

# Diminished Lipoxin Biosynthesis in Severe Asthma

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**Rationale and Objectives:** Severe asthma is characterized by increased airway inflammation that persists despite therapy with corticosteroids. It is not, however, merely an exaggeration of the eosinophilic inflammation that characterizes mild to moderate asthma; rather, severe asthma presents unique features. Although arachidonic acid metabolism is well appreciated to regulate airway inflammation and reactivity, alterations in the biosynthetic capacity for both pro- and antiinflammatory eicosanoids in severe asthma have not been determined.

**Methods:** Patients with severe asthma were identified according to National Heart, Lung, and Blood Institute Severe Asthma Research Program criteria. Samples of whole blood from individuals with severe or moderate asthma were assayed for biosynthesis of lipoxigenase-derived eicosanoids.

**Measurements and Main Results:** The counterregulatory mediator lipoxin A<sub>4</sub> was detectable in low picogram amounts, using a novel fluorescence-based detection system. In activated whole blood, mean lipoxin A<sub>4</sub> levels were decreased in severe compared with moderate asthma (0.4 [SD 0.4] ng/ml vs. 1.8 [SD 0.8] ng/ml,  $p = 0.001$ ). In sharp contrast, mean levels of proinflammatory leukotrienes were increased in samples from severe compared with moderate asthma (112.5 [SD 53.7] pg/ml vs. 64.4 [SD 24.8] pg/ml,  $p = 0.03$ ). Basal circulating levels of lipoxin A<sub>4</sub> were also decreased in severe relative to moderate asthma. The marked imbalance in lipoxigenase-derived eicosanoid biosynthesis correlated with the degree of airflow obstruction.

**Conclusions:** Mechanisms underlying airway responses in severe asthma include underproduction of lipoxins. This is the first report of a defect in lipoxin biosynthesis in severe asthma, and suggests an alternative therapeutic strategy that emphasizes natural counterregulatory pathways in the airways.

**Keywords:** biosynthesis; chromatography, eicosanoids; high-pressure liquid; inflammation mediators

(Received in original form October 26, 2004; accepted in final form June 14, 2005)

Supported in part by grants HL69349 and HL68669.

The Severe Asthma Research Program (SARP) is a multicenter asthma research group funded by the NHLBI and consisting of the following contributors (Steering Committee members are indicated with an asterisk): *Brigham and Women's Hospital*—Elliot Israel,\* Bruce D. Levy, Gautham Marigowda; *Cleveland Clinic Foundation*—Serpil C. Erzurum,\* Raed A. Dweik, Suzy A. A. Comhair, Abigail R. Lara, Marcelle Baaklini, Daniel Laskowski, Jacqueline Pyle; *Emory University*—W. Gerald Teague,\* Anne M. Fitzpatrick, Eric Hunter; *Imperial College School of Medicine*—Kian F. Chung,\* Mark Hew, Alfonso Torrego, Sally Meah, Mun Lim; *National Jewish Medical and Research Center*—Sally E. Wenzel,\* Diane Rhodes; *University of Pittsburgh*—William J. Calhoun,\* Bill T. Ameredes, Melissa P. Clark, Renee Folger, Rebecca Z. Wade; *University of Virginia*—Benjamin Gaston,\* Peter Urban; *University of Wisconsin*—William W. Busse,\* Nizar Jarjour, Erin Billmeyer, Cheri Swenson, Gina Crisafi; *Wake Forest University*—Eugene R. Bleeker,\* Deborah Meyers, Wendy Moore, Stephen Peters, Annette Hastie, Gregory Hawkins, Jeffrey Krings, Regina Smith; *Washington University in St. Louis*—Mario Castro,\* Leonard Bacharier, Iftikhar Hussain, Jaime Tarsi; *Data Coordinating Center*—James R. Murphy,\* Douglas Curran-Everett; *NHLBI*—Patricia Noel\*

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Am J Respir Crit Care Med Vol 172, pp 824–830, 2005

Originally Published in Press as DOI: 10.1164/rccm.200410-1413OC on June 16, 2005

Internet address: www.atsjournals.org

Most patients with asthma have intermittent or persistent symptoms that are readily controlled by standard asthma therapies, including  $\beta_2$ -agonists, low doses of inhaled corticosteroids, or leukotriene modifiers (1). However, 5 to 10% of individuals with asthma have poorly controlled asthma that is refractory to standard therapies, including daily systemic corticosteroids (2). These patients with severe asthma experience frequent daily symptoms despite the use of multiple therapies and account for a large proportion of the hospitalizations, emergency room visits, health care costs, and mortality attributable to asthma (3).

One distinguishing feature of severe asthma is persistent airway inflammation in the face of corticosteroid therapy (4). This inflammation differs from that observed in mild and moderate asthma, which has been characterized as driven by Th2 lymphocytes with a predominance of eosinophils (5). In contrast, the persistent inflammation of severe asthma is characterized by a neutrophil (polymorphonuclear leukocyte [PMN])-rich inflammatory response in addition to Th2-type inflammation (6). During status asthmaticus, the number of PMNs in the airways is several times greater than the number of eosinophils, and there is an association between PMN number and duration of intubation (7). Even when not in the midst of an exacerbation, PMNs are present in higher quantities in the airways of patients with severe compared with mild asthma (4). This chronic airway inflammation in severe asthma also increases the risk of developing persistent airflow limitation (8). Together, these findings suggest that the inflammation of severe asthma is the result of unique pathobiological mechanisms and not just a more profound extension of processes responsible for mild to moderate asthma.

Although this persistent airway inflammation in severe asthma may result from a microenvironmental excess of proinflammatory molecules, a similar pathologic state could derive from a loss of counterregulatory molecules that serve to restrain the inflammatory response. Lipid mediators are potent regulators of airway tone and inflammation (9). Distinct in structure and function from prostaglandins and leukotrienes (LTs), lipoxin A<sub>4</sub> (LXA<sub>4</sub>), and LXB<sub>4</sub> are lipoxigenase (LO) interaction products that are also derived from arachidonic acid (C<sub>20:4</sub>) (10). Unlike proinflammatory lipid mediators, LXs play key roles in promoting resolution of acute inflammation. After tissue injury or inflammation, LXs modulate both innate and adaptive immunity by regulating leukocyte trafficking (including that of both PMNs and eosinophils) (10), T-lymphocyte activation (11), and dendritic cell function (12). As autacoids, LXs act at specific receptors to transduce their antiinflammatory effects, which include inhibition of the formation and *in vivo* actions of LTs, cytokines, and chemokines (10, 13) and downregulation of allergic airway inflammation and hyperresponsiveness in experimental asthma (14). Low levels of LXs and a defect in LX signaling have been linked to excess PMN-rich inflammation in the airways of patients with cystic fibrosis (15). Because LXs can regulate both airway inflammation and reactivity, we sought to determine whether asthma severity was related to a decreased biosynthetic capacity for these counterregulatory mediators.

Here, we present evidence of reduced LX generation in whole blood from individuals with severe asthma, a novel disease mechanism that distinguishes severe from moderate asthma. Some of the results of these studies have been previously reported in the form of an abstract (16).

## METHODS

### Participants

Asthma severity was determined on the basis of guidelines developed by the Severe Asthma Research Program of the National Heart, Lung, and Blood Institute, National Institutes of Health (Bethesda, MD) as outlined in Table 1. Subjects with severe asthma ( $n = 17$ ) had to be more than 18 years of age, nonsmokers, have a normal diffusing capacity for carbon monoxide, and at least 12% reversibility of their FEV<sub>1</sub> or a methacholine PC<sub>20</sub> less than 8 mg. Subjects with moderate asthma ( $n = 15$ ) were at least 18 years of age and met the criteria outlined in Table 1. Healthy subjects ( $n = 4$ ) had no clinical history of asthma, were at least 18 years of age, and had not been ill or taking medications for at least 2 weeks preceding phlebotomy.

### Blood Collection and Sample Extraction

Peripheral venous blood (20 ml) was collected by venepuncture from volunteer subjects who had given written, informed consent to a protocol approved by the Brigham and Women's Hospital Committee for the Protection of Human Subjects in Research. Blood samples were drawn into four 5-ml tubes containing heparin and processed immediately. The materials in one tube (5 ml) were added directly to 5 volumes of iced methanol for lipid extraction. Whole blood samples (5 ml/reaction) were also exposed (37°C for 30 minutes) to the calcium ionophore A23187 (50 μM) or to vehicle (17). Note that this concentration of A23187 is required to activate eicosanoid generation in whole blood (17, 18). After stimulation, reactions were stopped with 5 volumes of iced methanol. Prostaglandin B<sub>2</sub> was added to each sample as an internal control and materials were kept overnight at -20°C. Eicosanoids were extracted with C<sub>18</sub> Sep-Pak cartridges (Waters, Milford, MA) (19). Materials in the methyl formate eluate (i.e., LX, 15-*epi*-LX, LTB<sub>4</sub>, and hydroxyeicosatetraenoic acids [HETEs]) and materials in the methanol eluate (i.e., cysteinyl LTs [CysLTs]: LTC<sub>4</sub>, LTD<sub>4</sub>, and LTE<sub>4</sub>) were

brought to dryness under a gentle stream of N<sub>2</sub> and kept at -80°C until eicosanoids were measured by HPLC or ELISA. The HPLC-LIF (laser-induced fluorescence) limit of detection for LXA<sub>4</sub> was 5 pg. The sensitivity (80% B/B<sub>0</sub>) and IC<sub>50</sub> (50% B/B<sub>0</sub>) for LXA<sub>4</sub> by ELISA (Neogen, Lexington, KY) were 30 and 250 pg/ml, respectively, and for CysLTs by ELISA (Cayman Chemical, Ann Arbor, MI) the sensitivity and IC<sub>50</sub> were 30 and 57 pg/ml, respectively. The physical properties for authentic LXs were determined with a fluorescence spectrophotometer (F-2500; Hitachi, Tokyo, Japan).

### Measurement of Lipid Mediators

The methyl formate fraction was applied to an HPLC (Agilent 1100 series; Agilent Technologies, Palo Alto, CA) equipped with a Hypersil ODS column (250 × 0.3 mm; Keystone Scientific/Thermo Electron, Bellefonte, PA), and coupled to either a photodiode array detector (ultraviolet and visible range) (20) or a helium-cadmium laser (325 nm, series 56; Melles Griot, Carlsbad, CA)-induced fluorescence detector (ZETALIF, Model LIF-SA-03; Picometrics, Ramonville, France) to collect emissions between 400 and 800 nm. The mobile phase was methanol-doubly distilled H<sub>2</sub>O-glacial acetic acid (70:30:1, vol/vol/vol) with a flow rate of 400 μl/minute that was reduced to 4 μl/minute with a precolumn flow splitter (100:1, series 620; Analytical Scientific Instruments, El Sobrante, CA) before LIF detection. The criteria used for identification of LXs were fluorescence, retention time, and coelution with authentic material on coinjection. CysLTs present in the methanol fraction were identified by a sensitive ELISA (Cayman Chemical).

### 15-LO Expression

Total RNA was extracted from whole blood (QIAamp RNA Blood Mini Kit; Qiagen, Valencia, CA) and treated with DNase (Qiagen). Two micrograms of total RNA were reverse transcribed (Ready-To-Go RT-PCR beads; Amersham Biosciences/GE Healthcare, Piscataway, NJ). One microgram of cDNA was used for semiquantitative gene expression by polymerase chain reaction, using specific primers for human 15-LO-1 mRNA (sense primer, 5'-CCG ACC TCG CTA TCA AAG AC-3'; antisense primer, 5'-GGA TGA CCA TGG GCA AGA G 3') and for β-actin mRNA (sense primer, 5'-GGT GGC TTT TAG GAT GGC AAG-3'; antisense primer, 5'-ACT GGA ACG GTG AAG GTG ACA G-3'). The annealing temperatures and number of cycles used for 15-LO-1

TABLE 1. CLINICAL PROFILE OF PATIENTS WITH MODERATE AND SEVERE ASTHMA\*

	Moderate Asthma	Severe Asthma	p Value
Clinical data			
Age, yr	33.5 (11.1)	46.3 (17.2)	0.03
Sex, F:M ratio	9:6	11:6	NS
Race	4 black, 11 white	5 black, 12 white	NS
Treatment			
Patients taking inhaled steroids	15	17	NS
Mean daily dose	556.8 (261.9)	1,235.3 (528.7)	p < 0.01
Patients taking prednisone	0	2	NS
Patients taking CysLT <sub>1</sub> receptor antagonist	4	8	NS
Clinical testing			
FEV <sub>1</sub> , % predicted	90.5 (16.1)	63.1 (11.2)	p < 0.01
PBL differential			
Eosinophils, %	4.3 (3.0)	8.8 (5.6)	NS
Neutrophils, %	59.9 (13.0)	64.0 (10.0)	NS
Lymphocytes, %	29.9 (10.6)	24.1 (7.4)	NS
Monocytes, %	5.9 (3.1)	3.1 (0.4)	NS

Definition of abbreviations: CysLT<sub>1</sub> receptor = cysteinyl leukotriene type 1 receptor; F = female; M = male; NS = not significant; PBL = peripheral blood leukocyte.

\* Moderate asthma was defined as an FEV<sub>1</sub> exceeding 80% with inhaled corticosteroids (at least 176 μg of fluticasone propionate or equivalent), well-controlled asthma symptoms with fewer than eight puffs of a short-acting β<sub>2</sub>-adrenergic agonist per day, a less than 5-pack-year smoking history, and at least 12% reversibility of FEV<sub>1</sub> or a methacholine PC<sub>20</sub> less than 8 mg. Severe asthma was defined as continuous or nearly continuous treatment (more than 50% of year) with oral corticosteroids or high-dose inhaled corticosteroids (at least 880 μg of fluticasone propionate or equivalent), an FEV<sub>1</sub> not greater than 70% predicted on at least two occasions in the past year, at least 12% reversibility of their FEV<sub>1</sub> or a methacholine PC<sub>20</sub> less than 8 mg, asthma symptoms requiring a short-acting β<sub>2</sub>-adrenergic agonist on a daily or nearly daily basis, a normal diffusing capacity for carbon monoxide, and a less than 5-pack-year smoking history.

Values represent means (SD).

and  $\beta$ -actin mRNA amplification were 58°C, 35 cycles, and 62°C, 25 cycles, respectively. The polymerase chain reaction product sizes were 402 bp for 15-LO-1 and 159 bp for  $\beta$ -actin. After electrophoresis (1% agarose gel), densitometry was performed with Scion Image software (Scion, Frederick, MD).

### Statistical Analysis

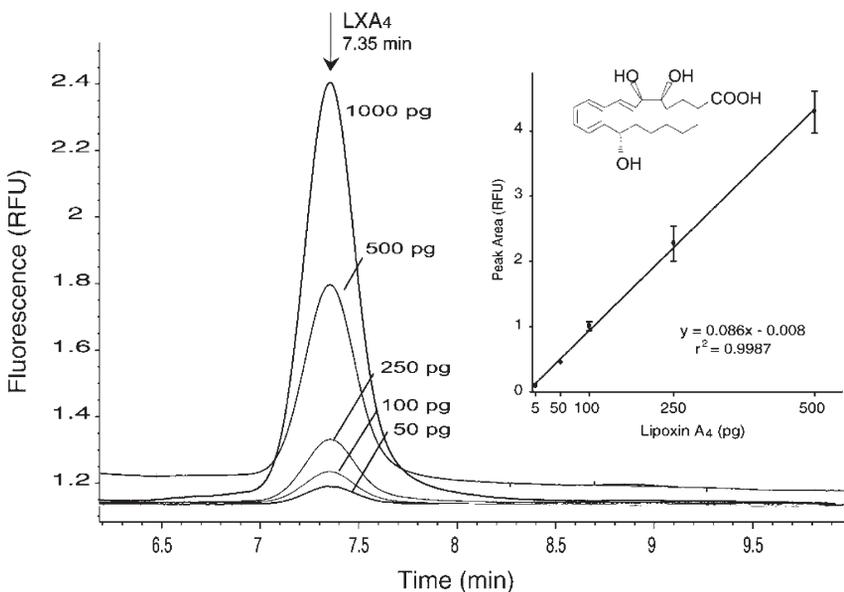
Samples were deidentified before analysis. For the purposes of these analyses, lipid mediator values below the lower limit of detection were assigned a value of one-half the limit of detection. Bivariate analysis was based on the nonparametric Mann-Whitney U test or Kruskal-Wallis test to compare the ranks of continuous variables across the levels of a categorical variable with two or three levels, respectively (21); the Spearman rank correlation test to compare the ranks of two continuous variables; and  $\chi^2$  tests or a hybrid approximation to the Fisher's exact test (22) on contingency tables for comparisons of categorical variables. S-Plus 6.1R3 (Mathsoft, Cambridge, MA) and LogXact version 4.1 (Cytel, Cambridge, MA) were used to manage and analyze the data. Statistical significance was defined at the standard 5% level.

## RESULTS

Lipoxins display counterregulatory actions in low picogram amounts (10), yet in complex biological matrices, such as whole blood, their measurement has been limited by methods with a lower limit of detection higher than their biologically active concentrations. To facilitate the identification of low amounts of LXs in activated whole blood, we devised a new, more sensitive physical means for LX detection that coupled online laser-induced fluorescence detection to an HPLC system (HPLC-LIF). Distinct from LTs, the presence of a conjugated tetraene in LXs provides fluorescence properties that are unique among eicosanoids (23). At room temperature, the excitation maximum for LXs is 320 nm and the emission maximum in methanol is 410 nm. To provide increased sensitivity for compounds with endogenous fluorescence, materials eluting in this new HPLC-LIF system were excited by a helium-cadmium laser at 325 nm to approximate the LX excitation maximum and emission spectra collected. With this system, detection of LXA<sub>4</sub> was quantitative to the low picogram range (Figure 1), representing increased sensitivity compared with immunologically based detection by ELISA and other HPLC-based physical means, including ultraviolet absorbance and mass spectrometry.

Characteristics of the subjects with moderate and severe asthma on enrollment are presented in Table 1. Subjects with severe asthma were older. Differences in CysLT<sub>1</sub> receptor antagonist ( $p = 0.29$ ) and inhaled corticosteroid use ( $p = 0.49$ ) were also present, although not significant (Table 1). Mean daily dose of inhaled corticosteroid and FEV<sub>1</sub> percent predicted were significantly lower in the subjects with severe asthma (Table 1); this was related to the criteria used to ascertain the diagnostic groups. There were no other significant differences in treatment variables or peripheral leukocyte differential counts. No patients were experiencing asthma exacerbations at the time of phlebotomy.

To assess maximal eicosanoid biosynthetic capacity in these clinically characterized individuals with moderate or severe asthma, samples of their whole blood were activated with the divalent cation ionophore A23187. After incubation, lipids were extracted from whole blood and analyzed with an HPLC coupled to either the new LIF detector or a photodiode array ultraviolet-visible wavelength detector (HPLC-PDA) (20). Routinely, LXs were detected by LIF that were not evident by PDA. Activated whole blood from patients with moderate asthma generated substantial amounts of LXA<sub>4</sub> (mean, 1.8 [SD 0.8] ng/ml) (Figure 2a). LXB<sub>4</sub> was also detectable, but interference with coeluting fluorescent materials precluded accurate quantitation. Samples of activated whole blood from individuals with severe asthma generated significantly less LXA<sub>4</sub> (mean, 0.4 [SD 0.4] ng/ml) than did samples from subjects with moderate asthma (mean, 1.8 [SD 0.8] ng/ml;  $p = 0.001$ ). LXA<sub>4</sub> was not detectable in control samples from nonasthmatic subjects. For comparison, we also determined the biosynthetic capacity of subjects for other 5-LO-derived lipid mediators, including the proinflammatory LTB<sub>4</sub> and bronchoconstrictive CysLTs. LTB<sub>4</sub> was detected by HPLC-PDA and generated in activated whole blood by all subjects with asthma. LTB<sub>4</sub> biosynthesis increased with asthma severity, but this trend did not reach statistical significance ( $p = 0.33$ ; Figure 2b). In contrast, levels of CysLTs were increased in those with moderate asthma (mean, 64.4 [SD 24.8] pg/ml) compared with nonasthmatic subjects (mean, 25.5 [SD 13.2] pg/ml;  $p = 0.02$ ), and markedly increased in severe asthma (mean, 112.5 [SD 53.7] pg/ml) compared both with subjects with moderate asthma ( $p = 0.05$ ) and nonasthmatic subjects ( $p = 0.007$ ; Figure 2b). The ratio of LT (i.e., LTB<sub>4</sub> plus CysLTs) to LXA<sub>4</sub> production



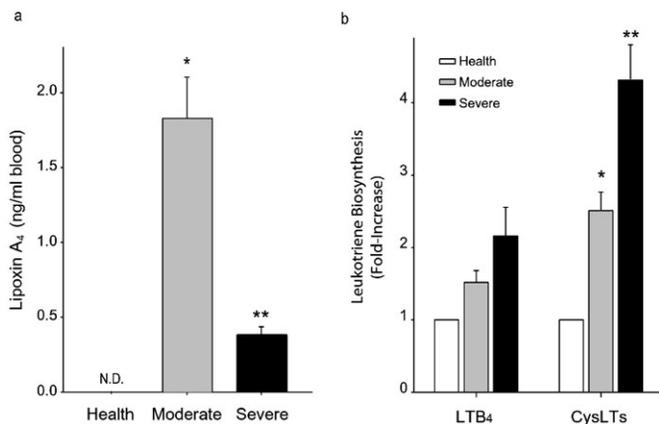
**Figure 1.** Fluorescence-based detection of lipoxin A<sub>4</sub> (LXA<sub>4</sub>). A new HPLC system, coupled online to a 325-nm helium-cadmium laser to enhance fluorescence, enabled detection of LXA<sub>4</sub> in the low picogram range and (inset) the assay was validated with authentic LXA<sub>4</sub> for quantitation over 3 log orders of concentration (mean  $\pm$  SEM,  $r^2 = 0.9987$  with a 5-pg limit of detection). The structure of LXA<sub>4</sub> is shown to demonstrate the unique conjugated tetraene that is responsible for its endogenous fluorescence. Results are representative of  $n \geq 3$  experiments.

was approximately 10-fold less in moderate asthma (1.42 to 1) compared with severe asthma (10.39 to 1), with an even more dramatic difference in the ratio of LXA<sub>4</sub> to CysLT production in subjects with moderate (34.15 [SD 23.68]) and severe (4.50 [SD 5.19]) asthma ( $p < 0.03$ ), providing a striking change in eicosanoid biosynthesis in severe asthma with a relative increase in conversion of C<sub>20:4</sub> to proinflammatory lipid mediators. To determine whether the relative conversion of C<sub>20:4</sub> to LTs and LXs was stimulus specific or truly reflective of a difference in asthma clinical severity, we also determined the amounts of CysLTs and LXA<sub>4</sub> in nonstimulated whole blood. Basal LXA<sub>4</sub> levels were decreased in severe compared with moderate asthma (0.22 [SD 0.14] vs. 0.40 [SD 0.09] ng/ml, respectively;  $p < 0.01$ ) and the ratio of LXA<sub>4</sub> to CysLTs was significantly lower in nonstimulated whole blood from subjects with severe (16.14 [SD 8.02]) compared with moderate (35.93 [SD 8.80]) asthma ( $p < 0.0003$ ). Together, these findings indicate that the generation of LO-derived eicosanoids in whole blood is a regulated process that differs with asthma clinical severity. Moreover, those with the most severe asthma preferentially convert C<sub>20:4</sub> to LTs rather than to LXs.

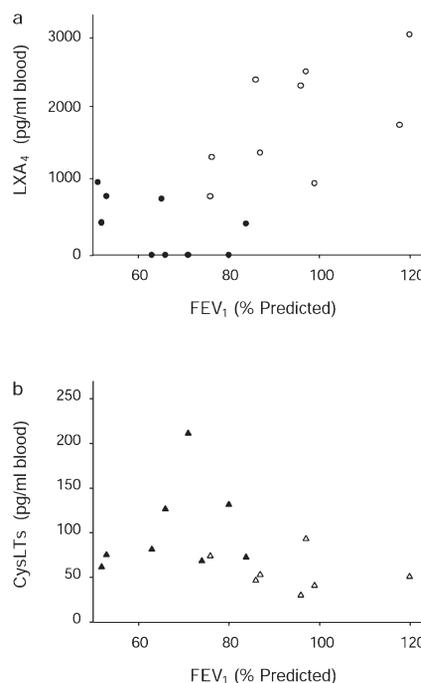
Major routes of LX formation in activated whole blood are established during cell-cell interactions between leukocyte 5-LO and 15-LO as well as leukocyte 5-LO and platelet 12-LO (20). To identify the biochemical mechanism for decrements in LX formation in severe asthma, we next monitored levels of the mono-oxygenation products of C<sub>20:4</sub> as markers of LO activity. Levels of the 5-LO product 5-HETE were increased in activated samples from patients with severe asthma and, similar to LTs, increased with asthma severity (17.1 [SD 13.8] and 35.8 [SD 24.7] ng/ml in moderate and severe asthma, respectively;  $p = 0.07$ ). Of interest, levels of the 15-LO-catalyzed product 15-HETE were significantly decreased in samples from patients with severe asthma (2.6 [SD 1.5] ng/ml) compared with patients with moderate asthma (8.7 [SD 6.0] ng/ml;  $p = 0.01$ ). No significant differences

in 12-HETE were identified in samples from individuals with and without asthma. The relationship between 5-LO and 15-LO activity in the samples, as monitored by mono-HETE formation, was also markedly different in severe asthma (17.6 [SD 14.2], 5-HETE:15-HETE ratio) than in moderate asthma (2.1 [SD 0.9], 5-HETE:15-HETE ratio;  $p = 0.001$ ). In addition, expression of 15-LO-1 mRNA was significantly decreased in severe asthma (0.17 [SD 0.16], ratio of 15-LO to  $\beta$ -actin expression) compared with moderate asthma (0.30 [SD 0.10];  $p < 0.05$ ). Together, these findings indicate that activated whole blood from patients with severe asthma had increased 5-LO and decreased 15-LO, leading to low levels of both 15-HETE and LXs and accounting for the different biosynthetic patterns of LO-derived eicosanoids observed in severe and moderate asthma.

Because CysLTs are potent bronchoconstrictors and LXs can block CysLT-mediated bronchial hyperresponsiveness in asthma (9, 24), we next examined the relationship between lipid mediator biosynthetic capacity and the severity of airflow obstruction. Most of the subjects with severe asthma did not generate substantial amounts of LXA<sub>4</sub>, especially those with an FEV<sub>1</sub> less than 80% predicted at the time of assessment (Figure 3a). Among subjects with asthma, increased LXA<sub>4</sub> levels were associated with increased FEV<sub>1</sub> (percent-predicted values;  $p = 0.006$ ). In sharp contrast to LXA<sub>4</sub> levels, subjects with severe asthma generated high levels of CysLTs relative to those with moderate asthma (Figure 3b). For the subjects with asthma, increased CysLT formation was associated with decreased FEV<sub>1</sub> (percent-predicted values), although this difference did not reach formal statistical significance ( $p = 0.16$ ; Figure 3b). The relative amounts of LXA<sub>4</sub> and CysLTs in nonstimulated blood also correlated



**Figure 2.** LXA<sub>4</sub> and leukotriene (LT) generation in activated whole blood from individuals with moderate and severe asthma. Samples of whole blood were obtained from healthy individuals and from subjects with clinically characterized asthma and activated (30 minutes at 37°C) with A23187. After extraction, materials were analyzed by HPLC-LIF for LXA<sub>4</sub> determination (a), by HPLC-PDA for LTB<sub>4</sub> determination (b), and by ELISA for CysLT determination (b). Results are expressed as means  $\pm$  SEM ( $n = 9$ ) for LXA<sub>4</sub> and the change in LT biosynthesis in subjects with asthma relative to the mean value in nonasthmatic volunteers (2.3 ng of LTB<sub>4</sub>/ml, 25.5 pg of CysLTs/ml,  $n = 4$ ). N.D. = not detected. \* $p < 0.05$  for subjects with moderate asthma versus nonasthmatic subjects and \*\* $p < 0.05$  for subjects with severe compared with moderate asthma.



**Figure 3.** Relationship between lipid mediator generation and airflow obstruction. The mean value for (a) LXA<sub>4</sub> (circles) and (b) CysLT (triangles) biosynthetic capacity for each subject with asthma was compared with their FEV<sub>1</sub> (percent-predicted values). Values for patients with severe asthma are solid, and values for patients with moderate asthma are open.

with FEV<sub>1</sub> ( $p = 0.003$ ; Figure 4). No significant association was observed for levels or ratios of lipid mediators with peripheral blood eosinophils or PMNs, age, sex, race, or treatment modalities, including steroids, in patients with either moderate or severe asthma.

## DISCUSSION

Our results are the first to describe a new method to detect LXs in low picogram quantities and to demonstrate that individuals with severe asthma have a reduced capacity in whole blood to convert C<sub>20:4</sub> to 15-LO-catalyzed products, including both 15-HETE and LXA<sub>4</sub>. Markedly distinct from these decrements in 15-LO activity, 5-LO-derived products, including 5-HETE, LTB<sub>4</sub>, and CysLTs, were all increased in our cohort of patients with severe asthma. Moreover, relationships between FEV<sub>1</sub> percent-predicted values and LXA<sub>4</sub> and CysLTs suggest a link between biosynthetic capacity for these bioactive lipid mediators and airflow obstruction in asthma. Decrements in LX generation and increases in CysLT production in severe asthma would create an imbalance favoring the persistent airway inflammation and airflow obstruction typical of this condition.

Lipoxins are a unique class of eicosanoids with counterregulatory properties for inflammatory responses *in vitro* in subnanomolar concentrations (10). These compounds promote resolution of cytokine-driven acute inflammation (25) and reduce airway inflammation in experimental asthma (14). During allergic airway inflammation, LXA<sub>4</sub> is generated and LXA<sub>4</sub> receptor signaling decreases the generation of interleukin (IL) 13, IL-5, and CysLTs; the formation of IgE; as well as eosinophil trafficking (14). LXs modulate leukocyte and tissue-resident cellular inflammatory responses via diverse mechanisms (reviewed in Reference 9) that include inhibition of NF- $\kappa$ B activation (26), which, notably, has been linked to overproduction of inflammatory cytokines in severe asthma (27). LXs also promote clearance of inflammation by stimulating macrophage-mediated phagocytosis of apoptotic leukocytes (28). Together, these properties are consistent with LXs carrying biological actions with the capacity to regulate the airway inflammation of asthma.

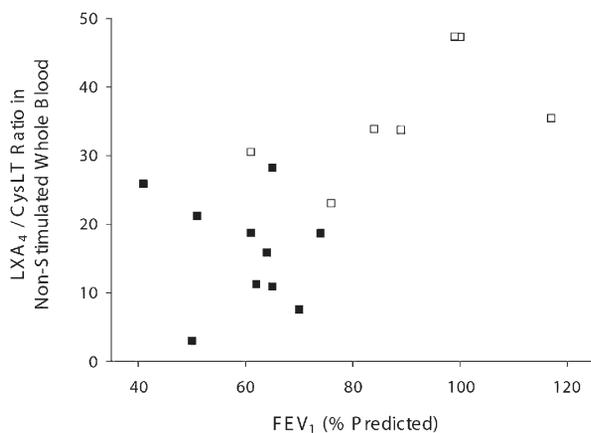
Lipoxins are also generated at times and in quantities *in vivo* that are commensurate with a role in asthma pathophysiology. LXA<sub>4</sub> and LXB<sub>4</sub> are present in nanogram quantities in bron-

choalveolar lavage fluids from patients with respiratory inflammation (29). Their biosynthesis *in vivo* is temporally dissociated from the early formation and impact of proinflammatory eicosanoids, such as prostaglandins and LTs (25). Aspirin challenge increases LXA<sub>4</sub> levels in nasal lavage fluid from individuals with aspirin-intolerant asthma, yet overall LX biosynthetic capacity in the peripheral blood of subjects with aspirin-intolerant asthma is decreased compared with individuals with aspirin-tolerant asthma and nonasthmatic individuals (17). Because individuals with aspirin-intolerant asthma can have protracted and severe clinical courses like those of patients with severe asthma (1), a diminished ability to generate LXs may represent a common mechanism that predisposes to the clinical expression of severe asthma. Decrements in the formation of endogenous mediators of antiinflammation would facilitate an influx of leukocytes to perpetuate the local inflammatory response and expose bronchial smooth muscle to relatively unopposed actions of bronchoconstricting substances, such as CysLTs.

For LX formation, 15-LO is capable both of initiating LX biosynthesis and of converting the LT intermediate LTA<sub>4</sub> to LXs (10). Here, levels of both 15-HETE, a 15-LO-derived C<sub>20:4</sub> metabolite, and LXs were decreased in activated whole blood from individuals with severe asthma. When inhaled *in vivo*, 15(S)-HETE does not have an immediate impact on airway caliber, the late asthmatic response, or bronchial hyperresponsiveness (30, 31). In some scenarios, 15-HETE can be a substrate for conversion to LXs during cell-cell interactions by leukocyte 5-LO. Transgenic rabbits with increased 15-LO expression in monocyte/macrophages display a local increase in LX formation at sites of inflammation and are protected from chronic inflammatory disease (i.e., atherosclerosis and periodontal disease) (32, 33). In addition, 15-LO gene therapy stimulates LX generation during acute glomerulonephritis and protects the kidneys from glomerular inflammation (34). 15-LO was originally purified from elicited rabbit peritoneal PMNs (35), yet human peripheral blood PMNs isolated from nonasthmatic individuals display predominantly 5-LO and little 15-LO activity (20). Cytokines produced during Th2 inflammation, including IL-4 and IL-13, induce 15-LO (36, 37) and, unlike PMNs from healthy volunteers, activated peripheral blood PMNs from patients with asthma acquire the capacity to generate LXs and other 15-LO-derived products (38). Also of note, the lungs of animals deficient in the murine homolog for 15-LO-1 display more exuberant inflammatory responses to antigen-dependent allergic airway inflammation (39).

15-HETE levels in the airways are increased in severe asthma, but correlate poorly with increased 15-LO-1 (40). Although eosinophils and PMNs can display 15-LO-1 activity, the majority of 15-HETE in asthmatic airways is generated by airway epithelia (41) and cytokine-primed alveolar macrophages (42) via the actions of multiple potential biosynthetic enzymes, including 15-LO-1, 15-LO-2, and cytochrome P-450 enzymes (10, 41-43). Because all cells capable of 15-HETE generation in the airways are not present in the peripheral circulation, 15-HETE and LX formation in activated whole blood may not be predictive of low levels in the respiratory tract (40). However, individuals with severe asthma also have lower concentrations of LXA<sub>4</sub> in the supernatants of induced sputum than do those with mild asthma and LXA<sub>4</sub> levels in the airways are correlated with levels of the PMN chemoattractant and agonist IL-8 (44, 45). Our findings add to these interesting observations by determining the biochemical relationship between leukotriene and lipoxin generation, identifying key differences in the regulation of eicosanoid biosynthetic enzymes, and uncovering a clinical relationship between these LO-derived eicosanoids and airflow obstruction.

In summary, our results address an important relationship between LX biosynthesis and asthma severity and provide evidence



**Figure 4.** Relationship between lipoxygenase-derived eicosanoids in whole blood and airflow obstruction. The value for the ratio of LXA<sub>4</sub> to CysLTs (squares) in nonstimulated whole blood for each subject with asthma was compared with their FEV<sub>1</sub> (percent-predicted values). Values for patients with severe asthma are solid, and values for patients with moderate asthma are open.

of dysregulated 15-LO as a biochemical mechanism for diminished LX production in severe asthma. Among individuals with severe asthma, the persistent inflammation, airflow obstruction, and frequent symptoms may result from decrements in the levels of these counterregulatory molecules that help to prevent bronchoconstriction and restrain excessive inflammation in those with mild to moderate asthma. Distinct from our current therapeutic approach in clinical asthma that is directed toward antagonizing proinflammatory mediators and leukocyte effectors, our findings suggest consideration of a new strategy that emphasizes natural counterregulatory pathways.

**Conflict of Interest Statement:** B.D.L. received \$5,000 in patent licensing fees from Schering AG. C.B. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. E.S.S. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. L.J.P. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. G.M. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. E.I. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

**Acknowledgment:** The authors thank Charles N. Serhan, and specifically Jeffrey vom Saal, Erin Lee, and Guangli Zhu, for technical assistance, and Caroline A. Owen and Steve S. Boukedes for assistance with measurement of LX fluorescence.

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