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Original article

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Differences in NO airway diffusion after $\dot{V}O_2$-max test in asthmatic and non-asthmatic elite junior cross-country skiers

Marieann Högman[1], Lars Wedholm[2,3], Tomas Carlsson[3], Magnus Carlsson[3], Michail Tonkonogi[3]

Affiliations: [1]Dept. of Medical Sciences, Respiratory, Allergy and Sleep Research, Uppsala University, University Hospital, S 751 85 Uppsala, Sweden,

[2]Dept- of Medicine, Falun Hospital, Lasarettsvägen 10, S 791 82 Falun, Region Dalarna, Sweden

[3]School of Education, Health and Social Studies, Dalarna University, Högskolegatan 2, S 791 88 Falun, Sweden

Correspondence: Marieann Högman, Dept. Medical Sciences, Respiratory, Allergy and Sleep Research, Uppsala University, University Hospital, S 751 85 Uppsala, Sweden

E-post: marieann.hogman@medsci.uu.se

Take home message

NO pulmonary dynamics are altered in elite junior cross-country skiers and subjects who declared asthma responded differently to the $\dot{V}O_2$-max test. Analysis of the airway inflammation should be considered before inhaled corticosteroids are prescribed.

Keywords

exhaled nitric oxide, asthma, cross-country skiing, maximum oxygen uptake

Abstract

Asthma is common in cross-country skiers and is often treated with ß$_2$-agonists and inhaled corticosteroids (ICS). Exhaled nitric oxide is often used to guide ICS treatment in asthma. This study investigated the change in the pulmonary NO dynamics before and after a maximum oxygen uptake ($\dot{V}O_2$-max) test.

An extended NO analysis was performed among Swedish elite junior cross-country skiers (n=25), with and without declared asthma, before and after a $\dot{V}O_2$-max test using roller skis. Asthma was declared by six boys and two girls among whom five occasionally used ICS.

There were no differences in the baseline NO parameters between those with and without declared asthma. The diffusion capacity over airway wall ($D_{aw}NO$) was 21 (17,25) mL/s (median, quartiles), which is much increased for this age group. After the $\dot{V}O_2$-max test, there were statistically significant differences from the baseline fraction of exhaled nitric oxide (F_ENO_{50}), NO-flux from airways, $D_{aw}NO$ and alveolar NO values; but not in the NO content in airway wall ($C_{aw}NO$) for all subjects together as one group. However, in the asthma group, differences were only seen in the F_ENO_{50} and in $C_{aw}NO$.

Interestingly, a majority of the subjects had an increase in the $D_{aw}NO$. An increase in $D_{aw}NO$ has been found with allergic asthma together with elevated $C_{aw}NO$. The skiers did not have elevated $C_{aw}NO$, which indicates an absence of inflammation in the airway wall. Modelling of lung NO production clearly shows that the asthma among our skiers is distinct from the allergic asthma in non-athletes.

Abbreviations

BMI = body mass index

C_ANO = alveolar NO

$C_{aw}NO$ = NO content in airway wall

$D_{aw}NO$ = diffusion capacity over airway wall

EIB = exercise induced bronchoconstriction

F_ENO_{50} = fraction of exhaled nitric oxide at the flow of 50 mL/s

HMA = Högman Meriläinen algorithm non-linear method

ICS = inhaled corticosteroids

$J_{aw}NO$ = NO-flux from airways

NO = nitric oxide

TG = Tsoukias & George linear method

$\dot{V}O_2$-max = maximum oxygen uptake

Introduction

Respiratory symptoms are common in elite athletes. The prevalence of asthma/airway hyperreactivity is as high as 15% [1]. In cross-country skiers, the prevalence is even higher. In a Swedish study among skiers that used a postal questionnaire, 29% in the age group 15-19 years self-reported a physician-diagnosed asthma [2]. In another Swedish postal questionnaire study among adolescent skiers that also included control subjects, the prevalence of physician-diagnosed asthma was 23% among the skiers and 12% in the control group [3]. Beta$_2$-agonists and inhaled corticosteroids (ICS), which are asthma medications, are often used among skiers for the treatment of respiratory symptoms. According to a 2008 joint task force of the European Respiratory Society (ERS) and the European Academy of Allergy and Clinical Immunology (EAACI), a diagnosis of exercise induced asthma (EIA) is not the same as exercise induced bronchoconstriction (EIB), and most of the elite athletes referred for respiratory problems do not suffer from asthma [4].

Elite cross-country skiers are exposed to cold dry air, and with a minute volume around 200 L during training or competition, their airways will be challenged to heat and to humidify the air. Stress on the airway also entails inflammatory cell attraction. However, these cells do not seem to be activated [5] so low values of exhaled nitric oxide (F_ENO) can therefore be expected. It has been shown that F_ENO was not elevated in a group of skiers when compared to a group of asthmatics even though significant bronchial responsiveness to inhaled methacholine was present [6].

F_ENO has been shown to decrease after a short exercise challenge [7], as well as after a marathon race [8]. A measurement of F_ENO at one flow will only reveal a sum of the changes in the pulmonary NO dynamics, while an extended NO analysis can localize the specific NO parameter changes. When assessing pulmonary NO dynamics, the non-linear method makes it possible to estimate the alveolar NO (C_ANO), the airway flux of NO ($J_{aw}NO$), the content of NO in the airway wall ($C_{aw}NO$) and the diffusing capacity of NO ($D_{aw}NO$). Published C_ANO, $C_{aw}NO$ and $D_{aw}NO$ data on winter sports athletes have not been found. This study investigated the pulmonary NO dynamics and the change before and after a maximum oxygen uptake ($\dot{V}O_2$-max) test in elite junior cross-country skiers.

Material and methods

Study Design and Subjects

Swedish elite junior cross-country skiers (n=25) were recruited during their yearly check-up to determine their maximum oxygen uptake. Among these were 17 boys and 8 girls. Our sample was taken from the top performing cross-country skiers, which was 12% of the total 210 from this age group in Sweden. The Regional Review Board in Uppsala, Sweden (Dnr 2018/349) approved the study on 28 November 2018. All subjects gave written informed consent.

The subjects filled out a questionnaire regarding their health that contained questions about illnesses, medications, training, diet, hydration, current injuries as well as any heart, airway, dizziness, blood pressure, head trauma or allergy issues. Exhaled NO was analysed before and within 5-10 minutes after a $\dot{V}O_2$-max test that used roller skis.

Testing procedures

An analyser (EcoMedics DLC 88, Eco Medics AG, Dürnten, Switzerland) was used to measure the NO levels. Calibration and maintenance of the analyser was performed according to the manufacturer's instructions. The zero-point off-set was controlled before the measurements. The subjects' statures (Harpenden Stadiometer, Holtain Limited, Crymych, Great Britain) and body masses (Midrics 2, Sartorius AG, Göttingen, Germany) were measured before a standardized warm-up that consisted of ten minutes roller-skiing on a treadmill. The roller-skiing tests to determine the subjects' $\dot{V}O_2$-max were performed on a motor-driven treadmill (Saturn 450/300rs; h/p/cosmos sports & medical GmbH, Nussdorf-Traunstein, Germany). The subjects used the laboratory's roller skis (Pro-Ski C2; Sterners Specialfabrik AB, Dala-Järna, Sweden) and their own poles with plastic tips (Black Plastic Tip; LEKI Lenhart GmbH, Kirchheim, Germany) provided by the laboratory. Throughout the $\dot{V}O_2$-max test, variables of expired air were continuously analysed using a metabolic cart in the mixing-chamber mode (Jaeger Oxycon Pro, Erich Jaeger GmbH, Friedberg, Germany). Before each test, the expiratory flow meter and the O_2 and CO_2 analysers were calibrated according to the manufacturer's specifications. Capillary blood samples were collected from a fingertip at two and five minutes after the $\dot{V}O_2$-max test. These samples were then analysed to determine blood-lactate concentrations (Biosen 5140, EKF-diagnostic GmbH, Barleben, Germany). Within ten minutes after the $\dot{V}O_2$-max test, the exhaled NO levels were re-analysed.

NO analysis

In conformity with the 2005 American Thoracic Society and the European Respiratory Society (ERS) recommendations for NO measurements, the exhaled nitric oxide was analysed at an exhalation flow rate of 50 mL/s (F_ENO_{50}). In accordance with the ERS technical standards of modelling NO dynamics two methods were used: the nonlinear method with exhalation flows of 20, 100 and 300 mL/s, and the linear method with exhalation flows of 100, 200 and 300 mL/s [9]. Flow resistors were used with the subjects to facilitate a constant exhaled flow. A visual feedback system was used to guide the subjects so they could achieve the targeted flow throughout the exhalation. The Högman-Meriläinen algorithm (HMA) non-linear method and the Tsoukias and George linear method (TG) [9] were both used with the software in the NO analyser. For quality control, a calculated F_ENO_{50} for the HMA and an *r*-value for the linearity of the TG method were derived for each subject on the two NO analysis occasions. The HMA method estimates the following NO parameters: C_ANO, $C_{aw}NO$, $D_{aw}NO$ and $J_{aw}NO$, while the TG method only estimates the C_ANO and $J_{aw}NO$. Since they could find it difficult to breathe after the $\dot{V}O_2$-max test and perform an exhalation at the low flow of 20 L/s, all of the subjects were to perform the HMA method and TG method on both test occasions. The two-compartment model of the lung NO dynamics is seen in Figure 1.

Maximum oxygen uptake test

The incremental treadmill roller-skiing test to determine the $\dot{V}O_2$-max was begun using the diagonal-stride technique. During the initial 30 seconds of the test, the treadmill inclination

was 2.4° and the treadmill speeds were 9.0 km/h for the females and 10.9 km/h for the males. Thereafter, the inclination was increased by 0.4° and the speed was increased by 0.1 km/h every 30 seconds until volitional fatigue. The $\dot{V}O_2$-max was defined as the highest mean oxygen uptake during a 60 second period when meeting the criterion of a plateau in oxygen uptake despite an increase in exercise intensity. Determination of the plateau was based on the recognition of data points that fell outside (and below) the extrapolated 95% confidence interval (CI) for the $\dot{V}O_2$-work rate relationship [10]. In addition to the oxygen uptake plateau, a blood-lactate concentration of ≥ 8 mmol/L was also required to confirm the participant achieved $\dot{V}O_2$-max.

Analysis

All statistical analyses were performed using SPSS, v. 26 for Windows (SPSS Inc., Chicago, MI, USA). Data are expressed as median as well as upper and lower quartiles (Q1, Q3). The Mann-Whitney *U* test was used to make comparisons between any two groups. For pairwise comparisons the Wilcoxon signed rank test was applied. Correlations were tested with Spearman rank order correlation. A *p*-value of <0.05 was considered significant. Plots were made with SigmaPlot graphic software, v. 14 for Windows (SYSTAT, Alfasoft AB, Göteborg, Sweden)

Results

Of the 25 elite junior cross-country skiers that participated, eight declared that they had asthma that was diagnosed by a physician. Of these, five used occasionally ICS together with a $ß_2$-agonist (short or long acting), one only used a short acting $ß_2$-agonist and two did not use any medication. Allergies (mite, furry animals, pollen or grass) were declared by seven subjects, four of whom had asthma that was treated with ICS together with a $ß_2$-agonist (short or long acting) and one that was treated with a short acting $ß_2$-agonist. All of the remaining three who declared allergies took an antihistamine. There were no statistically significant baseline differences in the NO parameters when comparisons were made between the non-asthma and the asthma groups, the non-allergic and the allergic groups and non-allergic asthmatic and the allergic asthmatic groups. Therefore, the subjects were divided into non-asthma (n=17) and asthma (n=8) groups. Subject characteristics are presented in Table 1. There were no differences in relation to age or body mass index (BMI) between the non-asthma and asthma groups. For the $\dot{V}O_2$-max test, all subjects used roller skies except for three male skiers who preferred to run. There was no statistically significant difference in their performance in regard to asthma. When those with asthma and allergy were treated as one group, their time to exhaustion was shorter than the non-asthma/non-allergy group: 6.72 (6.14, 7.52) versus 7.96 (6.91, 8.79) minutes, *p*=0.002.

TABLE 1 Characteristics expressed as median (upper and lower quartiles) for all the subjects together as a group and also divided into non-asthma and declared asthma groups. The *p*-values given are from a comparison of these groups

	All (n=25)	Non-asthma (n=17)	Asthma (n=8)	*p*-value
Age (years)	18 (17, 20)	19 (17, 20)	18 (17, 19)	0.75
Body Mass Index (kg/m^2)	22 (21, 24)	22 (22, 23)	22 (21, 26)	0.84
$\dot{V}O_2$-max (L/min)	4.25 (3.77, 4.79)	4.25 (3.74, 4.80)	4.20 (3.77, 4.79)	0.93
$\dot{V}O_2$-max (mL/min/kg)	60 (55, 64)	60 (55, 66)	61 (52, 64)	0.80
Blood lactate (mmol/L)	12.7 (11.7, 14.6)	12.7 (11.7, 14.1)	12.7 (11.1, 16.2)	0.67
Time to exhaustion (min)	7.37 (6.24, 8.21)	7.50 (6.51, 8.52)	6.50 (6.11, 7.73)	0.12

All subjects were able to perform the HMA and TG methods on both test occasions. The quality control measurement results were well within the ERS standardization recommendations [9]. For the HMA method, the calculated F_ENO_{50} in comparison to the measured F_ENO_{50} was -1 (-2, 0) ppb, and the *r*-value for the TG method was 0.99 (0.97, 1). There was no difference in the estimation of C_ANO between the methods: 1.9 (1.6, 2.4) and 1.9 (1.3, 2.3) ppb, respectively, p=0.71. The estimation of $J_{aw}NO$ using the HMA method was 0.63 (0.33, 0,90) nL/s, and with the TG method 0.67 (0.50, 1.0) nL/s, p=0.34. Since all subjects were able to perform the low flow exhalation, the results are from the data using the HMA method.

Before the $\dot{V}O_2$-max test, the F_ENO_{50} and NO-parameters were not significantly different in regard to sex, or treatment with ICS and/or ß$_2$-agonists. Neither were there any differences in F_ENO_{50} (p=0.37), $J_{aw}NO$ (p=0.59), $C_{aw}NO$ (p=0.10), $D_{aw}NO$ (p=0.19) or C_ANO (p=0.71) between those with and those without declared asthma, as shown in Table 2. There were no statistically significant differences between those with declared allergy and those without: F_ENO_{50} (14 (12, 29) ppb and 17 (10, 23) ppb respectively) p=0.57, $J_{aw}NO$ (0.9 (0.6, 1.7) nL/s and 0.8 (0.6, 1.5) nL/s) p=0.70), $C_{aw}NO$ (63 (38, 113) ppb and 52 (32, 72) ppb) p=0.46, $D_{aw}NO$ (19 (15, 26) mL/s and 20 (15, 25) mL/s) p=1.0 and C_ANO (1.6 (1.4, 1.7) ppb and 1.6 (1.0, 2.0) ppb) p=0.84. Additionally, there were still no statistically significant differences when those with declared asthma and allergy were combined as one group and compared to those without asthma or allergy: F_ENO_{50} (18 (13, 29) ppb and 14 (9, 22) ppb respectively) p=0.17, $J_{aw}NO$ (0.9 (0.7, 1.7) nL/s and 0.7 (0.5, 1.3) nL/s) p=0.32, $C_{aw}NO$ (63 (44, 113) ppb and 46 (25, 62 ppb) p=0.13, $D_{aw}NO$ (19 (15, 26) mL/s and 20 (15, 25) mL/s) p=0.85 and C_ANO (1.6 (1.4, 1.7) ppb and 1.5 (1.0, 2.0) ppb) p=0.94.

After the $\dot{V}O_2$-max test, there were statistically significant differences from the baseline values in F_ENO_{50}, $J_{aw}NO$, $D_{aw}NO$ and C_ANO (all p<0.05), but not in $C_{aw}NO$ for all subjects when grouped together as one group. These statistically significant differences were the same in the non-asthma group, but in the asthma group the differences could only be seen in the F_ENO_{50} and in $C_{aw}NO$ values as shown in Table 2. The $D_{aw}NO$ decreased in the non-asthma group, but no change was found in the asthma group. The $C_{aw}NO$ decreased in the asthma group, but no change was found in the non-asthma

group. This is illustrated with the data from the individual $D_{aw}NO$ values plotted in Figure 2 and the $C_{aw}NO$ values plotted in Figure 3.

TABLE 2 Exhaled nitric oxide, F_ENO_{50} and NO parameters expressed as median (upper and lower quartiles), before and after $\dot{V}O_2$-max test for all subjects together as a group and also divided into non-asthma and declared asthma groups

	All (n=25)			**Non-asthma** (n=17)			**Asthma** (n=8)		
	before	**after**	***p*-value**	**before**	**after**	***p*-value**	**before**	**after**	***p*-value**
F_ENO_{50} (ppb)	17 (11, 24)	11 (7, 17)	<0.001	17 (10, 24)	11 (7, 18)	<0.001	16 (13, 28)	13 (8, 16)	0.012
$J_{aw}NO$ (nL/s)	0.91 (0.60, 1.57)	0.63 (0.33, 0.90)	<0.001	0.74 (0.55, 1.57)	0.63 (0.26, 0.88)	<0.001	0.93 (0.68, 1.67)	0.69 (0.42, 0.94)	0.093
$C_{aw}NO$ (ppb)	52 (36, 81)	43 (31, 73)	0.427	51 (24, 64)	36 (31, 96)	0.554	64 (45, 131)	51 (33, 59)	0.036
$D_{aw}NO$ (mL/s)	20 (15, 25)	16 (8, 19)	0.032	21 (16, 25)	11 (7, 18)	0.004	16 (8, 24)	19 (11, 22)	0.401
C_ANO (ppb)	1.6 (1.1, 1.9)	1.9 (1.6, 2.4)	0.006	1.5 (1.0, 1.9)	2.0 (1.6, 2.8)	0.005	1.6 (1.4, 2.0)	1.7 (1.4, 2.0)	0.401

F_ENO_{50} is highly correlated to the NO flux ($J_{aw}NO$) from the airways (*rho*=0.96, $p \leq 0.001$). $J_{aw}NO$ is estimated from the $C_{aw}NO$ and $D_{aw}NO$ values using the non-linear method with an adjustment for C_ANO [11]. The $C_{aw}NO$ has a correlation to the F_ENO_{50} (*rho*=0.83, $p \leq 0.001$), but not to the $D_{aw}NO$ (*rho*=-0.19, *p*=0.37). In the non-asthma group the correlation between F_ENO_{50} and the $C_{aw}NO$ remains (*rho*=0.96, $p \leq 0.001$), but not in the asthma group (*rho*=0.50, *p*=0.21). This is possibly due to too few individuals, because when the numbers are increased with the allergy subjects, the correlation is present (*rho*=0.70, *p*=0.007).

Discussion

Elite junior cross-country skiers decreased their F_ENO_{50} after a $\dot{V}O_2$-max test. When modelling the NO production in the lung, a difference was seen between the subjects who declared asthma and in those who did not. The most prominent difference was in the change in the airway wall diffusion rate of NO; $D_{aw}NO$. It decreased in the non-asthmatics, while the asthmatics and those with allergies had no change.

The F_ENO values are in line with data from healthy subjects of the same age [12]. The same NO levels were also found by Sue-Chu *et al.* in skiers and control subjects [6]. They used a high flow of 250 mL/s, which resulted in a F_ENO_{250} of 6 (4-10) ppb for their skiers. Using the NO parameters to retrieve the same flow, we found the F_ENO_{250} to be 5 (4, 7) ppb, which is quite similar. After the $\dot{V}O_2$-max test, our skiers decreased their F_ENO levels by 35% and this is in line with the 34% decrease seen after a marathon race [8]. A short exercise challenge

with a work intensity designed to increase the minute ventilation 40-50%, gave only a 10% decrease in F_ENO [7]. This is most likely due to the difference in the degree of insult to the airways, and this should be investigated.

The most interesting finding in this study is the different response to the exercise test found in the diffusion rate of NO over the airway wall. Only among those subjects who declared asthma and/or allergy demonstrated no change in the diffusing rate of NO. This exercise test forces the subjects to breathe heavily and the dry air causes a reduction in the extravascular water in the airways. The airway mucosa becomes hyperosmotic. The hyperosmolarity in the airway epithelial cells, which is due to cell shrinkage, releases mediators that may directly or indirectly increase the airway blood flow [5]. These mediators may also be responsible for the EIB. After the cell shrinkage, there is a regulatory cell volume increase in healthy subjects and a transport of sufficient water to the mucosa to overcome the hyperosmolarity of the airway surface liquid. A result of this may also be airway wall oedema, which could be the cause of the decrease in the $D_{aw}NO$. In asthmatic subjects however, the rate of the transfer of water to the airway surface is slower due to airway inflammation [13]. This could possibly explain the lack of change in the $D_{aw}NO$ in our subjects with asthma and allergies. In addition, the majority of our asthmatics were treated with β_2-agonists that relax the bronchial smooth muscles and prevent EIB. β_2-agonists can also increase the rate of water transport to the airway surface by stimulating chloride ion secretion on the apical surface of the of epithelial cells [14]. Hence, the formation of oedema is prevented. The overuse of short acting β_2-agonists, which has recently been demonstrated among persons with asthma, is shown to be associated with an increased risk for exacerbations and asthma related mortality [15].

Persons with allergic asthma and rhinitis have an increased $D_{aw}NO$ and $C_{aw}NO$ [16]. In our skiers, we found a doubling of $D_{aw}NO$. This is more than we had previously reported for allergic asthmatics [16, 17]. Additonally, the $C_{aw}NO$ was half as much as in a healthy population [18]. In EIB, there is an increase of inflammatory cells and remodelling of the airway [19]. The inflammatory cells do not seem to be activated in athletes [5], which is a probable refection of the low NO content in the airway walls of our skiers. In atopic asthma, where there is an increased $C_{aw}NO$, ICS prove very effective in treating the inflammation. Treatment guidelines for asthma recommend the combination of a β_2-agonist together with ICS. The interpretation from the results obtained with our young skiers is that they do not have an inflammation of the airway wall. Therefore, they will not benefit from treatment with an anti-inflammatory drug. A lack of response to ICS by skiers has been shown by Sue-Chu *et al.* [20]. ICS treatment does not affect the $D_{aw}NO$ [21], only the $C_{aw}NO$. This explains why, for persons receiving ICS treatment for atopic asthma, the F_ENO_{50} level barely reaches the reference values and thereby a risk for over treatment with ICS exists. F_ENO_{50} is the product of $D_{aw}NO$ and $C_{aw}NO$ with adjustment for C_ANO [11]. A high F_ENO_{50} can be due to an increase in either $D_{aw}NO$ or $C_{aw}NO$. An individual's optimal F_ENO_{50} can therefore be

calculated when the level of $D_{aw}NO$ is taken into account [22]. In our skiers, the $D_{aw}NO$ was increased and the $C_{aw}NO$ was low. Hence, their F_ENO_{50} levels were not different from those found among the healthy controls. This does not mean, however, that their airways are unaffected by their strenuous training and competitions in cold dry air.

There are limitations to our study that need addressing. We did not test the skiers for bronchial hyperreactivity and did not perform lung function tests. Since we did not have access to the subject's medical records, we could not examine them and we could not determine the asthma phenotype among those who declared having physician diagnosed asthma. Also, we do not know how adherent the asthmatics were to their anti-inflammatory therapy. This additional information may have been useful when we were interpreting our NO results. The NO analysis could have been made with different time intervals, but the subjects needed to stabilize their breathing to be able to perform the different flows for the extended NO analysis. Additionally, the testing may have benefited from a third measurement, but the skiers' time was too limited.

In summary, we have shown that elite junior cross-country skiers included in the current study have an increase in the diffusion capacity of NO over the airway wall. Such an increase has been found in allergic asthmatics together with an elevated content of NO in the airway wall. The skiers did not have an elevated $C_{aw}NO$, which indicates an absence of inflammation in the airway wall. Modelling of lung NO production clearly shows that the asthma among our skiers is distinct from the allergic asthma in non-athletes and points to the need for an analysis of the airway inflammation in skiers before ICS are prescribed.

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Authors' contributions

MH, LW, TC, MC, and MT participated in the study design. MH and LW collected and together with TC, MC, and MT analysed the data. MH prepared the initial draft. All authors have discussed and adjusted the draft and have read and approved the final manuscript.

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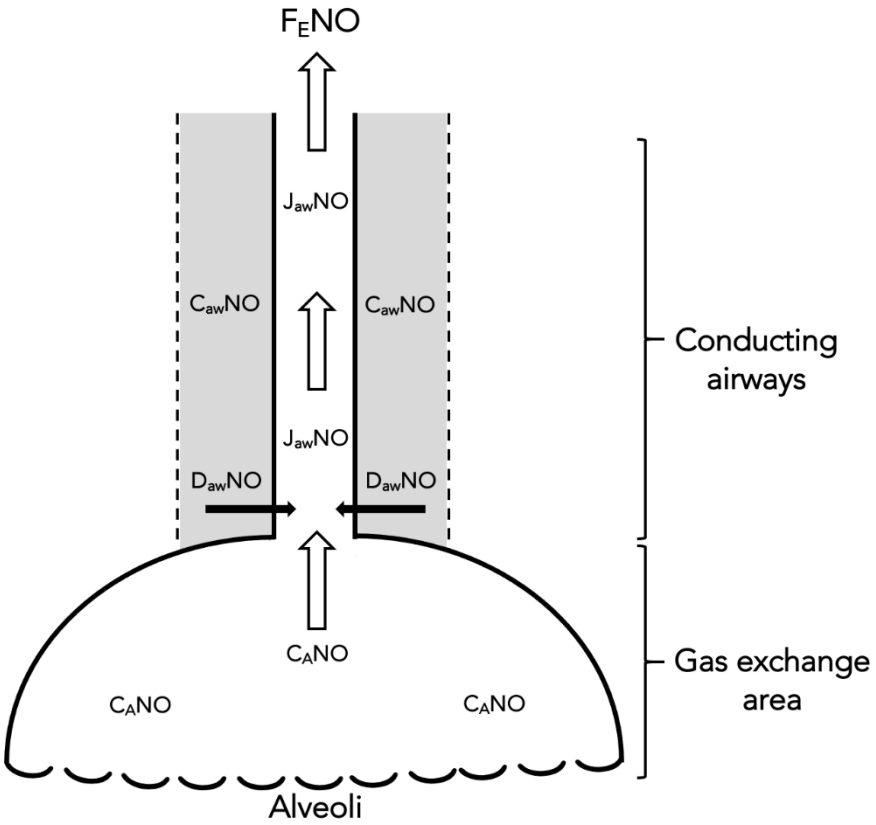

FIGURE 1 The two-compartment model consists of the conducting airways and the gas exchange area. In the conducting airways, all airways of the lungs are equally represented. The gas exchange area contains the alveolar or acinar compartment as well as the respiratory bronchiole. When the alveolar gas with its low content of NO (C_ANO) is expelled, the contribution of NO from the airways is driven by a concentration gradient of NO from the airway wall ($C_{aw}NO$). The rate is governed by the diffusion capacity of the airway wall ($D_{aw}NO$). The airway NO flux ($J_{aw}NO$) is therefore dependent on both the $C_{aw}NO$ and the $D_{aw}NO$. Exhaled NO (F_ENO) is dependent on all of the NO parameters.

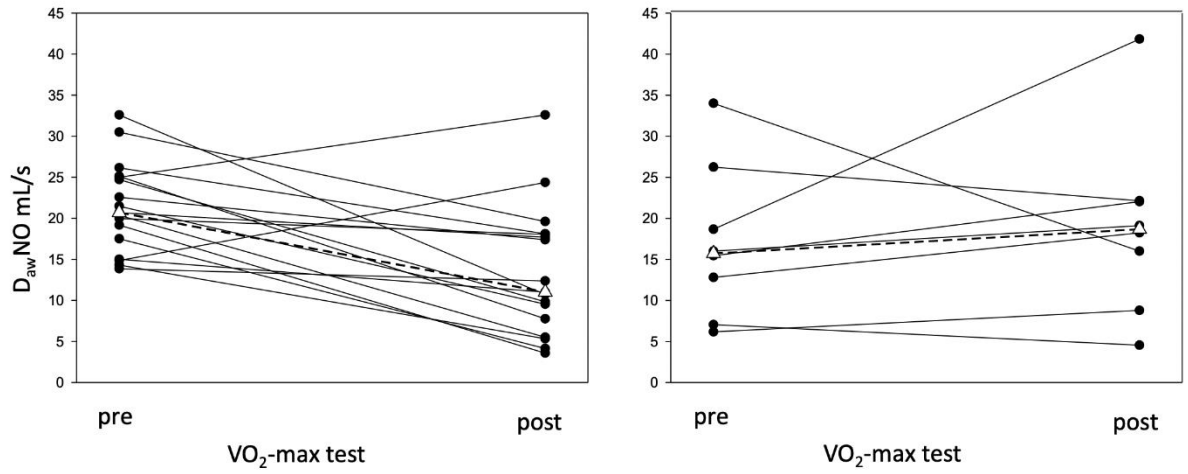

FIGURE 2 In the non-asthma group (left graph) the NO diffusion capacity over the airway wall ($D_{aw}NO$) is decreased (p=0.004) after the $\dot{V}O_2$-max test, while in the asthma group (right graph) there was no statistical change in the median value (p=0.40).

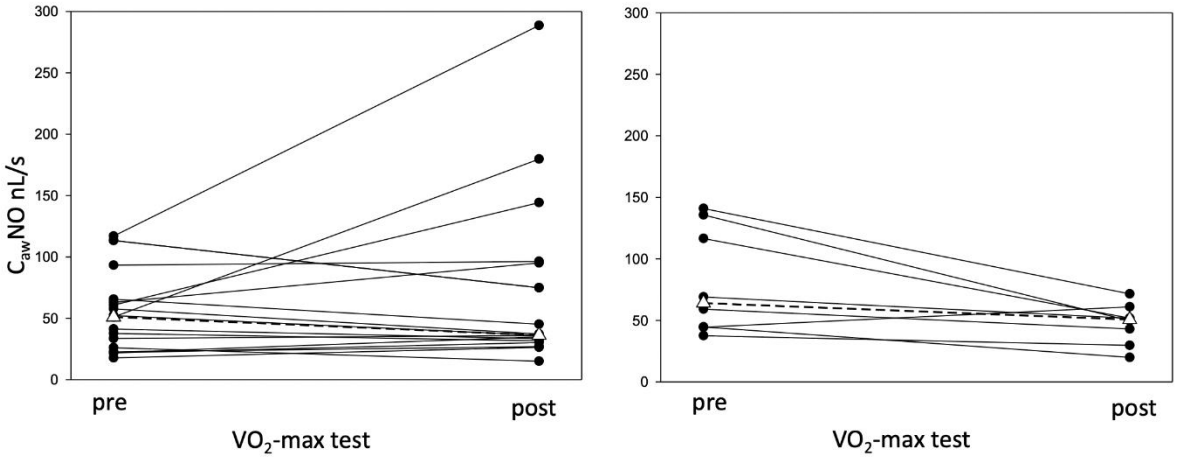

FIGURE 3 In the non-asthma group (left graph) the NO content in the airway wall ($C_{aw}NO$) did not change (p=0.55) after the $\dot{V}O_2$-max test, while in the asthma group (right graph) there was a statistically significant decrease in the median value (p=0.036).