

# Reduced asthma symptoms with n-3 fatty acid ingestion are related to 5-series leukotriene production<sup>1-3</sup>

K Shane Broughton, Cody S Johnson, Robin K Pace, Michael Liebman, and Kent M Kleppinger

**ABSTRACT** Asthma may respond to dietary modification, thereby reducing the need for pharmacologic agents. This study determined the effectiveness of n-3 polyunsaturated fatty acid (PUFA) ingestion in ameliorating methacholine-induced respiratory distress in an asthmatic population. The ability of urinary leukotriene excretion to predict efficacy of n-3 PUFA ingestion was assessed. After n-3 PUFAs in ratios to n-6 PUFAs of 0.1:1 and 0.5:1 were ingested sequentially for 1 mo each, patient respiratory indexes were assessed after each treatment. Forced vital capacity (FVC), forced expiratory volume for 1 s (FEV<sub>1</sub>), peak expiratory flow (PEF), and forced expiratory flow 25-75% (FEF<sub>25-75</sub>) were measured along with weekly 24-h urinary leukotriene concentrations. With low n-3 PUFA ingestion, methacholine-induced respiratory distress increased. With high n-3 PUFA ingestion, alterations in urinary 5-series leukotriene excretion predicted treatment efficacy. Elevated n-3 PUFA ingestion resulted in a positive methacholine bronchoprovocation dose change in > 40% of the test subjects (responders). The provocative dose to cause a 20% reduction (PD<sub>20</sub>) in FEV<sub>1</sub>, FVC, PEF, and FEF<sub>25-75</sub> values could not be calculated because of a lack of significant respiratory reduction. Conversely, elevated n-3 PUFA ingestion caused some of the patients (nonresponders) to further lose respiratory capacity. Five-series leukotriene excretion with high n-3 PUFA ingestion was significantly greater for responders than for nonresponders. A urinary ratio of 4-series to 5-series leukotrienes < 1, induced by n-3 PUFA ingestion, may predict respiratory benefit. *Am J Clin Nutr* 1997;65:1011-7.

**KEY WORDS** Asthma, n-3 fatty acids, leukotriene, FEV<sub>1</sub>, forced expiratory volume for 1 s, methacholine, n-6 fatty acids, 4-series leukotrienes, 5-series leukotrienes, fish oil

## INTRODUCTION

Asthma may affect ≤ 5% of the Western population (1) and is the most common chronic condition of childhood, with between 20% and 25% of all children experiencing wheezing at some point in their lives. Airflow obstruction associated with bronchial asthma is composed of four components: airway wall swelling, elevated luminal secretion, increased presence of inflammatory cells in the airway wall, and muscle contraction (2). Numerous chemical mediators released during degranulation, including leukotrienes, can elicit an allergic reaction.

Four-series sulfidopeptide leukotrienes (SPLTs: LTC<sub>4</sub>, LTD<sub>4</sub>, and LTE<sub>4</sub>) increase postcapillary permeability, are potent stimulators of airway smooth muscle cells, mediate pul-

monary asthma through their involvement in vasoconstriction and mucus secretion (3), and are ≤ 1000 times more potent than histamine on a molar basis. LTC<sub>4</sub> and LTD<sub>4</sub> can stimulate contraction of smaller airways of pulmonary parenchymal tissue (4) and smooth muscle of lobar and segmental bronchi in vitro (3). Leukotrienes have been detected in blood, bronchoalveolar lavage fluid, and urine of asthmatics and are produced by cells that mediate pulmonary inflammation in asthma (5).

The principal 5-lipoxygenase product of human eosinophils (6) and mast cells (7) is LTC<sub>4</sub>, the initial SPLT synthesized. LTC<sub>4</sub> appears to be involved in asthma through amplification of local processes and the induction of cooperating cells in the airways (8) to produce LTB<sub>4</sub> and 5S-hydroxy-6,8,11,14 (*E,Z,Z,Z*)-eicosatetraenoic acid. Although LTC<sub>4</sub> and LTD<sub>4</sub> are the most biologically potent SPLTs, LTE<sub>4</sub> and *N*-acetyl-LTE<sub>4</sub> are the primary urinary metabolites; there is no evidence of urinary LTC<sub>4</sub> and LTD<sub>4</sub> excretion (9).

The asthmatic response can be precipitated by numerous initiators, including aspirin ingestion and exercise. Urinary leukotriene concentrations are elevated after induction of asthma by exercise (10), antigen (11), or aspirin (12). It is unknown what role, if any, the 5-series SPLTs (LTC<sub>5</sub>, LTD<sub>5</sub>, and LTE<sub>5</sub>) play in this response. These leukotrienes originate from eicosapentaenoic acid (EPA, 20:5n-3), found in fish and fish oil.

The effect of leukotrienes in various disease processes (13), particularly asthma (6), has been reviewed extensively. Current clinical and biomedical research on asthma has focused on the development of pharmacologic agents that do the following: 1) inhibit the release of arachidonic acid (20:4n-6) to inhibit leukotriene formation (14); 2) inhibit 5-lipoxygenase, which is necessary for the synthesis of leukotriene precursors (15); or 3) serve as leukotriene receptor antagonists (16). Leukotriene receptor antagonists are designed to inhibit the action of LTC<sub>4</sub> and LTD<sub>4</sub> because these are the two most potent leukotrienes.

<sup>1</sup> From Nutrition/Department of Family and Consumer Science, University of Wyoming, and Laramie Physicians for Women and Children, Laramie, WY.

<sup>2</sup> Supported by a University of Wyoming Biomedical Research Support grant.

<sup>3</sup> Address reprint requests to KS Broughton, Nutrition/Department of Family and Consumer Science, College of Agriculture, University of Wyoming, Laramie, WY 82071. E-mail: BROUGHTO@UWYO.EDU.

Received April 17, 1996.

Accepted for publication November 7, 1996.

High dietary intake of linoleic acid (18:2n-6) may indirectly elevate leukotriene synthesis and contribute to leukotriene-induced exacerbation of the asthmatic response. Current 18:2n-6 intakes in much of the US population may be as high as 10% of total energy because of perceived health benefits associated with substitution of n-6 polyunsaturated fatty acids (PUFAs) for saturated fats in the diet. In the body, 18:2n-6 can be converted to 20:4n-6 and thus serve as a leukotriene precursor. It has been shown that n-3 PUFA ingestion results in a reduction of 4-series and total leukotriene biosynthesis, apparently induced through reductions in tissue 20:4n-6 concentrations (17).

This study focused on the potential effect of n-3 PUFAs in ameliorating asthmatic bronchial hyperresponsiveness induced by a methacholine challenge, possibly mediated via n-3 PUFA-induced alterations in leukotriene formation. Bronchial responsiveness to methacholine has been proven to be a valid means of assessing the tendency of airways to constrict with specific stimuli (18). Previous studies assessed n-3 PUFA effects on asthma (19-23) but did not examine relations with urinary metabolites that may predict efficacy in ameliorating bronchial hyperresponsiveness to a stimulus. Specifically, this study was designed to determine whether and to what extent the asthmatic response can be reduced by differing intakes of n-3 PUFAs.

## SUBJECTS AND METHODS

### Subjects

Twenty-six, nonsmoking atopic asthmatic subjects (mean age: 22 y; range: 19-25 y) were recruited from the Laramie, WY, community. Subjects showed nonspecific bronchial responsiveness to methacholine with a forced expiratory volume in 1 s (FEV<sub>1</sub>) > 70% of predicted. Subjects were screened for overall health status, use of drugs that affect asthma or eicosanoid synthesis, and fish (or fish oil) consumption. Potential subjects who ingested fish oil supplements in any amount or who consumed more than one fish meal per week were not selected. Individuals with bleeding disorders or a history of delayed clotting time were not considered. Subjects were taking various treatments, including inhalants such as salbutamol, steroids, and oral ingestion of theophyllines. No subject had had an upper respiratory tract infection or exacerbation of asthma in the 6 wk before study initiation. Nonsteroidal anti-inflammatory drugs were not allowed during the study. The study was approved by the Human Subjects Review Board at the University of Wyoming and informed consent was obtained from all participants after the study design and expectations were described to them. Nineteen subjects completed the study.

Seven-day food records for all participants were examined to determine normal n-6 PUFA consumption. Three random 3-d diet analyses were conducted throughout the study to monitor changes in diet patterns and n-6 PUFA intakes. Subjects participated in a 2-mo study examining the effect of variations in ratios of n-3 to n-6 PUFAs on respiratory measures and urinary metabolites. Treatment order for all participants was as follows: low fish-oil supplementation with a ratio of n-3 to n-6 PUFAs of 0.1:1 followed by high fish-oil supplementation with a ratio of n-3 to n-6 PUFAs of 0.5:1. A washout period between fish-oil treatments was not deemed necessary because

low n-3 PUFA intake was followed by high n-3 PUFA intake in all subjects. It was shown previously that tissues retain n-3 PUFAs even when no n-3 PUFA is consumed for an extended period (17). Thus, in this case, a washout period would have had to be inconveniently long and may not have resulted in a marked decrease in tissue n-3 PUFA content.

Each treatment period lasted 4 wk. Fish-oil regimens at both n-3 PUFA intakes were individualized for each participant. Individualization of n-3 PUFA ingestion was based on the dietary analysis of each subject's n-6 PUFA intake. Encapsulated n-3 PUFA was provided in the appropriate amounts, ie, in ratios with n-6 PUFAs of 0.1:1 and 0.5:1. Encapsulated fish oil was donated by the Shaklee Corporation (Hayward, CA). Subjects were given the appropriate number of oil capsules divided into daily allotments at the beginning of each study week. Subjects were questioned weekly regarding compliance with fish-oil treatments throughout the entire study. The average increase in energy was < 1.5% and thus of minimal significance.

Two 24-h urine samples were collected in urine collection bottles and transferred to refrigerated opaque bottles containing methanol and formic acid on the 2 d immediately preceding the study, to serve as baseline samples. After determination of volume, samples were further acidified with formic acid to a final concentration of 3 mmol/L and diluted with methanol to a final concentration of 10%. FEV<sub>1</sub>, peak expiratory flow (PEF), and forced expiratory flow 25-75% (FEF<sub>25-75</sub>), as assessments of large, medium, and small airway capacity, respectively, and forced vital capacity (FVC) were obtained immediately preceding the study to serve as baseline values. Twenty-four-hour urine samples were obtained the last day of each week during each 4-wk oil supplementation period to monitor potential progressive changes in eicosanoid metabolism. After determination of baseline and treatment urine volumes, leukotrienes were extracted from a 200-mL aliquot and frozen at -80 °C for subsequent analysis.

### Study protocol

At baseline and after each 4-wk treatment period, patients entered the participating physician's office for assessment of respiratory status via FVC, FEV<sub>1</sub>, PEF, and FEF<sub>25-75</sub> after a methacholine (Provocholine, Roche Laboratories, Nutley, NJ) challenge. Methacholine was administered sequentially through five inhalations in serial concentrations with a Salter Lab nebulizer (Series 8900; Salter Labs, Arvin, CA) at a flow rate of 7-8 L/min, such that the total administered was 0, 0.125, 1.375, 13.88, and 63.88 cumulative units. All respiratory indexes were determined within 5 min. The procedure was terminated if there was a ≥ 20% reduction in FEV<sub>1</sub> compared with a baseline saline (0.9% NaCl with 0.4% phenol, pH 7.0) solution or when 63.88 cumulative units had been administered. If there was a reduction of 15-19% in FEV<sub>1</sub>, the challenge was repeated at that concentration or the next higher concentration as long as the cumulative units did not exceed 63.88. Methacholine challenge values for FVC, FEV<sub>1</sub>, PEF, and FEF<sub>25-75</sub> were obtained by the attending physician using a Brentwood 2000 spirometer (Brentwood, CA).

### Leukotriene analysis

Leukotrienes were extracted from 200 mL freshly collected, acidified urine. Urine was selected for analysis because it has

been shown to provide a fairly accurate estimate of leukotriene biosynthesis (9, 11). One hundred nanograms prostaglandin B<sub>1</sub> was added to the 200-mL aliquot as an internal standard. Leukotrienes were isolated by solid-phase extraction over C-18 cartridges (Supelclean LC-18; Supelco, Inc, Bellefonte, PA) (24) that were prewashed sequentially with 10 mL methanol, 5 mL water, and 5 mL hexane. After application of urine, cartridges were washed sequentially with 10 mL methanol:water (1:9, by vol), 10 mL water, and 5 mL hexane, followed by elution of leukotrienes with 2 mL methanol. After elution, leukotriene extractions were stored in methanol at -80 °C for subsequent analysis.

For analysis (25), samples were evaporated to dryness under nitrogen; the resulting residue was reconstituted in an HPLC solvent system of methanol:water (65:35, by vol), pH 4.68, containing 5 mmol ammonium acetate/L and 1 mmol EDTA/L. All samples were analyzed in duplicate with averages being used for statistical analysis. The leukotrienes were separated by reversed-phase HPLC on a Partisphere C-18 column (6 mm × 12.5 cm; Whatman, Hillsboro, OR) with a flow rate of 1.0 mL/min and quantified by using a Hewlett-Packard spectrophotometer (10409A Diode Array; Hewlett-Packard, Liverpool, NY) by monitoring at 280 nm. All leukotrienes were identified by their distinctive ultraviolet absorption spectra and comparison of retention times with known standards. Leukotrienes were quantified against the internal PGB<sub>1</sub> standard by using extinction coefficients of authentic standards (26). Four-series leukotrienes, ie, LTE<sub>4</sub>, *N*-acetyl-LTE<sub>4</sub>, and their 11-*trans*-isomers were pooled for analysis. Five-series leukotrienes in the form of LTE<sub>5</sub> and its 11-*trans*-isomer were combined for analysis. LTE<sub>4</sub>, LTE<sub>5</sub>, and *N*-acetyl LTE<sub>4</sub> were purchased from Caymen Chemical (Ann Arbor, MI).

### Statistics

The minimal number of samples for each assay was determined by statistical analyses of the power to detect a physiologically significant difference between treatments. Estimates of variance used in the design were computed from previous experiments. As an example, urinary leukotriene concentrations were judged to be clinically different if a diet-induced decrease of 15% occurred. For 90% confidence of detecting a 15% change in urine leukotriene concentrations, the number of replicates required is seven, based on our experimental history. Differences between baseline, low n-3 PUFA, and high n-3 PUFA ingestion with respect to the effect on the respiratory factors FVC, FEV<sub>1</sub>, PEF, and FEF<sub>25-75</sub> at each methacholine dose and the provocative dose to cause a 20% reduction in each of these indexes (PD<sub>20</sub>) were assessed by one-way analysis of variance (ANOVA). Differences between baseline, low n-3 PUFA, and high n-3 PUFA ingestion with respect to effect on urinary total and leukotriene subfractions were assessed by two-way ANOVA (ie, responder or nonresponder and n-3 PUFA treatment) using a split-plot factorial design (27). All statistical analyses were conducted with SAS software (Statistical Analysis Systems Institute, Inc, Cary, NC). When overall differences were detected, specific treatment differences were assessed by using Duncan's protected least-significant-difference test. Significance was determined at  $P < 0.05$ . Values are expressed as means ± SEMs with a common  $n = 19$  in participant cases,  $n = 9$  in responder cases, and  $n = 10$  in nonresponder cases, unless stated otherwise.

## RESULTS

All participants tolerated the study well and body weights were relatively constant for the duration of the study. No side effects other than fishy hiccups and occasional mild gastrointestinal discomfort were reported on questioning. Problems with hiccups and gastrointestinal discomfort were alleviated by changing n-3 PUFA ingestion times from morning to evening before sleep.

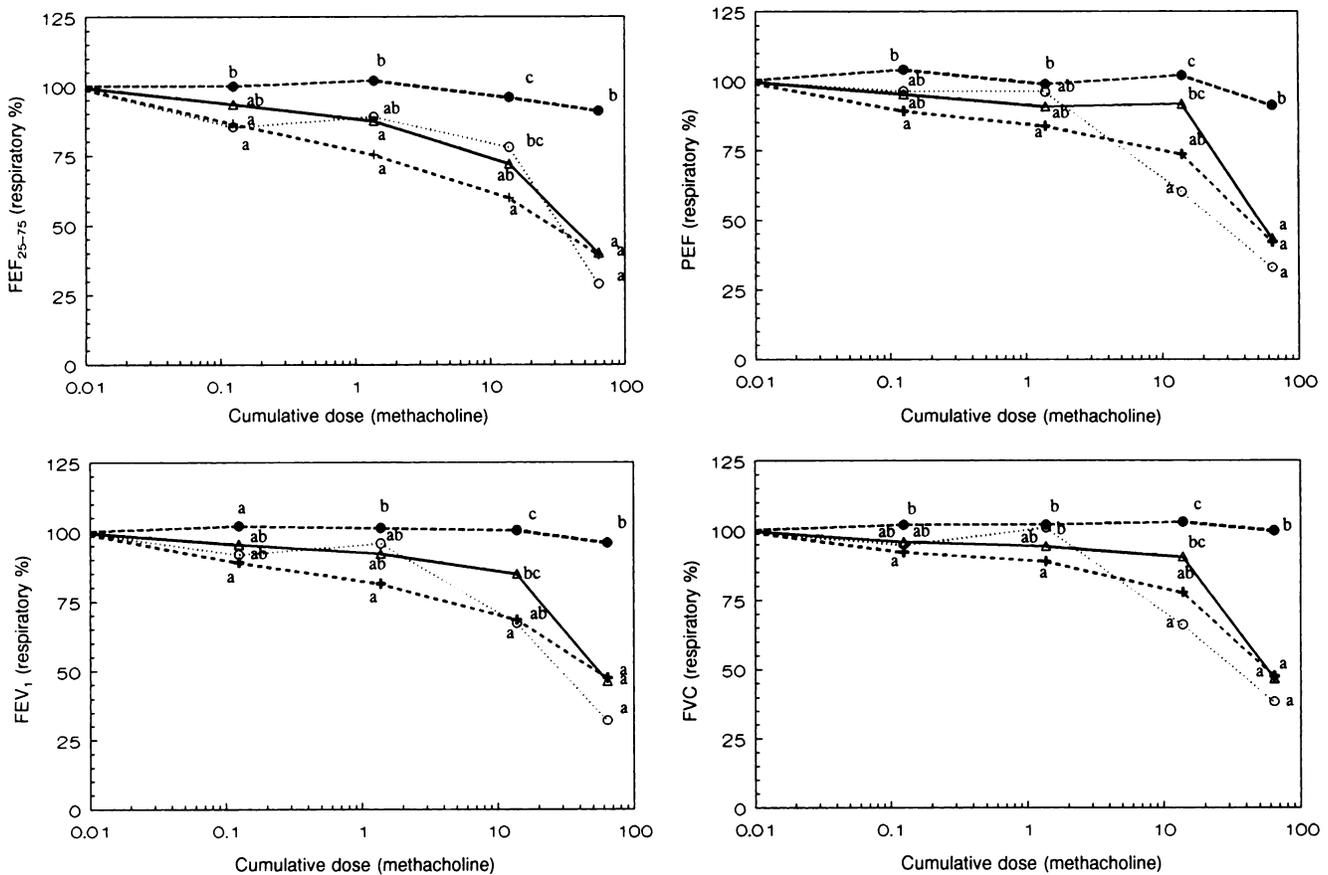
### Pulmonary function tests during methacholine bronchoprovocation

Ingestion of n-3 PUFAs at a dietary ratio of n-3 to n-6 PUFAs of 0.1:1 resulted in a pattern suggesting greater breathing difficulty compared with baseline at any methacholine dose. Respiratory measures generally decreased two- to three-fold faster (% change) with ingestion of n-3 PUFAs in this ratio to n-6 PUFAs (Figure 1). PD<sub>20</sub> values for FVC, FEV<sub>1</sub>, PEF, and FEF<sub>25-75</sub> were reduced by 51%, 89%, 65%, and 92%, respectively (Table 1).

When n-3 PUFA ingestion was increased to a ratio with n-6 PUFAs of 0.5:1, respiratory measures in the total sample population were not significantly different from baseline responses. On examination of these data, it became evident that breathing capacity was actually improved in 9 of the 19 participants with high n-3 PUFA ingestion. Those characterized by no reduction in respiratory measures with increased methacholine challenge after the period of elevated n-3 PUFA intake were referred to as responders. When data from the responders were summarized separately, there was virtually no decrease in any respiratory index with increasing methacholine challenge, with the exception of a minimal change in PEF and FEF<sub>25-75</sub> values at a cumulative dose of 63.88 units. PD<sub>20</sub> values with elevated n-3 PUFA ingestion in this subpopulation could not be calculated because of improvements in respiratory indexes (ie, respiratory responses were never reduced by 20%, regardless of methacholine dose) (Table 1). Note that at study initiation, respiratory indexes of responders were not significantly different from those of the other 10 participants. Those showing respiratory reductions with increased methacholine challenge, even with elevated n-3 PUFA intake were referred to as nonresponders. These individuals were generally unable to continue beyond a cumulative dose of 13.88 units methacholine, and had significantly greater breathing difficulty at 1.375 units methacholine that persisted when tested with 13.88 units.

The number of subjects tested with 63.88 units methacholine differed between baseline, low n-3 PUFA, and high n-3 PUFA ingestion (Table 2). Of the 19 subjects screened at baseline, only five were given this dose of methacholine. After high n-3 PUFA ingestion, 11 participants were tested with 63.88 units. Eight of nine responders were tested with 63.88 units after ingestion of the high fish-oil treatment compared with four of nine at baseline. Conversely, only 3 of 10 nonresponders were tested with 63.88 units methacholine with high n-3 PUFA ingestion.

Nonresponders' respiratory capacity appeared to be hindered by high n-3 PUFA ingestion (Figures 1). Compared with baseline, nonresponders' FEV<sub>1</sub>, FVC, and PEF values were significantly lower with high n-3 PUFA ingestion at a cumu-



**FIGURE 1.** Forced expiratory flow 25–75% ( $FEF_{25-75}$ ; small airway index) change, peak expiratory flow (PEF; medium airway index) change, forced expiratory volume for 1 s ( $FEV_1$ , large airway index) change, and forced vital capacity change in the asthmatic response to inhaled methacholine at baseline ( $\Delta$ ) and after ingestion of n-3 polyunsaturated fatty acids (PUFAs) in a ratio with n-6 PUFAs of 0.1:1 ( $n = 19$ ) and 0.5:1. +, low n-PUFAs; ●, responders to high n-3 PUFA ingestion; and ○, nonresponders to high n-3 PUFAs. See Table 2 and Methods for patient numbers and n-3 PUFA concentrations, respectively. Points with different letters are significantly different at the same cumulative methacholine dose,  $P < 0.05$ .

lative dose of 13.88 units methacholine. The only exception to this pattern occurred for  $FEF_{25-75}$  (Figure 1). Whereas the  $PD_{20}$  values for  $FEV_1$ , FVC, and PEF were reduced by 85%, 71%, and 79%, respectively, with high n-3 PUFA ingestion, small airway capacity—as assessed by  $FEF_{25-75}$ —actually improved by 10% (Table 1). Four nonresponders were eliminated at a cumulative dose of 1.375 units methacholine. At 63.88 units, the three remaining subjects were characterized by respiratory capacities not significantly different from those at baseline.

### Leukotriene quantitation

In all participants, the lower ratio of n-3 to n-6 PUFAs, ie, 0.1:1, was associated with an increase ( $13.2 \pm 4.5$  ng,  $P < 0.05$ ) in total 4-series leukotriene excretion and a nonsignificant increase ( $7.8 \pm 1.4$  ng) in  $LTE_5$  excretion. At low n-3 PUFA ingestion, there was no significant difference in 4-series, 5-series, or overall leukotriene excretion between responders and nonresponders (Figure 2).

**TABLE 1**

Calculated provocative dose to cause a 20% reduction in respiratory indexes<sup>1</sup>

Treatment	Respiratory index			
	FVC	$FEV_1$	PEF	$FEF_{25-75}$
Baseline ( $n = 19$ )				
Ratio of n-3 to n-6 PUFAs of 0.1:1 ( $n = 19$ )				
Ratio of n-3 to n-6 PUFAs of 0.5:1				
Responders ( $n = 9$ )	NC <sup>2</sup>	NC	NC	NC
Nonresponders ( $n = 10$ )	3.7 ± 1.4	4.9 ± 2.4	3.5 ± 3.9	9.9 ± 1.2

<sup>1</sup>  $\bar{x} \pm SEM$ . FVC, forced vital capacity;  $FEV_1$ , forced expiratory volume/s; PEF, peak expiratory flow;  $FEF_{25-75}$ , forced expiratory flow 25–75%.

<sup>2</sup> NC, not calculable; respiratory reduction did not exceed 20%.

**TABLE 2**  
Number of patients assessed at each cumulative methacholine dose

Treatment	Cumulative methacholine dose (units)				
	0	0.125	1.375	13.88	63.88
Baseline	19	19	15	9	5
Ratio of n-3 to n-6 PUFAs of 0.1:1	19	19	12	8	4
Ratio of n-3 to n-6 PUFAs of 0.5:1					
Responders	9	9	9	9	8
Nonresponders	10	10	7	6	3

With high n-3 PUFA ingestion, 4-series leukotriene excretion was not different from baseline for responders or nonresponders (Figure 2). However, in both groups, overall 4-series leukotriene excretion was significantly lower ( $P < 0.05$ ) with high than with low n-3 PUFA ingestion. High n-3 PUFA ingestion was associated with marked increases in LTE<sub>5</sub> excretion compared with baseline in both responders and nonresponders ( $P < 0.05$ ).

High n-3 PUFA consumption resulted in no changes in urinary 4-series leukotriene excretion in responders and nonresponders. The 5-series leukotriene excretion with high n-3 PUFA intake was 230% higher in responders ( $P < 0.05$ ).

## DISCUSSION

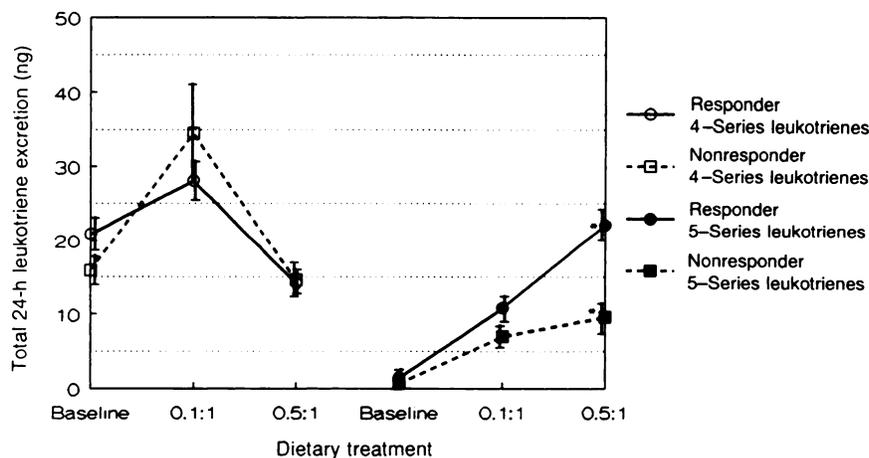
Ingestion of the n-3 PUFAs found in fish oil reduces total leukotriene formation  $\leq 40\%$  and 4-series leukotriene production by  $> 75\%$  after an inflammatory stimulus in mice (17). To date, studies examining the effect of dietary n-3 PUFA consumption on asthmatic symptoms have yielded equivocal results (19–23). Cells present in human bronchi are capable of metabolizing EPA to 5-series leukotrienes (25). Dry and Vincent (21) showed an elevation in FEV<sub>1</sub> values after feeding 1 g docosahexaenoic acid (DHA, 22:6n-3) plus EPA daily for 1 y. However, it is not clear whether they simply assessed FEV<sub>1</sub> every 3 mo or if an asthmatic response was elicited through the use of a specific agonist. Arm et al (19) showed that n-3 PUFA ingestion in asthmatic patients altered tissue phospholipid composition and neutrophil LTB<sub>4</sub> and LTB<sub>5</sub> metabolism

but did not cause any significant change in airway responsiveness to a histamine challenge. Additional studies are necessary to assess potential mechanisms by which n-3 PUFA ingestion may affect the course of asthma. A specific balance of n-3 with n-6 PUFAs, rather than absolute consumption of n-3 PUFAs, may be the critical factor that can alter synthesis of inflammatory metabolites (28).

The eicosanoids may have a role in the rate of progression of numerous disease processes, with the SPLTs implicated in inflammatory conditions of the skin (psoriasis) (29), lung (allergic asthma) (30), and joints (rheumatoid arthritis) (31). Recent recommendations to increase fish consumption are motivated by research results that show that when n-3 PUFAs are present at a dietary ratio of between 1:5 and 1:2.5 with dietary n-6 PUFAs (found in vegetable oil) there is a reduction in overall eicosanoid production that may reduce the risk of a host of pathophysiologies (17, 24, 32).

In the present study dietary supplementation with fish oil at a ratio of n-3 to n-6 PUFAs of 0.1:1 for 1 mo tended to diminish respiratory capacity in asthmatics. In contrast, when the ratio of n-3 to n-6 PUFAs was increased to 0.5:1,  $> 40\%$  of those tested showed significant respiratory benefit after methacholine challenge. The average low and high dose of fish oil provided  $\approx 0.7$  and 3.3 g EPA and DHA daily, respectively.

Previously, it was reported that consumption of 1 g DHA + EPA/d for 1 y beneficially altered FEV<sub>1</sub> values in allergic asthmatic patients (21). A similar study that used 5.4 g EPA + DHA/d for 10 wk did not show any effect in the total subject population after histamine challenge (19). However, 6 of the 11



**FIGURE 2.** Total 24-h leukotriene excretion by dietary treatment. \*Significantly different from one another,  $P < 0.05$ . Dietary treatments of n-3 and n-6 polyunsaturated fatty acids (PUFAs) were given in ratios of 0.1:1 and 0.5:1. Four-series leukotrienes: LTE<sub>4</sub>, 11-*trans*-LTE<sub>4</sub>, *N*-acetyl LTE<sub>4</sub>, and 11-*trans*-*N*-acetyl LTE<sub>4</sub>; 5-series leukotrienes: LTE<sub>5</sub> and 11-*trans*-LTE<sub>5</sub>,  $n = 9$  responders,  $n = 10$  nonresponders.

subjects showed minor respiratory improvements. In a subsequent study (20), subjects who consumed n-3 PUFAs as Max-EPA (Seven Seas, Marfleet Hall, United Kingdom) did not show respiratory improvements immediately after histamine challenge, but showed significant improvements in their recovery periods. These findings, when considered in conjunction with the present data, indicate that n-3 PUFAs, when consumed long enough or in high enough amounts, may help to ameliorate asthmatic symptoms in a portion of the asthmatic population. Further studies will be necessary to determine why some asthmatics benefit from n-3 PUFA ingestion whereas others do not.

Rather than the absolute amount of n-3 or n-6 PUFA ingested, the ratio of n-3 to n-6 PUFAs is the critical factor for altering eicosanoid biosynthesis in rats (28). In previous human studies the investigators did not indicate whether n-6 PUFA ingestion was controlled for (19, 20). If the critical ratio of n-3 to n-6 PUFAs necessary to markedly alter leukotriene biosynthesis is not achieved, there may be no respiratory benefit from n-3 PUFA ingestion, even among responding individuals.

The beneficial change in respiratory indexes documented in responders may be attributable primarily to an overall increase in 5-series SPLT production. A necessary shift in leukotriene biosynthesis (ie, an increase in 5-series leukotriene coupled with a reduction in 4-series leukotriene synthesis) to a point where the ratio of 5- to 4-series leukotrienes is  $> 1$  seems to be critical for mediating an improved response to methacholine challenge. Furthermore, the ineffectiveness of n-3 PUFA ingestion in nonresponders was associated with a blunted increase in 5-series leukotriene production. Because urinary leukotriene excretion was not reported in the previous studies cited (20, 21), the relation we report between diet-induced elevations in 5-series SPLT production and improvements in respiratory indexes may be a new finding of key physiologic significance. The 5-series leukotrienes have been shown to be less biologically potent in guinea pig ileum (33) and EPA has been shown to inhibit the release of anaphylactic cyclooxygenase products in guinea pig lung parenchymal strips while enhancing SPLT release (34). The respiratory benefit associated with n-3 PUFA ingestion in responders could be associated with the inability of 5-series leukotrienes to elicit an asthmatic response, or by competitive inhibition by 5-series leukotrienes at the 4-series leukotriene receptors.

In summary, the incorporation of a source of n-3 PUFA in the diet, ie, fish or fish oil, could alleviate minor respiratory problems in asthmatics or decrease the degree of respiratory problems among a subset of severe asthmatics who respond to diet. Increased consumption of n-3 PUFAs can produce beneficial alterations in 4- and 5-series leukotriene synthesis. These findings raise the possibility that dietary supplementation with marine oils or highly enriched sources of n-3 PUFAs may be another viable treatment modality for asthma. □

We thank Alan Moore, University of Wyoming, for his assistance with the statistical analysis.

## REFERENCES

- Fleming DM, Crombie DL. Prevalence of asthma and hay fever in England and Wales. *Br Med J* 1987;294:279-83.
- Kaliner M, Eggleston PA, Mathews KP. Rhinitis and asthma. *JAMA* 1987;258:2851-71.
- Dahlén SE, Hedquist P, Hammarstrom S, Samuelson B. Leukotrienes are potent constrictors of human bronchi. *Nature* 1980;288:484-6.
- Chagnon M, Gentile J, Glada M, Sirios P. The mechanism of action of leukotrienes A<sub>4</sub>, C<sub>4</sub> and D<sub>4</sub> on human lung parenchyma in vitro. *Lung* 1985;163:55-62.
- Henderson WR. Role of leukotrienes in asthma. *Ann Allergy* 1994;72:272-8.
- Weller PF, Lee CW, Foster DW, Corey EJ, Austen KF, Lewis RA. Generation and metabolism of 5-lipoxygenase pathway leukotrienes by human eosinophils: predominant production of LTC<sub>4</sub>. *Proc Natl Acad Sci U S A* 1983;80:7626-30.
- Peters SP, MacGlashan DW, Schulman ES, et al. Arachidonic acid metabolism in purified human lung mast cells. *J Immunol* 1984;132:1972-9.
- Chavis C, Godard P, Michel FB, Crastes de Paulet A, Damon M. Sulfidopeptide leukotrienes contribute to human alveolar macrophage activation in asthma. *Prostaglandins Leukot Essent Fatty Acids* 1991;42:95-100.
- Maltby NH, Taylor GW, Ritter JM, Moore K, Fuller RW, Dollery CT. Leukotriene C elimination and metabolism in man. *J Allergy Clin Immunol* 1990;85:3-9.
- Kikawa Y, Hosoi S, Inoue Y, et al. Exercise-induced urinary excretion of leukotriene E<sub>4</sub> in children with atopic asthma. *Pediatr Res* 1991;29:455-9.
- Westcott JY, Smith HR, Wenzel SE, et al. Urinary leukotriene E<sub>4</sub> in patients with asthma. Effect of airways reactivity and sodium cromoglycate. *Am Rev Respir Dis* 1991;143:1322-6.
- Knapp HR, Sladek K, Fitzgerald GA. Increased excretion of leukotriene E<sub>4</sub> during aspirin-induced asthma. *J Lab Clin Med* 1992;119:48-51.
- Lewis RA, Austen KF, Soberman RJ. Leukotrienes and other products of the 5-lipoxygenase pathway: biochemistry and relation to pathology in human diseases. *N Engl J Med* 1990;323:645-55.
- Nagai H, Yakuo I, Arimura A, Choi SH, Inagaki N, Koda A. Antiasthmatic activity of newly synthesized calcium antagonists: 2-n-butyl-1-(N-methyl-n-[2-(N',N'-dimethylamino)ethyl]amino)-5,6-methylenedioxy-indene and indane. *Int Arch Allergy Appl Immunol* 1989;90:137-43.
- Kreutner W, Sherwood J, Rizzo C. The effect of leukotriene antagonists, lipoxygenase inhibitors and selected standards on leukotriene-mediated allergic bronchospasm in guinea pigs. *Agents Actions* 1989;28:173-84.
- Dinh-Xuan AT, Regnard J, Similowski T, Rey J, Marsac J, Lockhart A. Effects of SK&F 104353, a leukotriene receptor antagonist, on the bronchial responses to histamine in subjects with asthma: a comparative study with terfenadine. *J Allergy Clin Immunol* 1990;85:865-71.
- Broughton KS, Whelan J, Hardardottir I, Kinsella JE. The effect of increasing the dietary n-3 to n-6 polyunsaturated fatty acid ratio on murine liver and peritoneal cell fatty acids and eicosanoid metabolism. *J Nutr* 1991;121:155-64.
- Hargreave FE, Ryan G, Thomson NC, et al. Bronchial responsiveness to histamine or methacholine in asthma: measurement and clinical significance. *J Allergy Clin Immunol* 1981;68:347-55.
- Arm PJ, Horton CE, Mencia-Huerta JM, et al. Effect of dietary supplementation with fish oil lipids on mild asthma. *Thorax* 1988;43:84-92.
- Arm JP, Horton CE, Spur BW, Mencia-Huerta JM, 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;139:1395-400.
- Dry J, Vincent D. Effect of a fish oil diet on asthma: results of a 1-year double-blind study. *Int Arch Allergy Appl Immunol* 1991;95:156-7.
- Kirsch CM, Payan DG, Wong MYS. The effect of eicosapentaenoic acid in asthma. *Clin Allergy* 1988;18:177-87.
- Picado C, Castillo JA, Schinca N, et al. Effects of a fish oil diet on

- aspirin intolerant asthmatic patients: a pilot study. *Thorax* 1988;43:93-7.
24. Whelan J, Broughton KS, Lokesh B, Kinsella JE. In vivo formation of leukotriene E<sub>5</sub> by murine peritoneal cells. *Prostaglandins* 1991;41:29-42.
25. Broughton KS, Morgan LJ. Frequency of (n-3) polyunsaturated fatty acid consumption induces alterations in tissue lipid composition and eicosanoid synthesis. *J Nutr* 1994;124:1104-11.
26. Orning L, Bernstrm K, Hammarstrom S. Formation of leukotrienes E<sub>3</sub>, E<sub>4</sub> and E<sub>5</sub> in basophilic leukemia cells. *Eur J Biochem* 1981;120:44-5.
27. Kirk BE. *Experimental design*. 2nd ed. Belmont, CA: Brooks/Cole Publishers, 1982.
28. Boudreau MD, Chanmugam PS, Hart SB, Lee SH, Hwang DH. Lack of dose response by dietary n-3 fatty acids at a constant ratio of n-3 to n-6 fatty acids in suppressing eicosanoid biosynthesis from arachidonic acid. *Am J Clin Nutr* 1991;54:111-7.
29. Brain S, Camp R, Dowd P, Kobza-Black A, Greaves M. The release of leukotriene-B<sub>4</sub>-like material in biologically active amounts from the lesional skin of patients with psoriasis. *J Invest Dermatol* 1984;83:70-3.
30. Chanarin N, Johnston SL. Leukotrienes as a target in asthma therapy. *Drugs* 1994;47:12-24.
31. Kremer JM, Lawrence DA, Jubiz W, et al. Dietary fish oil and olive oil supplementation in patients with rheumatoid arthritis. *Arthritis Rheum* 1990;33:810-20.
32. Hwang DH, Boudreau M, Chanmugam P. Dietary linolenic acid and longer-chain n-3 fatty acids: comparison on arachidonic acid metabolism in rats. *J Nutr* 118:427-37.
33. Hammarstrom S. Leukotriene C<sub>5</sub>: a slow reacting substance derived from eicosapentaenoic acid. *J Biol Chem* 1980;255:7093-4.
34. Simmet T, Aissa J, Sutter D, Juan H, Peskar BA. Modulation of the contractile activity of the guinea-pig lung parenchymal strip by exogenous 5,8,11,14,17-eicosapentaenoic acid. *Arch Pharm (Weinheim)* 1987;335:652-9.

