Nair, MD, PhD; Sven-Erik Dahlén, MD, PhD; and Paul M. O'Byrne, MB, FCCP

CHEST 2015; 147(2):397-405

e-Appendix 1.

METHODS

Subjects

Fifty-four subjects were screened and 28 subjects were entered. (Figure 1) Five subjects withdrew from the study leaving 23 subjects. There was one serious adverse event that was unrelated to the study treatments or procedures however the subject was not withdrawn. Five subjects from the original 28 recruited withdrew from the study; one subject experienced fishy reflux and could only tolerate two weeks of the 3-weeks treatment period; one subject changed asthma medication dose during the study violating protocol; two subjects were withdrawn due to exacerbations at the end of the first treatment period (one on placebo, one on the omega-3 supplements); one subject withdrew due to personal reasons. This left 23 subjects of which 12 were steroid-naïve taking $beta_2$ agonists only as needed (p.r.n), and 11 were taking regular inhaled corticosteroids (ICS) with or without long-acting $beta_2$ agonists (ICS dose <1000 mcg fluticasone/day).

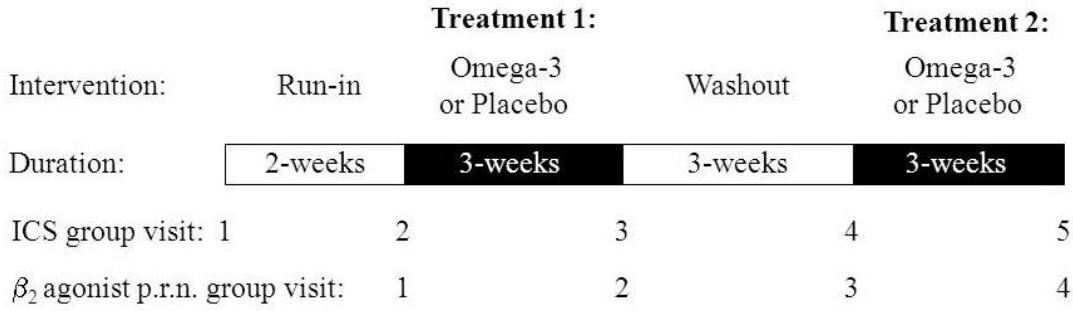
The study was performed at the Firestone Institute for Respiratory Health and subject recruitment commenced in October 2007 with the last subject completing the study in January 2009. Subsequent *in vitro* analyses were performed at the Karolinska Institute and were completed in June 2012.

 DOI: 10.1378/chest.14-1214

CHEST™ Online Supplement

Study Design

e-Figure 1: Schematic of the study design



Mannitol bronchial provocation challenge

A mannitol challenge (Aridol™, Pharmaxis Ltd., Australia) was performed as previously described[1], and FEV_1 was used as the index of change in airway calibre (VMax, Jaeger, Germany). A low resistance dry powder inhaler (Plastiape, Osanago, Italy) was used to administer progressively increasing doses of mannitol; the dose protocol consists of 0 (empty capsule acting as a placebo), 5, 10, 20, 40, 80, 160, 160 and 160 mg mannitol. The challenge was completed when a ≥ 20% reduction in FEV_1 was documented or when the maximum cumulative dose (635 mg) of mannitol had been administered. Subjects were entered into the study if they had a provoking dose of mannitol to cause a 15% fall (PD_{15}) < 315 mg on the initial study visit. Spontaneous recovery of FEV_1 was monitored following mannitol at 5-min and then at 10-min intervals to 20-min following the challenge.

Treatment randomisation

Randomisation was computer generated using an online random number generator and treatments were blinded by the same single investigator who was not involved in performing the tests on the study participants (JO). All other investigators and study participants were blinded throughout the trial. The random numbers were allocated so there were even numbers of subjects who commenced with the placebo and active treatments. All treatments were labelled with a study participant number and a sequence for the first and second treatment periods. This was performed separately for the group taking $beta_2$ agonists p.r.n and those taking regular ICS.

Sputum induction and examination

Immediately following the mannitol challenge and airway recovery subjects were administered 200mcg salbutamol and FEV_1 was measured 10-min later until minimum of within 5% of the pre-mannitol FEV_1 was achieved. Subjects were then administered 7-min exposures of 3, 4 and 5% hypertonic saline using an ultrasonic nebuliser. Between each exposure subjects were asked to expectorate into a sterile

 DOI: 10.1378/chest.14-1214

container an adequate sample of sputum as described.[2] Sputum induction and examination for total and differential cell counts were performed.

Symptoms

Subjects were asked to complete the self-administered Asthma Quality of Life Questionnaire (AQLQ)[3] at baseline and the Asthma Control Questionnaire (ACQ) at all visits.[4] Permission has been obtained for the use of the ACQ and AQLQ.

Blood triglycerides and free fatty acid analysis

Omega-3 fatty acid supplements are known to increase the free fatty acids of omega-3s in the blood and to lower blood triglycerides.[5] This was used as a measure of adherence to the treatment. Following overnight fasting for 12 hrs, blood samples were taken from each subject on arrival to the laboratory. Analysis was performed using a Johnson & Johnson Vitros 700 Analyzer (Raritan, NJ, USA). The full data on lipid mediator serum levels are reported by Lundström et al.[6] The measured levels in serum are in rough agreement with the levels found in a larger study on plasma levels in healthy controls.[7]

Enzyme Immune Assay

Enzyme immunoassay of PGD_2 metabolites (11β-PGF_2 and 2,3-dinor-11β,$PGF_{2\alpha}$) and LTE_4 was performed in serially diluted urine samples, using a rabbit polyclonal antiserum and acetylcholinesterase-linked tracer (Cayman Chemical Company, Ann Arbor, MI, USA) essentially as described previously.[8,9] The antibody developed against 9α,11β-PGF_2 cross-reacted with 2,3-dinor-11β-$PGF_{2\alpha}$ (10%), 13.14-dihydro-15-keto-prostaglandin$F_{2\alpha}$ (0.5%) and all other primary eicosanoid metabolites (below 0.01%). The specificity of the antiserum for LTC_4 was 100%, for LTD_4 100% and for LTE_4 67%. Analysis of creatinine were performed using a modification of Jaffe's creatinine protocol as described previously.[10] The results are expressed as nanograms (ng) of excreted mediator per mmol of creatinine.

Mass Spectrometry

The oxylipins in urine were analyzed by modification of published methods.[11,12] Metabolites in urine were extracted by solid phase extraction (60mg HLB Oasis, Waters, MA, USA). An Acquity UPLC separation module coupled to a Xevo triple quadrupole mass spectrometer equipped with an electrospray source was used for analyses (Waters, MA, USA)**.** Compounds were quantified by external and deuterated internal standards (Cayman Chemicals, Ann Arbour, MI, USA). The methods have been described in greater detail by Lundström et al.[13,14] and Balogma et al.[15]

 DOI: 10.1378/chest.14-1214

RESULTS

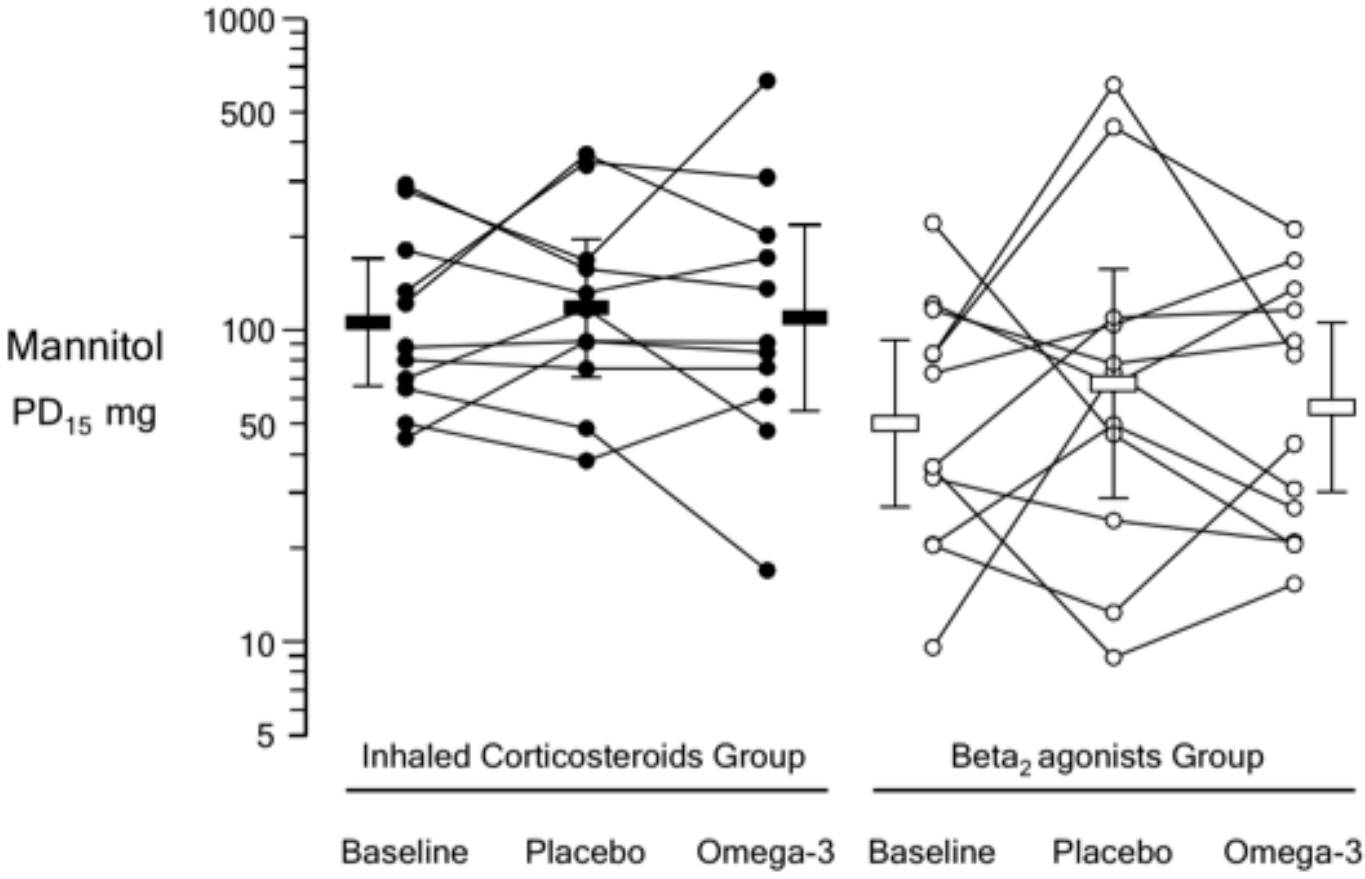
e-Table 1. Urinary mediators LC/MS/MS, n=5 (ng/mmol creatinine)

Mediator	***Placebo***	*Omega-3*	
Tetranor-Prostaglandin DM	82±37	47±12	ns
2,3-dinor-11β-Prostaglandin $F_{2\alpha}$	86±19	51±6	ns
Prostaglandin E_2	141±52	29±19	p=0.048
Tetranor-Prostaglandin EM	449±236	211±72	ns
Prostaglandin $F_{2\alpha}$	122±23	98±19	ns
11-dehydro-Thromboxane B_2	36±11	21±8	ns
8-isoProstaglandin $F_{2\alpha}$	36±5	31±7	ns
2,3-dinor-8-isoProstaglandin $F_{2\alpha}$	452±84	337±42	ns
8,12-iso-iPF $F_{2\alpha}$-VI	730±129	581±103	p=0.0117
Leukotriene E_4	3±1	3±1	ns
Lipoxin A_4	41±15	44±8	ns
8,9-DiHETrE	25±16	9±3	ns
9-HODE	16±6	7±3	ns
13-HODE	55±34	29±12	ns
9-KODE	17±7	9±3	ns
13-KODE	40±30	18±4	ns
9,10,13-TriHOME	52±29	43±22	ns
9,12,13-TriHOME	44±12	24±4	ns
9(10)-EpOME	14±6	7±3	ns
9,10-DiHOME	107±54	49±33	ns
12,13-DiHOME	7±3	3±1	ns

 DOI: 10.1378/chest.14-1214

CHEST™ Online Supplement

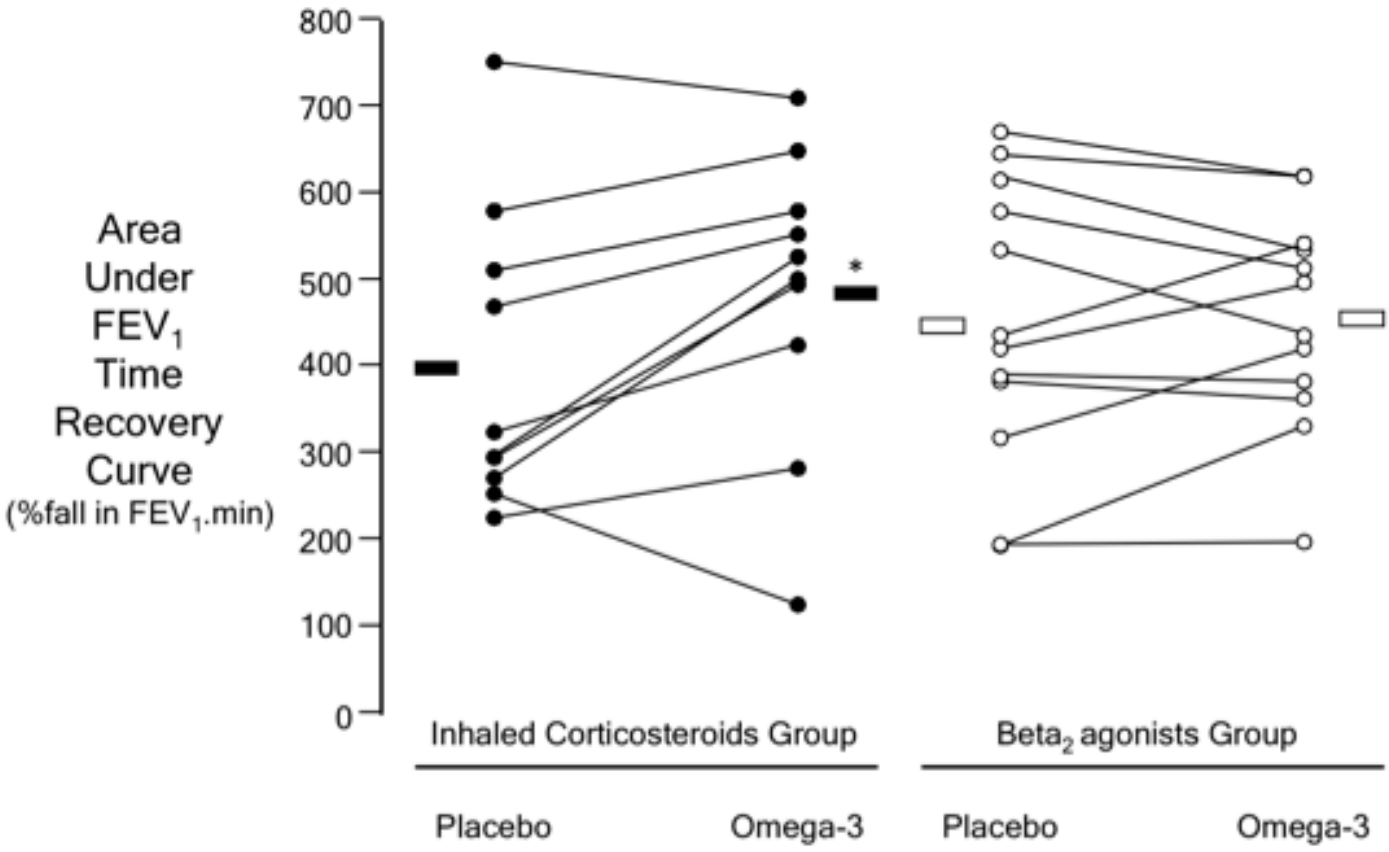
e-Figure 2. Individual PD_{15} values for Omega-3 and placebo in relation to baseline PD_{15}



There was no significant difference in PD_{15} between the Baseline (screening visit), Placebo and Omega-3 visits. The baseline values are the first laboratory visit of each subject; the Run-in visit values for the inhaled corticosteroid group and the first visit of the beta2 agonist group. Superimposed on the individual data points are the Geometric mean (95% CI).

 DOI: 10.1378/chest.14-1214

e-Figure 3. Area under the FEV_1 vs time (30 min) post challenge recovery period separated by treatment group



There was a significantly longer time to recover for those on inhaled corticosteroids (p=0.04), however there was no difference in the % fall in FEV_1 or the maximum cumulative dose of mannitol (mg) administered following placebo or omega-3: For those on inhaled corticosteroids; placebo 26±7%, vs omega-3 27±10% (p=0.51) and placebo 271±173mg vs omega-3 290±207mg (p=0.67); For those on $beta_2$ agonists alone; placebo 24±5% vs omega-3 27±4% (p=0.29) and placebo 171±173mg vs omega-3 148±114mg (p= 0.65).

 DOI: 10.1378/chest.14-1214

e-Table 2. PD_{15} values before and following each treatment period

Treatment Sub-groups	n	$Visit_{run\text{-}in}$	Pre-Treatment 1	*Pre-Treatment 2	Placebo	Omega-3
		PD_{15} (mg)				
Visit (weeks)		-2	0	6	3 or 9#	3 or 9#
No.						
1		71.1	18.0	161.8	118.9	49.3
2		50.6	35.9	35.5	38.0	61.6
3		181.6	194.0	358.2	130.9	169.2
4		88.4	84.9	91.9	92.5	92.4
5		126.6	82.4	161.4	370.2	204.9
6		136.4	202.7	277.8	346.5	310.8
7		65.5	20.2	16.3	48.6	17.1
8		81.5	150.4	100.1	75.6	74.9
9		293.4	195.2	202.1	156.1	135.3
10		45.4	33.7	58.6	92.8	84.6
11		282.0	173.7	147.5	169.2	635^
12			85.8	241.8	462.5	214.7
13			20.9	58.9	51.2	26.9
14			20.6	18.9	12.4	43.5
15			34.9	9.1	15.3	8.8
16			70.9	224.3	635^	85.7
17			73.7	92.1	104.6	167.5
18			122.6	134.8	67.1	135.9
19			33.4	19.7	24.5	21.2
20			116.0	58.4	79.0	92.2
21			9.9	36.4	73.8	31.3
22			37.4	25.0	110.9	116.7
23			220.2	49.4	46.7	20.6
		Geometric mean PD_{15} (95% CI)				
Total Group	23		62 (41, 92)	74 (47, 114)	88 (56, 139)	78 (51, 119)
Inhaled Cortico-steroids	11	107 (69, 164)	78 (41, 147)	108 (57,200)	118 (73, 191)	110 (57, 211)
$Beta_2$ agonists p.r.n.	12		50 (28, 89)	52 (27, 99)	67 (30, 151)	57 (31, 102)

*Pre-Treatment 2 is following 3-week washout of Treatment 1, # randomised thus occurred at either 3 or 9 weeks, ^ No PD_{15} obtained and arbitrary value of 635mg assigned. $Visit_{run\text{-}in}$ was performed in the sub-group taking ICS only.

 DOI: 10.1378/chest.14-1214

REFERENCES

1 Brannan JD, Anderson SD, Perry CP, et al. The safety and efficacy of inhaled dry powder mannitol as a bronchial provocation test for airway hyperresponsiveness: a phase 3 comparison study with hypertonic (4.5%) saline. *Respir Res* 2005;6144

2 Pizzichini MM, Popov TA, Efthimiadis A, et al. Spontaneous and induced sputum to measure indices of airway inflammation in asthma. *Am J Respir Crit Care Med* 1996;154(4 Pt 1):866-869

3 Juniper EF, Guyatt GH, Epstein RS, et al. Evaluation of impairment of health related quality of life in asthma: development of a questionnaire for use in clinical trials. *Thorax* 1992;47(2):76-83

4 Juniper EF, O'Byrne PM, Guyatt GH, et al. Development and validation of a questionnaire to measure asthma control. *Eur Respir J* 1999;14(4):902-907

5 Harris WS. n-3 fatty acids and serum lipoproteins: human studies. *Am J Clin Nutr* 1997;65(5 Suppl):1645S-1654S

6 Lundstrom SL, Balgoma D, Wheelock AM, et al. Lipid mediator profiling in pulmonary disease. *Current pharmaceutical biotechnology* 2011;12(7):1026-1052

7 Psychogios N, Hau DD, Peng J, et al. The human serum metabolome. *PLoS ONE* 2011;6(2):e16957

8 O'Sullivan S, Roquet A, Dahlén B, et al. Evidence for mast cell activation during exercise-induced bronchoconstriction. *Eur Respir J* 1998;12(2):345-350

9 Kumlin M, Stensvad F, Larsson L, et al. Validation and application of a new simple strategy for measurements of urinary leukotriene E4 in humans. *Clin Exp Allergy* 1995;25(5):467-479

10 Larsson J, Perry CP, Anderson SD, et al. The occurrence of refractoriness and mast cell mediator release following mannitol-induced bronchoconstriction. *J Appl Physiol* 2011;110(4):1029-1035

11 Yang J, Schmelzer K, Georgi K, et al. Quantitative profiling method for oxylipin metabolome by liquid chromatography electrospray ionization tandem mass spectrometry. *Anal Chem* 2009;81(19):8085-8093

12 Newman JW, Watanabe T, Hammock BD. The simultaneous quantification of cytochrome P450 dependent linoleate and arachidonate metabolites in urine by HPLC-MS/MS. *Journal of lipid research* 2002;43(9):1563-1578

13 Lundstrom SL, D'Alexandri FL, Nithipatikom K, et al. HPLC/MS/MS-based approaches for detection and quantification of eicosanoids. *Methods Mol Biol* 2009;579161-187

14 Lundstrom SL, Levanen B, Nording M, et al. Asthmatics exhibit altered oxylipin profiles compared to healthy individuals after subway air exposure. *PLoS ONE* 2011;6(8):e23864

15 Balogma D, Larsson J, Rokach J, Lawson JA, Daham K, Dahlén B, Dahlén SE, Wheelock CE. Quantification of lipid mediator metabolites in human urine from asthma patients by electrospray ionisation mass spectometry: controlling matrix effects. Anal Chem. 2013;85(16):7866-7874.

 DOI: 10.1378/chest.14-1214