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Respiratory symptoms and bronchial responsiveness in competitive swimmers

Short title: Bronchial responsiveness in elite swimmers

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Abstract

Purpose: Swimmers show high prevalence of bronchial hyperresponsiveness (BHR) and respiratory symptoms. From Winter Olympics 2002 measurements of BHR or bronchodilator reversibility are required for the approved use of β_2-agonists in sports.

Aims of the study: To evaluate the relationship between respiratory symptoms in young elite swimmers, eucapnic voluntary hyperventilation (EVH) and the inhaled dose of methacholine causing 20% decrease in FEV_1 ($PD_{20methacholine}$). Secondly, to assess repeatability of the EVH test.

Methods: 15 male, 9 female adolescent elite swimmers, 15 to 25 years, performed one $PD_{20methacholine}$ test and two EVH tests in randomised order. Dry air containing 5% CO_2 was inhaled for 6 minutes with a target ventilation of ≥ 85 % MVV (minimum 65%). $PD_{20methacholine}$ ≤2µmol and EVH with FEV_1-reduction ≥10% were considered positive. Respiratory symptoms, medication were reported in the modified $AQUA_{2008}$ questionnaire.

Results: Twenty swimmers (83%) reported respiratory symptoms, 13 of these (65%) had a positive provocation test. Fourteen (58%) had at least one positive test to either EVH or $PD_{20\ methacholine}$, three had only one positive EVH test. One athlete had BHR without symptoms. The sensitivity of $PD_{20\ methacholine}$ ≤2µmol for respiratory symptoms was 50% versus 60% and 47.37% for the two EVH tests, respectively, and 75% for $PD_{20\ methacholine}$ ≤4µmol. Bland-Altman plot of the two EVH tests showed a consistent distribution with only one subject outside limits of agreement.

Conclusions: Respiratory symptoms and BHR were very frequent among adolescent competitive swimmers. $PD_{20\ methacholine}$ ≤2µmol and EVH ≥ 10% agreed, but $PD_{20\ methacholine}$ ≤4µmol showed highest sensitivity for respiratory symptoms. The EVH test has high repeatability, but is very expensive and uncomfortable to perform.

Introduction

Paragraph Number 1: The prevalence of exercise induced asthma (EIA), other respiratory symptoms and bronchial hyperresponsiveness (BHR) is especially high amongst elite endurance athletes and has been reported to markedly increase over the last three decades (13, 15, 18, 26). This high prevalence is found in both summer and winter athletes. Whereas cross country skiers develop BHR with increasing age (25), this relationship to age has not been reported in swimmers. From 2002 and on the international Olympic Committee-Medical Commission (IOC-MC) has required a positive bronchial provocation test for the approval of the use of β_2-agonists or alternatively a positive reversibility to inhaled bronchodilators (12) as consequence of the increased use among athletes in and outside the Olympic Games (4).

Paragraph Number 2: The optimisation of laboratory provocation tests in the diagnosis of BHR and EIA in relationship to reported respiratory symptoms is therefore important. Eucapnic voluntary hyperventilation (EVH) is an indirect test for BHR and has its effect through the dehydrating effect on extra cellular fluid of the respiratory mucous membranes causing mediator or transmitter release effecting on bronchial smooth muscle, bronchial vessels and glands (3). EVH has been recommended by IOC-MC to diagnose BHR among athletes (4). Reported for the first time in 1984 (23) EVH became well standardized and has been shown to be sensitive to identify BHR among athletes (4, 10, 24). Methacholine bronchial provocation measuring PD_{20}

$_{methacholine}$ is a direct method with the inhaled transmitter substance, methacholine, acting directly on the effector cells (bronchial smooth muscle, vessels and glands). Direct provocation tests are generally reported to be more sensitive in asthmatic patients than indirect provocation tests (8), except for EVH which has been used preferably in athletes (17).

Several studies assessed $PD_{20\ methacholine}$ as compared to other indirect tests in athletes, but only two direct comparisons of $PD_{20\ methacholine}$ and EVH exist, both concluding that EVH was more often positive using the IOC-MC limits (16, 20).

Paragraph Number 3: However, the relationship between EVH and $PD_{20\ methacholine}$ and especially as compared to subjective respiratory symptoms in competitive athletes needs further exploration. The main aims of the present study were therefore to determine the relationship between reported respiratory symptoms and the airway responses to EVH and $PD_{20\ methacholine}$ in a group of adolescent elite swimmers. The secondary aim was to assess the repeatability of the EVH test.

Subjects and methods

Design

Paragraph Number 4: The present study is a cross-sectional study comparing indirect bronchial responsiveness, as measured by eucapnic voluntary hyperventilation (EVH) with direct bronchial responsiveness ($PD_{20\ methacholine}$). By measuring EVH twice on separate days, we assessed the repeatability of the EVH-test.

Paragraph Number 5: The athletes attended two visits for the two EVH tests including maximum voluntary ventilation measurement (MVV) and one visit for measuring $PD_{20\ methacholine}$ and performing skin prick test (SPT). Lung function and NO in exhaled air (FeNO) were measured at all three visits. At their first visit the subjects filled in the Aqua questionnaire modified for the Olympic study (mAQ) (6). The three visits were in randomized order at least 24 hours apart. The subjects were randomised consecutively to one of the three test blocks according to random order generated by a computer programme. Six swimmers performed $PD_{20\ methacholine}$ as first, seven as second and eleven as third test. The study could not be blinded because the two test procedures are completely different.

Subjects

Paragraph Number 6: Twenty-four elite competitive swimmers were included in the study, 15 male and 9 female swimmers, 15 to 25 years of age, from two

swimming clubs (Asker and Bærum). All were competing on high national and/or international level and trained 2-3 hours daily. Demographic data are given in Table 1. All 24 swimmers were non smokers and did not consume snuff, 20 used food supplements (vitamins, amino acids, creatine). Four subjects had a diagnosis of asthma and five a diagnosis of allergy. Nine swimmers used anti-allergic or anti-asthma drugs before the study. (Two used anti histamines, two used allergy vaccination (immunotherapy), four used bronchodilators, two of them in combination with a leukotriene antagonist). One swimmer performed $PD_{20\ methacholine}$ and one EVH test, only, due to intercurrent illness.

Paragraph Number 7: The study was performed according to the principles stated in the Declaration of Helsinki and approved by the Regional Medical Ethics committee. The swimmers signed an informed consent form after being given oral and written information about the study objectives and methods. In addition for subjects below 18 years of age, one of their parents gave the written consent.

Methods

Paragraph Number 8: For the conduct of the study anti-asthmatic medication was withheld according to European Respiratory Society (ERS) guidelines (19). Inhaled short-acting β_2-agonists were withheld for 8 hours prior to testing; inhaled long-acting β_2-agonists, theophylline and leukotriene antagonists for the last 72 hours, anti histamines for the last seven days and

orally administered glucocorticosteroids for the last month. Inhaled steroids were not to be used on the day of testing.

Lung function measurements

Paragraph Number 9: Lung function was measured by maximum expiratory flow-volume loops by use of Masterscreen Pneumo Jaeger® (Würzburg, Germany). The predicted values used are according to the European Respiratory Society (22). The following variables were recorded; forced vital capacity (FVC), forced expiratory volume in one second (FEV_1) and forced expiratory flow at 50% of vital capacity (FEF_{50}).

Maximal voluntary Ventilation (MVV)

Paragraph Number 10: MVV was measured by voluntarily breathing as rapidly and deeply as possible over a time period of 10 seconds by use of Masterscreen Pneumo Jaeger® (Würzburg, Germany). The value was multiplied by six to get the maximal voluntary ventilation per minute. The best of 3 trials was used to assess maximum minute ventilation.

Eucapnic voluntary hyperventilation (EVH)

Paragraph Number 11: The subjects hyperventilated voluntarily a dry gas mixture consisting of dry air at room temperature containing 5% carbon dioxide (CO_2), 20.93% oxygen (O_2), balanced with nitrogen (N_2) by use of Aiolos Asthma test [AIOLOS medical AB (Karlstad, Sweden)] using Hans Rudolph valves (Hans Rudolph Inc. 2700 series, large 2-way NRBV). The target ventilation rate was 85% of the maximum voluntary ventilation (MVV) which is said to be equivalent to $30 \times FEV_1$, over a time period of 6 minutes.

The equipment was connected to a Douglas bag system to collect the expired air for measuring ventilation. The exhaled air was sampled every minute for 30 seconds, starting with the first measurement after half a minute. The 6 bags were analysed by a Flow Transducer ("Ventilation measuring system", model K-520, KL-Engineering, Northridge, CA, USA). Lung function was measured before and 1, 3, 5, 10 and 15 minutes after the EVH challenge. A reduction in FEV_1 of 10% or more from before to after EVH was considered as a positive test. The procedure was according to Anderson et al. (2).

Methacholine challenge ($PD_{20\ methacholine}$)

Paragraph Number 12: The methacholine challenge was performed with an inspiration triggered nebuliser Aerosol Provocation System Jäger® (Würzburg, Germany). Methacholine was inhaled in doubling doses from a starting dose of 0.51 µmol (0.1 mg). Lung function measurements were performed one minute after every delivered dose until FEV_1 decreased 20% from baseline. Baseline lung function was set after inhaled nebulised isotonic saline (0.9%). The maximum cumulative dose was 24.48 µmol (4.8 mg). A positive response to $PD_{20\ methacholine}$ was defined as a 20% fall in FEV_1 at a cumulative dose of 2 µmol methacholine or less, calculated by linear interpolation of the dose-response curve. All tests were performed according to current guidelines from American Thoracic Society (9). After the methacholine provocation all subjects were given salbutamol inhalations (0.1 mg/ml×10 kg bodymass^{-1}) to reverse bronchial obstruction. Clinical bronchial hyperresponsiveness (BHR) was defined as $PD_{20\ methacholine} \leq 8$ µmol (1.6 mg) and a positive bronchial provocation test $PD_{20\ methacholine} \leq 2$ µmol (0.4 mg) according to the IOC-MC (12).

Skin prick test (SPT)

Paragraph Number 13: The Skin Prick test (SPT) was performed with 10 allergens (cladosporium herbarum, dermatophagoides pteronyssinus, dog dander, cat dander, birch, timothy, mug worth, pollen, cow's milk, shrimp and egg) (Soluprick, ALK, Copenhagen, Denmark) according to the European guidelines (11). A positive SPT was defined as a wheal of at least 3 mm in diameter as reaction to one or more allergens. The size was recorded by measuring [maximum + minimum diameter (mm)] × 2^{-1}.

Fractured exhaled Nitric Oxide (FeNO)

Paragraph Number 14: Fractured exhaled NO was measured with a EcoMedics Exhalyzer® CLD 88sp with DENOX 88 (ECO ME DICS AG, Duernten, Switzerland) according to published guidelines (1). Deep inhalation through the mouthpiece provides NO-free air, followed by full exhalation for 10 seconds with a constant flow of 50 ml×min^{-1} against resistance of 5 cm H_2O. The mean of three technically acceptable measurements was used.

Modified $AQUA_{2008}$ questionnaire (mAQ)

Paragraph Number 15: The questionnaire developed for the assessment of asthma, allergy and other respiratory symptoms was used in the athletes participating in the summer Olympic Games in Beijing August 2008 (6).

Statistical analysis

Paragraph Number 16: Demographic data are expressed as mean values with

standard deviation (SD) and results as means with 95% confidence intervals (CI). Correlation was calculated by Pearson's correlation coefficient (r_p) for normally distributed and by Spearman's correlation coefficient (r_s) for not normally distributed data. The agreement between the two EVH tests was assessed by Bland-Altman Plot through determination of limits of agreement. Differences were analyzed by standard paired sampled t-tests when normally distributed. The response to the EVH tests was recorded as the maximum percent fall in FEV_1 from before to after the test, in accordance with the rules given by IOC-MC and were used in the Olympic Games after 2002 for obtaining approval for therapeutic use of the use of inhaled β_2-agonists. P-values of $p \leq 0.05$ were considered statistically significant. Statistical analyses were performed with Statistical Package for Social Sciences (SPSS) version 15.0 and MedCalc statistical system version 10.4.6.0.

Results

Paragraph Number 17: Twenty swimmers (83%) reported lower respiratory symptoms in the mAQ (cough, heavy breathing, wheeze, phlegm and expectoration) (Table 2). Fifteen of these swimmers (75%) had at least one positive provocation test, and 10 (50%) felt that these symptoms had an impact on their sports performance. The sensitivity, specificity, positive and negative predictive values are given in Table 3. The highest sensitivity in relation to respiratory symptoms was achieved with $PD_{20\ methacholine} \leq 4$ µmol. The specificity was similar for all tests, most probably due to the high frequency of reported symptoms, leaving out erroneously diagnosed subjects when the tests were compared to reported symptoms.

Paragraph Number 18: Fourteen of the 24 swimmers (58%) had at least one positive test result to either one of the EVH tests or to $PD_{20\ methacholine} \leq 2$ µmol. Of the 14 BHR-positive subjects, 8 (57%) had a positive reaction to all three tests including 4 of the 5 subjects with a previous asthma diagnosis. Three swimmers (21%) had a negative response to $PD_{20\ methacholine}$ and were only identified by at least one positive EVH test. One subject had only a positive response to $PD_{20\ methacholine}$ and not to either of the EVH tests (Figure 1a and b, Table 4). We found a statistically significant correlation between the maximal reduction in FEV_1 in both EVH tests and the cumulative dose of methacholine causing 20% reduction of FEV_1 [Dose response slope (r_s = 0.542 and 0.453, p = 0.01 and 0.05, respectively)].

Paragraph Number 19: The two EVH tests also correlated significantly (r_p = 0.873, p = 0.01). The repeatability as assessed by Bland-Altman plot (Figure 2) was acceptable and only one subject was outside the limits of agreement. The limits of agreement were found to be 6%, and the distribution of agreement was not dependent upon reduction in FEV_1 (Fig. 2). Only two EVH-positive subjects in the first EVH test and three EVH-positive subjects in the second EVH test had a reduction in FEV_1 ≥ 10% in only one of the five lung function measurement after EVH.

Paragraph Number 20: Baseline lung function (FEV_1, FEF_{50}, MVV) did not differ significantly between the three test days. Measured MVV and FEV_1 were significantly higher than their predicted values (p < 0.001)(table 1). There was no significant correlation between the achieved ventilation (% target ventilation) and the maximal reduction in FEV_1 from before to after the EVH tests.

Paragraph Number 21: The target ventilation of 30×FEV_1 was in both tests significantly lower than 85% MVV (p = 0.04 and 0.002, respectively) (Table 1). In the first EVH test 10 of 24 swimmers (41%) had a ventilation rate ≥ 85% MVV and 13 of 24 swimmers (54%) obtained a ventilation rate higher than 30×FEV_1 ≥ 100%. In the second EVH test 8 of 23 swimmers (35%) obtained a ventilation rate ≥ 85% MVV and 13 of 23 swimmers (57%) a ventilation rate higher than 30×FEV_1 ≥ 100%. When using the EVH test with the greatest reduction in FEV_1, 11 of 24 swimmers (46%) had a ventilation rate ≥ 85% MVV and 18 of 24 (75%) a ventilation rate of 30×FEV_1 ≥ 100%.

Paragraph Number 22: Eight swimmers (33%) had allergic sensitization as assessed by the skin prick test. Six of the subjects with allergic sensitization (75%) were also positive to one of the tests for BHR, four of them (67%) to all three provocation tests (Table 2).

Discussion

Paragraph Number 23: In the present open randomised study in adolescent elite swimmers we found a surprisingly high prevalence of BHR with 58% of the subjects having at least one positive response to either $PD_{20\ methacholine}$ or one of two EVH tests. The prevalence of reported lower respiratory symptoms (83%) was even higher, and $PD_{20\ methacholine} \leq 4$ µmol had the highest sensitivity (75%), specificity (75%), positive predictive value (93.75%) and negative predictive value (37.5%) of all tests. All tests had low negative predictive values (37.5% - 23.08%) indicating that several subjects with respiratory symptoms still had negative tests, reflecting the high frequency of reported symptoms in this group of subjects.

Paragraph Number 24: The results of EVH and $PD_{20\ methacholine}$ agreed mostly very well. The repeatability of the EVH tests was found to be very satisfying, well within limits of agreement.

Paragraph Number 25: The high prevalence of BHR among swimmers has already been reported in other studies and supports the hypothesis that swimmers are at great risk to develop BHR rapidly during their swimming careers, possibly enhanced through their exposure to chlorine and chlorine products while swimming (5). This underlines the importance of the environment and environmental control where sports activities are being performed. The high ventilation of swimmers while performing their sport activity and their high baseline lung function (Table 1) most probably increase their exposure to these provoking environmental factors (14).

In the present study 20 swimmers (83%) reported lower respiratory symptoms in the mAQ, 13 (65%) also had at least one positive provocation test. Both tests had satisfactory sensitivity and specificity, also positive predictive values, whereas all had low negative predictive values, reflecting the frequent report of respiratory symptoms. This demonstrates that positive tests well identifies subjects with lower respiratory symptoms, whereas subjects with symptoms may still have negative tests. This may infer that the symptoms occurring in swimmers may to some extent be unspecific and not always related to BHR, alternatively that the requirements set up by IOC-MC may be too strict. However, the combination of high sensitivity and high specificity of these tests support their usefulness, and supports the continuous use of these tests in competitive sports. This also demonstrates that the frequent symptoms reported by these athletes, cannot alone identify bronchial hyperresponsiveness. The present study confirms that the presence of respiratory symptoms needs verification by objective measures of bronchial hyperresponsiveness or reversibility to bronchodilators. However, as recommended by the combined taskforce on asthma and allergy in sports set up by European Academy of Allergy and Clinical Immunology, and by European Respiratory Society (33), to use a cut-off of $PD_{20\ methacholine}$ of 4 µmol is supported by the present study with the highest sensitivity and the highest negative predictive value found by using a $PD_{20\ methacholine} \leq 4$ µmol. Eleven subjects (55%) would have been diagnosed by $PD_{20\ methacholine} \leq$ 2µmol, only, and 13 (65%) by EVH only. If 4 µmol was used as limit for positive results in $PD_{20\ methacholine}$ instead of 2 µmol, we would have diagnosed 17 subjects (85%) and five more subjects with respiratory symptoms would

have been tested positive, while only one of these subjects was positive to EVH (Figure 1a and b, Table 4).

Paragraph Number 26: The concordance between EVH and $PD_{20\ methacholine}$ in defining BHR-positive subjects in the present study is higher than reported previously (16, 21). Two other studies have been performed comparing the efficacy of EVH against $PD_{20\ methacholine}$ in athletes both showing a much higher number of positive provocation tests results to EVH. Holzer et al. (16) found 50% of the summer sport athletes positive to EVH and only 18% positive to $PD_{20\ methacholine}$, and in Pedersen's study on swimmers, 31% had a positive response to EVH and none reacted to $PD_{20\ methacholine}$ (21). However, these studies did not compare the test results to the presence of respiratory symptoms. In determining which test to use, it should also be considered that the athletes in the present study felt that the EVH test is much more uncomfortable to do, and also that the gas-mixture used during the EVH test is extremely expensive.

Paragraph Number 27: We found a very high repeatability for EVH (Figure 2). In Bland-Altman Plot only one subject was outside the limits of agreement between the two tests. This subject had only one positive EVH test (reduction in FEV_1 of 17.8% and 7.6%), but was also positive to $PD_{20\ methacholine}$ (1.71 µmol). Although a sample size of 50 subjects was calculated necessary to assess the reproducibility of EVH, the repeatability is remarkable. This supports the reliability of the EVH test (16).

Paragraph Number 28: Furthermore, we found that using 85% $MVV_{measured}$ for the target ventilation during the EVH test was not equal 30×FEV_1 in these swimmers. To perform an EVH test using 30xFEV_1 as target ventilation, will reduce the needed ventilation and reduce the uncomfortable condition caused by the test. A quality characteristic of the present study is that in comparison with the study of Pedersen et al (20) none was excluded due to low ventilation rate (Table 1). Like Brummel et al. (7), we found that females are less likely to achieve 85% $MVV_{measured}$ (Table 1), as also was demonstrated by the low ventilation rate in Pedersen's female swimmers (20). The variation in the ventilation rate between subjects on the different test days was, however, not related to the level of reduction in FEV_1 after EVH, suggesting that the recommended high ventilation rate may not be decisive for obtaining positive results, also agreeing with the high number of positive responders despite inadequate ventilation rates in other studies (7, 20). Also the finding that 77% of the girls tested positive, compared to 47% of the boys supports the assumption that the achieved ventilation rate is not quite needed to obtain a positive test result.

Paragraph Number 29: In conclusion, the present study demonstrates a very high prevalence of both lower respiratory symptoms and BHR as measured both by methacholine bronchial provocation and by EVH. Furthermore, the high specificity and sensitivity of both methacholine bronchial provocation test and EVH test related to respiratory symptoms demonstrates the usefulness of both test methods, although a cut off level of 4 µmol for $PD_{20\ methacholine}$ seems the most optimal when also the negative predictive value is considered. The

EVH test demonstrated a high reproducibility, but was expensive and uncomfortable to perform.

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Paragraph Number 30: **References**

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Figure 1a Results of EVH and $PD_{20\ methacholine}$. Positive result definition: ≥ 10% reduction in FEV_1 after EVH_1 and ≤ 2 µmol $PD_{20\ methacholine}$ (n=24)

FEV_1: Forced expiratory volume in one second; EVH: Eucapnic voluntary hyperventilation; $PD_{20\ methacholine}$: The dose of inhaled methacholine causing 20% reduction in FEV_1

Figure 1b Results of EVH and $PD_{20\ methacholine}$. Positive result definition: ≥ 10% reduction in FEV_1 after EVH_2 and $PD_{20\ methacholine}$ ≤ 2 µmol (n=23)

FEV_1: Forced expiratory volume in one second; EVH: Eucapnic voluntary hyperventilation; $PD_{20\ methacholine}$: The dose of inhaled methacholine causing 20% reduction in FEV_1

Figure 2 Bland-Altman Plot of the maximal reduction in FEV_1 from before to after EVH_1 and EVH_2 including 23 subjects.

FEV_1: Forced expiratory volume in one second; EVH: Eucapnic voluntary hyperventilation