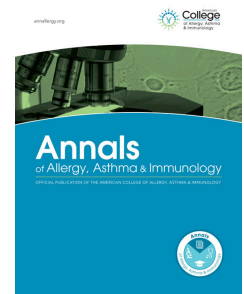


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Exhaled nitric oxide as a marker of chlorine exposure in young asthmatic swimmers

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Abbreviations: ATS (American Thoracic Society); CPET (Cardiopulmonary exercise testing); EIB (Exercise-induced bronchoconstriction); ERS (European Respiratory Society); FeNO (Fractional exhaled nitric oxide); FEV1 (Forced expiratory volume in one second), FVC (Forced vital capacity), IgE (Immunoglobulin E); Ppb (Parts per billion); RER (Respiratory Exchange Ratio); SD (standard deviation); THMs (trihalomethanes); Th2 (T helper 2); WHO (World Health Organization),

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1 Introduction

2 Allergic asthma is a Type 2 (T2) inflammatory disease commonly presented in
3 childhood. A complex interplay between genetic and environmental factors has been
4 implicated in its pathogenesis.¹ Environmental exposures seem to play an important
5 role in the rising trend of the disease prevalence.^{1,2} There are several preventable
6 environmental factors known to increase the risk of asthma in children such as damp
7 and moldy housing or cigarette smoke.^{1,2,3}

8 There is evidence that exercise improves daily symptoms,³⁻⁶ the overall quality
9 of life as well as limiting the need for high-dose glucocorticoids in asthmatic
10 children.⁷⁻⁹ Besides, one of the goals of asthma treatment is to maintain a normal and
11 healthy lifestyle, which includes regular exercise, as low physical activity is
12 independently associated with faster decline in lung function.¹⁰⁻¹⁴

13 Aerobic physical exercise such as swimming has been reported to improve
14 nocturnal asthma in children¹⁵ and adults by reducing the prevalence and frequency of
15 nighttime symptoms.¹⁵ Swimming is proposed to be superior to other forms of
16 exercise for children with asthma due to the warm humidified air and low pollen
17 count exposure that reduce bronchoconstriction as well as due to the hydrostatic
18 pressures on the chest wall that improve elastic recoil of the thoracic wall.¹² Inhalation
19 of moist air has been supported to have a protective role against the onset of exercise-
20 induced bronchoconstriction (EIB),¹² a frequent phenomenon presented among elite
21 athletes.¹³ Significant beneficial effects of swimming have been reported regarding
22 peak expiratory flow, cardiorespiratory fitness¹⁴⁻¹⁶ and body composition in analyses
23 including children/adolescents with asthma compared to healthy controls.^{18,19}

24 However, swimming in indoor chlorinated swimming pools involves
25 inevitable exposure to disinfectants and its byproducts. Chlorination is the most

26 preferred method used for swimmers' safety. Chlorine is added to swimming pool
27 water as chlorine gas or as sodium hypochlorite or calcium hypochlorite.²⁰
28 Hypochlorite, in turn, reacts with organic matter derived from swimmers (sweat,
29 urine, saliva) to generate inorganic chloramines (monochloramine, dichloramine,
30 trichloramine). Trichloramine, in particular, is responsible for the characteristic odour
31 of the swimming pool and irritant symptoms.²⁰ Early life exposure to chlorinated
32 swimming pool environments has been associated with pre-school asthma onset,^{13,21-23}
33 especially among the youngest children and those with an atopic predisposition.^{24,25}
34 Furthermore, endurance swimming coupled with disinfection by-products exposure
35 has been associated with increased airways dysfunction and neurogenic inflammation
36 in elite swimmers.²⁶⁻²⁸ Chloramines were also described as the most frequent cause of
37 occupational asthma frequently seen in swimming pool employees and healthcare
38 workers.²⁹ Specifically highly exposed to indoor swimming pool environments
39 asthmatic lifeguards for more than 500 hours had a significantly higher risk of
40 suffering from asthma exacerbation(s) compared to non-exposed asthmatic
41 lifeguards.³⁰ Swimming pool attendance appears to be associated with autonomic
42 changes and increased baseline airway smooth muscle constriction even in children
43 without asthma.^{30,31} Specifically, chlorinated pool induces perturbations of the
44 immune response via acute alterations of serum cytokine, damage associated
45 molecular pattern and chemokine secretion.^{30,31}

46 Fractional exhaled nitric oxide (FeNO) testing constitutes a non-invasive,
47 inexpensive and easy to perform assay, used for asthma monitoring and treatment
48 management.³²⁻³⁶ FeNO correlates with airway hyperresponsiveness and acts as a
49 marker of airway inflammation.³²⁻³⁶ Previous studies attempted to study FeNO
50 immediate and delayed changes after exercise.³⁶ However, it is not known whether the

51 FeNO measurement could efficiently monitor airway inflammation in young
52 swimmers.

53 The aim of the present study was to evaluate FeNO levels as a marker of
54 airway inflammation due to chlorine exposure in young asymptomatic swimmers with
55 controlled asthma attending an indoor chlorinated swimming pool compared to a set
56 of healthy swimmers. To that end, we assessed pre and post-exercise FeNO levels in
57 asthmatic and non-asthmatic children in two distinct exercise fields; in an indoor
58 chlorinated swimming pool and an in-hospital, stationary cycle ergometer.

59 **Methods**

60 *Participants and study design*

61 146 volunteer young male and female swimmers aged 8 to 18 years old
62 constantly attending an indoor chlorinated swimming pool in Larissa, the largest city
63 of the Thessaly region in central Greece, were enrolled for this study from 2012 to
64 2014. Since allergies can trigger asthma, this study was conducted from September to
65 February of each year, to avoid exposure to allergic stimuli.

66 Exclusion criteria included a history of upper or lower respiratory infection,
67 antibiotic treatment within the last month, use of tobacco products (including e-
68 cigarettes), a cardiovascular history, and a baseline post-bronchodilator FEV1/FVC
69 ratio of less than 70%. Furthermore, asthmatic subjects were steroid naïve; had not
70 been treated with systemic or inhaled corticosteroids within the last 3 months.

71 All athletes completed a validated structured survey questionnaire.^{27,28} This
72 questionnaire included information on demographics, athletes' training history in
73 indoor swimming pools, a detailed medical history of asthma or atopic disorders such
74 as allergic rhinitis and/or atopic dermatitis, as well medication history, as previously

75 described in competitive swimmers.^{27,28} All questions were explained simply and
76 answered with the help of parents.

77 The ethics committee of the University Hospital of Larissa approved the study
78 protocol. All subject's voluntary confirmation of willingness to participate in this
79 study was further approved by their parents who also gave written informed consent.

80 ***Spirometry procedure***

81 Spirometry test was performed with a dry spirometer (Spirolab III MIR)
82 according to the American Thoracic Society (ATS) and European Respiratory Society
83 (ERS) guidelines.^{37,38} The closed circuit method was followed. Calibration checks
84 were carried out daily, before the beginning of the spirometry program. A respiratory
85 physician who had undergone a special training program performed spirometry
86 testing. All spirometries were done in a sitting position.

87 At least 3 acceptable maneuvers which met repeatability criteria were
88 performed. An acceptable maneuver was defined as follows: 1) an explosive start (no
89 hesitation or sigmoid curve) with a back-extrapolation volume <150 mL, 2) the
90 maneuver was performed with a maximal inspiration and expiration, 3) no glottis
91 closure or cessation of airflow occurred during the maneuver, 4) no coughs
92 (particularly during the first second), inspirations during the trace or evidence of
93 leaks, and 5) the maneuver met the end-of-test criteria (exhaling for ≥ 6 seconds with
94 <50 mL being exhaled in the last 2 seconds. Forced expiratory maneuvers were
95 repeated until three acceptable reproducible tests were obtained and the best. Forced
96 expiratory volume in one second (FEV1), forced vital capacity (FVC), their ratio
97 FEV1/FVC (FEV1%) were recorded and further analyzed.^{37,38} The results obtained
98 were expressed as a percent of predicted values. Short-acting bronchodilators were
99 withheld for at least 4 hours in case of a previously known history of asthma.³⁸

Diagnosis of asthma

A bronchodilator reversibility test with 400 µg of salbutamol was used. A positive response to bronchodilators was defined as an increase in FEV₁ of 200 mL and 12% from the pre-bronchodilator value (baseline value) according to ATS/ERS guidelines.³⁹

FeNO measurement

Earlier studies used FeNO as an appropriate tool for evaluating the environmental effect on airways in indoor swimming pools.⁴⁰⁻⁴² Accordingly, FeNO was measured using a portable analyzer (Nobreath, Bedfont, United Kingdom). The testing standards for FeNO followed the guidelines recommended by the ATS and the ERS.⁴⁰⁻⁴² Measurements were taken before spirometry in a sitting position, and the obtained values were expressed in parts per billion (ppb). All measurements were performed thirty minutes after children arrived at the swimming pool and immediately after the end of one-hour exercise. Similarly, FeNO and spirometry were measured at the beginning of the cycle ergometer exercise and the end of the exercise. Calibrations were performed regularly, and the reliability of the test was confirmed with three values that varied < 10%.

Cardiopulmonary exercise testing (CPET)

Cardiopulmonary exercise testing was performed on a cycle ergometer. All asthmatics and non-asthmatic CPET-participants were randomly selected from among the population of asthmatic and non-asthmatic swimmers, respectively. The protocol involved a gradual increase of the exercise load until the athlete reached maximum exhaustion. All subjects inhaled dry air (10 mg H₂O/L) at room temperature through a

two-way valve and mask. During the first three minutes, at rest, registration of VO_2 was performed and then for three consecutive minutes, the athlete was cycling initially without resistance and then with a constantly increasing load (5-25 Watt/min) until exhaustion was reached. A valid test was considered when one of the following criteria was met 1) the Respiratory Exchange Ratio (RER) was >1.10 , 2) heart rate was $> 90\%$ of the predicted maximal heart rate of each individual and 3) plateau of VO_2 was observed.⁴³

Exposure measurements pool

The Hery et al. method⁴⁴ was used to determine the levels of airborne trichloramine in the swimming pool.

Statistical analyses

Quantitative variables were expressed as mean values with standard deviation (SD), while qualitative variables were presented as frequencies with percentages. Chi-square test was used to explore any associations between qualitative variables. To identify differences between two independent groups when the dependent variable was either ordinal or continuous, unpaired *t*-tests or Mann–Whitney *U* tests were used for normal and skewed data, respectively. Repeated measurements analysis of variance (ANOVA) was adopted to evaluate the changes observed in FeNO and all spirometry measurements with regard to the presence of asthma over the follow-up period. All reported *p* values are two-tailed. Statistical significance was set at $p < 0.05$ and analyses were conducted using SPSS statistical software (version 19.0).

Results

146 children with a mean age of 13.1 ± 2.8 years participated in this study. The demographic and clinical characteristics of the subjects are presented in **Table 1**.

were boys and 74 were girls with a mean age of 13.4 ± 2.6 and 12.9 ± 2.9 years, respectively. The average swimming period was 6.5 ± 3 years. 10 children self-reported with asthma; half of whom reported a periodic inhaled corticosteroid use not extended within the last three months, according to exclusion criteria of the study. All of them were medicated with as-needed SABA and 4 children with daily use of anti-leukotriene agents. 2 of them also had a known medical history of allergic rhinitis based on the presence of specific nasal symptoms accompanied with the proper laboratory investigation both reported a sensitivity to pollen. Remarkably, bronchodilator reversibility testing revealed 23 children (15.8% of the sample) with asthma. Hence, 13 children were newly diagnosed and they were never medicated for asthma. There was no difference detected regarding the length of exposure to chlorinated water between the athletes with a known history of asthma and the newly-diagnosed asthma group (7.92 ± 2.75 vs. 6.18 ± 1.89 , $p=0.095$). None of the children in the study was detected with atopic dermatitis based on skin examination and medical history. A randomly chosen sample of ten asthmatics (7 males and 3 females) and four healthy children (2 males and 2 females) from among the same population of swimmers underwent CPET. Furthermore, no difference was observed for FeNO values in terms of gender, and body mass index in both exercise fields.

Training environments

The swimming squad trained in an indoor pool with air temperatures of 28°C and relative humidity above 60%. The mean airborne concentration of trichloramine was less than 0.1 mg/m^3 . In contrast, CPET measurements were performed at room temperature $24 \pm 0.5^\circ\text{C}$, humidity $37 \pm 1\%$, barometric pressure $1002 \pm 10 \text{ mmHg}$ and height 90m.

Pre and post-swimming FeNO and spirometric values of study participants

As shown in **Table 2**, asthmatic children (n=23) had significantly higher pre-exercise FeNO values compared to non-asthmatics (n=123), (40.1 ± 19.6 ppb vs. 9.2 ± 7.6 ppb, $p < 0.001$). In both groups, post-swimming FeNO values were significantly lower compared to pre-swimming FeNO values. However, in the asthmatic group, post-exercise FeNO values remained significantly higher compared to post-exercise FeNO values of the non-asthmatic group (26.5 ± 15.3 vs. 6.7 ± 6.2 ppb, $p < 0.001$). The FeNO levels in 2/23 asthmatic athletes who suffered from allergic rhinitis were among the highest in the study group. There were no significant variations of spirometric parameters (FVC, FEV1, FEV1/FVC) in both groups before and after swimming. The swimming training lasted an hour.

Pre and post-cycle ergometer exercise FeNO and spirometric values of study participants

Comparisons of healthy (n=4) and asthmatic children (n=10) before and after exercise (CPET) regarding FeNO and spirometric values are presented in **Table 3**. Pre-exercise FeNO values were not varied among groups. Similarly, there was no significant difference in post-exercise FeNO values between the two groups. In both asthmatic and non-asthmatic groups post-exercise FeNO values were significantly decreased compared to pre-exercise FeNO values. Pre and post-cycle ergometer exercise spirometric parameters (FVC, FEV1, FEV1/FVC) showed no significant difference between the two groups (**Table 3**). The mean of VO_{2max} for the two groups was 34.2 ± 5.5 mL/kg/min, did not significantly differ between males (n=9) and females (n=5), (34.1 ± 5.5 vs. 34.3 ± 6.2 , respectively). However, asthmatics had significantly lower mean VO_{2max} values compared to healthy children (**Table 3**).

In 10/20 asthmatic swimmers who underwent CPET testing, pre-CPET FeNO values were significantly lower compared to pre-swimming FeNO values (18.1 ± 9.5 vs. 33.6 ± 21.9 ppb, $p < 0.001$). Likewise, in this sub-group, post-swimming FeNO values were significantly higher compared to post-cycle ergometer exercise FeNO values (23.6 ± 15.5 vs. 13.1 ± 7.2 ppb, $p < 0.002$) (**Table 4**). The mean CPET exercise lasted approximately 15-25 minutes including recovery.

Discussion

In this study, we evaluate pre and post-training FeNO levels in young asthmatics swimmers attending indoor chlorinated swimming pools compared to a set of healthy swimmers. To examine the potential risk of exposure to chlorine as a factor associated with increased FeNO levels in asthmatics, we further assessed pre and post-exercise FeNO levels in a randomly selected subgroup of the initial study population that trained in a non-pool based exercise field, *i.e.*, in an in-hospital, stationary cycle ergometer.

Interestingly, 16% of the study group detected with asthma, 56.6% (13/23) of whom were newly diagnosed. This is in agreement with other research reporting a high prevalence of asthma and airway hyper-responsiveness among pool-based endurance swimmers as well as a higher frequency of current asthma than non-pool-based athletes.⁴⁵ Swimming pool attendance has been previously associated with increased airway inflammation and smooth muscle constriction even in children without asthma.²² Notably, a dose-response relationship between swimming pool exposure and asthma has been indicated in children with asthma onset after the first year of age according to previous data.²³ The association of asthma with indoor pool attendance during childhood is still unclear.²⁵ However, it is supported that children

who were both sensitized and exposed seem to have a particularly high risk of developing it.²³

One of the main findings of this study was that the mean concentration of pre-swimming FeNO in asthmatics was not only significantly above the upper limit of the normal range for healthy children but also greater than 30 ppb, a level which has been associated with uncontrolled asthma, implicating that the presence of an irritating factor at the swimming pool such as chlorine might be responsible for increased airway inflammation in asthmatics.

17% (4/23) of the asthmatics were medicated with leukotriene antagonists which are known that affect FeNO levels.⁴⁶ Furthermore, 9% (2/23) of the asthmatics suffered from allergic rhinitis an atopic condition that is correlated with elevated FeNO levels; however, the small sample sizes did not allow the detection of some level of effect with inferential statistics. In the present study, multiple potential influencing factors that can modify FeNO were also evaluated. Male gender and height have been reported as independent factors of increased FeNO levels.⁴⁶ Nevertheless, no difference was observed in FeNO values in terms of gender, and body mass index between groups.

Importantly, by comparing FeNO levels of the ten same asthmatic athletes in distinct exercise fields (i.e. in an indoor chlorinated swimming pool and a non-pool-based sporting background) we showed that FeNO measurements before CPET were significantly lower compared to FeNO values before swimming, further emphasizing that the presence of a provoking factor in pool water might increase pre-swimming FeNO values.

The mean trichloramine exposure levels determined in this study were below the recommended air concentration level of 0.5 mg/m^{-3} , a World Health Organization

(WHO) reference value based on stationary sampling.⁴⁷ Therefore, these levels could not be surely associated with the high FeNO levels in asthmatics. However, it has been reported that long term exposed swimming pool workers have approximately half as high exposure to trichloramine compared with stationary measurements performed in parallel.^{48,49} Furthermore, according to Pedersen et al. trichloramine levels between 0.1 and 0.57 mg/m⁻³ showed an increased prevalence of asthma in swimming pool staff.⁴⁷ Moreover, a following study determined irritative symptoms among swimming pool employees at trichloramine levels of 0.2–0.3 mg/m⁻³.⁵⁰ A significantly increased risk of at least one ocular symptom has been previously attributed to trichloramine exposure above 0.02 µg/m³. Furthermore, high air concentrations of other disinfection by-products such as trihalomethanes (THMs) which had not been measured in this study might be implicated in the persistent post-exercise airway inflammation observed in young asthmatic swimmers.

In this study, we showed that spirometric parameters were normal after one-hour pool chlorine exposure. Similar to our results, lung function has been previously reported to be unchanged in competitive swimmers.⁵¹ In addition, there were no significant differences in mean spirometric (FEV1, FVC and FEV1/FVC) values between the two distinct exercise fields and at the end of both types of activity. However, FeNO values remained elevated in asthmatic children. Hence, FeNO constitutes a sensitive marker of airway inflammation compared to spirometry in young swimmers. In detail, in asthmatic athletes, post-swimming FeNO values remained significantly increased compared to non-asthmatic swimmers and significantly elevated compared to their post-CPET FeNO values. Chlorine could be a responsible agent for a certain degree of airway inflammation remained fairly constant in asthmatic swimmers; however, based on the fact that FeNO levels in both

cohorts (asthmatics and non-asthmatics) dropped by approximately $1/3^{\text{rd}}$, independently of the presence of chlorine, we speculate that there was no real difference in post-exercise NO responses between two groups. In asthmatics, pre-exercise NO values started much higher and had further to drop compared to non-asthmatics. Similarly, the post-exercise difference in FeNO observed between different sporting fields might be attributed to higher initial pre-swimming FeNO values versus pre-CPET FeNO values.

Notably, we observed that post-exercise FeNO values were significantly decreased by approximately $1/3^{\text{rd}}$ in both healthy and asthmatic children in both sporting backgrounds in line with the results of previous work which documented that FeNO decreased at the end of exercise in both healthy and asthmatic subjects.^{35,52} Specifically, Terada et al. reported that FeNO significantly decreased in asthmatic children with EIB compared to healthy non-EIB children and further supported that NO production in response to exercise may be impaired in patients with asthma.³⁵ It is widely accepted that NO has a crucial bronchoprotective role that represents not only airway inflammation but also a protective function of NO against EIB acting as an airway smooth muscle relaxant in humans.³⁵ Hence, decreased NO levels might act as a counter-regulator of bronchospasm.^{35,52} In a more recent study, Font-Ribera L et al. found that although FeNO levels were unchanged overall among healthy adults after swimming pool exposure, they tended to decrease among atopics.⁵³ Furthermore, Carbone S et al. reported a FeNO increase in healthy non-asthmatic individuals who trained in a non-chlorinated pool, not observed in a chlorinated pool, suggesting that chlorination might inhibit NO-induced vasodilation observed during exercise.⁵⁴

Contradictory data reported that the FeNO concentration did not change in asthmatic children after a treadmill exercise challenge.⁵⁵ Another study suggests that

aerobic exercise training increased FeNO generation.⁵⁶ Possible explanations of the
aforementioned discrepancy might be the method of NO measurement, online versus
off-line, different patient backgrounds, or different methods for exercise challenge, as
well as the use of an inhaled steroid. For instance, in the study by Scollo et al.,⁵⁵ the
majority of patients with EIB had been previously treated with inhaled steroid
whereas none of the asthmatic athletes had been on inhaled steroid in our study.
Importantly, according to Terada et al., a decrease in FeNO in asthmatics after an
exercise challenge is observed only in steroid-naïve patients, not in steroid-treated
patients.³⁵ On the other hand, Tsukiyama et al. did not investigate the effects of
exercise training on FeNO in asthmatic young athletes but in adult non-asthmatic
workers.⁵⁶

In this study, both swimming sessions and CPET consisted of a submaximal
and maximal exercise test. As far as the potential impact of exercise intensity in
FeNO is concerned, there is only one study reported significant decreases in post-
exercise FeNO levels between different training blocks, with moderate load training,
resulting in the worst lung in competitive youth swimmers.⁵⁷ There was a difference
in the average time spent swimming versus the average time of the CPET. So far,
however, there has been no reliable evidence that differences in exercise endurance
time had an acute effect on FeNO levels. Particularly, it has been supported that
adolescent elite swimmers do not have significant signs of airway damage after only a
few years of intense training and competition. They develop respiratory symptoms,
airway inflammation, and airway hyperresponsiveness only during their swimming
careers.⁵⁸ Besides, athletes' asthma which may be developed during and subside after
an active sports career, is partly reversible.^{58,59} In support of this, another study
reported that there was no acute effect of a swimming training session in an indoor

chlorinated pool on lung function or airway composition in adolescent swimmers.^{59,60} FeNO was found to be unchanged among healthy non-smoking adults before and after they swam for 40 min in a chlorinated indoor swimming pool (short term change).⁶¹ However, intense, long-term swimming training in indoor chlorinated swimming pools has been associated with airway changes similar to those seen in mild asthma, with higher mucin expression.⁶¹ There is a growing body of evidence illustrating that athletes who regularly attend indoor swimming pools are acutely and repeatedly exposed to high concentrations of inhaled surface irritants, such as chlorine gas derivatives which can induce airway inflammation and remodeling process that may lead to the development of asthma/EIB.^{26,62} Hence, the increased occurrence of asthma/EIB in swimmers may be caused by the combined effects of the inhalation of by-products arising from disinfection and the high number of training hours.²⁶

Previous research has shown that FeNO measured in the expired gas of asthmatic patients decreased after repeated spirometry efforts, but that bronchoprovocation may counteract this fall in expired FeNO.⁶² In this study, we found that although the mean swimming time of the young athletes was relative low (6.5 ± 3 years), one-fifth of the study group was documented suffering from asthma, having persistently higher FeNO levels after repeated spirometry efforts before and after swimming training compared to non-asthmatics. This result is indicative of the presence of a provocative factor of airway inflammation in the pool. Conversely, normal FeNO levels were measured in a non-chlorinated exercise field.

FeNO measurement is reported to be a very useful parameter for the management of asthma, which is able to maintain a treatment sufficient to obtain a meaningful improvement of asthma. What is more, asthmatic swimmers with repeated exposure to airborne irritants and sensitizing agents may be at risk of a progressive

loss of asthma control and should be monitored with caution. More precisely, high FeNO levels have been proposed as a marker of respiratory disease in professional divers who should be scheduled for further clinical-laboratory investigation and more careful management.⁶² Likewise, FeNO measurement has been considered a useful marker for the asthmatic children/adolescents to master exercise-induced asthma and EIB to be able to participate in physical activity on an equal level with their peers.⁶² A precise early diagnosis with optimal treatment follow-up is vital in this aspect.⁶² Besides, there are perspectives for moderate exercise as a potential therapeutic tool for asthmatics.⁶² Training reduces breathlessness and asthma symptoms and attenuates T2-mediated inflammatory responses. Taken together, the benefits of swimming training far outweigh the potential hazards.

One of our study limitations is that swimming and CPET are distinct forms of exercise, but our study protocol focused on the presence and absence of chlorine factor respectively, rather than the exercise itself. The main limitation of the study is the low number of subjects with CPET although the CPET-participants were randomly selected from among the study population of asthmatic and non-asthmatic swimmers and were used to document the existence of stability of FeNO measurement in a non-pool-based sporting background; thus the comparisons surely need to be further investigated. In this study, individuals with asthma had very mild disease. The results may not be relevant to those with more severe disease taking inhaled corticosteroids or other controller medications, given that FeNO could be decreased in a dose-dependent manner by inhaled corticosteroids. Moreover, air concentrations of THMs that might be implicated in the persistent airway inflammation observed in young asthmatic swimmers had not been measured in this study.

369 In conclusion, FeNO compared to spirometry, constitutes a more sensitive and
370 easy to perform assay in detecting airway inflammation in young swimmers,
371 consequently, discriminating population at risk of loss of asthma control. FeNO levels
372 were higher in asthmatic swimmers and remained significantly increased after an-hour
373 pool workout in the chlorinated indoor environment compared to healthy athletes.
374 Further monitoring of the subgroup of asymptomatic young children with controlled
375 asthma and persistently high FeNO levels for a possible loss of asthma control and
376 asthma exacerbation is recommended.

Table 1. Demographic and laboratory characteristics of study participants (N=146)

Characteristics	Total (N=146)	Males (n=72)	Females (n=74)	<i>p-value</i>
Age(years)	13.1±2.8	13.4±2.7	12.9±2.9	0.179
BMI	20.3±3.0	20.9±3.0	19.7±2.9	0.180
Height (cm)	162.1±14.2	166.0±14.4	158.3±12.9	0.084
History of Asthma Yes (%)	23 (15.8)	15 (20.8)	8 (10.8)	0.075
Swimming history (years)	6.5±3.0	6.8±2.8	6.1±3.1	0.064

**Data are expressed as mean ± standard deviation or as frequency (percentage).*

Table 2. Comparisons of healthy (n=123) and asthmatic children (n = 23) before (pre) and after (post) exercise (swimming) regarding FeNO and spirometric values

	Pre	Post	Change		
	Mean±SD	Mean±SD	Mean±SD	p**	p‡
FeNO (ppb)					
<i>Healthy children</i>	9.2±7.6	6.7±6.2	-2.5±3.8	<0.001	0.001
<i>Asthmatics</i>	40.1±19.6	26.5±15.3	-13.6±12.2	0.003	
p*	<0.001	<0.001			
FEV1 (mL)					
<i>Healthy children</i>	107.0±14.4	107.2±14.4	0.2±6.2	0.812	0.700
<i>Asthmatics</i>	104.5±10.0	103.9±11.4	-0.6±5.0	0.695	
p*	0.552	0.449			
FVC (mL)					
<i>Healthy children</i>	110.2±14.8	110.1±13.9	-0.1±6.2	0.546	0.923
<i>Asthmatics</i>	109.3±12.3	109.8±13.0	0.5±2.7	0.541	
p*	0.853	0.944			
FEV1/FVC					
<i>Healthy children</i>	86.3±7.0	86.8±7.2	0.5±4.8	0.397	0.597
<i>Asthmatics</i>	86.8±5.4	86.4±6.9	-0.4±3.5	0.702	
p*	0.804	0.843			

^aData are expressed as mean ± standard deviation *p-value for group effect; **p-value for time effect; ‡Repeated measurements ANOVA. Effects reported include differences between the groups in the degree of change over the follow-up period

Table 3. Comparisons of healthy (n=4) and asthmatic children (n=10) before (pre) and after (post) exercise on cycle ergometer (CPET) regarding FeNO and spirometric parameters

	Pre	Post	Change		
	Mean±SD	Mean±SD	Mean± SD	p**	P‡
FeNO (ppb)					
Healthy children	13.8±6.4	9.5±4.2	-4.3 ±2.6	0.048	0.654
Asthmatics	19.8±10.3	14.6±7.8	-5.2± 3.7	0.002	
p*	0.302	0.246			
FEV1 (mL)					
Healthy children	98.8±5.2	99.5±8.3	0.7 ±3.8	0.767	0.262
Asthmatics	104.5±11.3	101.8±9.4	-2.7±5.3	0.111	
p*	0.354	0.677			
FVC (mL)					
No asthma	107.8±5.4	107.5± 3.8	-0.3±3.3	0.856	0.927
Asthma	108.1±14.5	108.0±13.9	-0.1±2.5	0.909	
p*	0.964	0.946			
FEV1/FVC					
Healthy children	87.5±5.5	88.3±5.5	0.8±2.5	0.453	0.684
Asthmatics	84.7±3.4	85.0±3.5	0.3±1.9	0.663	
p*	0.264	0.200			
VO₂max (mL/kg/min)					
Healthy children		41.5±3.3			
Asthmatics		31.3±2.7			
p*		<0.001			

^aData are expressed as mean ± standard deviation *p- value for group effect; **p- value for time effect; ‡Repeated measurements ANOVA. Effects reported include differences between the groups in the degree of change over the follow-up period

Table 4. Comparisons of pre and post-exercise FeNO, FEV1 and FVC values between swimming and cycle ergometer exercise in the randomly selected asthmatic group who had been trained in both exercise fields.

	Swimming pool (n=10)	CPET (n=10)	p
	Mean± SD	Mean± SD	
FeNO pre	33.6±21.9	18.1±9.5	0.001
FeNO post	23.6±15.5	13.1±7.2	0.002
FEV1 pre	104.3±11.8	102.9±10.1	0.615
FEV1 post	103.1±13.1	101.1±8.8	0.381
FVC pre	106.3±12.2	108±12.4	0.944
FVC post	105±13.4	107.9±11.7	0.875

^aData are expressed as mean ± standard deviation