

## Serum Lipoxin A4 Decreases in Exercise Induced Asthma

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## Abstract

**Background:** Lipoxin A4 is a potent anti- inflammatory and several lipoxin forming multicellular interactions occur during strenuous exercise**Aim of the study:** to evaluate diagnostic value of lipoxin A4 as a biomarker of exercise induced asthma**Subject& Methods:** This cross sectional study was conducted on forty- five asthmatic children divided in two groups; EIA group: 25 children with history of exercise- induced symptoms of asthma confirmed by spirometry, Non EIA group: 20 patients who did not have history of exercise induced symptoms of asthma. Forty- five apparently healthy children were included as a control group with no history of asthma or atopic conditions. Their age and sex were matched with the patients group. For all patients, pulmonary function tests were done before and after a free running test using portable spirometry. Serum levels of lipoxin A4 were estimated for patients (after exercise) and controls using ELISA**Results:** A significant difference was detected between the levels of lipoxin A4 in EIA and control groups (p value 0.000). There was significant difference between Non EIA and control groups regarding the level of lipoxin A4 with (P value0.000). The mean level of lipoxin A4 in EIA patients was lower than that of Non EIA patients; however, the difference was statistically insignificant (P value 0.973).**Conclusion:** Lipoxin A4 is a promising anti- inflammaory factor in management of asthma. It correlates with degree of reduction in FEV1 in EIA. Future pharmaceutical studies should consider lipoxin as a new therapeutic treatment in EIA.**Keywords:** Lipoxin A4- exercise induced asthma- bronchial asthma

## تغيير مستوى الليبوكسين أ٤ فى الدم فى حالات الربو الشعبى الناتج عن المجهود

يمثل الليبوكسين أ٤ إشارة تنظيميه مضاده قويه لوسائط الالتهاب التى تشمل اليوكوتريينات وعامل تنشيط الصفائح الدمويه والذى يودى الى تثبيط عملية الالتهاب التى تقوم بها كرات الدم البيضاء.

**الهدف:** كان الهدف من هذه الدراسة هو محاولة إيجاد العلاقة بين نسبة الليبوكسين أ٤ فى الدم والإصابة بالربو الشعبى المصاحب للمجهود. المرضى وطرق البحث: ضمت هذه الدراره تسعون طفلا تم تقسيمهم المجموعتين، المجموعه الأولى: ضمت خمسة وأربعون طفلا ممن يعانون من الربو الشعبى تتراوح اعمارهم بين (٦- ١٢) سنه تم اختيارهم من عيادة الحساسيه والمناعه وعيادة الصدر بمستشفى الأطفال- جامعة عين شمس، ثم تقسيمهم الى مجموعتين: مجموعه الربو الشعبى غير مصاحب للمجهود وتضم ٢٥ طفلا يعانون من الربو الشعبى غير مصاحب للمجهود. مجموعه الربو الشعبى المصاحب للمجهود وتضم ٢٠ طفلا ليس لديهم اعراض الربو الشعبى المصاحب للمجهود. والمجموعه الثانيه: تضم خمس واربعون طفلا من نفس العمر والجنس ولا يعانون من اى امراض وقد تم اخضاع جميع الأطفال الى قياس وظائف التنفس قبل وبعد المجهود وقياس نسبة الليبوكسين أ٤.**النتائج:** بعد تحليل النتائج لوحظ أن: وظائف التنفس كانت اقل من فى الأطفال اللذين يعانون من الربو الشعبى المصاحب للمجهود بالمقارنه مع باقى الأطفال، ونسبه الليبوكسين أ٤ كانت اقل بنسبه ملحوظه فى الأطفال اللذين يعانون من الربو الشعبى المصاحب للمجهود بالمقارنه مع الأطفال اللذين يعانون من الربو الشعبى، ولكن لم يكن هناك فرق ذو دلالة إحصائية فى نسبة الليبوكسين أ٤ بين الأطفال اللذين يعانون من الربو الشعبى المصاحب للمجهود والأطفال اللذين يعانون من الربو الشعبى بدون اعراض مصاحبه للمجهود.**الاستنتاج:** يعتبر الليبوكسين أ٤ عامل واعد فى علاج الربو الشعبى وخاصة الربو الشعبى المصاحب للمجهود.

## Introduction:

Exercise induced asthma (EIA) can be demonstrated in up to 70% of patients with asthma, and in other individuals who have EIA in the absence of additional features of asthma. The pathogenesis of EIA is poorly understood. Although conditioning of the inspired air, leading to drying and cooling of the intrathoracic airways, may serve as the initial trigger for EIA, the subsequent events in the airways are unclear.<sup>(3)</sup>

Airway cooling and vasoconstriction may contribute to EIA via respiratory heat loss, followed by secondary reactive congestion, edema and further narrowing of the airways.<sup>(11)</sup> The severity of obstruction in asthmatic patients is determined by the amount of heat loss from the respiratory tract during exercise.<sup>(1)</sup>

Lipoxin A4 is a biological active lipooxygenase interaction product derived from arachidonic acid. Lipoxins and 15 epilipoxins are lipid mediators that modulate leucocyte trafficking and promote inflammation potential mediators or modulators of inflammation within the lungs.<sup>(4)</sup> Lipoxin A4 is a potent counter regulator signal for endogenous proinflammatory mediators including leukotriens and platelet activating factor resulting in inhibition of leukocyte dependent inflammation.<sup>(10)</sup>

As Lipoxin A4 is a potent anti inflammatory and several lipoxin forming multicellular interactions occur during strenuous, an urge is needed to evaluate the lipoxin A4 after exercise.

## Subjects And Methods

This cross sectional study was conducted on forty- five asthmatic children, with mild intermittent asthma diagnosed according to GINA.<sup>(7)</sup> they were selected from both Pediatric Allergy and Immunology clinic and Pediatric Chest clinic Children's Hospital Ain Shames University, during the period from September 2011 to August 2014. They comprised 25 females and 20 males. Their ages ranged from (6- 12) years.

The patients' group was divided into 2 groups; Exercise induced asthma group This group included 25 children with history of exercise induced symptoms of asthma in the form of cough, wheezing and shortness of breath after exercise. Their mean age was (8± 2.3) years with range from (6- 12) years. 14 (56%) of them were females and 11 (44%) were males. Their mean absolute weight was (34.4± 8.5) kg ranging from (23- 50) kg, mean absolute height was (132.5± 12.2) ranging from (116- 153) cm, and BMI were (19.2± 2). They were neither obese nor underweight.

Non- exercise induced asthma: This group included 20 patients who didn't have history of exercise induced symptoms of asthma. Their mean age was (8.6± 2.4) years with range from (6- 12) years. 11 (55%) of them were females and 9 (45%) were males. Their mean absolute weight was (31.7± 10.9) kg with range from (19- 52) kg. Their mean absolute height was (130.3± 13.3) cm with range from (114- 150) cm, and their BMI was (17.8± 2.8). None of them was either obese or underweight.

Control group: It consisted of 45 of apparently healthy children with no history of asthma or atopic conditions. Their age and sex were matched with the patients group. Their mean age was (8.7± 2.2) years

range from (6- 12) years. 23 (51%) of them were females and 22 (48%) were males. Their mean absolute weight was (30.2± 8.8) ranging from (19- 46) kg, their mean absolute height was (130.3± 13.2) cm ranging from (110- 154) cm, and their mean BMI was (17.4± 1.8). They were selected from Outpatients' clinic visits to Ophthalmology clinics.

### Exclusion Criteria:

1. Children with moderate to severe asthma were excluded, to avoid exacerbation of their asthma during exercise test.
2. Presence of upper respiratory or lower respiratory tract infection or an asthma exacerbation within the last six weeks, or those receiving controller therapy for asthma.
3. Wheezing at physical examination prior to exercise test.
4. Baseline FEV1 of less than 80% of the predicted and physical exercise limitation.
5. Obesity as a cofactor for exercise induced asthma was excluded among both patients and control groups.
6. During the test some of the patients couldn't finish the challenge test they got severe attack of asthma exacerbation and we gave them rescue medications so we couldn't get the sample.
7. Others start to feel fatigue and the parents refused to continue, so we excluded them from the study

## Methods:

1. History And Clinical Examination: Full medical history was taken from all patients laying stress on frequency of acute attacks, nocturnal symptoms, wheezing, episodic symptoms after exercise, predisposing factors, drug therapy, degree of clinical severity and positive family history of asthma.

The patients were examined clinically including BMI centiles were estimated according to the Egyptian growth curves none of them was obese or underweight. Vital data body temperature in Celsius, respiratory rate/ min and heart rate/ min were recorded and evaluated against normal values for age.

2. Laboratory Investigations: Blood samples were collected from all the patients and examined for Complete blood count including white blood cell count and absolute eosinophilic count using (Coulter Microdiff 18, Fullerton, CA, USA), Serum IgE by (Medix Biotech, Inc. Agenzyme company. Industrial Road, San Carlos; USA)
3. Pulmonary Function Tests: Spirometry tests were done before and after free running test. It was performed using portable spirometry (Jaeger, Germany).

The time of the test was in in Autumn to ensure proper temperature between (22- 30)°C. it was performed in a 50 meters corridor with signs on each 15 meters. The caregivers were asked to put comfortable clothes and shoes for the patient and to provide a light meal in the morning for child.

Steps were explained to the child and the caregiver who attend the testing. Patient data (Name, Sex, Age, Bodyweight and Height).

Physical examination was done for the patient to exclude signs of

bronchospasm. The baseline respiratory rate and heart rate were monitored by portable pulse oximetry

4. Procedure of Spirometry: The nasal clip was applied to the patient's nose who would breathe by the mouth. The test was started by breathing at rest for a few moments then when ready to start the patient inspired slowly as much air as possible and then made a complete expiration as fast as possible.

The child then started run freely back and forth in a 50 meters long corridor for 6 minutes, with monitoring of the heart rate every 15 meters to achieve 80% increase in the heart rate.

The pulmonary function test was repeated after completion of the running test.

5. Estimation of serum level of Lipoxin A4: Blood sample was collected within (5- 10) minutes after exercise in serum separator tubes. Serum was collected and stored in aliquots at -20°C for (1- 3) months according to manufacturer instructions. Serum levels of lipoxin A4 were estimated using General Lipoxin A4 ELISA kit (Catalog No. E1452Ge), (EIAab, China). The test procedure was performed according to the manufacturer. The optical density of each well was determined at once, using a microplate reader set to 450 nm.

**Statistical Methods:**

Data were analyzed using standard computer program SPSS window

Table (1) Comparison between the two patient groups regarding socio- demographic and the Anthropometric measures (Mann- Witney test).

	Exercise Induced N= 25	Non Exercise induced N= 20	t Value	P value	Sig
Age (Years) (Mean± Sd)	8.88± 2.28	8.55± 2.42	-0.721	0.0471	NS
Risk Factors:	1. Crowding Index (Mean± SD)	2.79± 1.90	2.5± 1.12	1.7± 1.26	NS
	2. Parental smoking index (Mean± SD)	-0.344	-0.3 89	0.0731	0.0697
Weight (Kg) (Mean± SD)	34.44± 8.49	31.7± 10.98	- 1.19	0.0234	NS
Weight Centiles	67± 19.20	60.75± 13.87	- 1.29	0.0196	NS
Height (Cm) (Mean± Sd)	132.52± 12.25	130.3± 13.26	-0.9 61	0.0337	NS
Height Centiles	58.2± 15.67	56± 15.27	-0.5 08	0.0612	NS
BMI	19.28± 2.07	17.8± 2.84	- 1.86	0.062	NS
Bmi Centiles	70.80± 16.37	51.75± 23.56	-2.87	0.004	S

Table (2) Comparison between the two groups of patients and control regarding FEV1 before and after exercise and percentage of reduction and Lipoxin A4 (Independent samples t test).

	Exercise Induced Asthma N= 25 Mean± Sd	Non Exercise Induced Asthma N= 20 Mean± Sd	Control Groups N= 45 Mean± Sd	P1 value	P2 value	P3 value
FEV1% of expected before exercise	83± 9.79	87.75± 10.57	92.22± 8.01	0.000	0.0553	0.061
FEV1% of expected after exercise	65± 5.59	86.25± 10.37	90.33± 7.26	0.000	0.0668	0.000
Percentag of reduction in FEV1% of expected after exercise	19.2± 5.33	1.5± 2.86	1.8± 2.87	0.000	0.0616	0.000
Serum Lipoxin A4 (Ng/MI)	71.12± 48.78	78.05± 89.39	223± 215.32	0.000	0.000	0.973

P1 Between EIA and Control groups, P2 Between Non EIA and Control groups, P3 Between EIA and Non EIA

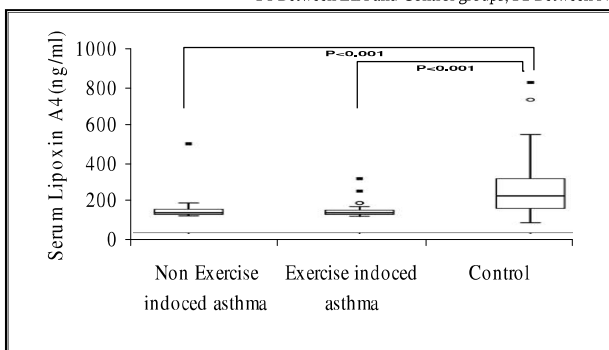


Figure (1) Serum lipoxin A4 in both patients groups and control.

version (SPSS Corporation, USA).<sup>(13)</sup> Continuous data were expressed in the form of Mean± SD. Categorical data we expressed in the form of number and percent. For comparison of continuous data we used student t test while categorical data we recompared used chi- square test. Anova test was used for comparing more than two groups in quantitative data.

Linear regression analysis was used to determine the value of certain variables as predictors of the study outcome. Correlation between variables was done using correlation coefficient (r). This test detects if the change in one variable was accompanied by corresponding change in the other variable or not. ROC curve had been used to estimate the cutoff point.

**Results:**

Comparative sociodemographic data between the two patients' group were plotted in table (1). Serum lipoxin levels and pulmonary function tests in two patients groups and control are shown in table (2), figure (1) show lipoxin A4 in both patient groups and control. Correlations between serum lipoxin A4 and other parameters are demonstrated in table (3). Cutoff value were calculated through ROC curve figure (2). It was found that the best cutoff value of serum Lipoxin A4 was 100 ng/ ml with 84% sensitivity and 33% specificity. So the levels below 100 ng/ ml were considered to have EIA.

This figure showed significant difference between the two patients groups and the control group regarding the Lipoxin A4 level with no significant difference between the two patients groups.

Table (3) Correlation between serum Lipoxin A4 and clinical parameters among Exercise induced Asthma patients group

	Serum Lipoxin- A4	r <sup>2</sup>	P Value	Sig.
Smoking Index	2± 1.10	0.531	0.062	NS
Crowding Index	2.79± 1.90	- 0.139	0.507	NS
BMI	19.28± 2.07	0.151	0.470	NS
Bmi Centiles	70.80± 16.37	0.247	0.233	NS
Ige (IU)	573.40± 287.89	- 0.139	0.397	NS
Eosinophil (cells/ml)	343.32± 316.4	- 0.193	0.205	NS
Fev1 Before	83± 9.79	- 0.056	0.790	NS
Fev1 After	65± 5.59	0.364	0.074	NS
Percentage of reduction of expected FEV1	19.2± 5.33	-0.227	0.040	S

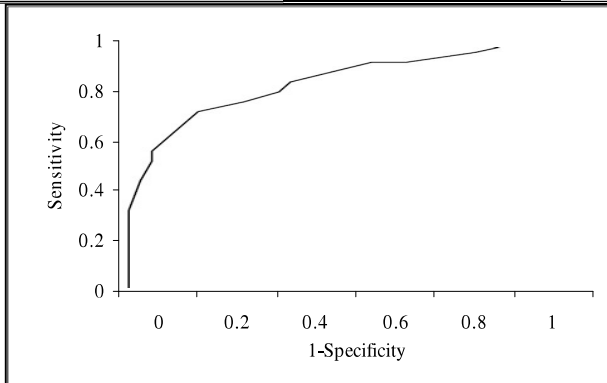


Figure (2) Receiving Operating Characteristic (Roc) curve to define the best cutoff value of serum Lipoxin

### Discussion:

Exercise induced bronchospasm (EIA) refers to the condition where exercise precipitates airway obstruction, but the person has normal lung function at rest. The term EIA is sometimes used to refer to persons who have exacerbation of their chronic asthma during exercise. We use the term EIA to encompass both this latter condition as well as EIB.<sup>(12)</sup>

Gangemi et.al.<sup>(6)</sup> evaluated urinary excretion of LXA4 and related compounds in healthy volunteers. It showed that maximal physical exertion is accompanied by a rapid increase in the urinary excretion of LXA4, which is likely to reflect in vivo cell- to- cell interactions and to represent a defense mechanism against stress- induced inflammation.

In our study there were 71% of patients atopic with a mean of lipoxin level is (69.06 ng/ml), although there was no significant difference between the atopic and non- atopic patients regarding the lipoxin A4 level but the mean values were less in atopic than in non- atopic patients.

In the present study, LxA4 showed a statistically significant positive relation with pulmonary function tests, specially FEV1 and percentage of reduction in predicted FEV1. Celik<sup>(5)</sup> found that LxA4 level correlated positively with pulmonary function tests, suggesting a link between biosynthetic capacity of this bioactive lipid mediator and air- flow obstruction, especially in asthma patients, while, the association between LxA4 and pulmonary function tests is significant only with FEV1 and not with other tests.

In this series, there was a significant correlation between the percentage of reduction in FEV1 and BMI centiles with p value of 0.02, which reflects the effect of obesity on EIB asthmatic patients.

There was a significant decrease in the level of lipoxin A4 between

asthmatic patients with mean (74.20± 68.99) ng/ ml and in control group with mean (223± 215.32) ng/ ml with highly significant p value 0.000.

Regarding the level of lipoxin A4 in between EIB and control groups there was a highly significant difference with decrease in the level of lipoxinA4 after exercise in EIB with mean (71.12± 48.78) ng/ ml in comparison to control with mean (223± 215.32) ng/ ml, with P value 0.000.

Also between Non EIB with mean (78.05± 89.39) ng/ ml and control group (223± 215.32) ng/ ml with mean (there was significant difference with p value 0.000.

An increase in LX biosynthesis during exercise may have relevant pathophysiological implications. LX function as stop signals during inflammation, and their role in the resolution of the inflammatory response has been recently elucidated. Moreover, LX possesses vasodilatory properties. Thus, LX production in the course of physical exercise may, on one side, counterbalance the action of exercise- induced proinflammatory mediators.<sup>(9)</sup>

On the other side there was no significant difference in between EIB with mean (71.12± 48.78) ng/ ml and Non EIB asthmatic patients with mean (78.05± 89.39) ng/ ml with P value 0.937 although there was decrease in the mean in between the two groups and this could be explained by the small number of both groups and if this study could be done on large number we will find a significant difference. Also our asthmatic patients are mild asthmatic and there was a study revealed that the level of lipoxin A4 is not decreasing too much like in severe asthma.

A study by Mahmoud et.al.<sup>(8)</sup> revealed that increased level of LxA4 in asthmatic groups more than the control, more- ever, there was significant progressive decline of LxA4 from mild to severe form of asthma, and this could be explained by the patients with mild asthma phenotype are considered fighters, having capability of producing anti- inflammatory mediator LxA4 to counter- balance the pro- inflammatory ones, thus abating the inflammatory process, resulting in a mild form of asthma, while patients with severe asthma are weak fighter seems incapable of achieving enough levels of LxA4 to resolve the inflammation, resulting in a severe asthma phenotype.<sup>(2)</sup>

Sterneous exercise may induce lipoxin biosynthesis which observed within 10 seconds and > 60% of it will be metabolized within 30seconds by peripheral monocytes. Increase in lipoxin A4 biosynthesis has an important role in resolution of inflammation response and their production in course of physical exercise may suppress the actin of EIB.<sup>(5)</sup>

This might explain why there was no significant difference between EIA and non- EIA as we need to go through more sterneous exercise and to try to take the sample within 30 seconds after the stoppage of exercise to ensure accurate estimation of lipoxin A4.

In the present study, there was a negative correlation between percentage of reduction in FEV1 and lipoxin A4 and this could be explained by the anti- inflammatory role of Lipoxin A4 so can protect against the occurrence of EIA, and airway hyperresponsiveness which will

improve the FEV1 after exercise. The effect of lipoxinA4 as anti-inflammatory is a promising factor to help in decrease the EIA.<sup>(2)</sup>

**Conclusion:**

Lipoxin A4 is a promising anti inflammatory factor in management of asthma as the decreased capability of lipoxin A4 in exercise asthma could be the cause of occurrence of exercise induced bronchoconstriction. Lipoxin mimetic and compounds could provide a novel therapeutic approach in the treatment of Exercise induced Asthma.

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