Allergol Immunopathol (Madr). 2012;40(3):181–186

Allergologia et immunopathologia

www.elsevier.es/ai

ORIGINAL ARTICLE

Urinary leukotriene excretion profile in children with exercise-induced asthma compared with controls: A preliminary study

P.E. Brockmann[a,*], J.A. Castro-Rodriguez[a], N.L. Holmgren[a], J. Cerda[b], A. María Contreras[c], A. Moya[d], P.J. Bertrand[a]

[a] *Department of Pediatrics, Pontificia Universidad Catolica de Chile, Santiago, Chile*
[b] *Department of Public Health, Pontificia Universidad Catolica de Chile, Santiago, Chile*
[c] *Center of Medical Investigation, Pontificia Universidad Catolica de Chile, Santiago, Chile*
[d] *Pediatric Lung Function Laboratory, Pontificia Universidad Catolica de Chile, Santiago, Chile*

Received 13 February 2011; accepted 29 March 2011
Available online 23 June 2011

KEYWORDS
Inflammation;
Exercise;
Paediatric;
Asthma;
Lung function

Abstract
Background: Leukotrienes are among the most important mediators associated with inflammatory responses in patients with exercise induced asthma (EIA). The aim of this study was to investigate the impact of exercise on the urinary leukotriene profile. Hence, we compared post exercise changes of urinary leukotriene E4 (LTE4) concentration between children with EIA and healthy controls.
Methods: Ten children with EIA and 15 controls were enrolled. Both groups underwent a standardised exercise challenge test (ECT). LTE4 concentration was measured in urine samples obtained pre and post ECT, using enzyme immunoassay and adjusted by urinary creatinine concentrations.
Results: Median (minimum–maximum) pre ECT concentration of LTE4 was 17.82 (7.58–90.23 pg/ml) in EIA and 17.24 (4.64–64.02 pg/ml) in controls, $p = 0.86$. LTE4 concentration post ECT were 23.37 (4.02–93.00 pg/ml) in EIA and 11.74 (0.13–25.09 pg/ml) in controls, $p = 0.02$. Changes of LTE4 concentration post ECT were 2.54 (−31.98 to 43.31 pg/ml) in cases and −13.53 (−46.00 to 11.02 pg/ml) in controls, $p = 0.03$. There was no significant correlation between basal predicted FEV_1 [%] and changes in LTE4 concentration in cases (i.e., $r_s = 0.14$) nor controls (i.e., $r_s = 0.12$). There was a tendency towards more pronounced changes in LTE4 concentration post ECT in children with moderate/mild persistent asthma compared to those with mild but intermittent asthma.

Abbreviations: EIA, exercise-induced asthma; ECT, exercise challenge test; FEF 25-75, forced expiratory flow 25–75%; FEV_1, forced expiratory volume in 1st second; FVC, forced vital capacity; LTE 4, leukotriene E 4; Max, maximum; Min, minimum; r_s, Spearman's correlation test.

* Corresponding author.
E-mail address: pbrockmann@med.puc.cl (P.E. Brockmann).

doi:10.1016/j.aller.2011.03.012

Conclusions: Children with EIA had significantly higher changes of urinary LTE4 concentrations post ECT compared to healthy controls. Urinary measurement of LTE4 may be an interesting and non-invasive option to assess control of EIA in children.

Introduction

Exercise-induced symptoms are common among children with asthma. Coughing is the most common symptom associated with exercise, with a frequency of approximately 70–80% in untreated patients.[1] Exercise-induced symptoms produce limitations in daily activity[2–4] and a poorer quality of life.[5] Therefore, early recognition of non-controlled exercise-induced asthma (EIA) may be important for adjustment of treatment and prevention of physical and psychological effects.[5,6] Nevertheless, this assessment may be especially difficult in children using conventional methods like spirometry, peak flow or clinical symptoms.[7] In recent years, markers of airway inflammation, such as fractional exhaled nitric oxide, induced sputum eosinophils and urinary leukotriene E4 (LTE4), have been studied and proposed for better assessment of the inflammatory state of children with asthma.[8–10]

Leukotrienes are among the most important cytokines involved in exercise-induced inflammatory response.[11] They are products of arachidonic acid metabolism by the cyclooyxgenase 1/2 pathways.[12–14] Since leukotriene E4 (LTE4) is the end product of all cysteinyl-LT metabolisms in the lung,[11] measuring urinary leukotrienes may be an interesting option for assessing the inflammatory state in children with asthma.

The role of leukotrienes in EIA has been evidenced by a sustained increase in their concentration 30–90 min after exercise challenge in adults.[15] However, in children with asthma, the specific profile of urinary leukotriene excretion and its association with the clinical severity of asthma has not yet been clarified.

We aimed to determine if urinary LTE4 concentration shows a disease-specific pattern and could therefore be useful as a non-invasive marker to compare post exercise LTE4 urinary excretion in children with EIA versus healthy controls.

Methods

Children between 6 and 17 years of age with EIA (cases) and healthy children (controls) were enrolled prospectively in 2008. Cases were enrolled among children with physician diagnosed EIA (symptoms like wheezing, cough, or dyspnoea clearly associated with exercise[16]) who were routinely sent to our respiratory laboratory to perform an exercise challenge test (ECT). All children who presented any disease other than asthma; admitted active tobacco smoking; or had received long-term treatment with inhaled or oral corticosteroids, montelukast or antihistamines (the latter for at least seven days before the ECT), were excluded from this group. As controls, healthy volunteers (children of hospital co-workers) who were age-matched to cases were selected. In this group, children who presented any chronic underlying disease; admitted active smoking; or had used inhaled or oral corticosteroids, montelukast or antihistamines were also excluded. In both groups, children with any acute respiratory infection in the last three weeks were excluded.

After explaining the purpose of the study, informed consent was obtained from parents and children. Age, gender and reported weekly hours of physical activity were assessed for both groups. Parents of cases were also asked about the use of medications, the date of the EIA diagnosis, visits to the emergency department and hospitalisation. Cases were classified as suffering from mild intermittent, mild persistent, moderate persistent or severe asthma according to the Global Initiative for Asthma Guidelines (http://www.ginasthma.org).

The ECT was performed at the paediatric pulmonary function laboratory according to guidelines.[17] In both groups (cases and controls) the ECT was conducted between 9 am and 4 pm under controlled environmental conditions (21 °C and 45% relative humidity), in order to achieve standardisation of known factors that influence bronchial response to exercise.[17] Spirometry (Schiller SP 100®, Switzerland) was carried out with children standing and wearing a nose clip. At least three baseline spirometric measurements were performed in order to ensure appropriate acceptability and reproducibility of the test.[17] Subsequently, subjects performed a sub maximum effort, defined as a high intensity test of six minutes on a treadmill (Stracrac model ST-1600®) at 3.6 miles/h and 10° elevation. Heart rate reached at least 80% of theoretical maximum for patient age (220 - age). This variable was monitored with a Polar F1™ fitness monitor placed around the thorax. Arterial saturation was monitored with Masimo RAD9® (Irvine, CA, USA) equipment. A nose clip was worn to ensure breathing through the mouth. After each spirometry, the children's breathing was monitored with a stethoscope to detect wheezing.

Once the stimulus (exercise) was completed, spirometry was repeated at 1, 3, 5, 7, 10, 15 and 30 min. Two or three spirometric manoeuvres were performed for each measurement, with a maximum difference of ±5% in forced expiratory volumen 1st second (FEV_1). FEV_1 and forced expiratory flow 25-75% (FEF_{25-75}) was registered for each measurement, choosing the best corresponding FEV_1 manoeuvre. After obtaining a fall in FEV_1 from the baseline, we analysed the flow volume curve shape, which allowed testing for quality control of the spirometric effort. After the ECT, every child received 200 mcg of salbutamol MDI using a breathing mask, and performed spirometry again to ensure the return to baseline values. ECT was considered positive if: (i) the decrease of FEV_1 was >10% of baseline; or (ii) if wheezing and dyspnoea were determined.[16]

Urine samples were collected immediately before starting the ECT. A second urine sample was collected

Table 1 Comparison of demographic and pulmonary function variables between cases and controls.

Variable	Cases ($n = 10$)	Controls ($n = 15$)	p-Value
Girls/boys	4/6	4/11	0.79
Age [years]	10.1 (7.6–15.5)	10.6 (7.2–17.6)	0.37
Weekly hours of physical activity	1.5 (0–8)	2.0 (0–12)	0.85
FEV_1 predicted [%]	118 (85–150)	123 (88–136)	0.53
FVC predicted) [%]	116 (88–155)	116 (85–135)	0.73
FEF25–75 predicted [%]	99 (67–139)	97 (68–144)	0.73
Maximum fall of FEV_1 after ECT [%]	14.0 (8–59)	1.0 (0–7)	<0.0001
Time for maximum fall in FEV_1 [min]	3.0 (1–3)	1.0 (0–7)	0.17

Gender is expressed as *n*; all other variables as median (min–max).
ECT: exercise challenge test; FEF 25–77: forced expiratory flow 25–75%; FEV_1: forced expiratory volume in 1st second; FVC: forced vital capacity.

30 min after finishing the ECT. Urine samples were then refrigerated at −70 °C and saved until all samples were processed. LTE4 in urine was measured by ACETM Enzyme Immunoassay Kit® (Cayman Chemical, Ann Arbor, MI, USA), according to recommendations.[18] Urine LTE4 concentration was reported in pg/ml and standardised per mg/ml of creatinine. Therefore, all urinary LTE4 concentrations were reported as pg/mg of creatinine.

This protocol was approved by the Ethics Committee of the Pontificia Universidad Católica de Chile and parents authorised their children's participation by signing informed consent forms.

Statistical analysis

In this preliminary phase of the study, the objective was to compare LTE4's excretion profile in cases and controls. Statistical analyses were performed using statistical software (Statistical Package for the Social Sciences, Version 15 for Windows; SPSS; Chicago, IL, USA). A comparison of demographic and lung function variables between cases and controls was performed using the X^2 test for categorical variables and the Mann–Whitney *U*-test for continuous variables. The distribution of urinary LTE4 concentrations was analysed before and after performing ECT. These distributions were compared between cases and controls using the Mann–Whitney *U* test. For each subject the change in urinary LTE4 concentrations after ECT (post ECT concentration − basal concentration) was calculated and its distribution was compared between groups using the Mann–Whitney *U* test. the correlations (non-parametric correlations) according to Spearman [r_s] were estimated between changes in urinary LTE4 concentrations post ECT and pulmonary function variables or reported hours of physical activity. The Kruskal–Wallis test was used to investigate associations between changes in urinary LTE4 concentrations post ECT and clinical asthma severity. p-Values <0.05 were considered statistically significant.

Results

Of the 29 children initially recruited, four were excluded from the analysis. Two patients with EIA and one control were excluded because they were found to have previously received inhaled corticosteroids. An additional control subject was excluded for being an ex-smoker. Twenty-five subjects were finally analyzed, 10 with EIA (six boys) and 15 controls (11 boys). All of them completed the ECT successfully and reached the target heart rate. There were no differences between cases and controls in terms of age, weekly hours of physical activity, nor basal lung function. However, there were significantly higher decreases of FEV_1 (%) after ECT among cases than controls (14 [8–59] vs. 1 [0–7], respectively, $p < 0.0001$) (see Table 1). Among cases, four wheezed after full completion of ECT and required bronchodilator therapy in situ. After the ECT, all cases either developed a fall in FEV_1 >10%, or had wheezing and had therefore, a positive ECT. In contrast to that, none of the controls developed such findings and all had therefore a negative ECT.

Children with EIA were classified as: mild intermittent ($n = 4$); mild persistent ($n = 4$); and moderate persistent ($n = 2$). In this group, median (min–max) time of the asthma diagnosis was 0.23 (0–13) years; the number of asthma crises in the last year that needed treatment was one (1–6); the number of visits to the emergency department in the last year was one (0–8); and hospitalisations were zero (0–5).

Baseline median (min–max) values of LTE4 concentrations [pg/mg creatinine] were similar between cases and controls (17.8 [7.6–90.2] vs. 17.2 [4.6–64.0], respectively, $p = 0.86$). However, LTE4 concentrations post ECT were significantly higher among cases versus controls (23.8 [4.0–93] vs. 11.7 [0.1–25.1], respectively, $p = 0.02$). Also, changes of LTE4 concentration pre and post ECT were significantly different among cases versus controls (2.5 [−32 to 43.3] vs. −13.5 [−46 to 11] in controls, $p = 0.03$), Fig. 1. Individual changes in LTE4 concentrations in cases and controls are shown in Figs. 2 and 3, respectively. There was a tendency towards higher changes in LTE4 concentration post ECT in children with more severe asthma ($p = 0.11$), Table 2.

There was no significant correlation between basal % predicted FEV_1 and changes in LTE4 concentration in cases ($r_s = 0.14$) and controls ($r_s = 0.12$). Also, there was no significant correlation between absolute or predicted values of FVC, FEF25-75 and FEV_1 and changes in LTE4 concentration pre and post ECT (all $r_s < 0.20$). There was a significant cor-

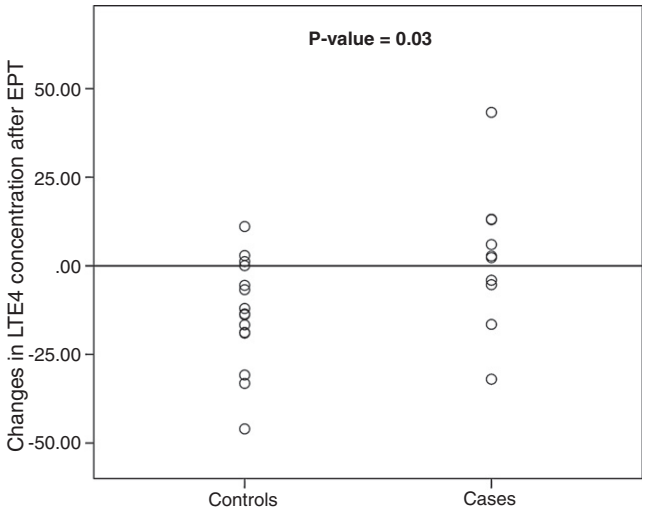

Figure 1 Comparison of changes in urinary LTE4 concentration post-ECT between cases and controls.

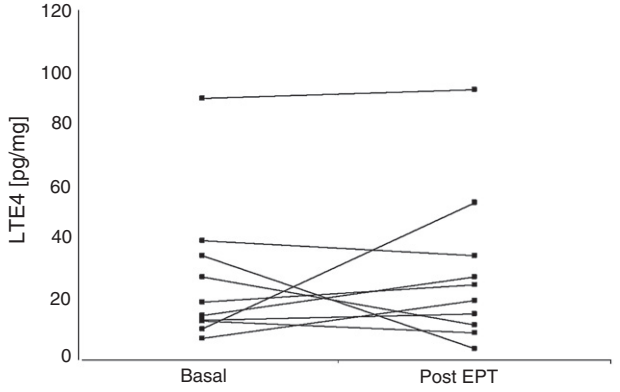

Figure 2 Intraindividual changes in urinary LTE4 concentrations in cases.

relation between the maximum fall in FEV_1 and changes in LTE4 concentration ($r_s = 0.69$) in cases but not in controls ($r_s = 0.19$). Correlation between changes in LTE4 concentration post ECT and weekly hours of physical activity was $r_s = 0.09$ ($p = 0.80$) in cases, and $r_s = 0.49$ ($p = 0.05$) in controls.

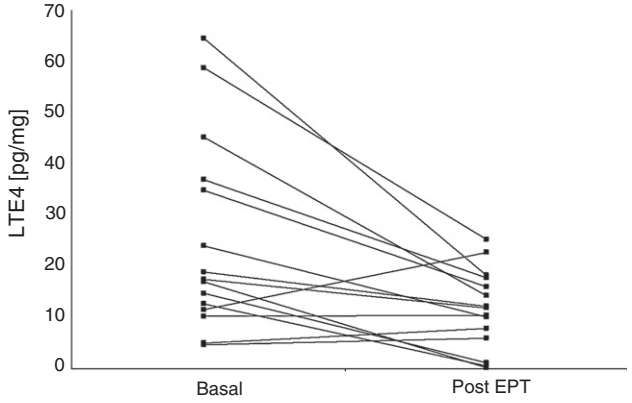

Figure 3 Intraindividual changes in urinary LTE4 concentrations in controls.

Table 2 Comparison of changes in urinary LTE4 concentration after ECT stratified by asthma severity.

Asthma severity classification	Change in urinary LTE4 concentration [pg/mg] after exercise test
No asthma ($n = 15$)	−15.3 (−81.3; +13.0)
Mild intermittent ($n = 4$)	−4.1 (−17.7; +109.6)
Mild persistent ($n = 4$)	+9.1 (−103.4; +15.8)
Moderate persistent ($n = 2$)	+12.6 (+8.4; +16.8)

All results given as median (min–max).
Kruskal–Wallis test between asthma severity groups: p-value = 0.11.
LTE4: leukotriene E4; ECT: exercise challenge test.

Discussion

This study demonstrated a different pattern in urinary LTE4 concentration post ECT in subjects with asthma compared to healthy children. As this response was observed within 30 min of the running test, this may suggest a different and rapid pattern of LTE4 excretion after exercise in children with EIA compared to healthy controls. Interestingly, this response seems rapid enough to be assigned to the preceding exercise and could possibly be useful for the assessment of non-controlled inflammation in EIA.

This pattern of LTE4 changes after exercise may reflect a specific and rapidmetabolism of inflammatory mediators in children with EIA. Interestingly, intraindividual changes showed that most of the healthy controls had a decrease in LTE4 concentration after ECT. In contrast to that, LTE4 concentration was stable in children with EIA, and increased in some cases.

Nevertheless, there is conflicting evidence about changes in LTE4 after exercise. Kikawa et al. showed no significant differences between basal LTE4 concentration in children with asthma compared to healthy peers, but a significant increase only among asthmatics.[19] On the other hand, others have shown no increased urinary excretion of LTE after exercise, nor an association with exercise-induced bronchospasm.[15]

In adults, Reiss et al. showed an increase in urinary leukotrienes and an attenuation of that response using a leukotriene receptor antagonist in non-smoking asthmatic subjects.[20] Also in adults, Taylor et al. showed no significant increase in urinary leukotrienes after exercise.[21]

However, in children with EIA the specific profile of urinary leukotrienes has not yet been clarified and studies that have assessed urinary excretion of leukotrienes after exercise are sparse. For example, Severien et al. showed that there was significantly higher urinary excretion of LTE4 in subjects with asthma compared to healthy controls.[22] Our results, similar to those of Kikawa et al.,[19] using another technique for LTE4 measure (i.e., radioimmunoassay), showed no significant changes in basal concentration of LTE4, and a significant difference in post-exercise change in LTE4 concentration between children with EIA and controls.

An association of LTE4 concentrations and lung function has been summarized by a recent review.[10] One study showed an association among LTE4 concentrations, a fall in FEV_1, and a protection using leukotriene receptor

antagonist in children.[23] In contrast, we did not find a correlation between LTE4 concentration and FEV_1. Interestingly, Kikawa et al. did not find any correlation either between LTE4 concentration after exercise and the degree of bronchoconstriction, although using maximum percentage decrease in the peak expiratory flow. This may be explained by either the presence of milder forms of asthma in the studied subjects, or even by a more precise recognition of non-controlled asthma by the LTE4 than FEV_1. We also showed no correlation between hours of physical activity and changes in LTE4 concentration in children with EIA. This could be explained by diverse reasons, like different basal inflammatory conditions of the subjects or the subjective and possibly imprecise self-estimation of weekly hours of physical activity.[3]

We hypothesize that under certain conditions, like exercise, leukotriene-associated inflammation has a specific pattern in children with EIA, and this may mark a difference with healthy children, among whom there is no such inflammation. Furthermore, we found in the present study, that children with more severe EIA, according to a widely used clinical classification (i.e., Global Initiative for Asthma Guidelines), showed higher levels of change in urinary concentration of LTE4 post ECT. While some have found similar results,[22,24] others have found no correlation between leukotriene concentration and asthma severity.[9,13,25] This fact may be of special clinical interest, considering that using montelukast may be useful in patients with EIA with such high leukotriene excretion levels.

In order to better understand the role of leukotrienes in asthma, different metabolites (i.e., leukotriene B4, C4, D4 or E4) have been investigated in sputum, exhaled condensate and urine.[10,26] For the present study, urinary LTE4 was chosen as a marker of inflammation, as it has recently shown specific functions in patients with asthma, suggesting an important role for this molecule in inflammation.[13] In addition, urinary LTE4 could be easily obtained in school-aged children from single urine samples. Previously, techniques for assessing urinary LTE4, such as reversed-phase high-performance liquid chromatography, radioimmunoassay, or enzyme immunoassay have been used.[19,21] Qiu et al. showed that enzyme immunoassay significantly correlated with what was usually considered the gold standard (i.e., reversed-phase high-performance liquid chromatography).[27] To our knowledge, the present study represents the first comparison in LTE4 concentration after exercise using enzyme immunoassay in children with this specific form of asthma (i.e., EIA).

The present study has some potential limitations. First, we measured only one sample of LTE4 from all subjects 30 min after the ECT. It is possible that obtaining more samples of each subject, in order to establish the optimal excretion time, would have shown further differences in LTE4 excretion between the groups. Notwithstanding that, we demonstrated a significant difference in the concentration of LTE4 post ECT under the same sampling conditions between cases and healthy controls. Another limitation of this preliminary phase may have been the sample size, although it was based on previous studies.[19] However, we hypothesize that associations obtained between urinary LTE4 concentration and lung function or clinical severity could be strengthened with a larger sample, which may be designed based on this results. Moreover, intraindividual changes showed that LTE4 concentrations after ECT in subjects with EIA often remained stable and increased in some cases.

Further studies should confirm if assessment of changes in LTE4 concentration after exercise could be helpful in directing a specific treatment in children with EIA. A strength of the present study was the use of an ECT that strictly followed the recommendation of the American Thoracic Society (ATS) recommendation, considering that only one out of nine protocols of ECT published after 2000 (and none published before) followed these guidelines.[17] Due to the pattern of urinary LTE4 excretion in subjects with EIA in our study, we think that methods for its detection in a clinical setting could be helpful for adjusting short-term and long-term treatment. We speculate that assessment of changes in personal concentration of LTE4 could be more reliable than the absolute value of a single measurement.

In conclusion, urinary LTE4 excretion after exercise has a different pattern (i.e. higher changes) in children with EIA compared to controls. Assessment of urinary LTE4 using enzyme immunoassay could be a promising non-invasive alternative for optimising therapy in children with EIA.

Conflict of interest

The authors have no competing interests to declare. The authors alone are responsible for the content and writing of the paper.

Acknowledgments

This study was supported by a research grant from the Centro de Investigaciones Medicas (CIM), Pontificia Universidad Católica de Chile, Chile.

References

1. Carlsen KH, Carlsen KC. Exercise-induced asthma. Paediatr Respir Rev. 2002;3(June):154–60.
2. Lang DM, Butz AM, Duggan AK, Serwint JR. Physical activity in urban school-aged children with asthma. Pediatrics. 2004;113(April):e341–6.
3. Brockmann P, Fodor D, Caussade S, Campos E, Bertrand P. Exercise-induced asthma as perceived by pediatric patients and their parents. Rev Med Chil. 2006;134(June):743–8.
4. Chiang LC, Huang JL, Fu LS. Physical activity and physical self-concept: comparison between children with and without asthma. J Adv Nurs. 2006;54(June):653–62.
5. Kojima N, Ohya Y, Futamura M, Akashi M, Odajima H, Adachi Y, et al. Exercise-induced asthma is associated with impaired quality of life among children with asthma in Japan. Allergol Int. 2009;58(June):187–92.
6. Basaran S, Guler-Uysal F, Ergen N, Seydaoglu G, Bingol-Karakoc G, Ufuk Altintas D. Effects of physical exercise on quality of life, exercise capacity and pulmonary function in children with asthma. J Rehabil Med. 2006;38(March):130–5.
7. Dundas I, McKenzie S. Spirometry in the diagnosis of asthma in children. Curr Opin Pulm Med. 2006;12(January):28–33.
8. Singer F, Horak Jr F, Friesenbichler W, Schneider B, Szepfalusi Z, Frischer T. Cysteinyl-leukotrienes in nasal lavage fluid in children with asthma. Pediatr Allergy Immunol. 2008;19(May):227–32.

9. Baraldi E, Carraro S, Alinovi R, Pesci A, Ghiro L, Bodini A, et al. Cysteinyl leukotrienes and 8-isoprostane in exhaled breath condensate of children with asthma exacerbations. Thorax. 2003;58(June):505–9.
10. Gogate S, Katial R. Pediatric biomarkers in asthma: exhaled nitric oxide, sputum eosinophils and leukotriene E4. Curr Opin Allergy Clin Immunol. 2008;8(April):154–7.
11. Bisgaard H. Role of leukotrienes in asthma pathophysiology. Pediatr Pulmonol. 2000;30(August):166–76.
12. Carlsen KH. Exercise induced asthma in children and adolescents and the relationship to sports. Pediatr Allergy Immunol. 1998;9(November):173–80.
13. Hallstrand TS, Henderson Jr WR. An update on the role of leukotrienes in asthma. Curr Opin Allergy Clin Immunol. 2010;10(February):60–6.
14. Henderson Jr WR, Lewis DB, Albert RK, Zhang Y, Lamm WJ, Chiang GK, et al. The importance of leukotrienes in airway inflammation in a mouse model of asthma. J Exp Med. 1996;184(October):1483–94.
15. O'Sullivan S, Roquet A, Dahlen B, Larsen F, Eklund A, Kumlin M, et al. Evidence for mast cell activation during exercise-induced bronchoconstriction. Eur Respir J. 1998;12(August):345–50.
16. Weiler JM, Bonini S, Coifman R, Craig T, Delgado L, Capao-Filipe M, et al. American Academy of Allergy, Asthma & Immunology Work Group report: exercise-induced asthma. J Allergy Clin Immunol. 2007;119(June):1349–58.
17. Crapo RO, Casaburi R, Coates AL, Enright PL, Hankinson JL, Irvin CG, et al. Guidelines for methacholine and exercise challenge testing-1999. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, July 1999. Am J Respir Crit Care Med. 2000;161(January):309–29.
18. Kumlin M. Measurement of leukotrienes in humans. Am J Respir Crit Care Med. 2000;161(February):S102–6.
19. Kikawa Y, Miyanomae T, Inoue Y, Saito M, Nakai A, Shigematsu Y, et al. Urinary leukotriene E4 after exercise challenge in children with asthma. J Allergy Clin Immunol. 1992;89(June): 1111–9.
20. Reiss TF, Hill JB, Harman E, Zhang J, Tanaka WK, Bronsky E, et al. Increased urinary excretion of LTE4 after exercise and attenuation of exercise-induced bronchospasm by montelukast, a cysteinyl leukotriene receptor antagonist. Thorax. 1997;52(December):1030–5.
21. Taylor IK, Wellings R, Taylor GW, Fuller RW. Urinary leukotriene E4 excretion in exercise-induced asthma. J Appl Physiol. 1992;73(August):743–8.
22. Severien C, Artlich A, Jonas S, Becher G. Urinary excretion of leukotriene E4 and eosinophil protein X in children with atopic asthma. Eur Respir J. 2000;16(October):588–92.
23. Rabinovitch N. Urinary leukotriene E4. Immunol Allergy Clin North Am. 2007;27(November):651–64, vii.
24. Kikawa Y, Hosoi S, Inoue Y, Saito M, Nakai A, Shigematsu Y, et al. Exercise-induced urinary excretion of leukotriene E4 in children with atopic asthma. Pediatr Res. 1991;29(May): 455–9.
25. Henderson Jr WR. Role of leukotrienes in asthma. Ann Allergy. 1994;72(March):272–8.
26. Shibata A, Katsunuma T, Tomikawa M, Tan A, Yuki K, Akashi K, et al. Increased leukotriene E4 in the exhaled breath condensate of children with mild asthma. Chest. 2006;130(December):1718–22.
27. Qiu DW, Hui KP, Lee CW, Lim TK, Tan WC. Simplified method for measuring urinary leukotriene E4. J Chromatogr B Biomed Appl. 1996;677(February):152–5.