

## ORIGINAL PAPER

# Lipoxin A<sub>4</sub> levels in asthma: relation with disease severity and aspirin sensitivity

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## Clinical and Experimental Allergy

### Summary

**Background** Lipoxin (LX) A<sub>4</sub>, an endogenous anti-inflammatory eicosanoid, has been found to be low in patients with severe asthma. However, few studies also suggested more diminished LX A<sub>4</sub> levels in aspirin-exacerbated respiratory disease (AERD) when compared with aspirin-tolerant asthma (ATA). It is, therefore, currently not clear whether the asthma severity or the presence of AERD has a primary role in the disturbed LX metabolism.

**Objective** To detect LX A<sub>4</sub> and 15-epi-LX A<sub>4</sub> levels in asthma patients with and without AERD of comparable severity.

**Methods** The study groups consisted of 22 subjects with AERD, 22 subjects with ATA and 10 volunteers without asthma and aspirin sensitivity. Whole-blood samples were stimulated with calcium ionophore, A23187 ( $5 \times 10^{-5}$  M) and A23187 ( $5 \times 10^{-5}$  M) + aspirin ( $10^{-4}$  M).

LX A<sub>4</sub> and 15-epi-LX A<sub>4</sub> levels were analysed by the enzyme immune assay method.

**Results** Severe asthma patients in both AERD [0.5 (0.8)] ng/mL and ATA [0.5 (0.45) ng/mL] groups showed diminished generation for LX A<sub>4</sub> to stimulation with A23187 in comparison with other severity degrees in their groups ( $P = 0.02$  and  $0.046$ , respectively). LX A<sub>4</sub> generation in both severe groups was comparable with each other ( $P > 0.05$ ). Although severe cases with AERD showed a diminished capacity to generate 15-epi-LX A<sub>4</sub>, this did not reach statistical significance.

**Conclusion** This study indicated that diminished LX A<sub>4</sub> generation was unique to severe asthma phenotype regardless of comorbid aspirin sensitivity.

**Clinical Implications** Lower LX A<sub>4</sub> levels in severe asthma would suggest a possibility for LX analogues as future treatment options in these patients.

**Keywords** aspirin, aspirin-exacerbated respiratory disease, asthma, 15-epi-lipoxin A<sub>4</sub>, inflammation, lipoxin A<sub>4</sub>, severity

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### Introduction

It is well known that aspirin (ASA) and other non-steroidal anti-inflammatory drugs (NSAIDs) cause asthma flares in a particular group of asthma patients and this phenotype, 'aspirin-exacerbated respiratory disease' (AERD), is related to more severe disease presentation [1–3]. Although several issues in the pathogenesis of AERD remain to be explained, arachidonic acid (AA) pathway metabolites or enzymes have always been a subject of interest in the pathogenesis of AERD. Both therapeutic and adverse effects of aspirin and other NSAIDs are believed to be caused by inhibition of

cyclooxygenase, an enzyme that is responsible for the synthesis of prostaglandins (PG) from AA [1, 2, 4, 5]. Increased cysteinyl leukotriene (CysLT) levels have been suggested to be one of the main mechanisms involved in this disease. Although *in vitro* studies reveal conflicting results, *in vivo* studies performed on human secretions such as bronchoalveolar lavage (BAL) and urine provided good evidence for CysLT enhancement and possible involvement in this disease [6–11]. Decreased levels of PGE<sub>2</sub>, increased levels of PGD<sub>2</sub>, and 15-hydroxyeicosatetraenoic acid (HETE) and overexpression of LTC<sub>4</sub> synthase are other mechanisms suggested to be involved in the disease [12–14].

As a member of the AA pathway, the role of lipoxins (LX) in the pathogenesis of AERD is a current topic of interest. LX, a structurally unique class of eicosanoids, are produced by a variety of cells including airway epithelium, eosinophils, neutrophils, platelets and others [15–18]. Strong evidence supports that LX exert endogenous anti-inflammatory actions by inhibition of both nuclear factor kappa beta (NF $\kappa$ B) and leucocytic inflammation, reduction in oedema formation, as well as removal of apoptotic leucocytes by phagocytes [15, 18–20]. ASA has also been shown to cause acetylation of COX-2, thereby leading to the formation of 15 R-HETE and finally, 15-epi-LXA<sub>4</sub>, so-called 'aspirin-triggered lipoxins' (ATL) with well-characterized anti-inflammatory properties [15–18].

Regarding the role of LXA<sub>4</sub> in AERD, two studies suggested diminished LXA<sub>4</sub> generation in AERD when compared with aspirin-tolerant asthma (ATA) in which severity was not controlled for [21, 22]. Moreover, another study conducted in only ATA patients showed diminished LXA<sub>4</sub> in severe patients compared with mild asthmatics [23]. We have previously noted the paucity of the literature in drawing definitive conclusions whether the asthma severity or the presence of AERD has a primary role in the disturbed LX metabolism [24]. Therefore, in order to test our hypothesis of whether asthma severity or co-morbid aspirin sensitivity has a primary role in disturbed LX metabolism, we conducted an *in vitro* study on patients with AERD and ATA having matched asthma severity degrees, aimed primarily at investigating LXA<sub>4</sub>, and secondarily 15-epi-LXA<sub>4</sub> generation.

## Materials and methods

### Patients selection

This study was performed in our tertiary care clinic located in Ankara, Türkiye. The study consisted of three different groups: Group 1: patients with AERD diagnosed by a clinically documented asthmatic response after ingestion of ASA or other NSAID; Group 2: patients with asthma having no problem in taking ASA and/or NSAIDs; and Group 3: healthy volunteers without asthma and analgesic intolerance as controls. Asthma diagnosis was based on a history of recurrent symptoms of wheezing, shortness of breath, cough and demonstration of objective signs of reversible airway obstruction by means of at least a >12% increase in forced expiratory volume in 1 s (FEV<sub>1</sub>) after 15 min with an inhalation of 200 µg salbutamol according to Global Initiative for Asthma guidelines [25]. A positive bronchoprovocation test was also accepted for asthma diagnosis. Exclusion criteria were chronic obstructive pulmonary disease indistinguishable from asthma, and unstable asthma. The patients under regular asthma treatment were allowed to use their medicines. None of the asthmatic patients was on specific immu-

notherapy. The subjects who had taken any NSAIDs or ASA during the last 2 weeks were not included in the study. This study was approved by the local ethics committee of Ankara University School of Medicine (approval date: 4 October 2004, number: 58-1431), and each subject gave written informed consent.

### Study design

At the beginning of the study, asthma severity was determined by the frequency of daytime and night-time asthma symptoms, pulmonary function tests and medication requirements according to the Global Initiative for Asthma Guidelines by chest disease and allergy specialists, and were classified as mild intermittent and persistent, moderate persistent and severe persistent asthma [25]. Using the guideline recommended by American Thoracic Society, subjects with severe asthma were also required to meet one of the two major criteria – (1) the use of high-dose inhaled corticosteroids and/or (2) the requirement for very frequent oral corticosteroid use – as well as two of seven minor criteria: the use of additional controller medications, the presence of daily symptoms requiring rescue inhaler, reduced lung function, urgent care by a physician, recurrent exacerbations requiring oral corticosteroids, clinical deterioration with steroid withdrawal and a history of near-fatal events [26].

After obtaining demographic and clinical data, the atopic status of the asthmatic subjects was recorded from their charts according to skin prick tests (SPTs) and/or specific IgE results.

### In vitro evaluation

The methodology defined by Sanak et al. was applied in this experiment [23]. Briefly, 10 mL of peripheral venous blood was drawn into a tube containing EDTA. This amount was divided into two separate tubes and after adding recombinant IL-3 (20 ng/mL) (Biosource International, Camarillo, CA, USA) to both tubes, 10<sup>−4</sup> M of lysine-aspirin (Bayer, Leverkusen, Germany) was also added to the first tubes and whole-blood samples were maintained at 37 °C with gentle shaking for 30 min. Then, 5 × 10<sup>−5</sup> M A23187 (Sigma, Istanbul, Turkey) was added to the first and second tubes and maintained for 45 min at 37 °C. After stimulation, plasma was separated from blood by centrifugation (3000 g, 10 min, 24 °C) and stored at −80 °C until analysis.

### Extraction of lipoxin A<sub>4</sub> and 15-epi-lipoxin A<sub>4</sub>

The samples were first diluted by methanol and then by water and were acidified by 1 N HCl. C18 Sep-Pak light columns (Waters Associates, Milford, MA, USA) were preconditioned by washing with 2 mL of methanol,

followed by water. Then, samples were applied into the columns and were washed by 5 mL water, followed by 5 mL hexane. LXA<sub>4</sub> and 15-epi-LXA<sub>4</sub> were eluted by 2 mL of methyl formate. Methyl formate was evaporated by vacuum centrifugation (MaxiDry Plus, Heto Holten, Allerød, Denmark). Residue was reconstituted by 1 mL of diluted extraction buffer and stored at  $-80^{\circ}\text{C}$  until ELISA.

#### *Determination of lipoxin A<sub>4</sub> and 15-epi-lipoxin A<sub>4</sub> levels*

LXA<sub>4</sub> and 15-epi-LXA<sub>4</sub> were measured by ELISA according to the manufacturer's instructions (Neogen Corp., Lexington, KY, USA). The detection limit was 20 pg/mL for LXA<sub>4</sub> and 15-epi-LXA<sub>4</sub>. There was no cross-reactivity between 15-epi-LXA<sub>4</sub> and LXA<sub>4</sub>.

#### *Statistical analysis*

The overall data analysis was carried out by SPSS (Statistical Package for Social Sciences v11.0, Chicago, IL, USA). Owing to the distribution characteristics of the main study parameters of LXA<sub>4</sub> and 15-epi-LXA<sub>4</sub>, non-parametric methods were chosen for analysis: Inter-group comparisons were made by Kruskal–Wallis (KW) analysis and in case of significant *P*-values after the KW test, post hoc Mann–Whitney *U*-tests, with downward-adjusted *P*-values, in order to avoid Type II error, were used. LXA<sub>4</sub> levels were expressed as median [interquartile range (IQR)].

For intergroup comparisons of numeric parameters including age, disease duration and FEV<sub>1</sub> levels, parametric tests were used and values are expressed as mean  $\pm$  SEM. The  $\chi^2$  test was used for comparison of ordinal and nominal values and were expressed as *n* (%).

The Pearson correlation test was used for determination of factors related to LXA<sub>4</sub> levels. A *P*-value less than 0.05 was considered to be significant.

## **Results**

### *Subjects*

The study groups consisted of 22 subjects with AERD (mean age:  $46.3 \pm 2.3$  year, female/male 17/5), 22 subjects with ATA (mean age:  $43.9 \pm 2.3$  years, female/male: 17/5) and 10 volunteers without asthma and analgesic intolerance (mean age:  $40.3 \pm 3.1$  years, female/male: 7/3) with comparable age and sex distribution ( $P > 0.05$ ).

Regardless of the underlying asthma severity, the patients with AERD had a higher asthma duration ( $222.0 \pm 22.6$  months vs.  $126.4 \pm 16.2$  months,  $P < 0.001$ ), more chronic rhinosinusitis/nasal polyps (CRS/NP) (90.9% vs. 72.7%,  $P < 0.001$ ) and more polypectomy ( $4.4 \pm 0.8$  vs.  $1.8 \pm 1.1$ ,  $P = 0.029$ ) when compared with

the patients with ATA. The atopy rate was similar in both asthma groups (40.9% vs. 31.8%,  $P > 0.05$ ). Regarding the severity of asthma, six, six and 10 patients with AERD had mild, moderate and severe asthma, respectively, whereas the corresponding numbers were seven, eight, and seven for the ATA group. The patients with AERD had usually reacted to both aspirin and NSAIDs ( $n = 9$ , 40.9%). The mean time to the first reaction to aspirin or other NSAID was  $153.0 \pm 2.0$  months, with a mean of  $3.9 \pm 0.7$  episodes to aspirin. The disease characteristics of the asthma patients with different degrees of severity are given in Table 1. Platelet, eosinophil and white blood cell counts were similar in each group ( $P > 0.05$ ).

#### *A23187-stimulated lipoxin A<sub>4</sub> levels*

Mild asthma patients with AERD had higher LXA<sub>4</sub> generation with A23187 stimulation when compared with healthy controls [1.8 (1.8) vs. 0.7 (0.8) ng/mL,  $P = 0.04$ ]. Although moderate asthma with AERD [1.1 (1.4) ng/mL] and mild-moderate asthma with ATA [1.1 (0.9) and 1.3 (1) ng/mL, respectively] also revealed a higher LXA<sub>4</sub> level in comparison with controls, this finding was not statistically significant (Fig. 1).

Severe asthma patients in both AERD [0.5 (0.8) and ATA 0.5 (0.4) ng/mL] groups showed a diminished generation of LXA<sub>4</sub> to stimulation with A23187 in comparison with other severity degrees in their groups ( $P = 0.02$  for AERD and  $P = 0.046$  for ATA) (Fig. 1). These diminished levels were not different from those of healthy controls ( $P > 0.05$ ). LXA<sub>4</sub> levels with A23187 stimulation revealed similar results for mild and moderate asthmatics in both the AERD and ATA groups ( $P > 0.05$ ).

#### *A23187+ aspirin-stimulated lipoxin A<sub>4</sub> levels*

No significant differences were observed between groups after A23187+lysine aspirin stimulation regarding LXA<sub>4</sub> levels [control: 1.1 (0.8) ng/mL, AERD; mild: 1 (2.3) ng/mL, moderate: 1.0 (1.1) ng/mL, severe: 0.7 (0.5) ng/mL; ATA; mild: 0.7 (0.92) ng/mL, moderate: 1 (0.4) ng/mL; severe: 0.5 (0.6) ng/mL,  $P > 0.05$ ] (Fig. 2).

#### *A23187+ aspirin-stimulated 15-epi-lipoxin A<sub>4</sub> levels*

Severe cases with AERD showed a diminished capacity to generate 15-epi-LXA<sub>4</sub>, which did not reach statistically significant levels [control: 40.0 (25.0) ng/mL, AERD; mild: 45.7 (16.7) ng/mL, moderate: 37.0 (33.0) ng/mL, severe: 31.5 (25.7) ng/mL, ATA; mild: 39.5 (22.7) ng/mL, moderate: 43.2 (14.1) ng/mL; severe: 39.5 (13.0) ng/mL,  $P > 0.05$ ] (Fig. 3).

Table 1. Demographic and disease characteristics of study groups according to the asthma severity

| Variables                                       | AERD             |                |                  | ATA             |                |                   |
|-------------------------------------------------|------------------|----------------|------------------|-----------------|----------------|-------------------|
|                                                 | Asthma severity  |                |                  | Asthma severity |                |                   |
|                                                 | Mild             | Moderate       | Severe           | Mild            | Moderate       | Severe            |
| N                                               | 6                | 6              | 10               | 7               | 8              | 7                 |
| Age (years, mean $\pm$ SEM)                     | 41.6 $\pm$ 4.4   | 53.0 $\pm$ 5.7 | 45.2 $\pm$ 2.7   | 40.3 $\pm$ 3.3  | 46.6 $\pm$ 2.7 | 44.3 $\pm$ 6.0    |
| Gender (F/M, <i>n</i> )                         | 4/2              | 5/1            | 8/2              | 6/1             | 7/1            | 4/3               |
| Asthma duration (months, mean $\pm$ SEM)        | 194.0 $\pm$ 53.6 | 272 $\pm$ 36.8 | 208.8 $\pm$ 31.5 | 72.3 $\pm$ 5.9  | 153 $\pm$ 18.9 | 150.8 $\pm$ 29.0* |
| Atopy rate                                      | 3                | 3              | 3                | 4               | 2              | 1                 |
| <i>N</i> (%)                                    | (50)             | (50)           | (30)             | (57.1)          | (25)           | (14.3)            |
| Current use of oral steroids ( <i>n</i> )       | –                | –              | 2                | –               | –              | 2                 |
| Use of inhaled steroids ( <i>n</i> )            | 4                | 6              | 10               | 6               | 8              | 7                 |
| Doses of inhaled steroids                       |                  |                |                  |                 |                |                   |
| < 500 mcg                                       | 4                | 1              | 1                | 6               | 4              | 4                 |
| 500–1000 mcg                                    | –                | 5              | 6                | –               | 4              | 3                 |
| > 1000 mg                                       | –                | –              | 3                | –               | –              | –                 |
| Presence of chronic rhinosinusitis/nasal polyps | 6                | 4              | 10               | 3               | 8              | 5                 |
| <i>N</i> (%)                                    | (100)            | (66.7)         | (100)            | (42.9)          | (100)          | (71.4)            |
| FEV <sub>1</sub> (L/s, mean $\pm$ SEM)          | 2.8 $\pm$ 0.2    | 2.2 $\pm$ 0.2  | 1.9 $\pm$ 0.1**  | 2.6 $\pm$ 0.2   | 2.2 $\pm$ 0.1  | 1.8 $\pm$ 0.1*    |
| FEV <sub>1</sub> (% mean $\pm$ SEM)             | 92.7 $\pm$ 3.6   | 85.2 $\pm$ 5   | 70.1 $\pm$ 3.6** | 88.8 $\pm$ 3.5  | 77.5 $\pm$ 4.2 | 59.4 $\pm$ 4.6*   |

\*Significant difference in ATA group (asthma duration:  $P = 0.04$ , FEV<sub>1</sub>  $P = 0.001$ , FEV<sub>1</sub>%  $P < 0.0001$ ).

\*\*Significant difference in AERD group (FEV<sub>1</sub>  $P = 0.018$ , FEV<sub>1</sub>%  $P = 0.002$ ).

ATA, aspirin-tolerant asthma; AERD, aspirin-exacerbated respiratory disease; FEV<sub>1</sub>, forced expiratory volume in 1 s; SEM, standard error of the mean; F, female; M, male.

### Correlation analysis

There was a significant correlation between A23187-stimulated LXA<sub>4</sub> levels and FEV<sub>1</sub> ( $P < 0.0001$ ,  $r = 0.644$ ) (Fig. 4). There were no correlations between A23187-stimulated LXA<sub>4</sub> levels with asthma duration or age ( $P > 0.05$ ).

### Discussion

This study involved cases with matched severity degrees of mild, moderate and severe asthma with and without AERD and, therefore, provided a good opportunity for comparison of LXA<sub>4</sub> generation according to not only the severity of asthma but also to the presence of aspirin sensitivity. Our results documented that severe asthmatics in both asthma groups with and without AERD had a diminished capacity to generate LXA<sub>4</sub> compared with other severity degrees in their groups. Impaired LXA<sub>4</sub> generation in both severe groups was comparable with each other, with this pattern also being evident in other matched severity degrees.

Finding comparable LXA<sub>4</sub> generation in both asthma groups with and without AERD was in contrast with previous studies arguing more diminished capacity of LXA<sub>4</sub> formation in patients with AERD than in patients with ATA [21, 22]. The differences in the methodology and the patients' characteristics may explain this inconsistency. First of all, the severity, by itself, has not been

mentioned in these studies. If one extracts the inhaled steroid use and doses and FEV<sub>1</sub>% of the study groups, the former study [21] included mainly mild and some moderate asthma patients with comparable degrees in both asthma groups with and without aspirin sensitivity. The latter study [22], which was also performed with a different methodology, included more severe asthmatics in the AERD group than in the ATA group. In the current study, we included all severity degrees of asthma in both the AERD and ATA groups to make a matched comparison. Our data conclusively demonstrated that a deficiency of LXA<sub>4</sub> was related to severe asthma phenotype regardless of the presence of aspirin sensitivity. Regarding the relation between LXA<sub>4</sub> particularly in asthma without AERD, two recent publications also provided strong evidence for inadequate production of LXA<sub>4</sub> in severe cases with ATA [27, 28]. Moreover, another finding of strongly correlated LXA<sub>4</sub> levels with FEV<sub>1</sub> values in all asthmatics regardless of co-morbid aspirin sensitivity and stable LXA<sub>4</sub> levels after aspirin plus A23187 stimulation are supporting evidence of the consistency of the overall results.

Recent studies propose that severe asthma is a 'different phenotype' with a unique inflammatory cell profile such as neutrophil dominance, and cytokines such as IL-8 and TNF $\alpha$  [29–32]. Regarding the importance of diminished capacity of LX in severe asthma, LXA<sub>4</sub> has been shown to have a variety of anti-inflammatory properties in airways and this may be the key importance of diminished

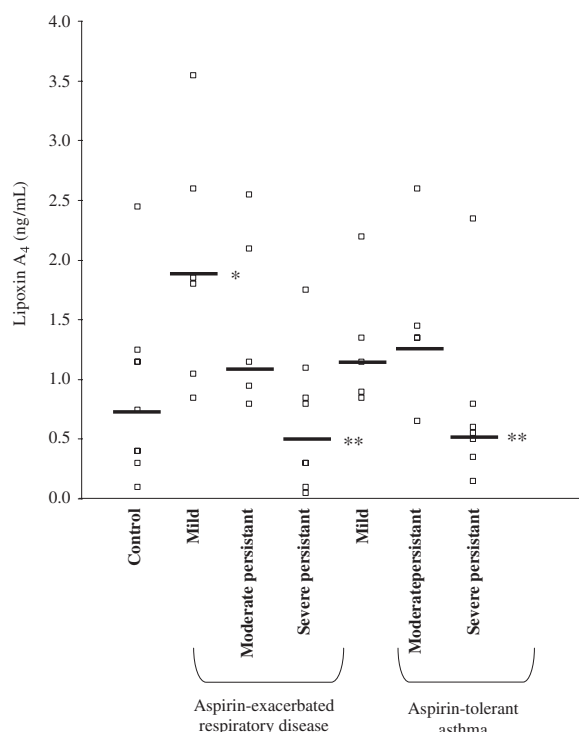


Fig. 1. Lipoxin (LX) $A_4$  levels (ng/mL) in peripheral venous blood of aspirin-exacerbated respiratory disease (AERD) and aspirin-tolerant asthma (ATA) patients after stimulation with A23187 ( $5 \times 10^{-5}$  M). Bars represent median levels. \* $P=0.028$ , higher in mild asthma patients with AERD in comparison with controls. \*\* $P=0.02$  for AERD group and  $P=0.046$  for ATA group, lower in severe asthma patients in comparison with mild and moderate asthma patients in their own groups.

capacity of LX in severe asthma. It binds not only to its 'specific receptor, ALX' but also to the cysLT receptor 1 [15–17]. The inflammatory actions of cysLTs in the asthmatics are well defined [33]. The interaction of LXA $_4$  with cysLT receptor 1 on the cell surface provides a functional antagonism of cysLTs. In contrast to cysLTs, LXA $_4$  inhibits bronchoconstriction and produces anti-inflammatory signals [34, 35]. Such an effect is beneficial for asthma and LX are called 'good lipids' for asthma and their therapeutic use is also a current topic of interest [36]. A recent trial in a murine model of asthma demonstrated that administration of a stable LXA $_4$  analogue had blocked airway hyperresponsiveness and pulmonary infiltration [37]. Hence, removal of all these beneficial effects might have a role in the presentation of a more severe asthma phenotype.

It is not clear why LXA $_4$  generation is diminished in severe asthma. LXA $_4$  formation requires cell-to-cell interactions and interactions between several lipoxygenases such as 15-LO (airway epithelium, eosinophils PMNs), 5-LO (leucocytes), 12-LO (platelets) and 5-LO (leucocytes) [15–18]. Decreased 15-HETE level, a product of 15-LO, in severe asthma patients was shown to decrease LXA $_4$  capacity by Levy et al. [28]. Regarding the cell source for LXA $_4$ , airway epithelium, eosinophils, PMNs (15-LO) and

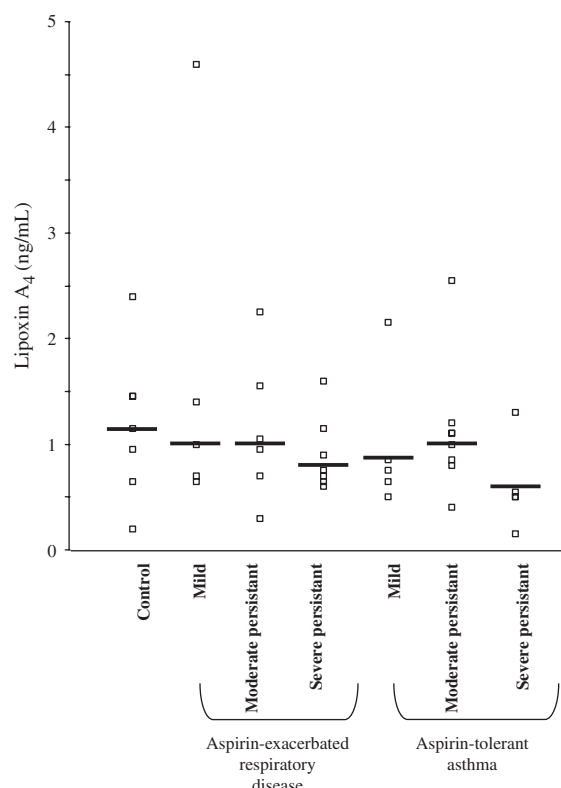


Fig. 2. Lipoxin (LX) $A_4$  levels (ng/mL) of aspirin-exacerbated respiratory disease (AERD) and aspirin-tolerant asthma (ATA) patients after stimulation with A23187 ( $5 \times 10^{-5}$  M) + aspirin ( $10^{-4}$  M). Bars represent median levels.

leucocytes (5-LO) and platelets (12-LO) all cause the production of LXA $_4$ . In our study, there was no difference between cell counts including eosinophil, platelets and leucocytes among groups in accordance with Levy et al. On the other hand, major activity for 15-LO in airways has been reported to have originated from airway epithelium and cytokine-primed alveolar macrophages, which is not expected to be detected in whole-blood samples.

In accordance with our findings, previous studies have shown increased levels for LXA $_4$  in mild asthma patients, suggesting an intact counterbalanced endogenous anti-inflammatory response in this group of patients keeping the disease status in mild form [27]. We also demonstrated that LXA $_4$  levels positively correlated with FEV $_1$  values in accordance with Levy et al. [28] suggesting an association between LXA $_4$  and airway obstruction. As the lipid mediators are potent regulators of airway tone and inflammation, this finding also raises the question about the effect of LXA $_4$  on the remodelling process in lower airways in addition to anti-inflammatory properties, as FEV $_1$  was known to be directly related to the airway characteristics.

The production of 15-epi-LXA $_4$  via COX-2 acetylation is another beneficial effect of aspirin on human beings

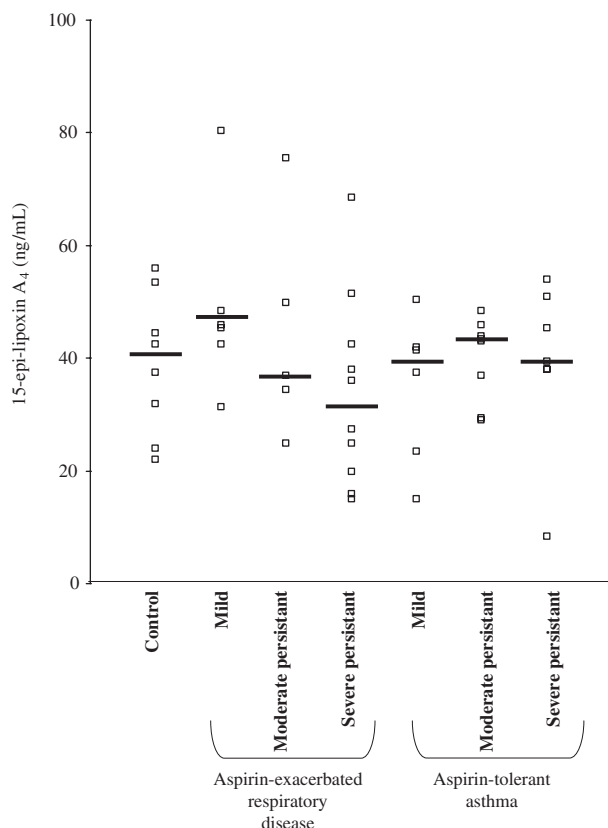


Fig. 3. 15-epi-lipoxin (LX) $A_4$  levels (ng/mL) of aspirin-exacerbated respiratory disease (AERD) and aspirin-tolerant asthma (ATA) patients after stimulation with A23187 ( $5 \times 10^{-5}$  M) + aspirin ( $10^{-4}$  M). Bars represent median levels.

[15]. Although a polymorphism in COX-2 has been reported in <5% of the population, the usual response to aspirin in a healthy subject is to produce ATLs [38]. For the first time, Sanak et al. [21] showed a diminished response to aspirin in terms of 15-epi-LXA $_4$  production in asthmatic subjects with AERD. Our data support this evidence while also demonstrating the tendency to become lower in severe patients with AERD. Current data suggest that the decrement in response was blocked in these particular groups of patients. Recent studies showed a consistent decreased mRNA expression of COX-2 enzyme on nasal polyp tissue of patients with AERD, which could explain the inhibited response to aspirin in terms of generation of 15-epi-LXA $_4$  [39–41]. Decreased NF $\kappa$ B activity has been shown to be a reason for decreased expression of COX-2 in this group of patients [42]. Contrary to this finding, Vachier et al. [27] demonstrated an increased level for 15-epi-LXA $_4$  without aspirin stimulation in induced sputum samples of patients with aspirin-intolerant asthma. As this value was obtained without aspirin stimulation, the increased activity of cytochrome P450, which is highly expressed in the human lung and able to produce 15-R-HETE from AA regardless of aspirin use, has been suggested for this increment. The differences in the

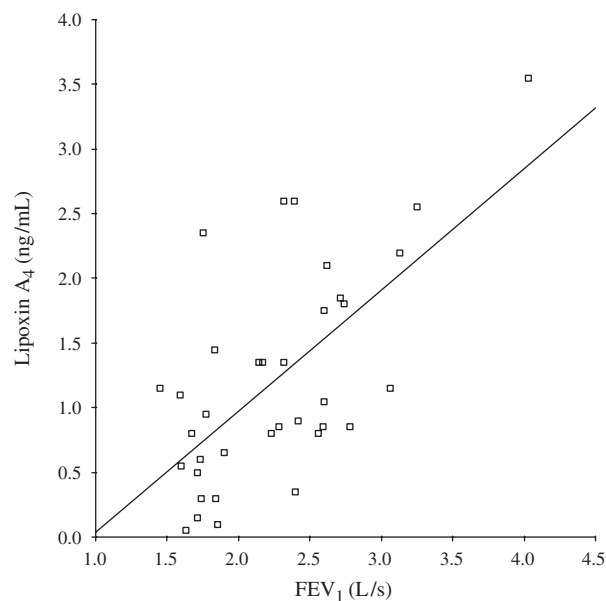


Fig. 4. Correlation analysis of forced expiratory volume in 1 s (FEV $_1$ ) and A23187 ( $5 \times 10^{-5}$  M) stimulated lipoxin (LX) $A_4$  levels ( $P < 0.0001$ ,  $r = 0.644$ ).

samples analysed (induced sputum vs. whole blood) and in the methods used (unstimulated vs. aspirin stimulated) in Vachier's and our studies, respectively, could explain this discrepancy.

In conclusion, although current guidelines recommend 'asthma control' for long-term evaluation, and the 'severity' phenotype only for research purposes, severe asthma with distinct inflammatory features and remodelling properties still has its place as a different asthma phenotype rather than being a simple 'grade' of the disease. In this theme, regardless of accompanying aspirin sensitivity, inadequate generation of LXA $_4$  seems to be one of the underlying mechanisms for the pathogenesis of 'severe asthma'. These findings might also help to provide a basis to develop and rationalize the use of new LX analogues for the critical management of these asthma patients.

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