

Plasma adenosine concentration increases during exercise: A possible contributing factor in exercise-induced bronchoconstriction in asthma

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We investigated whether the level of plasma adenosine (ADO) changed during exercise and whether this could be related to exercise-induced bronchoconstriction. Baseline levels of ADO did not differ, but exercise resulted in higher ADO in patients with asthma than in healthy subjects (86 ± 35 vs 59 ± 16 nmol/L; $P < .001$). In patients with asthma, the increase in ADO was related to decreases in FEV_1 ($r^2 = 0.475$; $P < .05$) and SAO_2 ($r^2 = 0.693$; $P < .05$). These data suggest that adenosine might be involved in the development of exercise-induced bronchoconstriction. (J Allergy Clin Immunol 2002;109:446-8.)

Key words: Exercise-induced bronchoconstriction, adenosine, asthma

Exercise induces bronchoconstriction in many patients with asthma. The mechanism of exercise-induced bronchoconstriction (EIB) is not completely understood, but available data indicate that cooling and dehydration of the airways might stimulate pulmonary inflammatory cells to release bronchoconstrictor mediators.¹

Adenosine produces a dose-related airway obstruction when it is inhaled by patients with asthma but not when it is inhaled by healthy volunteers.² It is likely that binding of adenosine to A3 and/or A2b adenosine receptor subtypes on immunologically activated lung inflammatory cells induces the release of bronchoconstrictor mediators.^{3,4} Allergen-induced bronchospasm is known to be associated with an elevation in levels of plasma adenosine (ADO) in patients with asthma.⁵ Furthermore, such an increase in ADO can enhance the release of bronchoconstrictor mediators during the allergen-induced immediate airway response.⁶ Adenosine itself is produced by and released

Abbreviations used

ADO: Plasma adenosine
EIB: Exercise-induced bronchoconstriction
HXN: Plasma hypoxanthine
INO: Plasma inosine
 SAO_2 : Hemoglobin oxygen saturation

from exercising tissues; thus it might play a homeostatic role through its effect as a potent vasodilator.⁷

The aims of the present study were to examine whether exercise is associated with increases in ADO in patients with asthma and in healthy control subjects and to determine whether changes in ADO are related to the degree of EIB observed in patients with asthma. We measured ADO and FEV_1 values in subjects at rest and after they had undergone treadmill exercise testing. To estimate the total amount of adenosine released, the plasma concentrations of the metabolites of adenosine, including inosine and hypoxanthine, were also determined. To monitor oxygen availability, hemoglobin oxygen saturation (SAO_2) and plasma lactate levels were also measured in these subjects.

METHODS

Subjects

Nine nonatopic healthy volunteers (age, 18 to 42 years; 4 male and 5 female) and 9 patients with stable mild asthma (age, 20 to 32 years; 6 male and 3 female) participated in the study. All patients met the American Thoracic Society diagnostic criteria for bronchial asthma, and all had histories of EIB. All patients were atopic, as demonstrated by at least 2 positive results to skin tests with the following common aeroallergens: house dust mite, cat dander, dog dander, grass pollen, and *Aspergillus fumigatus* (Soluprick; ALK Abello, Copenhagen, Denmark). Treatment was limited to inhalation of short-acting β_2 agonists and corticosteroids. β_2 agonists were withdrawn at least 12 hours and steroids (4 of 9 patients) 24 hours before the study.

The protocol was approved by the local ethics committee, and written informed consent was obtained from each subject before the study. The subjects, all of whom were nonsmokers, were instructed to avoid taking caffeinated drinks and foods and refrain from exercise for at least 12 hours before the study. No patients were studied within 6 weeks of an upper respiratory tract infection or an exacerbation of asthma.

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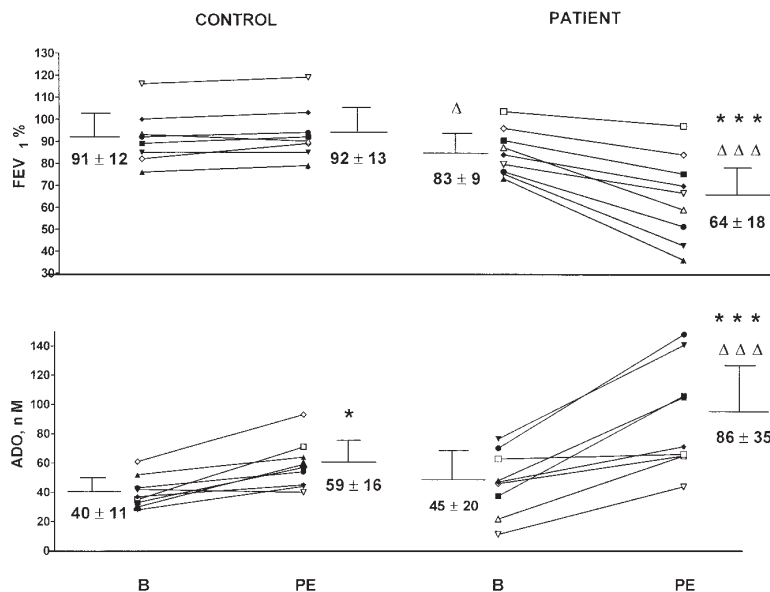


FIG 1. Individual and mean (± 1 SD) values of FEV₁ and concentrations of ADO in healthy volunteers (*Control*) and patients with asthma (*Patient*) before (B) and after (PE) exercise. Differences in FEV₁% and/or ADO within groups: * $P < .05$; *** $P < .001$. Differences in FEV₁% and/or ADO between groups: $\Delta P < .05$; $\Delta\Delta\Delta P < .001$.

Study design and protocol

All subjects completed a standardized treadmill exercise test at room temperature; the tests were administered at the same time of day, and nose clips were worn. The speed, gradient, time, and heart rate were displayed continuously during running. The exercise intensity was set to raise the subjects' heart rate to 90% of the age-adjusted maximum target level. Each subject exercised for 6 minutes under these target conditions. There were no significant differences in fitness levels between the 2 groups.

SaO₂ was determined by means of a pulse oxymeter before and at the termination of exercise. A venous blood sample was taken and FEV₁ was measured as soon as technically possible after the determination of post-exercise SaO₂ (within 6 minutes). Healthy volunteers and asthmatic subjects did not differ with respect to exercise measurement time.

Lung function test

FEV₁ was measured by means of an electronic spirometer (Medicor MS-11; Budapest, Hungary). The best of 3 consecutive maneuvers was accepted for evaluation. Each pre-exercise FEV₁ value was expressed as a percentage of the predicted normal value for the subject's height and age, according to European Community for Coal and Steel reference values for FEV₁.

Determination of ADO, plasma inosine, plasma hypoxanthine, and plasma lactate concentrations

Analysis of plasma samples for adenosine and its metabolites were carried out through use of an isocratic reverse-phase HPLC method, as described previously.⁸ Plasma lactate concentrations were determined by enzymatic analysis (Boehringer Mannheim, Mannheim, Germany). UV absorption was measured at 340 nm.

Each result is expressed as the mean ± 1 SD. Two-by-two repeated-measures ANOVA ([SUBJECT: control/patient] $\times$ [EXERCISE: before/after]) followed by Tukey-Kramer multiple-comparison testing was performed to compare baseline and postexercise data from healthy and asthmatic subjects within and between groups. Multiple regression analysis was performed to determine the intercorrelations between changes in ADO and FEV₁ or SaO₂. All statistical

analyses were performed through use of an NCSS 2000 computer software package (Statistical Solutions Ltd, Cork, Ireland).

RESULTS

Physiologic variables before exercise

Resting FEV₁ was significantly lower in patients with asthma than in healthy volunteers (Fig 1). There were no differences in pre-exercise values for ADO concentration (Fig 1), plasma inosine and plasma hypoxanthine (INO + HXN) concentration, plasma lactate concentration, or SaO₂ between patients with asthma and healthy volunteers.

Effects of exercise

After exercise, FEV₁ did not change in control subjects; however, there was a decrease in patients with asthma (83% $\pm$ 9% vs 64% $\pm$ 18%; Fig 1). ADO and plasma lactate levels were increased after exercise in both healthy subjects ($P < .05$ and $P < .001$ for ADO and lactate, respectively) and patients with asthma ($P < .001$ for both ADO and lactate). INO + HXN was elevated in the patients with asthma ($P < .001$) but not in the healthy controls. These elevated levels of postexercise plasma concentrations were significantly higher in patients with asthma than in healthy controls (ADO, 86 $\pm$ 35 nmol/L vs 59 $\pm$ 16 nmol/L [Fig 1]; INO + HXN, 8.5 $\pm$ 2.9 vs 4.4 $\pm$ 2.4 μ mol/L [$P < .01$]; lactate, 9.6 $\pm$ 3.9 vs 5.8 $\pm$ 2.0 mmol/L [$P < .01$]). There was a decrease in SaO₂ in patients with asthma ($P < .001$) after exercise. This was reflected by lower postexercise SaO₂ in patients with asthma than in control subjects (91.1% $\pm$ 4.5% vs 95.3% $\pm$ 1.4% [$P < .01$]).

ANOVA main effects

Because there were only 2 levels of the repeated-measures variable, there were no concerns regarding the nonsphericity assumption. Significant main effects of the

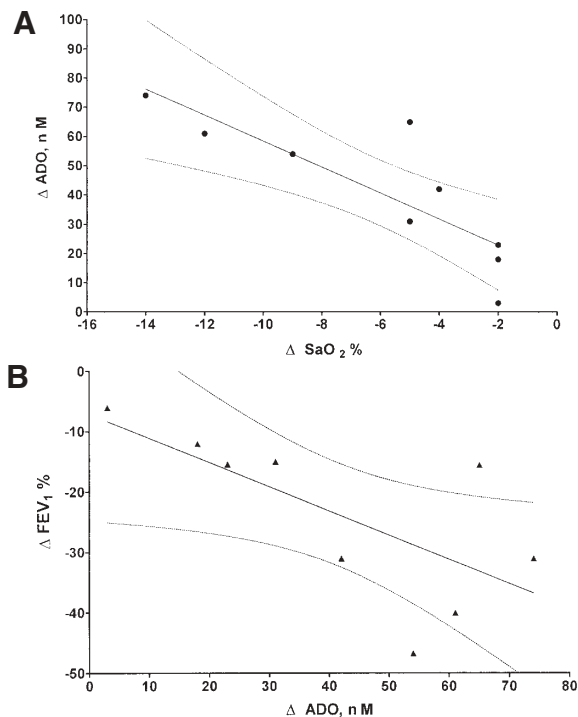


FIG 2. **A**, Relationship between increase in ADO and percent fall in SaO_2 in patients with asthma ($r^2 = 0.693$; $P < .05$). **B**, Relationship between percent fall in FEV_1 and increase in ADO in patients with asthma ($r^2 = 0.475$; $P < .05$).

SUBJECT factor were observed in all variables except ADO, and mean effects of the EXERCISE factor were significant in all variables (with power values [$1 - \beta$] between 0.61 and 0.99). The interactions of these 2 factors (SUBJECT/EXERCISE) were significant as follows: for FEV_1 : $F(1,16) = 32.47$; $P < .00003$; $1 - \beta = 0.99$ for ADO: $F(1,16) = 6.28$; $P < .023$; $1 - \beta = 0.65$ for INO + HXN: $F(1,16) = 12.10$; $P < .003$; $1 - \beta = 0.90$ for SaO_2 : $F(1,16) = 7.14$; $P < .0167$; $1 - \beta = 0.71$ for lactate: $F(1,16) = 6.91$; $P < .018$; $1 - \beta = 0.69$.

Relations between changes in SaO_2 , ADO, and FEV_1

In healthy subjects, there were no correlations between exercise-induced changes in SaO_2 , ADO, and FEV_1 . In patients with asthma, however, the increase in ADO was proportional to the percent fall in SaO_2 (Fig 2, A). Similarly, the percent fall in FEV_1 was proportional to the increase in ADO (Fig 2, B).

DISCUSSION

Our results indicate that exercise-induced increases in ADO and INO + HXN are significantly higher in patients with asthma than in healthy subjects. In patients with asthma, the increase in ADO was related to the decrease in SaO_2 ; however, the degree of airway obstruction was related to the increase in ADO.

The larger increase in ADO and its metabolites in response to exercise in asthmatic subjects was probably the result of enhanced adenosine production in these sub-

jects in comparison with healthy volunteers. It seems likely that reduced oxygen availability might contribute to the facilitated adenosine production, inasmuch as both the fall in SaO_2 and the increase in lactate accumulation were greater in patients with asthma than in healthy volunteers. Moreover, the fall in SaO_2 was also related to the increase in ADO. Elevation in ADO, together with all of the other changes associated with exercise, did not cause airway obstruction in healthy controls; in contrast, FEV_1 decreased both significantly and in proportion to the increase in ADO in patients with asthma.

Although association between FEV_1 and ADO does not necessarily mean a causative relationship, this finding supports the hypothesis that adenosine might participate in the development of EIB. It can be hypothesized that the increased adenosine concentration enhances the effect of physical triggers activated by exercise and that the summated effect of adenosine and physical stimulus on primed inflammatory cells induces the release of bronchoconstrictor mediators and leads to the development of airway obstruction.^{9,10} This proposed mechanism is supported by our previous data obtained from guinea pigs, in which we found that ovalbumin challenge produced a greater bronchoconstrictor mediator release and airway narrowing after adenosine administration.⁶

In conclusion, EIB is closely related to the increase in ADO concentration, indicating a possible role for adenosine in the development of EIB.

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