

Endurance Training and the Risk of Bronchial Asthma in Female Cross-Country Skiers

A. Żebrowska, B. Głuchowska, D. Jastrzębski,
A. Kochańska-Dziurawicz, A. Stanjek-Cichoracka, and I. Pokora

Abstract

Exercise is one of the crucial factors responsible for asthma development and exacerbation. The purpose of the present study was to assess the risk of bronchial asthma in female athletes. Spirometric evaluations and physical exercise test were performed and exhaled nitric oxide (eNO) levels were measured in 12 female elite cross-country skiers. Serum concentrations of interleukin 1 beta (IL-1 β), tumor necrosis factor-alpha (TNF- α), and interleukin 6 (IL-6) were measured in all subjects before exercise, immediately after it, and after 15 min of recovery. Peak eNO values were 18.7 ± 4.8 (ppb) and did not confirm the risk of early bronchial asthma symptoms. A graded exercise test caused significant increases in TNF- α and IL-1 β concentration ($p < 0.05$) after 15 min of recovery. A significant negative correlation was found between resting and post-exercise eNO and IL-6 levels ($p < 0.01$). Our study did not confirm an increased risk of bronchial asthma or respiratory tract inflammatory conditions among female cross-country skiers exposed to physical exertion.

Keywords

Athletes • Exercise training • Lung function • Nitric oxide • Skiers

A. Żebrowska (✉), B. Głuchowska, and I. Pokora
Department of Physiological and Medical Sciences,
The Jerzy Kukuczka Academy of Physical Education,
72A, Mikołowska St., 40-065 Katowice, Poland
e-mail: olazebrowska@yahoo.com

D. Jastrzębski
Department of Lung Diseases and Tuberculosis, Medical
University of Silesia, Zabrze, Poland

A. Kochańska-Dziurawicz and A. Stanjek-Cichoracka
Department of Isotope Diagnostics and
Radiopharmaceuticals, Medical University of Silesia,
Sosnowiec, Poland

1 Introduction

Asthma is a chronic inflammatory disease of the lower respiratory tract. It is characterized by bronchial hyperresponsiveness to various stimuli inducing reversible airway obstruction and bronchospasm. Chronic inflammation can be caused by an allergen (atopic asthma), infection, physical exertion, and several unknown factors (non-atopic asthma). According to previous

research, physical exertion is one of the crucial factors provoking the development or exacerbation of asthma (Poon et al. 2012; Parsons and Mastronarde 2009). In some asthmatics, exercise may be the only symptom-provoking factor or it may induce bronchospasm. Increased incidence of asthma is often observed among endurance athletes, frequently exposed to unfavorable weather conditions. Low temperature, low air humidity in combination with very high exercise intensity may cause damage to the airways or mixed neutrophilic-eosinophilic inflammation. This accounts for a high rate of asthma morbidity among cross-country skiers (Elers et al. 2011).

The precise mechanism responsible for initiating of exercise-induced asthma (EIA) is still not well understood, but it has been suggested that the cooling of the airway mucosa may be important (Anderson and Kippelen 2012; Haahtela et al. 2008). Other factors that may influence bronchial hyperresponsiveness include water loss and its potential to change the osmotic environment of the airways, chronic exposure to poorly ventilated wax rooms in Nordic sport, and exposure to high NO₂ concentrations in ice arenas (Rundell et al. 2000). It should be noted that athletes often report asthma symptoms while exercising; they seldom have symptoms at rest (Elers et al. 2011). A lot of evidence has indicated that baseline and functional spirometry performed under laboratory conditions has a low sensitivity to identify EIA, particularly in cold weather athletes (Miller et al. 2005; Rundell et al. 2000, 2004). Therefore, in recent years a number of investigations have focused on the relationships between training intensity, frequently under extreme weather conditions, and the risk of early symptoms of bronchial asthma in athletes (Anderson and Kippelen 2012; Elers et al. 2011; Grob et al. 2008).

Exhaled nitric oxide (eNO) measurement is a non-invasive test which can be used for diagnosis and monitoring of bronchial asthma as well as seasonal allergic rhinitis and chronic obstructive pulmonary disease (Lim and Mottram 2008; De Gouw et al. 2001; Alving et al. 1993). Thus, eNO measurement along with the evaluation of pro-inflammatory cytokines might help

assess the risk of inflammation in the lower respiratory tract. Asthma causes imbalance between pro- and anti-inflammatory cytokines resulting in a chronic inflammatory condition. Interleukin 1 beta (IL-1 β) and tumor necrosis factor-alpha (TNF- α) are the main overexpressed pro-inflammatory cytokines which attract and activate neutrophils through chemotaxis (Berry et al. 2007). They also stimulate phagocytic cells and NADPH oxidase which, in turn, catalyzes reactive oxygen species (ROS) generation. Increased ROS induce interleukin 6 (IL-6) which stimulates inflammatory processes but also, in negative feedback manner, inhibits TNF- α and IL-1 β secretion. Therefore, IL-6 acts as both pro- and anti-inflammatory cytokine. Cytokines and exposure to allergens and oxidants increase the expression of inducible nitric oxide synthase (iNOS), leading to NO production (Chung and Barnes 1999; Robbins et al. 1994). NO is a highly reactive molecule expressed by bronchial epithelial cells, blood vessel endothelium, type 2 pneumocytes, and nerve endings. It has a beneficial effect on the respiratory tract (Grob et al. 2008). However, in bronchial asthma, excessive iNOS-derived NO and ROS intensify the inflammatory process, increase small vessel permeability as well as mucus secretion, damage bronchial epithelium cells, and compromise oxygen uptake by type 2 pneumocytes (Lim and Mottram 2008; Renaud 2001). As cytokines and NO are among the most active asthma mediators, in the present study we attempted to establish whether intensive exercise training in a cold environment could affect the level of these biomarkers in the blood. The aim of the study also was to assess the risk of bronchial asthma in a group of female cross-country skiers on the basis of NO concentration in exhaled air.

2 Methods

2.1 Subjects

The study was approved by the Ethics Committee of The Jerzy Kukuczka Academy of Physical Education in Katowice, Poland and was

Table 1 Somatic characteristic of the subjects

Variables (<i>n</i> = 12)	Women (<i>n</i> = 12)
Height (m)	1.6 ± 0.1
Weight (kg)	56.6 ± 5.4
BMI (kg/m ²)	21.4 ± 1.7
PBF (%)	18.2 ± 3.5
BFM (kg)	9.8 ± 1.9
FFM (kg)	46.3 ± 4.2

Values are means ±SD
BMI body mass index, *PBF* percentage of body fat, *BFM* body fat mass, *FFM* fat-free mass

conducted in a group of 12 elite female cross-country skiers, aged 22.3 ± 2.1 years. The participants were members of the Polish biathlon and cross-country skiing teams and had been training for approximately 7.6 ± 2.1 years. Somatic characteristics of the subjects are presented in Table 1.

2.2 Exercise Protocols

Prior to the study, the participants were training for 6 weeks in cold or hypobaric hypoxic conditions (2,015 m above sea level). The investigations were carried out after the preparatory phase of the annual training cycle. A few days before the examination, the subjects were asked to abstain from strenuous exercise and were put on a standardized normocaloric diet. All subjects were evaluated in the laboratory (ambient conditions: 21 °C, 60 % relative humidity) after an overnight fast. Blood samples were taken from the cubital vein in the morning at rest (between 8:00 and 9:00 a.m.), immediately after exercise and after 15 min of post-exercise recovery. The level of pro-inflammatory cytokines was measured by Enzyme-Linked Immunosorbent Assay kit (ELISA) (Quantikine; R&D System, MN). Exhaled nitric oxide measurement was performed using HYPAIR eNO apparatus (MediSoft, Leeds, UK). The obtained eNO values (ppb) were compared with normal ranges (Dweik et al. 2011). The following spirometry parameters were measured: vital capacity (VC), forced vital capacity (FVC), forced expiratory volume in 1 s (FEV₁), mid-expiratory phase

(FEF_{25–75}), and maximal voluntary volume (MVV). Baseline eNO measurements and spirometry were obtained from each athlete (Lungtest; MES, Cracow, Poland). Each subject then performed graded treadmill exercise test (HP Cosmos; Nussdorf-Traunstein, Germany). Pulmonary ventilation (VE) and oxygen uptake (VO₂) were measured continuously from minute 6 before the test and at different phase of the exercise using an Oxycon Pro apparatus (Jaeger, Hoechberg, Germany).

2.3 Statistical Evaluation

Descriptive statistics were performed to analyze the data. The results were presented as means ±SD. Correlations between maximal oxygen uptake (VO_{2max}), VE, eNO, and, additionally, between eNO and TNF-α, IL-1β, and IL-6 were verified. Repeated measures analysis of variance was used to determine any significant changes in post-exercise cytokines compared with resting levels thereof. Differences were considered statistically significant at *p* < 0.05. The Statistica package ver. 10 (StatSoft Poland, 10.0) was used for data processing and analyses.

3 Results

The analysis of the somatic indices demonstrated low body fat mass and body mass index (BMI); the latter was however within low-normal range. Half of the study participants had the percentage of body fat (PBF) below the normal range (17–24 %). All subjects exhibited a high fat-free mass index (FFM) (Table 1).

The measurement of eNO was performed to assess the risk of early bronchial asthma symptoms. All investigated female athletes exhibited eNO levels below 25 ppb, which falls within normal range (Dweik et al. 2011; Grob et al. 2008) (Table 2). The levels of pro-inflammatory cytokines TNF-α, IL-1β, and IL-6 in blood samples obtained at rest, immediately after exercise, and after 15 min of recovery period are presented in Table 3. Resting levels of

Table 2 Exhaled nitric oxide measurement, lung function indices, and maximal oxygen uptake in female athletes ($n = 12$)

Variable	
eNO (ppb)	18.7 ± 4.8
eNO (mL/s)	45.7 ± 2.4
FVC (%pred)	112.5 ± 6.3
FEV ₁ (%pred)	108.6 ± 6.8
PEF (L/s)	7.0 ± 1.0
(FEF _{25–75}) (L/s)	4.9 ± 0.7
FEV ₁ /VC (%)	89.6 ± 4.6
MVV (L/min)	136.9 ± 25.4
VO _{2max} (mL/kg/min)	54.9 ± 3.0
VE _{max} (L/min)	109.6 ± 15.3

Values are means $\pm$ SD

eNO exhaled nitric oxide, FVC forced vital capacity, FEV₁ forced expiratory volume in 1 s, PEF peak expiratory flow, FEF_{25–75} forced expiratory flow of 25–75 %, FEV₁/VC Tiffeneau index, MVV maximal voluntary ventilation, VO_{2max} maximal oxygen uptake, VE_{max} maximal ventilation

Table 3 Cytokine levels at rest, immediately after graded maximal exercise (Max) and after a 15-min recovery period (Rec) ($n = 12$)

Variable	Rest	Max	Rec	Δ Ex%
TNF- α (pg/mL)	2.3 ± 1.0	$5.2 \pm 4.7^*$	$5.3 \pm 2.6^*$	187.7
IL-1 β (pg/mL)	1.1 ± 0.9	0.9 ± 0.3	$2.2 \pm 1.6^*$	168.8
IL-6 (pg/mL)	1.1 ± 0.4	1.4 ± 0.4	1.4 ± 0.6	30.7

Values are means $\pm$ SD. Ex% Δ percent change from baseline to recovery

* $p < 0.05$ significant difference between Max vs. Rest and Rec vs. Rest

interleukins IL-1 β and IL-6 were comparable (1.1 ± 0.9 vs. 1.1 ± 0.4 pg/ml). These levels were low as also was that of TNF- α , which is in accord with other data reported in endurance-trained subjects (Larsen et al. 2002; Suzuki et al. 2002). Maximal graded exercise led to a significant increase in TNF- α compared with the resting level ($p < 0.05$). TNF- α level remained increased after a 15-min recovery period. Post-exercise IL-6 concentrations were also increased, but the differences between pre- and post-exercise levels were not significant. Interestingly enough, the level of IL-1 β decreased during exercise and then increased significantly during a

15-min post-exercise recovery ($p < 0.05$) (Table 3). A significant negative correlation was revealed between eNO (mL/s) and IL-6 levels at rest ($r = -0.66$), and immediately after the exercise test ($r = -0.62$) ($p < 0.01$). The skiers demonstrated a high level of aerobic capacity as confirmed by high VO_{2max} and VE_{max} (Table 2). The mean VC was within normal range (4.4 ± 0.3 L and 108 % of predicted) in all females examined. The mean value of forced expiratory volume in 1 s (FEV₁), peak expiratory flow (PEF), forced expiratory flow of 25–75 % (FEF_{25–75}), and maximal voluntary ventilation (MVV) exceeded the physiological range for healthy population (Table 2). No significant correlation was found between baseline eNO and spirometric variables or eNO and VO_{2max} ($p > 0.01$).

4 Discussion

Chronic inflammation of the airways may lead to adverse changes in the structure of the bronchial walls and cause significant impairment of the respiratory system function (Poon et al. 2012). Therefore, early diagnosis and proper medical treatment seem to be crucial (Popov 2011). Nowadays, the diagnosis of bronchial asthma is based on a detailed interview, spirometry, and bronchial hyperresponsiveness examination (Porsbjerg et al. 2009; Miller et al. 2005). A reduction in forced expiratory flow at 25–75 % of the pulmonary volume, with otherwise normal spirometry, might indicate early allergic or inflammatory involvement of the airways. Monitoring of asthma activity includes the measurements of PEF, FEV₁, and the assessment of use of inhaled medications (β_2 -mimetics). However, these parameters do not allow sufficient estimation and prediction of asthma course in athletes. Traditional assessment of exercise-induced asthma requires the measurement of FEV₁ before and after exercise, or a surrogate of exercise such as eucapnic voluntary hyperpnea of dry air or mannitol dry powder. Laboratory spirometry testing has been shown to have a low sensitivity to detect exercise-induced asthma,

and exercise testing in the field can be challenging, particularly in athletes from winter sport disciplines (Anderson and Kippelen 2012; Rundell et al. 2000).

A growing body of evidence supports the utility of the concentration of NO in the exhaled air at a constant exhalation flow as a marker of inflammation in asthma (Porsbjerg et al. 2009; Kharitonov et al. 1994, 1995; Alving et al. 1993). Therefore, our study aimed to evaluate eNO exchange in female cross-country skiers. The findings demonstrate that endurance training positively affected functional capabilities of the respiratory system. Both spirometry and eNO data clearly indicate that female biathlon and cross-country skiing athletes were rather unlikely to develop inflammatory conditions in the lower respiratory tract. The literature data show a high incidence of bronchial asthma among swimmers, ice-hockey players, and cross-country skiers (Elers et al. 2011; Haahtela et al. 2008; Miller et al. 2005). Cross-country skiers seem to be the most exposed to extreme weather conditions, which causes strong and repeated hyperventilation. Hyperventilation-induced heat and water loss promotes an increase in airway osmolarity. This may lead to inflammation onset. Changes in eNO during and after exercise raised hopes of finding a noninvasive, more accurate method for the evaluation of exercise-induced inflammatory conditions within the respiratory tract. It has been found that increased eNO in asthma is due to an upregulation of one or more of NOS isoforms and extension of NO-producing cells in the airways (Lim and Mottram 2008; Robbins et al. 1994). Much evidence suggests that epithelial cells express inducible NOS in response to both pro-inflammatory cytokines, including TNF- α , IL-1 β , and interferon- γ (IFN- γ), and reactive oxygen species (Berry et al. 2007; Chung and Barnes 1999; Kharitonov et al. 1995). Since intensive physical exercise is associated with systemic cytokine increases to the levels observed during severe infections, it has been hypothesized that exercise training, particularly in cold environment, increases NO production.

eNO values of cross-country female skiers indicated a low risk of developing bronchial

asthma. Nonetheless, normal amounts of NO in exhaled air do not exclude asthma, and especially its non-atopic form. As NO is a nonspecific inflammatory mediator, we also decided to assess the levels of pro-inflammatory cytokines. Recent studies have demonstrated that strenuous exercise induces an increase in pro-inflammatory cytokines secretion leading to increase susceptibility to infections (Suzuki et al. 2002; Pedersen et al. 1998). However, a decrease in cytokine production resulting from regular training has also been documented (Petersen and Pedersen 2005; Larsen et al. 2002). Noticeable adaptation to physical exertion was also observed in the present study as the female skiers were characterized by low levels of cytokines after exercise and recovery period. The main inducers of acute phase reactions are the proinflammatory cytokines TNF- α and IL-1 β . The majority of studies have shown that the circulating level of these cytokines is not significantly changed following exercise. Small changes observed in these cytokines could be mediated by anti-inflammatory cytokines, such as IL-6 and IL-10, or by cytokine inhibitors. Increased cytokine secretion is observed during high intensity physical exercise inducing micro-damages to muscle fibers. We did observe a significant increase in TNF- α level immediately and shortly after a high intensity exercise test ($\Delta\text{Ex} = 187.7\%$). Plasma IL-1 β levels increased significantly after exercise ($\Delta\text{Ex} = 168.8\%$) during a 15-min post-exercise recovery. This might have been caused by an increase in anti-inflammatory cytokines or high exercise levels of TNF- α , which, according to numerous studies, stimulates the production of IL-1 β . This finding could also confirm a decrease in the athletes' body response to the exercise test. Negative correlation seen between the level of IL-6 at rest and immediately after graded maximal exercise and respective eNO concentrations might be associated with IL-6 exhibiting both pro- and anti-inflammatory activities.

There are some limitations to this study. Firstly, we did not perform repeated measurements of exercise-induced eNO levels. However, in healthy subjects, exercise increases NO elimination due to increased ventilation rates. Secondly, our study was carried out in the

laboratory which could have influenced the inflammatory response in a different way than there is during strenuous exercise training in the field. Nevertheless, there is a need for further research to clarify the relationship between eNO and the level of cytokines during sports competitions.

5 Conclusions

In conclusion, the present study failed to confirm an increased risk of bronchial asthma or respiratory tract inflammation among female cross-country skiers. Nevertheless, it should be emphasized that the evaluation of exhaled nitric oxide and pro-inflammatory cytokines might still prove to be a good adjunct tool for diagnosing bronchial asthma or respiratory tract inflammation in professional athletes.

Conflicts of Interest The authors declare no conflicts of interest in relation to this article.

References

- Alving K, Weitzberg E, Lundberg JM (1993) Increased amount of nitric oxide in exhaled air of asthmatics. *Eur Respir J* 6(9):1368–1370
- Anderson SD, Kippelen P (2012) Assessment and prevention of exercise-induced bronchoconstriction. *Br J Sports Med* 46:391–396
- Berry M, Brightling C, Pavord I, Wardlaw A (2007) TNF-alpha in asthma. *Curr Opin Pharmacol* 7(3):279–282
- Chung KF, Barnes PJ (1999) Cytokines in asthma. *Thorax* 54(9):825–857
- De Gouw HW, Marshall-Partridge SJ, Van Der Veen H, Van Den Aardweg JG, Hiemstra PS, Sterk PJ (2001) Role of nitric oxide in the airway response to exercise in healthy and asthmatic subjects. *J Appl Physiol* 90:586–592
- Dweik RA, Boggs PB, Erzurum SC, Irvin CG, Leigh MW, Lundberg JO, Olin AC, Plummer AL, Taylor DR (2011) An official ATS clinical practice guideline: interpretation of exhaled nitric oxide levels (eNO) for clinical applications. *Am J Respir Crit Care Med* 184:602–615
- Elers J, Pedersen L, Backer V (2011) Asthma in elite athletes. *Expert Rev Respir Med* 5(3):343–351
- Grob NM, Laskowski D, Dweik RA (2008) A technical report on exhaled nitric oxide measurement: asthma monitoring in athletes. *J Breath Res* 2(3):37027. doi:10.1088/1752-7155/2/3/037027
- Hahtela T, Malmberg P, Moreira A (2008) Mechanisms of asthma in Olympic athletes-practical implications. *Allergy* 63(6):685–694
- Kharitonov SA, Yates D, Robbins RA, Logan-Sinclair R, Shinebourne EA, Barnes PJ (1994) Increased nitric oxide in exhaled air of asthmatic patients. *Lancet* 343:133–135
- Kharitonov SA, Yates D, Barnes PJ (1995) Increased nitric oxide in exhaled air of normal human subjects with upper respiratory tract infections. *Eur Respir J* 8:295–297
- Larsen AI, Lindal S, Aukrust P, Toft I, Aarsland T, Dickstein K (2002) Effect of exercise training on skeletal muscle fiber characteristics in men with chronic heart failure. Correlation between skeletal muscle alterations, cytokines and exercise capacity. *Int J Cardiol* 83(1):25–32
- Lim KG, Mottram C (2008) The use of fraction of exhaled nitric oxide in pulmonary practice. *Chest* 133:1232–1242
- Miller MG, Weiler JM, Baker R, Collins J, D'Alonzo G (2005) National Athletic Trainers' Association position statement: management of asthma in athletes. *J Athl Train* 40(3):224–245
- Parsons JP, Mastronarde JG (2009) Exercise-induced asthma. *Curr Opin Pulm Med* 15(1):25–28
- Pedersen BK, Rohde T, Ostrowski K (1998) Recovery of the immune system after exercise. *Acta Physiol Scand* 162(3):325–332
- Petersen AMW, Pedersen BK (2005) The anti-inflammatory effect of exercise. *J Appl Physiol* 98(4):1154–1162
- Poon AH, Eidelman DH, Martin JG, Laprise C, Hamid Q (2012) Pathogenesis of severe asthma. *Clin Exp Allergy* 42(5):625–637
- Popov TA (2011) Human exhaled breath analysis. *Ann Allergy Asthma Immunol* 106:451–456
- Porsbjerg C, Lund TK, Pedersen L, Backer V (2009) Inflammatory subtypes in asthma are related to airway hyperresponsiveness to mannitol and exhaled NO. *J Asthma* 46(6):606–612
- Renauld JC (2001) New insights into the role of cytokines in asthma. *J Clin Pathol* 54:577–589
- Robbins RA, Barnes PJ, Springall DR, Warren JB, Kwon OJ, Buttery LDK, Wilson AJ, Geller DA, Polak JM (1994) Expression of inducible nitric oxide synthase in human bronchial epithelial cells. *Biochem Biophys Res Commun* 203:209–218
- Rundell KW, Wilber RL, Szmedra L, Jenkinson DM, Mayers LB, Im J (2000) Exercise-induced asthma screening of elite athletes: field versus laboratory exercise challenge. *Med Sci Sports Exerc* 32(2):309–316
- Rundell KW, Anderson SD, Spiering BA, Judelson DA (2004) Field exercise vs. laboratory eucapnic voluntary hyperventilation to identify airway hyperresponsiveness in elite cold weather athletes. *Chest* 125(3):909–915
- Suzuki K, Nakaji S, Yamada M, Totsuka M, Sato K, Sugawara K (2002) Systemic inflammatory response to exhaustive exercise. Cytokine kinetics. *Exerc Immunol Rev* 8:6–48