THE INTERACTION BETWEEN RESPIRATORY FUNCTION AND EXHALED NITRIC OXIDE IN EXERCISE-INDUCED BRONCHOCONSTRICTION IN SPORTSMEN

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The aim of the investigation was to study the features of exercise-induced bronchospasm revealed by laboratory tests under intensive training, to assess the relationship between this phenomenon and the level of expiratory NO fraction (FeNO) in asymptomatic winter sports athletes without asthma diagnosis.

Materials and Methods. Skiers and biathletes aged from 13 to 26 years were examined in two stages: stage 1 — standard laboratory treadmill test (n=52); stage 2 — outdoor exercise challenge test at subfreezing temperature (n=78). The FeNO level was measured before and after exercise at both stages.

Results. The prevalence of exercise-induced bronchospasm (EIB) in laboratory treadmill testing and outdoor exercise testing was 21 and 6.4% respectively. In these individuals no significant differences in baseline spirometry parameters were found compared to non-EIB athletes. The laboratory testing revealed significantly higher baseline FeNO levels in males compared to female athletes. We recorded the positive correlation of baseline spirometric parameters and FeNO, post-exercise FeNO and forced expiratory volume in 1 s after 1 and 5 minutes of the test, and the negative correlation of FeNO after the exercise and peak expiratory flow 5 minutes after the exercise.

Conclusion. EIB has low prevalence among winter sports athletes. However, the obtained results suggest that the excess NO production in respiratory ways of sportsmen can exacerbate the intensity of bronchial obstruction or is associated with the obstruction somehow.

Key words: exercise-induced bronchoconstriction; FEV1 in sportsmen; exhaled nitric oxide.

Exercise-induced bronchospasm (EIB) is the decrease of forced expiratory volume in one second (FEV1) by 10% and more compared to the initial value after exercise [1]. The phenomenon is the most important in winter sports (cross country skiing, biathlon, snowboard, etc) [1, 2]. In case of the increased minute pulmonary ventilation volume in at? low temperatures, the respiratory air is warmed and moistened with the involvement of the bronchi of the 12th order and less (<1 mm). The distinctive feature of these parts of the respiratory tract is a small volume of airway surface liquid , and a significantly greater number of mast cells compared to larger bronchi. Therefore, in these parts the most intensive processes of mucosal drying and cooling followed by degranulation of mast cells and release of pre-synthesized inflammatory mediators, as well as mucosal reactive hyperemia and edema [2, 3]. Thus, it is impossible to study the pathogenesis of EIB outside the context of lipid peroxidation and oxidative and nitrosative stress development. The activation of these processes in high class sportsmen is proved by the latest research findings, and may be an essential component in the maintaining of the respiratory mucosa inflammation [2, 4–6]. According to these authors, nitric oxide (NO) level can appear as EIB predictor [2, 4–6].

In appliance with joint clinical practice guideline of American Academy of Allergy, Asthma and Immunology

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(AAAAI) and American College of Allergy, Asthma and Immunology (ACAAI) [2], EIB diagnosis should be substantially based on objective diagnostic techniques including both direct and indirect provocation tests. In addition, the preference should be given to more sensitive indirect methods including eucapnic voluntary hyperventilation, mannitol inhalation, hypertonic solution or adenosine monophosphate nebulization, as well as an exercise testing [3].

The aim of the investigation was to study the features of exercise-induced bronchospasm revealed by laboratory tests (in room temperature) and at field-testing (intensive training in subfreezing temperature), to assess the interaction of this phenomenon with an exhaled nitric oxide level in asymptomatic winter sports athletes without asthma diagnosis.

Materials and Methods. The present work included two indirect methods to reveal EIB: a standard laboratory treadmill-test, as well as an intensive outdoor exercise challenge test at subzero temperature. The study was two-staged: the first stage — the examination of 86 adolescents and young adults engaged in winter sports (biathletes, skiers, hockey players, snowboarders), their mean age being 20.8 years (57 sportsmen and 29 sportswomen). The spirometry was performed initially, on the 1st, 5th, 10th and 15th minute after a standard 8-minute exercise treadmill-test using MasterScreenPneumo spirograph (Jaeger, Germany). Within the first four minutes of testing the sportsmen reached submaximal heart rate level, then the exercise activity was controlled in order to maintain the obtained rate at a constant level within 4 min (Guidelines for Methacholine and Exercise Challenge Testing, ATS, 1999). An exercise provocation test was estimated as positive if FEV1 decreased by 10% and more in two sequential measure points.

The second stage included 78 biathletes and skiers aged 15.8±0.3 (43 sportsmen and 35 sportswomen). At this stage EIB was screened after an intensive outdoor exercise at subzero temperature using the same time intervals in dynamic spirometry, as in the laboratory test.

Both stages included the measurement of fractional exhaled nitric oxide (FeNO) using analyzer CLD 88 in combination with Denox 88 device releasing inhaled air from NO (Eco Medics AG, Switzerland) initially and after exercise. The sportsmen were tested in accordance with ATS/ERS recommendations (2005) [7]. The prescreening excluded those who had had respiratory episodes for the last two weeks.

The results were statistically processed using “Statistica for Windows 10.0” program. The data were represented as arithmetic mean with standard error of mean. Mann-Whitney U test was used to assess the mean difference in pairwise independent samplings, and Wilcoxon criterion — in dependent samplings. The degree of interaction between the signs was assessed by calculating Spearman correlation coefficient. The differences were considered statistically significant at a significance level of $p<0.05$.

Results. In laboratory treadmill-test 21% of examined sportsmen demonstrated features of EIB [1]. Initial absolute values of FEV1 and forced vital capacity (FVC) in this group were significantly higher than those in non-EIB subjects (Table 1). In field-test at subzero temperature EIB was found in 6.4% of cases only. At this stage the examined sportsmen with recorded EIB had no statistically significant differences in initial spirometric parameters compared with non-EIB sportsmen .

Initial FeNO level in males undergoing laboratory tests was significantly higher than that in female athletes (Fig. 1). NO synthesis intensity in sportsmen of both genders when testing in a training session process at subzero temperatures was comparable. Post-exercise FeNO level in most examined male and female sportsmen was found to be decreased: a treadmill-test — up to 63.6 and 72.7%; outdoor exercises — up to 75.8 and 67.6%, respectively. It should be noted that in case of FeNO level increase,

Table 1

Respiratory function indices in EIB-positive and EIB-negative sportsmen

Indices	EIB, mean (95% CI*)	non-EIB, mean (95% CI)	p
Treadmill–test			
FEV1, initially, L	5.2 (4.7–5.7)	4.2 (3.9–4.4)	<0.01
FVC, initially, L	6.5 (5.5–7.6)	5.142 (4.8–5.5)	<0.01
PEF 1 min**, %	88.12 (80.1–96.1)	102.1 (96.8–107.4)	<0.01
ΔFEV1 1 min**, %	–15.98 (–21.9…–10.1)	2.38 (0.2–5)	<0.01
ΔFEV1 5 min***, %	–10.313 (–16.6…–4.0)	3.014 (0.6–6.6)	<0.01
Outdoor exercise			
ΔFEV1 1 min**, %	–12.6 (–19.0…–6.2)	–0.1 (–1.3…–1.2)	<0.01
ΔFEV1 5 min***, %	–11.8 (–24.7…–1.1)	–0.9 (–1.9…–0.1)	<0.01

Notes: * — 95% confidential interval; ** — 1st minute after exercise; *** — 5th minute after exercise. PEF — peak expiratory flow.

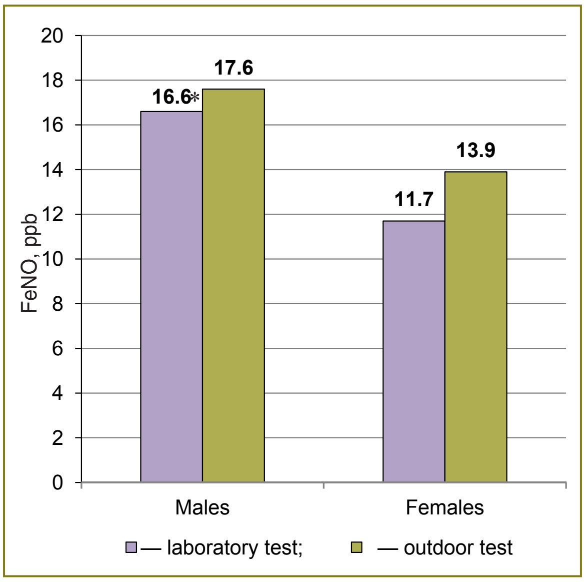

Fig 1. The distribution of the initial FeNO level depending on gender; * — p=0.04 compared to the index among female athletes

the differences of pre- and postexercise data were more significant rather than when the index decreased (+70 and –26%, respectively, in laboratory testing; +40 and –29% in testing under exercise conditions). Gender differences were found in pre- and post-exercise FeNO level dynamics both at the first, and the second stages (Fig. 2).

There were no significant differences of spirometric data in groups of sportsmen with decreased and increased/unchanged FeNO after a treadmill-test. However, significant differences of pre- and post-exercise forced expiratory flow rates during field-testing was noticed in these groups (Table 2). Table 3 demonstrates the revealed correlations of spirometry data with FeNO levels.

Discussion. The present survey enabled to obtain priority results on EIB incidence among sportsmen engaged in winter sports, as well as the interaction of nitrosative stress indices and spirometric parameters in Russian North. European and American research works are the sources

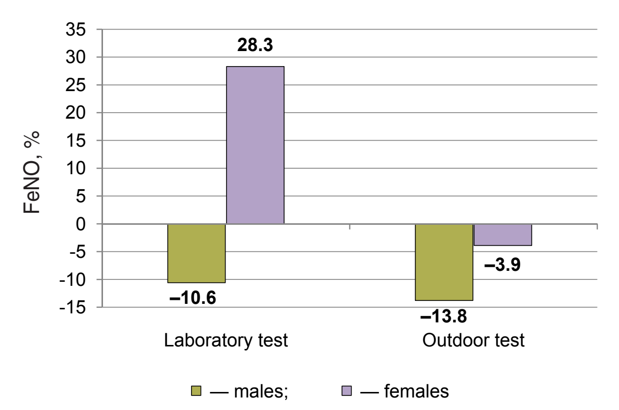

Fig. 2. The intensity of fractional exhaled NO in sportsmen depending on testing conditions

Table 2

Respiratory function data in sportsmen depending on pre- and post-exercise FeNO level dynamics, field-test

Index	FeNO decrease, mean (95% CI*)	Increased or not changed FeNO, mean (95% CI*)	p
$MMEF_{25–75}$, initially, L	4.2 (3.8–4.5)	3.2 (2.8–3.7)	<0.01
$MMEF_{25–75}$, initially, %	103.1 (95.7–110.4)	88.4 (78.5–98.3)	0.03
FEF_{50}, initially, L	4.7 (4.3–5.0)	3.9 (3.5–4.3)	0.01
FEF_{75}, initially, L	2.1 (1.9–2.3)	1.6 (1.3–1.9)	0.01
FEF_{50}**, L	4.6 (4.2–5.0)	3.9 (3.4–4.3)	0.02
FEF_{75}**, L	2.1 (1.9–2.4)	1.6 (1.4–1.9)	0.01
FEF_{75}**, %	97.7 (86.8–108.5)	77.6 (67.7–87.5)	0.03
$FEF_{25–75}$***, L	4.0 (3.7–4.4)	3.3 (2.9–3.8)	0.02
FEF_{50}***, L	4.6 (4.2–5.1)	3.9 (3.4–4.4)	0.04
FEF_{75}***, L	2.1 (1.9–2.4)	1.7 (1.4–1.9)	0.03

Note: * — 95% confidential interval; ** — 1st minute after exercise; *** — 5th minute after exercise. $MMEF_{25–75}$ — maximum mid-expiratory flow, $FEF_{25, 50, 75}$ – forced expiratory flow at 25, 50 and 75% of forced vital capacity.

Table 3

The correlations of respiratory function indices with exhaled NO level in sportsmen

FeNO	Indices	r_s	p
Treadmill–test			
FeNO, ppb, before exercise	FEV1*, L	0.41	<0.01
FeNO, ppb, after exercise	Δ FEV1**, %	0.38	0.04
	Δ FEV1***, %	0.58	0.001
	PEF 5 min after*, %	–0.56	0.002
Outdoor exercise			
FeNO, ppb	FEV1*, %	0.30	<0.01
	FVC*, %	0.33	<0.01
	PEF*, %	0.29	0.01
	FVC**, %	0.28	0.02
Δ FeNO, %	FVC*, %	0.31	0.01
	PFR_{50}**, L	–0.27	0.04
	PFR_{75}**, L	–0.25	0.04
	$PFR_{25–75}$***, L	–0.30	0.03
	$PFR_{25–75}$***, %	–0.28	0.04

Notes: * — 95% confidential interval; ** — 1st minute after

of the greater part of published epidemiological evidence on EIB and exercise induced asthma in representatives of various sports. Thus, according to the diagnostic findings of American Olympic Winter sport athletes in 1998, EIB incidence was 23%, and the subgroup of skiers had the maximum index (50%) when indirect diagnostic exercise methods (competition or intensive training) were used [8]. In the present work the total EIB incidence among skiers and biathletes according to the results of the both stages using various diagnostic procedures was 6.4–21%. It should be noted that only one skier with recorded FEV1 decrease in response to exercise had had anamnesis of asthma. In this context it is worth to remind the results of recent epidemiological study of EIB in 144 athletes of American National Collegiate Athletic Association [9].Using eucapnic voluntary hyperventilation test EIB was revealed in 4 sportsmen (2.7%) only. Only 2 of 64 examined sportsmen reported respiratory symptoms during sport activitywere found to have objective EIB signs. FeNO index in subgroups of EIB-positive and negative athletes was 13.25 ppb and 24.5 ppb, respectively. According to the authors, FeNO level as well as respiratory symptoms are not informative as EIB predictors.

Many works deal with the study of EIB mechanisms, its connection with the markers of oxidative and nitrosative stress, in particular, with FeNO level [2, 4–6]. It is worth noting that in most cases the object of the researches is patients with exercise induced asthma, where physical exertion appeared as a trigger of the disease symptoms. So, I. Chinellato et al. [10] studied the correlation between EIB severity and FeNO level in children with atopic asthma. Positive correlation between the degree of FEV1 decrease and $MMEF_{25–75}$ rate as a result of an exercise testwas found T. Grzelewsky et al. [11] studied FeNO level as a possible predictor of EIB and uncontrolled asthma in children and adolescents — FeNO for EIB diagnosis was 16 ppb. In the authors' opinion, this index is an independent EIB predictor and can be used for its screening in patients with atopic asthma.

The present work for the first time has represented the study of FeNO production in asymptomatic sportsmen, 21 and 6.4% of whom demonstrated EIB according to the results of a laboratory test and an exercise test at subzero temperature respectively. There were no any statistically significant differences in FeNO levels of EIB-positive and EIB-negative sportsmen both initially and after exercise test.

The most part of the athletes demonstrated an expected FeNO level decrease related to exercise-related hyperventilation and NO removing from the respiratory tract. However, 21% of the sportsmen examined in the laboratory and 28% of those who did outdoor exercises in low temperature had this index increased. In addition, the analysis of pre- and post-exercise spirometry (outdoor test) showed the sportsmen with increased FeNO level to have statistically significantly decrease of maximum mid-expiratory flow rate ($MMEF_{25–75}$) and forced expiratory flow at 50 and 75% of forced vital capacity (FEF_{50}, FEF_{75}), both initially, and after exercise (See Table 2). This is interesting due to the fact that $MMEF_{25–75}$ index along with FEV1 also indicates the bronchial obstruction and can be used for EIB diagnosis. Moreover, according to G. Ciprandi et al. [12], the decrease of $MMEF_{25-75}$ was associated with the marked bronchial hyperreactivity in metacholine challenge test and with NO production intensity in airways.

By the correlation analysis we determined the positive correlation of initial EIB and FeNO level. This correlation is likely to be explained by chronic respiratory inflammation provoked by intensive training at low temperatures. Inflammatory reaction in response to exercise and cold air can include mast cell degranulation, the activation of cysteinyl leurotrienes synthesis, and the granulocytes infiltration of mucosa resulting in its damage [2]. All these consequences will both contribute to the development of bronchoconstriction and bronchial hyperreactivity, and promote inducible NO-synthase in bronchial epithelium and in other cells [13].

The negative correlation of postexercise FeNO with PEF (peak expiratory flow) 5 min after treadmill-test, as well as the positive correlation of this index with ΔFEV1 at 1st and 5th minutes after exercise indicate the excess NO production to be able to worsen the respiratory obstruction or someway related to it. In this view it is worth mentioning the negative correlation of ΔFeNO and postexercise levels of $MMEF_{25–75}$, FEF_{50}, FEF_{75}. The rationale of the found correlations can be the inflammation of respiratory mucosa in the examined sportsmen with high FeNO associated with desquamation of bronchial ciliated epithelium, the increase in microvasculature permeability and mucosal edema resulted from increased osmolarity of bronchial secretion and degranulation of mast cells. In this case the decrease of spirometric parameters of broncho-obstruction after exercise is nothing else but the result of bronchial hyperreactivity in response to a triggering factor. Actually, the research findings of the last years give evidence that the content of exhaled NO in bronchial asthma patients with EIB before an exercise test exceeds this index in healthy subjects and remains high after provocation testing [13, 14]. In this regard, it seems reasonable to suggest NO participation in EIB pathogenesis due to the activation of free radical oxidation with the development of nitrosative stress and bronchial mucosal damage, epithelial desquamation, etc. In support of the hypothesis there is a number of Russian [15] and foreign researchers' [14] works on a key role of active nitric forms (NO, peroxynitrite, etc.) in the realization of lipid peroxidation cascade and the respiratory tract inflammation in broncho-obstructive pathology. Ö. Kasimay et al. conducted one of a few investigations dealing with the role of NO in EIB genesis in sportsmen [16]. In 8 of 43 examined soccer players EIB was found according to the results of a standard treadmill-test. This group of sportsmen was characterized by an increase of NO, blood endothelin-1, malon dialdehyde in response to exercise. In a group of healthy soccer players an exercise resulted in significant decrease of these parameters that was accompanied by statistically significant increase of glutathione level [16]. Thus, the findings prove the hypothesis for the presence of the signs of systemic oxidative stress with the participation of active nitric forms in professional soccer players with EIB.

In the presented study the absence of statistically significant differences of FeNO level in EIB-positive and EIB-negative sportsmen makes it difficult to interpret the role of NO in EIB. Nevertheless, EIB with no clinical presentation of asthma is the manifestation of nonspecific bronchial hyperreactivity [2], in which the expression of inducible NO-synthase is most likely to be not so high, as in persistent atopic eosinophilic inflammation.

Conclusion. Exercise-induced bronchospasm incidence among the examined sportsmen is not high. The determination of FeNO is sure to be an attractive method to study the state of the respiratory tract in sportsmen. The technique has a number of advantages: it is noninvasive, fast in execution, and enables to make an early dynamic assessment of airways condition. However, the characteristics of NO synthesis activation in response to exercise in EIB can differ from those in combination of EIB and asthma.

It is obvious that there is the necessity for further study of NO synthesis, cell composition of sputum and bronchoalveolar lavage in isolated subclinical EIB in sportsmen in order to optimize its diagnosis, prophylaxis and pharmacological correction.

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