



## ORIGINAL ARTICLE

# Effects of physical exercise on lung injury and oxidant stress in children with asthma

Ozlem Gunay<sup>a,\*</sup>, Ece Onur<sup>a</sup>, Ozge Yilmaz<sup>b</sup>, Pinar E. Dundar<sup>c</sup>,  
Canan Tikiz<sup>d</sup>, Ahmet Var<sup>a</sup>, Hasan Yuksel<sup>b</sup>

<sup>a</sup> Celal Bayar University Medical Faculty, Dept of Biochemistry, Manisa, Turkey

<sup>b</sup> Celal Bayar University Medical Faculty, Dept of Allergy and Pulmonology, Manisa, Turkey

<sup>c</sup> Celal Bayar University Medical Faculty, Dept of Public Health, Manisa, Turkey

<sup>d</sup> Celal Bayar University Medical Faculty, Dept of Physical Medicine and Rehabilitation, Manisa, Turkey

Received 22 July 2010; accepted 26 October 2010

Available online 19 February 2011

### KEYWORDS

Asthma;  
exercise training;  
Lung injury;  
Oxidant stress

### Abstract

**Background:** The aim of this study was to investigate the influence of exercise training on oxidative stress and markers of lung inflammation in children with asthma.

**Methods:** Thirty children aged 8–13 years diagnosed with asthma were enrolled in the study as well as 13 healthy children. One group received only pharmacological treatment and the other group was also enrolled in an exercise programme. Venous blood and 24-hour urine samples were obtained from the children enrolled in the study at the beginning and end of the study. Leukotriene E4 and creatinine levels were measured in the urine and matrix metalloproteinase (MMP-9), endothelin-1 (ET-1), malondialdehyde (MDA), IgE and specific IgE levels were measured in blood samples.

**Results:** Leukotriene E4, MDA and MMP9 levels decreased significantly with treatment in both groups ( $p < 0.001$ ). However, ET-1 levels decreased significant only in the exercise group ( $26.5 \pm 3.6$  vs  $21.3 \pm 2.4$  pg/ml respectively,  $p = 0.001$ ). Moreover, ET-1 levels were found to be significantly lower in the exercise group compared to the only pharmacotherapy group ( $24.2 \pm 3.1$  vs  $21.3 \pm 2.4$  pg/ml,  $p = 0.007$ ).

**Conclusions:** Positive influences of exercise training in children with asthma may be mediated by decrease in ET-1 levels.

© 2010 SEICAP. Published by Elsevier España, S.L. All rights reserved.

## Introduction

Children and adults with asthma may avoid exercise because of their respiratory symptoms and they may have reduced exercise tolerance due to the increased sensation of dyspnoea.<sup>9,10,20</sup> Exercise programmes in asthma have been

\* Corresponding author.

E-mail address: ece.onur@bayar.edu.tr (O. Gunay).

demonstrated to be associated with improved fitness levels and quality of life as well as a reduction in the need for medications, fewer visits to the emergency department, and decreased absenteeism from school.<sup>6,11,15,26,28</sup> Moreover, physical training was found to increase cardiorespiratory fitness and work capacity in patients with asthma.<sup>24</sup> Most of these previous studies have enrolled adult patients but a recent study on children demonstrated that eight weeks of basketball training had beneficial effects on exercise capacity and quality of life.<sup>4</sup> However, the results of previous studies about the influence of exercise training on spirometry are contradictory.<sup>28</sup> Training may increase exercise capacity without leading to a major change in rest lung function tests.<sup>18</sup> The basic pathogenetic mechanisms underlying these changes in asthma with exercise training is unclear.

When oxidative stress overwhelms anti-oxidant mechanisms, reactive oxygen species interact with proteins, lipids and DNA leading to pathological consequences.<sup>8</sup> Oxidative stress is thought to initiate airway inflammation; therefore it is an important component of asthma pathogenesis.<sup>25</sup> It has been demonstrated that endothelin-1 was associated with oxidative stress in lung diseases like emphysema and that it increased MMP-9 activity leading to destruction of parenchyma.<sup>7</sup> Moreover, blockage of ET-1 receptors has been reported to down regulate pulmonary inflammation.<sup>7</sup>

Therefore, the aim of this study was to investigate the influence of exercise training on levels of MDA as a marker of oxidative stress and on ET-1, MMP-9, leukotriene E4 (LTE4) levels as markers of lung inflammation and damage in children with asthma.

## Materials and methods

### Subjects

Thirty children aged 8-13 years diagnosed with asthma in the Paediatric Allergy and Pulmonology Department were enrolled in the study as well as 13 healthy children. Diagnosis of asthma was based on history of recurrent cough and wheezing with prolonged expiration time which demonstrated clinical reversibility with short acting bronchodilator therapy, beta-2 agonist.<sup>16</sup> Healthy children were the ones who presented to the Paediatrics outpatient department for healthy follow-up. History of any chronic disease was absent in the healthy control group and they were examined by a paediatrician to rule out any current disease.

### Study design

One group of children with asthma received only pharmacological treatment and the other group was enrolled in an exercise programme as well as the pharmacological treatment. Venous blood and 24-hour urine samples were obtained from the children enrolled in the study, at the beginning and the end of the study. Blood samples were centrifuged for five minutes at 4000 rpm. Both blood and urine samples were stored in  $-80^{\circ}\text{C}$  until biochemical analysis. Leukotriene E4 and creatinine levels were measured in the urine and MMP9, endothelin, MDA, IgE and specific IgE levels were measured in blood samples.

The study was approved by the Institutional Ethical Review Board and informed consent was taken from the parents and children.

### Exercise programme

The exercise programme was carried out by the Physiotherapy and Rehabilitation Department. Children exercised one hour twice a week on a bicycle for eight weeks. They were called in groups of four. The rate of exercise was determined according to the rest heart rate of the children. Submaximal heart rate was calculated as 50% higher than the rest heart rate. Target heart rate during exercise was calculated as 80% of the submaximal heart rate. Pedalling rate required to reach the target heart rate was determined using a pulse oximeter during exercise. During the eight week exercise programme, 15 minutes of warm-up exercise was followed by 45 minutes of cycling at the target heart rate.

### Biochemical measurements

LtE4 was measured in 24-hour urine samples with ELISA (LTE4 EIA kit, Cayman chemical company, Ann Arbor, MI 48108). Creatinine was measured spectrophotometrically in 24-hour urine sample with the Jaffe method using Cobas Integra 800 (Roche). Serum levels of MMP-9 and endothelin were determined by ELISA (Human MMP-9 Assay Kit, Bender MedSystems GmbH, A-1030 Vienna, Austria, Europe, and Endothelin EIA kit, Cayman chemical company, Ann Arbor, MI 48108 respectively). IgE and specific IgE levels were measured with the chemiluminescence method using Immulite 2000. Serum MDA levels were determined via quantification of the pink colour obtained with the reaction of MDA with thiobarbituric acid spectrophotometrically at 532 nm.

### Statistical analysis

Statistical analyses were performed by SPSS 10.0 (Chicago IL) computer program. Groups were compared using the Mann Whitney U test and Wilcoxon signed ranks test for continuous variables. Chi square test was used for frequency comparisons.

## Results

### Sociodemographic characteristics of the study population

Mean age of the children in the asthma group (22 male, 8 female) was  $9.8 \pm 1.8$  and mean age of the children in the control group (6 male, 7 female) was  $10.3 \pm 2.0$  years ( $p=0.33$ ). All children in the asthma group had positive skin prick tests.

### Comparison of LtE4, MDA, ET-1 and MMP-9 levels between asthma and control groups

Leukotriene E4 levels in the asthma group were significantly higher than the control group before initiation of treat-

**Table 1** Levels of LTE4, MDA, ET-1, and MMP-9 in asthma and control groups before intervention.

	Asthma	Control	p
LTE4 (pg/ml)	679.00 ± 505.75	326.69 ± 170.33	0.020
MDA (nmol/ml)	3.40 ± 0.96	2.46 ± 0.58	<0.001
ET-1 (pg/ml)	25.82 ± 3.78	19.82 ± 3.73	<0.001
MMP-9 (ng/ml)	67.62 ± 12.27	44.61 ± 6.66	<0.001

ment ( $679.0 \pm 505.8$  vs  $326.7 \pm 170.3$  respectively,  $p=0.02$ ). Similarly MDA levels were significantly higher in children with asthma when compared to the controls ( $3.4 \pm 0.9$  vs  $2.5 \pm 0.6$  respectively,  $p<0.001$ ). ET-1 and MMP-9 levels were also significantly higher in the asthma group when compared to the control subjects ( $p<0.001$  for both) (Table 1).

#### Comparison of levels of LTE4, MDA, ET-1 and MMP-9 in asthma patients who received only pharmacotherapy with the ones who received pharmacotherapy and exercise training before treatment

When the group who received only pharmacotherapy was compared with the group who received exercise training as well as pharmacotherapy, no significant differences were detected between levels of LTE4, MDA, ET-1 and MMP-9 ( $p>0.05$  for all) (Table 2).

#### Leukotriene E4, MDA, ET-1 and MMP-9 levels in children with asthma who received only pharmacotherapy

LTE4 levels were found to decrease significantly with treatment in children with asthma who received only pharmacotherapy ( $714.0 \pm 445.0$  vs  $300.1 \pm 158.0$  respectively,  $p=0.009$ ). Similarly MDA and MMP-9 levels were detected to decrease significantly ( $p=0.001$  and  $p=0.02$  respectively).

**Table 2** Comparison of levels of LTE4, MDA, ET-1, and MMP-9 in asthma patients who received only pharmacotherapy with the ones who received pharmacotherapy before treatment.

	Pharmacotherapy	Pharmacotherapy and exercise	p
LTE4 (pg/ml)	714.3 ± 445.7	643.6 ± 573.0	0.370
MDA (nmol/ml)	3.6 ± 1.3	3.2 ± 0.5	0.660
ET-1 (pg/ml)	25.1 ± 3.9	26.5 ± 3.6	0.130
MMP-9 (ng/ml)	65.5 ± 11.2	69.7 ± 13.3	0.340

**Table 3** Levels of LTE4, MDA, ET-1, and MMP-9 in the asthma patients who received only pharmacotherapy before and after treatment.

	Before treatment	After treatment	p
LTE4 (pg/ml)	714.0 ± 445.0	300.1 ± 158.0	0.009
MDA (nmol/ml)	2.5 ± 7.4	2.1 ± 0.6	0.001
ET-1 (pg/ml)	25.1 ± 3.9	24.0 ± 3.1	0.750
MMP-9 (ng/ml)	65.5 ± 11.2	59.4 ± 11.3	0.020

**Table 4** Levels of LTE4, MDA, ET-1, and MMP-9 in the asthma patients who received exercise training as well as pharmacotherapy before and after treatment.

	Before treatment	After treatment	p
LTE4 (pg/ml)	643.0 ± 573.0	346.1 ± 266.0	0.010
MDA (nmol/ml)	3.2 ± 0.5	2.2 ± 0.5	0.001
ET-1 (pg/ml)	26.5 ± 3.6	21.3 ± 2.4	0.001
MMP-9 (ng/ml)	70.0 ± 13.3	50.0 ± 14.4	0.001

However, ET-1 levels did not change significantly ( $25.1 \pm 3.9$  vs  $24.0 \pm 3.1$  respectively,  $p=0.75$ ) (Table 3).

#### Leukotriene E4, MDA, ET-1 and MMP-9 levels in children with asthma who received exercise training and pharmacotherapy

LTE4 levels were found to decrease significantly in children with asthma who received exercise along with pharmacotherapy ( $643.0 \pm 573.0$  vs  $346.1 \pm 266.0$  respectively,  $p=0.01$ ). Similarly, MDA levels decreased from  $3.2 \pm 0.5$  to  $2.2 \pm 0.5$  after therapy ( $p=0.001$ ). ET-1 and MMP-9 levels were also found to decrease significantly after pharmacotherapy and exercise training ( $p=0.001$  for both) (Table 4).

#### Comparison of levels of LTE4, MDA, ET-1 and MMP-9 in asthma patients who received only pharmacotherapy, with the ones who received pharmacotherapy and exercise training after treatment

LTE4, MDA, and MMP-9 levels were found to be similar after treatment in children with asthma who received only pharmacotherapy and who received exercise training along with pharmacotherapy ( $p>0.05$  for all). However, ET-1 levels were found to be significantly lower in children who received exercise training as well as pharmacotherapy compared to

**Table 5** Comparison of levels of LTE4, MDA, ET-1, and MMP-9 in asthma patients who received only pharmacotherapy with the ones who received pharmacotherapy after treatment.

	Pharmacotherapy	Pharmacotherapy and exercise	p
LTE4 (pg/ml)	299.5 ± 158.5	344.7 ± 267.0	0.900
MDA (nmol/ml)	2.1 ± 0.6	2.2 ± 0.5	0.400
ET-1 (pg/ml)	24.2 ± 3.1	21.3 ± 2.4	0.007
MMP-9 (ng/ml)	59.4 ± 11.3	50.1 ± 14.4	0.060

the ones who received only pharmacotherapy ( $24.2 \pm 3.1$  vs  $21.3 \pm 2.4$ ,  $p=0.007$ ) (Table 5).

## Discussion

Asthma, which is one of the most common chronic diseases of childhood, may be associated with fear of exercising due to increased sensation of dyspnoea.<sup>5,9</sup> Since functional status of the child influences his or her quality of life to a great extent, it is important to consider this problem during treatment. Asthma severity is an important characteristic to consider when advising patients to exercise. However, most cases of childhood asthma are mild-moderate and it has been demonstrated in adult patients that they achieve maximal heart rate similar to control subjects during exercise.<sup>9</sup> Additionally, exercise training has been shown to improve FEV1 levels, mean maximal oxygen uptake as well as the subjective exertion responses.<sup>9</sup> Nevertheless, previous studies have not identified the exact pathogenetic mechanism underlying these changes with exercises in children with asthma. Therefore, the aim of this study was to evaluate the influence of exercise training on oxidant stress, inflammation and lung parenchyma destruction parameters in children with asthma.

Leukotriene E-4, which is a cysteinyl leukotriene, may be considered as a biomarker of asthma.<sup>13</sup> It is the stable end product of the arachidonic acid metabolism and therefore can be measured.<sup>13</sup> It has been reported that a decrease in urinary LTE4 levels was associated with an increase in FEV1 levels.<sup>12</sup> Moreover, another research demonstrated that as daily urinary excretion of LTE4 increased, so FEV1 decreased in children with asthma.<sup>23</sup> Therefore, LTE4 may be considered as a marker of airflow limitation.<sup>12</sup> Moreover, LTE4 has been considered to be one of the main inflammatory mediators of exercise-induced asthma attacks.<sup>2</sup> Levels of LTE4 were found to be significantly higher in children with asthma when compared to the healthy children in our study. This finding is in concordance with previous studies that demonstrated correlation of this biomarker with airflow obstruction.<sup>12,23</sup> Moreover, LTE4 levels were found to decrease significantly with treatment in children with asthma. But end values in the groups who received only pharmacotherapy and who received exercise training as well as pharmacotherapy was similar. Therefore, exercise was not thought to have a significant influence on urinary LTE4 levels.

Reactive oxygen species generated depleted intracellular glutathione leading to oxidation of membrane phospholipids.<sup>27</sup> Malondialdehyde is one of the endogenously generated aldehydic lipid peroxidation products of membrane lipid peroxidation during this process.<sup>27</sup> Previous

studies have reported that MDA levels decreased somewhat but remained higher than healthy controls even after one to three months of inhaled corticosteroid treatment in patients with asthma, indicating the requirement of longer term treatment to observe values similar to healthy subjects.<sup>1,21</sup> Similar to these previous studies, MDA levels in children enrolled in our study were higher than controls at the beginning and improved significantly in both groups of pharmacotherapy alone and associated with exercise training at the end of eight weeks. However, the levels were similar between these two groups indicating that the addition of exercise training did not influence these levels significantly.

Matrix metalloproteinases are proteolytic enzymes that degrade all components of extracellular matrix and play a role in many physiological and pathological processes.<sup>14</sup> MMP-9, which is the most abundantly found MMP in asthmatic subjects, has been shown to play an important role in airway inflammation in asthma.<sup>3,14</sup> MMP-9 might also have been proposed to modulate airway remodelling in asthma by activating Transforming growth factor B, increasing collagen synthesis, or by increasing angiogenesis.<sup>3</sup> Serum MMP-9 levels in children with asthma were found to be higher than their healthy peers in our study as expected from the explanations above and the levels decreased with treatment in both treatment arms. However, end-treatment values were not different between the group which received exercise training and the one which received exercise training. This might indicate that exercise training does not have an influence on MMP-9 levels.

There are many actions of ETs, one which is smooth muscle contraction and immune system influenced.<sup>22</sup> Since lung is the primary organ of ET metabolism, ETs play an important role in asthma.<sup>22</sup> ET-1 was reported to increase smooth muscle mass and induce IL-6 in asthma.<sup>19</sup> It has been shown in a murine model of asthma that antagonism of ET-A receptor which has a high affinity for ET-1, upregulates lymphocyte infiltration and decreases eosinophils, hyper-reactivity and mucus.<sup>17</sup> Additionally ET-1 levels in exhaled breath condensate have been reported to be correlated with asthma severity.<sup>30</sup> Moreover, ET-1 release from the epithelium was proposed to play an important role in airway inflammatory response after exercise in patients with exercise induced bronchoconstriction.<sup>29</sup> The results of our study which demonstrated higher ET-1 levels in children with asthma compared to the healthy one were concordant with the above characteristics of ET-1. Treatment led to a significant decrease in these levels. Moreover, ET-1 levels at the end of the study were lower in the group who received exercise training compared to the ones who received only pharmacotherapy. This is an important finding suggesting that the positive influences of exercise training on asthma

might be modulated by its influence on ET-1 at least to some extent.

The major limitation of our study was the small number of subjects enrolled. However, this is a preliminary study which will be followed by more extensive studies about the influence of exercise training on childhood asthma. Moreover, measurement of MDA, MMP-9 and ET-1 in bronchoalveolar lavage would be more informative of levels of these markers of inflammation. However, ethical issues precluded us from taking bronchoalveolar lavage from children with asthma.

In conclusion, the results of our study indicate that children with asthma can undergo exercise training under medical supervision and the positive influences of exercise training may be mediated by the decrease in ET-1 levels.

## Conflict of interest

None of the authors have conflict of interest to declare.

## References

- Alzogaibi MA, Bahammam AS. Lipid peroxides in stable asthmatics receiving inhaled steroids and long-acting beta2-agonists. *Respirology*. 2007;12:439–42.
- Anderson SD. How does exercise cause asthma attacks? *Curr Opin Allergy Clin Immunol*. 2006;6:37–42.
- Atkinson JJ, Senior RM. Matrix metalloproteinase-9 in lung remodeling. *Am J Respir Cell Mol Biol*. 2003;28:12–24.
- 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;38:130–5.
- Blaiss MS, Hill B. Outcomes in pediatric asthma. *Curr Allergy Asthma Rep*. 2005;5:431–6.
- Cambach W, Chadwick-Straver RV, Wagenaar RC, van Keimpema ARJ, Kemper HC. The effects of a community-based pulmonary rehabilitation programme on exercise tolerance and quality of life: a randomized controlled trial. *Eur Respir J*. 1997;10:104–13.
- Chen Y, Hanaoka M, Droma Y, Chen P, Voelkel NF, Kubo K. Endothelin-1 receptor antagonists prevent the development of pulmonary emphysema in rats. *Eur Respir J*. 2010;35:904–12.
- Cieniewicz J, Trivedi S, Kleeberger SR. Oxidants and the pathogenesis of lung diseases. *J Allergy Clin Immunol*. 2008;122:456–68.
- Clark CJ. The role of physical training in asthma. *Chest*. 1992;101:293S–8S.
- Clark CJ, Cochrane LM. Assessment of work performance in asthma for determination of cardiorespiratory fitness and training capacity. *Thorax*. 1998;43:745–9.
- Cochrane LM, Clark CJ. Benefits and problems of a physical training program for asthmatic patients. *Thorax*. 1990;45:345–51.
- Green SA, Malice MP, Tanaka W, Tozzi CA, Reiss TF. Increase in urinary leukotriene LTE4 levels in acute asthma: correlation with airflow limitation. *Thorax*. 2004;59:100–4.
- Gogate S, Katial R. Pediatric biomarkers in asthma: exhaled nitric oxide, sputum eosinophils and leukotriene E4. *Curr Opin Allergy Clin Immunol*. 2008;8:154–7.
- Gueders MM, Foidart JM, Noel A, Cataldo DD. Matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs in the respiratory tract: potential implications in asthma and other lung diseases. *Eur J Pharmacol*. 2006;533:133–44.
- Hallstrand TS, Bates PW, Schoene RB. Aerobic conditioning in mild asthma decreases the hyperpnea of exercise and improves exercise and ventilatory capacity. *Chest*. 2000;118:1460–9.
- <http://www.ginasthma.com/GuidelinesResources.asp?l1=2&l2=0>.
- Landgraf RG, Jancar S. Endothelin A receptor antagonist modulates lymphocyte and eosinophil infiltration, hyperreactivity and mucus in murine asthma. *Int Immunopharmacol*. 2008;8:1748–53.
- Ludwick SK, Jones JW, Jones TK, Fukuhara JT, Strunk RC. Normalization of cardiopulmonary endurance in severely asthmatic children after bicycle ergometry therapy. *J Pediatr*. 1986;109:446–51.
- McWhinnie R, Pechkovsky DV, Zhou D, Lane D, Halayko AJ, Knight DA, Bai TR. Endothelin-1 induces hypertrophy and inhibits apoptosis in human airway smooth muscle cells. *Am J Physiol Lung Cell Mol Physiol*. 2007;292:L278–86.
- Orenstein DM, Reed ME, Grogan Jr FT, Crawford LV. Exercise conditioning in children with asthma. *J Pediatr*. 1985;106:556–60.
- Ozars R, Tahan V, Turkmen S, Talay F, Besirli K, Aydin S, Uzun H, Cetinkaya A. Changes in malondialdehyde levels in bronchoalveolar fluid and serum by the treatment of asthma with inhaled steroid and beta2-agonist. *Respirology*. 2000;5:289–92.
- Polikepahad S, Moore RM, Venugopal CS. Endothelins and airways—a short review. *Res Commun Mol Pathol Pharmacol*. 2006;119:3–51.
- Rabinovitch N, Zhang L, Gelfand EW. Urine leukotriene E4 levels are associated with decreased pulmonary function in children with persistent airway obstruction. *J Allergy Clin Immunol*. 2006;118:635–40.
- Ram FS, Robinson RM, Black PN. Effects of physical training in asthma: a systematic review. *Br J Sports Med*. 2000;34:162–7.
- Riedl MA, Nel AE. Importance of oxidative stress in the pathogenesis and treatment of asthma. *Curr Opin Allergy Clin Immunol*. 2008;8:49–56.
- Robinson DM, Egglestone DM, Hill PM, Rea HH, Richards GN, Robinson SM. The effects of a physical conditioning programme on asthmatic patients. *N Z Med J*. 1992;105:253–6.
- Rosias PP, Dompeling E, Hendriks HJ, Heijmans JW, Donckerwolcke RA, Jöbbsis Q. Exhaled breath condensate in children: pearls and pitfalls. *Pediatr Allergy Immunol*. 2004;15:4–19.
- Satta A. Exercise training in asthma. *J Sports Med Phys Fitness*. 2000;40:277–83.
- Zietkowski Z, Skiepkowski R, Tomasiak MM, Bodzenta-Lukaszyk A. Endothelin-1 in exhaled breath condensate of allergic asthma patients with exercise-induced bronchoconstriction. *Respir Res*. 2007;8:76.
- Zietkowski Z, Skiepkowski R, Tomasiak MM, Bodzenta-Lukaszyk A. Endothelin-1 in exhaled breath condensate of stable and unstable asthma patients. *Respir Med*. 2008;102:470–4.