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ORIGINAL ARTICLE

Fractional exhaled nitric oxide in the assessment of exercise-induced bronchoconstriction: A multicenter retrospective analysis of UK-based athletes

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Introduction: Exercise-induced bronchoconstriction (EIB) is not only highly prevalent in people with asthma, but can also occur independently, particularly in athletes. Fractional exhaled nitric oxide (FeNO) is an indirect biomarker of type 2 airway inflammation that has an established role in the assessment and management of asthma. The aim was to evaluate the value of FeNO in the assessment of EIB in athletes.

Method: Multicenter retrospective analysis. In total, 488 athletes (male: 76%) performed baseline FeNO, and spirometry pre- and post-indirect bronchial provocation via eucapnic voluntary hyperpnea (EVH). Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated for established FeNO thresholds—that is, intermediate (≥25 ppb) and high FeNO (≥40 ppb and ≥ 50 ppb)—and were evaluated against objective evidence of EIB (≥10% fall in FEV_1). The diagnostic accuracy of FeNO was calculated using receiver operating characteristics area under the curve (ROC-AUC).

Results: Thirty-nine percent of the athletes had a post-EVH fall in FEV_1 consistent with EIB. FeNO values ≥25 ppb, ≥40 ppb, and ≥ 50 ppb were observed in 42%, 23%, and 17% of the cohort, respectively. The sensitivity of FeNO ≥25 ppb was 55%, which decreased to 37% and 27% at ≥40 ppb and ≥ 50 ppb, respectively. The specificity of FeNO ≥25 ppb, ≥40 ppb, and ≥ 50 ppb was 66%, 86%, and 89%, respectively. The ROC-AUC for FeNO was 0.656.

Conclusions: FeNO ≥40 ppb provides good specificity, that is, the ability to rule-in a diagnosis of EIB. However, due to the poor sensitivity and predictive values, FeNO should not be employed as a replacement for indirect bronchial provocation in athletes.

KEYWORDS

airway inflammation, asthma, diagnosis, eucapnic voluntary hyperpnea, exercise, phenotype

1 | INTRODUCTION

Exercise-induced bronchoconstriction (EIB) is a condition characterized by temporary lower airway narrowing that occurs during and/or post-physical exertion. EIB is not only highly prevalent in people with asthma, but can also occur independently, particularly in athletic populations.[1] The prevalence of EIB is reported to be higher in athletic cohorts (21%)[2,3] in comparison to asthma in the general population (9%–12%).[4] The reason for this is thought to be related to the training demands associated with elite level sport; that is, sustained high ventilatory demand +/− exposure to environmental irritants or noxious pollutants.[5,6]

The diagnosis of EIB is challenging due to the limited value of self-reported respiratory symptoms and broad differential diagnosis.[7,8] In addition, there remains a lack of consensus regarding the optimal or "gold standard" test to confirm or refute a diagnosis of EIB. Nonetheless, it is currently recommended that diagnostic work-up should include a detailed clinical history and objective airway assessment.[9] Specifically, indirect bronchial provocation challenges, such as exercise challenge testing, eucapnic voluntary hyperpnea (EVH), and inhaled mannitol are recommended for this purpose, on the basis that they act to mimic the desiccating stimulus that promotes bronchoconstriction in susceptible individuals.[9]

Exercise challenges are typically considered to provide the greatest specificity to detect EIB; however, EVH is thought to offer greater sensitivity.[10,11] Although indirect bronchial provocation challenges provide objective evidence of EIB,[12] they offer limited insight into inflammatory mechanisms or disease subtypes. Airway inflammation is thought to contribute to the development of EIB via release of potent bronchoconstrictive agents, such as mast cell-derived prostaglandins,[13,14] and mast cell- and eosinophilic-derived leukotrienes.[14–16]

The measurement of fractional exhaled nitric oxide (FeNO) is a relatively accessible, simple, and cheap method to quantify type 2, eosinophilic-mediated airway inflammation (i.e., signaling activation of IL-4/IL-13 pathway).[17,18] Nitric oxide is present in exhaled breath due to nitric oxide synthase upregulation that occurs when eosinophils infiltrate the airways.[17] When utilized as part of standard asthma care, low FeNO in adults is considered to be less than 25 ppb.[18] The National Institute for Health and Care Excellence (NICE) and American Thoracic Society (ATS) have contrasting cut-offs for high FeNO (40 ppb and 50 ppb, respectively).[18,19] The European Respiratory Society (ERS) have recently suggested that FeNO ≥40 ppb is the optimal compromise between sensitivity and specificity; however, it recognizes that a FeNO ≥50 ppb has a particularly high specificity (>90%) to confirm an asthma diagnosis.[20]

A key limitation of "fixed" FeNO thresholds in the diagnosis of asthma is the failure to account for personal factors that have been shown to impact normal ranges. For example, females have been reported to have approximately 25% lower FeNO values compared to males.[21,22] Further, height,[23,24] age,[24,25] and atopic status[24,26] may all act as confounders. Personalized normal values for FeNO that account for these factors have therefore been proposed[24]; however, the diagnostic value of such personalized normal values in the context of EIB is yet to be evaluated.

From a practical point of view, it would be advantageous to use FeNO to predict EIB, as it would reduce the requirement to conduct often complex and time-consuming indirect bronchial provocation challenges. To date, however, there remains a lack of consensus regarding the value of FeNO to predict EIB in athletes. While some researchers have reported that a high FeNO (≥50 ppb) is predictive of EIB in children,[27,28] others have argued that FeNO should not be employed to detect EIB in adolescents.[29]

The purpose of this study was therefore to investigate the diagnostic value of FeNO in the assessment of EIB in a large cohort of elite and recreational athletes. A secondary aim was to evaluate whether athletic standard, self-reported respiratory symptoms, and/or sex influence the diagnostic value of FeNO.

2 | MATERIALS AND METHODS

2.1 | Study population and experimental design

The University of Kent, School of Sport and Exercise Sciences Ethical Committee provided ethical approval for this multicenter retrospective analysis (Ethics ID: 05_20_22). A total of 585 comprehensive respiratory assessments from six UK testing centers were collated into a collaborative database. All participants were assessed for EIB either as part of a screening intervention, referral based on symptoms or participation in a research study. Following data inspection for eligibility (which was based on established EVH testing guidelines and equipment used during testing[30,31]) and removal of missing data, $n = 488$ assessments were included (Figure 1).

Participants were divided into subgroups based on athletic standard: (i) recreational athletes (defined as competing and/or training at a local level) ($n = 159$; 33%) or (ii) elite level athletes (defined as competing at national or international level) ($n = 329$; 67%), self-reported respiratory symptoms and sex (Table 1). The cohort of athletes participated in a variety of sports including multidisciplinary sports ($n = 143$; 29%), football ($n = 135$; 28%), swimming

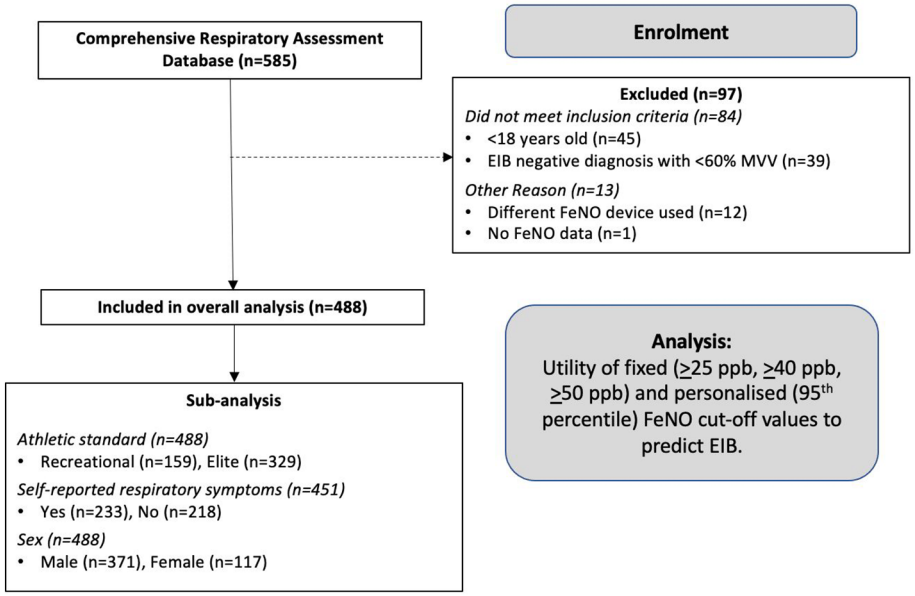

FIGURE 1 Consort flow diagram describing the study population and analysis. All athletes underwent a comprehensive respiratory assessment as part of a systematic screening or referral for suspected EIB. EIB, exercise-induced bronchoconstriction; MVV, maximum voluntary ventilation; FeNO, fractional exhaled nitric oxide; EVH, eucapnic voluntary hyperpnea.

TABLE 1 Participant characteristics.

	Recreational ($n=159$)	Elite ($n=329$)	Total ($n=488$)
Sex (M:F)	112: 47	259: 70	371: 117
Age (yrs)	34 ± 11	23 ± 5*	27 ± 9
Height (cm)	175.6 ± 8.2	179.6 ± 13.5*	178.3 ± 12.2
Body Mass (kg)	73.5 ± 12.7	75.5 ± 11.9	74.8 ± 12.2
FEV_1 (L)	3.86 ± 0.65	4.56 ± 0.83*	4.33 ± 0.84
FEV_1 (% of predicted)	100 ± 12	107 ± 13.0*	105 ± 13
FVC (L)	4.81 ± 0.87	5.62 ± 1.09*	5.36 ± 1.09
FVC (% of predicted)	105 ± 13	111 ± 14*	109 ± 14
FEV_1/FVC (%)	81.4 ± 7.2	81.1 ± 6.9	81.2 ± 7.0
Baseline FeNO (ppb)	35.8 ± 32.1	29.8 ± 27.5*	31.7 ± 28.9
EVH+ ΔFEV_1max (%)	−23.0 ± 13.3	−18.0 ± 9.6*	−19.7 ± 11.2
EVH- ΔFEV_1max (%)	−4.4 ± 2.9	−5.5 ± 2.3*	−5.1 ± 2.6
EIB Positive, *n* (%)	63 (40)	127 (39)	190 (39)
Achieved Ventilation %MVV (%)	72.4 ± 8.1	77.58 ± 12.4	77.5 ± 12.2

Note: Data presented as mean ± standard deviation.

Abbreviations: %MVV, percentage of maximum voluntary ventilation; EIB, exercise-induced bronchoconstriction; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; FeNO, fractional exhaled nitric oxide; ΔFEV_1max, maximum fall in FEV_1 from baseline following eucapnic voluntary hyperpnea (EVH) challenge.

*$p < 0.05$ compared to the recreational group.

($n=81$; 16%), cycling ($n=23$; 10%), boxing ($n=37$; 8%), athletics ($n=15$; <5%), canoeing ($n=3$; <5%), gymnastics ($n=3$ <5%), judo ($n=2$; <5%), netball ($n=1$; <5%), rowing ($n=8$; <5%), sailing ($n=1$; <5%), taekwondo ($n=1$; <5%), triathlon ($n=14$; <5%), and para-sports ($n=21$; <5%).

2.2 | Pulmonary function testing and fractional exhaled nitric oxide

All participants prescribed inhaler therapy ($n=127$) were asked to withhold from using medication prior to testing

in accordance with established methods.[30] Participants refrained from exercise for at least 4-hr prior to assessment and refrained from eating or drinking for 1-hr prior to assessment. Self-reported exercise respiratory symptoms including cough, wheeze, excess mucus, chest tightness, and/or dyspnea were evaluated via questionnaire. Baseline airway inflammation was assessed via FeNO (NIOX VERO).[18] FeNO measurements were obtained in accordance with international guidance with at least two FeNO measurements obtained within 10% and the mean of the two values used for analysis.[18]

Pulmonary function was assessed by maximal flow volume spirometry (Spiro-USB and MicroLab). Spirometry maneuvers were conducted in accordance with 2005 ATS/ERS recommendations.[31] At least three technically acceptable forced vital capacity (FVC) maneuvers were performed, with a minimum of two reproducible recordings (difference ≤150 mL for FEV_1 and FVC). Predicted values were calculated from Global Lung Initiative (GLI) 2012 equations.[32]

2.3 | Eucapnic voluntary hyperpnea

EVH was conducted as previously described by Anderson et al.[33] In brief, athletes were required to inspire medical grade, dry air (21% O_2, 5% CO_2 and 74%) for 6 mins at a target ventilation rate of 85% maximal voluntary ventilation (MVV). Target MVV was calculated as 30 x FEV_1, and minute ventilation ($\dot{V}_E$) was recorded. An EVH test was considered valid if athletes maintained >60% MVV throughout the test (or were positive for EIB despite not achieving 60% MVV). Maximal flow volume loops were measured in duplicate at 3, 5, 7, 10, and 15-min post-EVH, with the highest FEV_1 accepted at each time-point. A test was considered positive if FEV_1 fell by ≥10% over at least two consecutive time points post-challenge. The maximal fall in FEV_1 post-EVH (expressed as % difference from baseline) was calculated to quantify EIB severity. The severity of EIB was classified as mild, moderate, or severe depending on the fall in FEV_1 post-EVH (≥10% to <25%, ≥25% to <40% and ≥40% respectively).[30]

2.4 | Data analysis

Normality was tested using the Shapiro–Wilk test. Normally distributed data are expressed as mean ± standard deviation, unless otherwise stated. Recreational and elite athlete baseline characteristics were compared using an independent samples *t*-test ($p < 0.05$, Table 1). The relationship between FeNO and the maximum fall in FEV_1 (ΔFEV_1max) were analyzed using Spearman rank (r_s). Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated for established FeNO thresholds: intermediate ([≥25 ppb], high-NICE [≥40 ppb], high-ATS [≥50 ppb]). The sensitivity, specificity, PPV. and NPV for a personalized threshold (>95th percentile) adjusting for sex, age. and height was explored (19). Receiver operator characteristic (ROC) analysis was used to investigate the overall accuracy of FeNO to diagnose EIB. ROC area under the curve (ROC-AUC) is reported with 95% confidence intervals (95% CI). Data analyses were performed using SPSS Statistics Version 28.0 (IBM Corporation), and GraphPad Prism Version 9 (GraphPad Software Inc).

TABLE 2 Frequency and percentage of cohort according to FeNO thresholds.

FeNO threshold	Frequency/ (% of cohort)	EVH (EIB +: EIB-)
<25 ppb	283 (58%)	86: 197
≥25–<40 ppb	93 (19%)	33: 60
≥40 ppb	112 (23%)	71: 41
≥50 ppb	85 (17%)	53: 32
>95th Perc. (personalized)	170 (35%)	93:77

Abbreviations: EVH, eucapnic voluntary hyperpnea challenge; FeNO, fractional exhaled nitric oxide; ppb, parts per billion; Perc, percentile.

3 | RESULTS

3.1 | Respiratory symptoms and objective test outcome

At least one exercise-associated respiratory symptom (i.e., cough, wheeze, excess mucus, chest tightness and/or dyspnea) was reported in almost half of the cohort ($n = 233$; 48%). Data concerning respiratory symptoms was not obtained for $n = 37$ participants (8%). The majority of athletes (98%) had normal resting lung function (>80% FEV_1 pred). Of the 488 participants, 190 were EIB positive (39%) (mild: $n = 143$; moderate: $n = 40$; severe: $n = 7$) and 298 were EIB negative (61%). Of those with EIB, 104 (55%) had a FeNO value ≥25 ppb (i.e., type 2 high EIB phenotype) and 86 (45%) had a FeNO value <25 ppb (type 2 low EIB phenotype). FeNO values ≥25 ppb, ≥40 ppb, and ≥50 ppb were observed in 42%, 23%, and 17% of the cohort, respectively (Table 2).

3.2 | Predictive value of FeNO to detect EIB

A weak but significant rank correlation was observed between FeNO and ΔFEV_1max ($r_s = -0.32$, $p < 0.001$;

Figure 2). The sensitivity of FeNO (i.e., ability to rule out a diagnosis of EIB) at ≥25 ppb in the entire cohort was 55%, which decreased to 37% and 27% at ≥40 ppb and ≥ 50 ppb, respectively. The specificity of FeNO (i.e., ability to rule in a diagnosis of EIB) at ≥25 ppb, ≥40 ppb, and ≥ 50 ppb was 66%, 86%, and 89%, respectively. The sensitivity and specificity of the personalized threshold were 49% and 74%, respectively. Overall, the ROC-AUC for FeNO was 0.656 (95% CI, 0.605–0.706; Figure 3A). Sensitivity, specificity, PPV, and NPV are summarized for reference in Table 3.

The diagnostic value of FeNO improved in athletes with moderate-to-severe EIB (i.e., ≥25% fall in FEV_1 post-EVH). Specifically, the sensitivity of FeNO ≥25 ppb, ≥40 ppb, and ≥ 50 ppb was 74%, 70%, and 62%, whereas the specificity of FeNO ≥25 ppb, ≥40 ppb, and ≥ 50 ppb was 62%, 82%, and 87%, respectively. The ROC-AUC for FeNO in athletes with moderate-to-severe EIB was 0.813 (95% CI, 0.741–0.885; Figure 3A).

3.3 | Sub-analysis: athletic standard, respiratory symptoms and sex

Sensitivity, specificity, PPV, and NPV according to athletic standard, self-reported respiratory symptoms, and sex are presented in Table 4. For all sub-analyses, the increasing FeNO threshold resulted in reduced sensitivity, but increased specificity. The personalized FeNO threshold increased specificity compared to the intermediate FeNO threshold [≥25 ppb]. The ROC-AUC analysis revealed higher discrimination for FeNO to predict EIB in recreational athletes: 0.782 (95% CI, 0.707–0.856) versus elite athletes: 0.591 (95% CI, 0.528–0.655) (Figure 3B). However, this was not the case for the ROC-AUC for "self-reported respiratory symptoms" (symptomatic: 0.635, 0.562–0.708; asymptomatic: 0.684, 0.608–0.761; Figure 3c), or "sex" (male: 0.673, 0.616–0.730; female 0.624, 0.520–0.728; Figure 3D) sub-analysis.

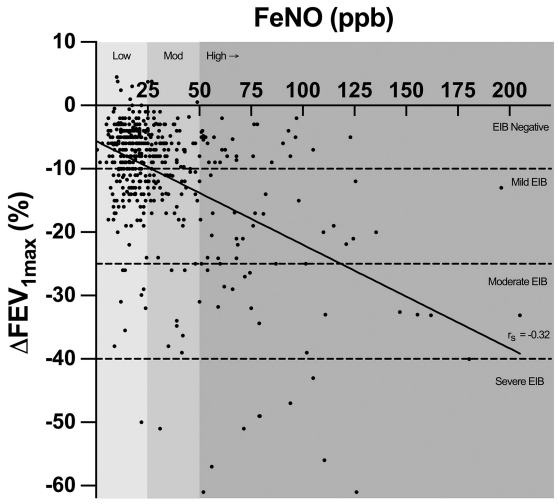

FIGURE 2 Relationship between FeNO and ΔFEV_1max post-EVH. Low, moderate, and high FeNO thresholds represented by vertical gray bands. Horizontal dotted lines denote mild, moderate, and EIB thresholds. EIB, exercise-induced bronchoconstriction; ΔFEV_1max, maximum fall in FEV_1; FeNO, fractional exhaled nitric oxide; EVH, eucapnic voluntary hyperpnea.

4 | DISCUSSION

Our findings indicate that an elevated FeNO (≥25 ppb) is a poor predictor of EIB in athletic individuals. This is the case across recreational and elite athletes, when comparing individuals with and without perceived respiratory symptoms, and when accounting for sex. It is important to note that whilst a high FeNO (≥40 ppb) provides good specificity (85%), a significant proportion (45%) of athletes with FeNO <25 ppb had a positive EVH challenge. The data from the present study therefore confirm that FeNO in isolation should not be employed as a replacement for indirect bronchial provocation to secure a diagnosis of EIB in athletes.

A recent meta-analysis demonstrated that a high FeNO (≥40 ppb) offers low sensitivity (0.65) but fair specificity (0.82) to predict asthma,[34] which is similar to our findings in athletes with EIB. A recent pilot study by Bonini et al.[35] reported similar sensitivity (55%) and specificity (78%) to our findings when using FeNO (≥50 ppb) to predict EIB using an exercise challenge test. The authors suggested that this data supported the use of FeNO to predict EIB. Our study focused specifically on athletes, and a significantly greater number of individuals were included in our analysis ($n = 488$) in comparison to the study by Bonini et al.[35] ($n = 40$). Bonini and colleagues also suggested that their findings provide evidence to use FeNO (≥50 ppb) when exercise testing is unavailable.[35] If we had adopted this strategy in the present study, 32 (7%) athletes would have been diagnosed inappropriately with EIB, and 137 (72%) athletes with a positive EVH challenge would have been missed. Our data therefore indicates that a high FeNO value may provide practitioners with reasonable confidence to confirm EIB, but a low-to-intermediate FeNO (<50 ppb) should not be employed to rule-out the condition.

The varied level of FeNO in athletes with a positive EVH challenge is likely related to differences in underlying pathophysiology. Indeed, previous research has suggested that EIB can be subdivided into two distinct phenotypes: "atopic (type 2 high) EIB" and sport asthma (type 2 low/non-atopic EIB).[36] Specifically, athletes who have atopic EIB typically have higher FeNO levels in comparison to athletes with sport asthma.[36] Furthermore,

multiple inflammatory cells have been implicated in EIB (e.g., eosinophils, neutrophils, and mast cells)[1]; however, FeNO is a specific indirect biomarker of type 2 inflammation/eosinophilia.[18] The discordance between FeNO and EIB is therefore likely attributable to the contribution of inflammatory cells other than eosinophils implicated in EIB. In our study, we did not evaluate atopy and thus we are unable to provide a detailed analysis concerning allergic or inflammatory biomarkers. Future work is therefore required to evaluate EIB sub-types with consideration for inflammatory mediators.

We observed no significant difference in the predictive value of FeNO for EIB between sex. It has previously been reported that females are likely to have lower FeNO values (by approximately 25%) in comparison to males.[21,22] Personalized reference values exist that stratify FeNO according to sex, while also accounting for age, height, and atopic status.[24] For example, using these personalized reference equations, we found that the highest upper limit (95th percentile) for FeNO was 42 ppb for a 52-year-old male with a stature of 166 cm. This is in contrast with a FeNO of 18 ppb for an 18-year-old female with a stature of 159 cm. The wide divergence in the 95th percentile values highlights the impact of adopting personalized normative values, rather than utilizing fixed thresholds. However, this approach only had a very small impact on the performance of FeNO as tool to predict EIB in our cohort. Indeed, the performance of the personalized reference values in predicting EIB was similar to the established fixed FeNO ≥25 ppb threshold across all cohorts, suggesting that factors other than sex, age, and stature account for the poor predictive value of FeNO for EIB. A key limitation of this approach is that the atopic status of the athletes was unknown. Indeed, atopy would add 16 ppb and 15 ppb to the 95th percentile for females and males, respectively,[19] thus increasing the specificity and reducing the sensitivity of the model.

We observed a high number of athletes ($n = 101$) with a negative EVH challenge but with an intermediate FeNO (≥25 ppb). Nitric oxide is present in exhaled breath due to nitric oxide synthase upregulation that occurs with inflammation.[17] It is important to acknowledge, however, that FeNO can be elevated due to a variety of factors, including atopic status, exposure to poor air quality, recent respiratory tract infection, or high dietary nitrate intake.[37–39] Although we were able to control for some of these variables (i.e., upper respiratory tract infection), we did not control for pre-test allergen or pollutant exposure in all cases. Likewise, we did not monitor dietary intake on the day of the test and thus it is plausible that some elevated FeNO measurements may relate to other factors (other than eosinophilic airway inflammation) which needs to be considered in future studies.

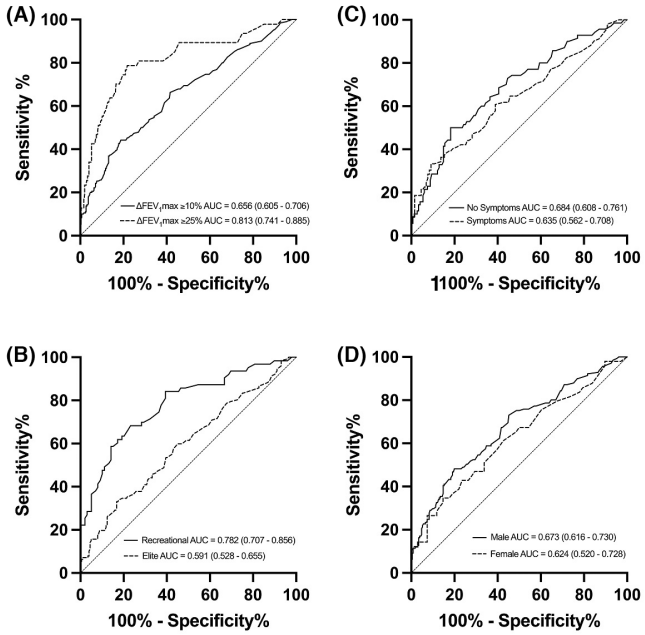

FIGURE 3 Multi-panel plot of receiver operating characteristic area under the curve (ROC-AUC) evaluating the predictive value of FeNO to detect EIB. (A) Mild and moderate severity EIB; (B) athletic standard; (C) self-reported respiratory symptoms; (D) sex. FeNO, fractional exhaled nitric oxide; EIB, exercise-induced bronchoconstriction; FEV_1max, maximum fall in FEV_1.

	FeNO ≥ 25 ppb	FeNO ≥ 40 ppb	FeNO ≥ 50 ppb	>95th Perc.
Sensitivity	55%	37%	27%	49%
Specificity	66%	86%	89%	74%
PPV	51%	63%	62%	55%
NPV	70%	68%	66%	70%
ROC-AUC	0.656 (95% CI, 0.605–0.706)			

Abbreviations: Fractional exhaled nitric oxide (FeNO); Perc, Percentile; positive predictive value (PPV) and negative predictive value (NPV); Receiver operating characteristic area under the curve (ROC-AUC); ΔFEV_1max, Maximum fall in FEV_1 from baseline following eucapnic voluntary hyperpnea (EVH) challenge.

TABLE 3 FeNO sensitivity, specificity, PPV, and NPV for the detection of EIB.

TABLE 4 FeNO sensitivity, specificity, PPV, and NPV for the detection of EIB according to athletic standard, self-reported respiratory symptoms and sex.

	Recreational ($n=159$; $-=96$, $+=63$)				**Elite ($n=329$; $-=202$, $+=127$)**			
	Cut off FeNO ≥ 25 ppb	**Cut off FeNO ≥ 40 ppb**	**Cut off FeNO ≥ 50 ppb**	**Cut off > 95th Perc.**	**Cut off FeNO ≥ 25 ppb**	**Cut off FeNO ≥ 40 ppb**	**Cut off FeNO ≥ 50 ppb**	**Cut off > 95th Perc.**
Sensitivity	71%	59%	41%	67%	46%	27%	21%	40%
Specificity	69%	84%	92%	78%	65%	87%	88%	72%
PPV	60%	71%	76%	67%	45%	57%	53%	48%
NPV	79%	76%	70%	78%	66%	65%	64%	66%
ROC-AUC	0.782 (95% CI, 0.707–0.856)				0.591 (95% CI, 0.528–0.655)			
	Symptomatic ($n=233$; $-=131$, $+=102$)				**Asymptomatic ($n=218$; $-=148$, $+=70$)**			
	Cut off FeNO ≥ 25 ppb	**Cut off FeNO ≥ 40 ppb**	**Cut off FeNO ≥ 50 ppb**	**Cut off > 95th Perc.**	**Cut off FeNO ≥ 25 ppb**	**Cut off FeNO ≥ 40 ppb**	**Cut off FeNO ≥ 50 ppb**	**Cut off > 95th Perc.**
Sensitivity	48%	33%	26%	44%	63%	43%	29%	54%
Specificity	69%	90%	92%	76%	64%	84%	88%	74%
PPV	55%	72%	73%	59%	45%	57%	53%	49%
NPV	63%	63%	62%	64%	78%	76%	72%	77%
ROC-AUC	0.635 (95% CI, 0.562–0.708)				0.684 (95% CI, 0.608–0.761)			
	Male ($n=371$; $-=230$, $+=141$)				**Female ($n=117$; $-=68$, $+=49$)**			
	Cut off FeNO ≥ 25 ppb	**Cut off FeNO ≥ 40 ppb**	**Cut off FeNO ≥ 50 ppb**	**Cut off > 95th perc.**	**Cut off FeNO ≥ 25 ppb**	**Cut off FeNO ≥ 40 ppb**	**Cut off FeNO ≥ 50 ppb**	**Cut off > 95th perc.**
Sensitivity	59%	41%	33%	50%	43%	27%	14%	47%
Specificity	64%	85%	88%	74%	77%	91%	93%	74%
PPV	50%	62%	63%	54%	57%	68%	58%	56%
NPV	71%	70%	68%	71%	65%	63%	60%	66%
ROC-AUC	0.673 (95% CI, 0.616–0.730)				0.624 (95% CI, 0.520–0.728)			

Abbreviations: Fractional exhaled nitric oxide (FeNO); Perc, Percentile; positive predictive value (PPV) and negative predictive value (NPV); receiver operating characteristics area under the curve (ROC-AUC).

In addition, we focused on the ability of FeNO to predict the outcome of an EVH challenge. Although the EVH challenge has previously been shown to have a high sensitivity to secure a diagnosis of EIB, the specificity may be compromised as the challenge involves inhaling dry medical grade air (<2% humidity).[10,40] It is likely that some athletes do not compete or train in a dry provocative environment, and therefore the test may be considered overly sensitive.[41] It is therefore possible that some of the athletes with a positive EVH challenge would not actually experience EIB during training or competition.[11] It is also important to note that EIB in athletes is not a stable condition and can fluctuate according to training status, environment, and time of year (e.g., cold climate and seasonal aeroallergens, etc.).[42,43] It is therefore often challenging to rule-out EIB in athletes with a mild or borderline response based on a solitary assessment[44] and thus repeat or "in-season" testing is recommended in this scenario.[12] Furthermore, a study by Bougault et al.[42] evaluated EIB severity status in swimmers when they were in an intensive phase of training and out of training. The authors reported no changes in FeNO measurements, despite fluctuations in airway caliber in response to EVH and methacholine, which further supports the disconnect between FeNO and EIB in athletes.

In our cohort, we had 127 athletes who reported a diagnosis of asthma and/or EIB and where using therapy. Athletes stopped asthma/EIB therapy following recommended practice in the days leading up to the EVH challenge.[30] On the day of testing, athletes were asked if they had followed this guidance. No testing would have taken place if they reported not following this guidance. Therefore, in that regards, there is limited risk that current medication would have interfered with our FeNO or EVH results. Although, not likely, it is possible that some athletes will not have followed this advice but stated that they had when questioned. Athletes who had not stopped therapy as instructed may have an altered FeNO and EVH compared to when they do not use asthma/EIB therapy. However, our author team agrees that the chances of the above scenario are minimal, and regardless, our data provide a thorough assessment of the diagnostic value of FeNO in the assessment of EIB.

Finally, although our findings support the concept that FeNO should not be used in isolation to predict EIB in athletes, it is important to acknowledge that FeNO has an established role in the assessment of type 2 airway inflammation and should therefore be considered as an adjunct measure to support diagnostic work-up and/or monitor the effectiveness of inhaler therapy.[45,46] Furthermore, the measurement of FeNO is simple and requires less patient effort when compared to measuring maximal spirometry and completing an EVH challenge. From a practical point of view, athletes with EIB are therefore likely to appreciate the inclusion of FeNO as part of long-term management as opposed to repeat indirect bronchial provocation.

5 | CONCLUSION

In conclusion, our findings confirm that an elevated FeNO (≥25 ppb) is a poor predictor of EIB in athletes. A high FeNO (≥40 ppb) provides good specificity but has poor sensitivity. Due to the poor sensitivity and poor predictive values, a high FeNO should not be used in isolation or as a replacement for an indirect bronchial provocation to secure a diagnosis of EIB in athletes. Future research evaluating mechanisms of inflammation and EIB phenotypes is required to improve the diagnosis and management of EIB in athletes.

6 | PERSPECTIVE

FeNO in isolation or in conjunction with resting lung function should not be used to confirm or refute EIB in athletes. Diagnostic work-up should include a form of indirect bronchial provocation such as exercise challenge testing, inhaled mannitol or EVH. Nonetheless, FeNO has an established role in identifying specific asthma phenotypes and monitoring the response to ICS therapy and should therefore be used as an adjunct to support the assessment of athletes with suspected asthma +/− EIB.

AUTHOR CONTRIBUTIONS

All authors contributed to the preparation of this manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors have no real or perceived interest in respect of this manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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