ERJ Express. Published on September 3, 2010 as doi: 10.1183/09031936.00049610

Relationships of BHR to methacholine/AMP to bronchodilator response in asthmatic children

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Running head: BHR to methacholine/AMP and BDR

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ABSTRACT

Bronchodilator response (BDR) is assessed to estimate the reversibility of airflow obstruction. Bronchial hyperresponsiveness (BHR) is a characteristic feature of asthma and is usually measured by bronchial challenges using direct or indirect stimuli. The aim of this study was to compare BHR to methacholine (direct) and that to adenosine 5'-monophosphate (AMP) (indirect) with regard to their relationships to BDR in asthmatic children.

Methacholine and AMP challenge tests were performed on 138 children with mild to moderate asthma, and a provocative concentration causing a 20% decline in FEV_1 (PC_{20}) was determined for each challenge. BDR was calculated as the change in FEV_1, expressed as % of the initial value, after inhalation of 400 μg salbutamol.

Methacholine PC_{20} correlated significantly but weakly with BDR ($r = -0.254$, $P = 0.003$). However, there was a significant and strong correlation between AMP PC_{20} and BDR ($r = -0.489$, $P = 0.000$). For AMP PC_{20}, the relationship was closer than for methacholine PC_{20} (comparison between correlation coefficients: $P = 0.024$). The same figures were observed, when BDR was expressed as % of the predicted value.

A stronger correlation of BDR with AMP PC_{20} than with methacholine PC_{20} suggests that BDR may be reflected better by BHR as assessed by AMP challenge than by methacholine challenge.

KEYWORDS

adenosine 5’-monophosphate

asthma

bronchial hyperresponsiveness

bronchodilator response

methacholine

INTRODUCTION

Measurement of the bronchodilator response (BDR) is widely applied to assess the acutely reversible component of airways obstruction [1]. Asthma is traditionally defined as reversible airflow obstruction, although clinicians have long recognized that sometimes the obstruction is not completely reversible [2]. In children and adults with asthma, the BDR is often used to indicate the degree of reversibility, to aid in confirming the diagnosis, to assess severity of the disease and to help make therapeutic decisions [3].

Bronchial hyperresponsiveness (BHR), defined as an exaggerated bronchoconstrictive response of the airways to a variety of stimuli, is considered to be a hallmark of asthma. BHR is most commonly evaluated using methacholine or histamine, which acts directly at the level of bronchial smooth muscle. However, BHR can also be assessed using indirect stimuli, such as adenosine 5'-monophosphate (AMP), which causes bronchoconstriction by stimulating or enhancing the release of mediators from mast cells [4]. There is increasing interest in the role of indirect bronchial challenges, because symptoms and bronchoconstriction occur in clinical asthma by means of indirect mechanisms [5].

It has been suggested that the assessment of BDR might be a useful guide to the presence of BHR [6]. In fact, provocation challenges, when contraindicated for severe airway obstruction, have been replaced by bronchodilator tests. However, studies on the relationship between BHR to histamine or methacholine and BDR have yielded conflicting results; some [7,8,9] found a significant correlation, whereas others [10,11] did not. On the other hand, there is no information on whether BDR correlates with BHR to indirect stimuli such as AMP.

In the present study, we have performed methacholine and AMP challenge tests and bronchodilator testing, and compared the degree of BHR to methacholine and that to

AMP with regard to their relationships to BDR in children with asthma.

MATERIAL AND METHODS

Children with mild to moderate asthma, aged 7 to 18 years, were enrolled in this study. They were attending the allergy clinic at Seoul National University Children's Hospital. All subjects had a physician-diagnosed asthma and a history of episodic wheezing and/or dyspnea during the previous year, which was resolved after using bronchodilators. They had been medicated with inhaled short-acting β_2-agonists on demand in order to relieve symptoms, with or without controller medications (i.e., inhaled corticosteroids or leukotriene receptor antagonists). Children were excluded if they had a history of near-fatal asthma, major exacerbations necessitating the use of systemic corticosteroids, or other respiratory diseases apart from asthma.

This study consisted of a 1-week observational period, followed by methacholine and AMP bronchial challenges in the second week and bronchodilator testing in the third week (Fig. 1). At the start of the observational period, the patients were asked to discontinue their controller medications, if used, and to only use inhaled β_2-agonists on demand during the entire study period. In the second week, each subject was evaluated by a battery of tests including blood eosinophil counts, serum total IgE, and skin prick tests. Atopy was defined as the presence of at least 1 positive skin reaction (wheal major diameter >3 mm) to a battery of 12 common airborne allergens. On each of the two days (at least 3 but no more than 6 days apart) during the second week, either a methacholine or an AMP challenge test was performed. The sequence of these challenges was randomized to preclude any bias related to potential carryover effects. To be eligible for the study, the subjects had to be able to undergo pulmonary function tests in a reproducible way (i.e., the 2 largest FEV_1 values were within 5% of each other after 3 acceptable spirograms had been obtained) and were required to have an $FEV_1 \geq 60\%$ of the predicted value [12]. During the third week, bronchodilator testing was performed. Subjects were excluded from the study if an exacerbation of asthma or a respiratory

tract infection occurred within 4 weeks prior to the tests, and if they showed unstable FEV_1 (difference in baseline $FEV_1 \geq 10\%$ of the predicted value between methacholine and AMP challenges).

Methacholine and AMP challenges tests

Methacholine inhalation tests were carried out using a modification of the method described by Chai et al. [13], and AMP challenge tests were performed using a modification of the method of the ERS [14]. Inhaled short-acting β_2-agonists were withheld for at least 8 hours, and other mediations were withheld for 3 days before each challenge. Fresh solutions of methacholine and AMP were prepared in buffered saline solution at concentrations (0.075, 0.15, 0.3125, 0.625, 1.25, 2.5, 5, 10, 25 and 50 mg/mL) for methacholine and at concentrations (3.125, 6.25, 12.5, 25, 50, 100, 200 and 400 mg/mL) for AMP. Lung function was measured using a computerized spirometer (Microspiro-HI 298, Chest, Tokyo, Japan), and the largest value of triplicate FEV_1 on each occasion was used for analysis. A Rosenthal–French dosimeter (Laboratory for Applied Immunology; Baltimore, MD, USA), triggered by a solenoid valve set to remain open for 0.6 seconds, was used to generate an aerosol from a DeVilbiss 646 nebulizer (DeVilbiss Health Care; Somerset, PA, USA), with air pressurized at 20 psi. Each subject inhaled 5 inspiratory capacity breaths of buffered saline solution and increasing concentrations of methacholine or AMP, respectively, at 5-minute intervals. This gave an output of 0.009±0.0014 mL (mean ± SD) per inhalation. FEV_1 was measured 90 seconds after inhalation at each concentration. The procedure was terminated when the FEV_1 decreased by more than 20% of its post-saline value or when the highest methacholine (50 mg/mL) or AMP (400 mg/mL) concentration was reached. The percentage decline of FEV_1 from the post-saline value was plotted against the log concentrations of the inhaled methacholine or AMP. PC_{20} values of methacholine and AMP were calculated by interpolating between two adjacent data points when the FEV_1

decreased by more than 20%. Censored values of 100 mg/mL for PC_{20} of methacholine and 800 mg/mL for that of AMP were given to those who did not show a 20% decline in FEV_1 after inhalation of the maximal concentration of methacholine (50 mg/mL) or AMP (400 mg/mL).

Bronchodilator testing

Bronchodilator testing was performed, as indicated by the ERS Task Force team [15]. Lung function was measured before and 15 minutes after inhalation of 400 μg salbutamol aerosol (Ventolin Evohaler, GlaxoSmithKline, London, UK) which was administered as 4 separate doses of 100 μg via a spacer (AeroChamber Plus, Trudell Medical International, Ontario, Canada). BDR was assessed in 2 ways: (1) ΔFEV_1%initial (postbronchodilator FEV_1 minus prebronchodilator FEV_1, expressed as a percentage increase over prebronchodilator FEV_1) and (2) ΔFEV_1%predicted (postbronchodilator FEV_1 minus prebronchodilator FEV_1, expressed as a percentage of the predicted value).

Parents gave written informed consent for their children to participate in the study. The study protocol was approved by the Institutional Review Board of our hospital.

Statistical analysis

Data are presented as means ± SD or as geometric means and a range of 1 SD. FEV_1 values are expressed as %predicted. Subjects were considered to have BHR to methacholine or AMP when their PC_{20} of methacholine was <16 mg/mL [16] or when their PC_{20} of AMP was <200 mg/mL [17]. PC_{20} values were log transformed before statistical analysis. Correlations between PC_{20} and BDR or blood eosinophil counts were calculated using the Spearman's rank-order method. Correlation coefficients were compared using a Fisher Z transformation and a 2 tailed Z test [18]. A P- value of 0.05 or less was considered to be statistically significant. All analyses were performed using the statistical software SPSS version 17.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

A total of 169 children with mild to moderate asthma were enrolled in this study. Thirty-one of these children were excluded subsequently because of the occurrence of asthma exacerbations or respiratory tract infections (n = 11), unstable or low FEV_1 (n = 6), failure to undergo methacholine or AMP challenges according to the schedule (n = 8), failure to undergo bronchodilator testing (n = 4), and incomplete data due to other causes (n = 2).

The characteristics of 138 patients whose data were complete are presented in Table 1. There was no significant difference in the baseline FEV_1s before methacholine and AMP challenges (90.2 ± 13.1%predicted vs. 91.0 ± 12.7%predicted). The geometric mean (95% CI) of methacholine PC_{20} was 1.99 mg/mL (1.57-2.52), and that of AMP PC_{20} was 37.6 mg/mL (27.6-51.2). One hundred and twenty-eight patients (92.8%) had a methacholine PC_{20} <16 mg/mL, the cut-off point for BHR to methacholine. On the other hand, 116 subjects (84.1%) exhibited BHR to AMP (PC_{20} <200 mg/mL). The prebronchodilator FEV_1 (88.9 ± 11.9%predicted) was not significantly different from the baseline FEV_1s before methacholine and AMP challenges : 105 subjects (76.1%) had a prebronchodilator FEV_1 ≥80%predicted. The postbronchodilator FEV_1 was 96.2 ± 11.6 %predicted: the majority (n = 127, 92.0%) had a value ≥80%predicted. The overall increase in FEV_1 following inhalation of salbutamol, expressed as a percentage of initial value (ΔFEV_1%initial) and the predicted value (ΔFEV_1%predicted), was 8.44 ± 5.13% and 7.29 ± 3.92%, respectively.

The relationship between methacholine PC_{20} and ΔFEV_1%initial or ΔFEV_1%predicted is shown in Fig. 2. Both ΔFEV_1%initial (Fig. 2a) and ΔFEV_1%predicted (Fig. 2b) significantly correlated with methacholine PC_{20} (r = -0.254, P = 0.003, and r = -0.212, P = 0.013, respectively).

The relationship between AMP PC_{20} and ΔFEV_1%initial or ΔFEV_1%predicted is shown in Fig. 3. Both ΔFEV_1%initial (Fig. 3a) and ΔFEV_1%predicted (Fig. 3b) correlated significantly with AMP PC_{20} (r = -0.489, P = 0.000, and r = -0.448, P = 0.000, respectively).

The correlation between AMP PC_{20} and ΔFEV_1%initial was significantly stronger than that between methacholine PC_{20} and ΔFEV_1%initial (P = 0.024 for the comparison of correlation coefficients of -0.489 and -0.254). The correlation between AMP PC_{20} and ΔFEV_1%predicted was also significantly higher than that between methacholine PC_{20} and ΔFEV_1%predicted (P = 0.029 for the comparison of correlation coefficients of -0.448 and -0.212).

When the analysis was confined to 109 subjects who were steroid naïve, both methacholine PC_{20} and AMP PC_{20} significantly correlated with ΔFEV_1%initial (r = -0.279, P = 0.003, and r = -0.543, P = 0.000, respectively). They also correlated significantly with ΔFEV_1%predicted (for methacholine PC_{20}, r = -0.242, P = 0.011 ; for AMP PC_{20}, r = -0.501, P = 0.000). The relationships of ΔFEV_1%initial and ΔFEV_1%predicted with AMP PC_{20} were significantly closer than with methacholine PC_{20}, respectively (P = 0.021 for the comparison of correlation coefficients of -0.543 and -0.279, and P = 0.028 for the comparison of correlation coefficients of -0.501 and -0.242, *data not shown*).

The ΔFEV_1%initial was calculated according to the presence/absence of BHR to methacholine and BHR to AMP, respectively, and the number of subjects with a positive and negative BDR, with a cutoff of 9% [19], in each category was presented in Table 2. The ΔFEV_1%initial was significantly (P = 0.032) higher in subjects with BHR to methacholine than those without. The same figures were observed between subjects with BHR to AMP and those without (P = 0.043). A positive BDR was associated with BHR to methacholine with a high positive predictive value (98%), but a negative BDR

does not exclude it (negative predictive value : 10%). Likewise, a positive and negative predictive value of BDR testing for BHR to AMP was 96% and 22%, respectively.

There was an inverse correlation between AMP PC_{20} and blood eosinophil counts (r = -0.237, P = 0.005), but not between methacholine PC_{20} and blood eosinophil counts (r = -0.059, P = 0.488) (*data not shown*).

DISCUSSION

In this study, we have investigated the relationship between BDR and bronchial responsiveness assessed by methacholine and AMP challenges. Although both methacholine PC_{20} and AMP PC_{20} correlated significantly with BDR, the correlation was stronger for AMP PC_{20} than for methacholine PC_{20}. To the best of our knowledge, this is the first study that has compared methacholine and AMP responsiveness with regard to their relationships to BDR in children with asthma.

BDR is usually measured by changes in airflow before and after the administration of beta-agonists. Most commonly, it is expressed as a percentage increase in FEV_1 over the initial value. The BDR of our asthmatic subjects, expressed in this manner, averaged 8.44%, which was comparable to that of other studies. Tantisira et al. [20] reported a mean BDR of 10.07% among the 1,041 participants in the Childhood Asthma Management Program. Galant et al. [21] observed various mean BDRs according to the clinical severity, ranging from 7.3% (mild intermittent group) to 10.1% (severe persistent group). There is no clear consensus about what constitutes a significant reversibility in subjects with airflow obstruction. A recent report suggested that a ≥9% BDR cutoff point best distinguishes children with asthma from those without [19]. According to this criterion, we found that 47 (34.1%) of 138 children with asthma had a positive BDR. This is in line with previous reports that a large proportion of patients with asthma do not show a positive BDR, which strengthens the suggestion that BDR provides only the modest sensitivity in confirming the diagnosis of asthma [21].

In the present study, methacholine and AMP challenge tests were performed during the second week in a randomized order. The low-dose inhaled corticosteroids administered to most of our subjects necessitating controller therapy are reported to have short-lived (within one week) effects on methacholine and AMP reactivity after

treatment is stopped [22,23]. On the other hand, the time course of the changes in BDR after inhaled corticosteroid withdrawal has not been studied. Therefore, bronchodilator testing was set to be performed during the third week in order to minimize the effect of corticosteroids, if it is.

It has been suggested that BDR is the physiological opposite of bronchoconstrictor responsiveness [6,9], and therefore bronchial challenge tests can be replaced by bronchodilator tests in subjects with airway obstruction. Several studies have shown that BDR is associated with histamine or methacholine responsiveness in both children and adults with asthma [7,8,9]. Similarly, we found a significant, albeit weak, correlation between BDR and methacholine PC_{20}. On the contrary, there are other reports that BDR is not related to methacholine responsiveness [10,11]. The reasons for these conflicting data are not clear, but are presumably due to confounding factors such as airway remodeling. It is hypothesized that airway wall thickening results in disproportionally severe airway narrowing and thus leads to an exaggerated BHR [24]. This hypothesis is supported by studies [25] showing a significant relationship between BHR to methacholine and the degree of airway wall thickening. On the other hand, airway remodeling may be an important mechanism that leads to fixed airflow obstruction in asthma [26].

It has not previously been studied whether BDR is related to BHR assessed by indirect challenge tests. Given that indirect challenges more closely reflect mechanisms via which clinical asthma manifests itself [5], it is surprising that little information is available on this relationship. In the present study, BDR correlated significantly with AMP PC_{20}. Furthermore, BDR more strongly correlated with AMP PC_{20} than with methacholine PC_{20} ($P = 0.024$ for comparison of correlation coefficients). The results of our study suggest that airway reversibility is more closely associated with bronchial responsiveness assessed by AMP than by methacholine in

asthma. To the best of our knowledge, this is the first study that has compared methacholine and AMP responsiveness with regard to their relationships to BDR.

When BDR is expressed as a percentage increase in FEV_1 over the initial value, small absolute changes in FEV_1 may be exaggerated to be larger in patients with a reduced baseline FEV_1. It has been suggested that relating the change in FEV_1 to the predicted value may be a more appropriate way [27], because this eliminates the influence of not only the initial value but also gender, age and height. In the present study, however, a closer relationship of BDR with AMP PC_{20} than with methacholine PC_{20} persisted ($P = 0.029$ for comparison of correlation coefficients), even when we evaluated the change in FEV_1 based on the predicted value instead of the initial value.

It is possible that inhaled corticosteroids used as a controller medication may have confounded the results, although they were discontinued at least 1 week before the study. However, when the analysis was restricted to steroid-naive subjects, the same figures were observed.

It is expected that individuals who at baseline are maximally bronchodilated will have minimal BDR, and vice versa. Thus, BDR, even expressed as % predicted, is dependent on the prebronchodilator value [28]. On the other hand, for bronchial challenge tests, a given stimulus provokes a larger bronchoconstrictor response in a subject with more severe obstruction than in a subject with less severe obstruction, resulting in lower PC_{20} [16]. As a result, both the severity of methacholine PC_{20} and AMP PC_{20} would be affected by baseline airway caliber. One may argue that our finding, that is, a stronger association of BDR with AMP PC_{20} than with methacholine PC_{20} may be due to a differential influence of baseline airway caliber on AMP PC_{20} and methacholine PC_{20}. However, this seems unlikely, because methacholine responsiveness is more strongly related to a diminished airway caliber than is AMP responsiveness [29].

The reason why BDR is more linked to AMP responsiveness than to methacholine responsiveness is not clear but speculative. Clinical studies in asthmatics have shown that BHR to AMP reflects an underlying bronchial inflammation more accurately than BHR to methacholine [30]. This hypothesis is supported by our observation of a significant correlation between blood eosinophil counts and AMP PC_{20} but not with methacholine PC_{20}. Several authors have investigated the association of BDR with biomarkers of inflammation including exhaled nitric oxide and bronchial eosinophilia. Covar et al. [31] reported that a level of exhaled nitric oxide was significantly higher in children who showed at least 12% BDR than that in those who did not. Faul et al. [32] reported that the changes in eosinophils at the bronchial biopsy specimens correlated with those in BDR 8 weeks after corticosteroid therapy in atopic children with asthma. Thus, a higher BDR would be associated with increased inflammatory events in the airways, the extent of which may be more specifically reflected by AMP responsiveness than by methacholine responsiveness.

The correlations between both AMP and methacholine responsiveness and BDR were not strong, albeit statistically significant. One should take into account that bronchodilator testing and the two challenge tests use different stimuli to elicit the response. Another factor that should be considered is the different nature of the tests. Bronchodilator testing is a “one-shot” test which is more analogous to exercise challenge, whereas the two challenge tests use a progressive dose-response method.

A positive BDR, defined as a ΔFEV1%initial $\geq$9%, was suggestive of BHR to methacholine or BHR to AMP, with a high predictive value. However, because many patients with BHR to methacholine or with BHR to AMP had a negative BDR result, the predictive value of BDR testing for BHR to methacholine or AMP is quite limited.

In summary, we found that BDR correlated significantly not only with methacholine responsiveness but also with AMP responsiveness. The comparison of correlation

coefficients revealed that BDR was more linked to AMP responsiveness than to methacholine responsiveness. The results of this study suggest that BDR may be better reflected by bronchial responsiveness as assessed by AMP challenge than by methacholine challenge.

ACKNOWLEDGMENTS

This study was supported in part by grant No. 04-2009-048-0 from the Seoul National University Hospital Research Fund.

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Table 1. Characteristics of the asthmatic children studied

Age (years)	11.3 ± 3.1
Sex (M/F)	99/39
Blood eosinophils (/μL)	455.8 ± 251.3
Serum IgE (IU/mL)	264.3 (213.2-327.8)
Atopy, n (%)	119 (86.2)
Controller therapy, n (%)	29 (21.0)
FEV_1*, methacholine challenge	90.2 ± 13.1
FEV_1*, AMP challenge	91.0 ± 12.7
Methacholine PC_{20} (mg/mL)	1.99 (1.57-2.52)
AMP PC_{20} (mg/mL)	37.6 (27.6-51.2)
Prebronchodilator FEV_1 (%predicted)	88.9 ± 11.9
Postbronchodilator FEV_1 (%predicted)	96.2 ± 11.6
ΔFEV_1%initial (%)	8.44 ± 5.13
ΔFEV_1%predicted (%)	7.29 ± 3.92

Mean ± SD or Geometric mean (95% CI)

*pre-test baseline values (% predicted)

AMP: adenosine 5'-monophosphate; PC_{20}: a provocative concentration causing a 20% decline in FEV_1; ΔFEV_1%initial: change in FEV_1, expressed as a percentage of the prebronchodilator value; ΔFEV_1%predicted: change in FEV_1, expressed as a percentage of the predicted value.

Table 2. The ΔFEV_1%initial according to the presence/absence of BHR to methacholine and BHR to AMP, respectively, and the number of subjects with a positive and negative bronchodilator response, with a cutoff of 9%, in each category.

	BHR to methacholine		BHR to AMP	
	+	–	+	–
ΔFEV_1%initial (mean±SD)	8.66±5.23	5.69±2.44	8.86±5.44	6.23±1.84
≥ 9% (n)	46	1	45	2
< 9% (n)	82	9	71	20

BHR : bronchial hyperresponsiveness ; AMP : adenosine 5'-monophosphate ; ΔFEV_1%initial : postbronchodilator FEV_1 minus prebronchodilator FEV_1, expressed as a percentage increase over prebronchodilator FEV_1

FIGURE LEGENDS

Fig. 1. Schematic flow chart of the study design. The order of methacholine and AMP (adenosine 5'-monophosphate) challenge tests was randomized. empty bars : methacholine challenge test ; gray bars : AMP challenge test ; solid bar : bronchodilator test.

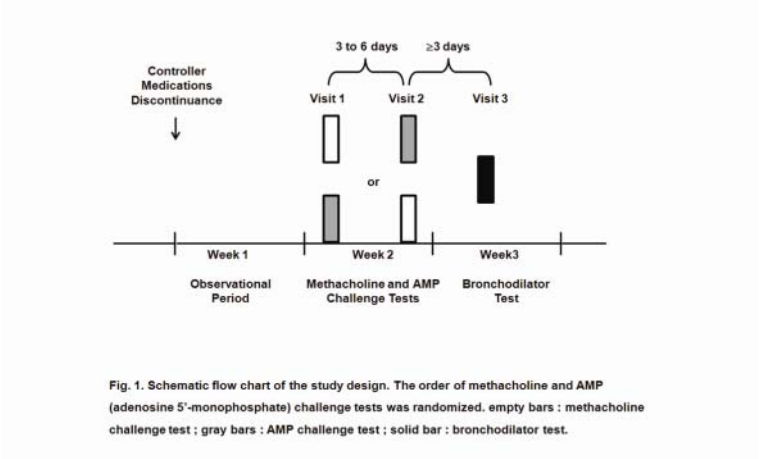

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Fig. 2. Scatter plots of the change in FEV_1, expressed as a percentage increase over the initial value (ΔFEV_1%initial), against methacholine provocative concentration causing a 20% decline in FEV_1 (PC_{20}) (Fig. 2a). Scatter plots of the change in FEV_1, expressed as a percentage increase over the predicted value (ΔFEV_1%predicted), against methacholine PC_{20} (Fig. 2b).

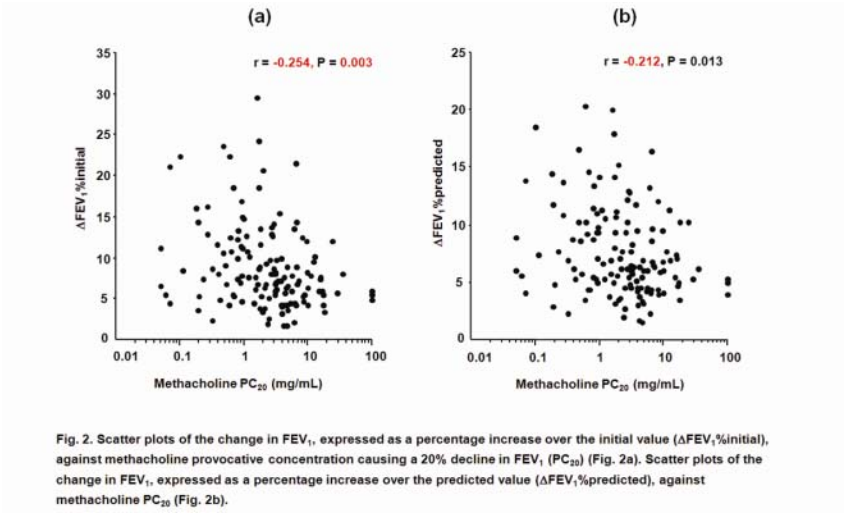

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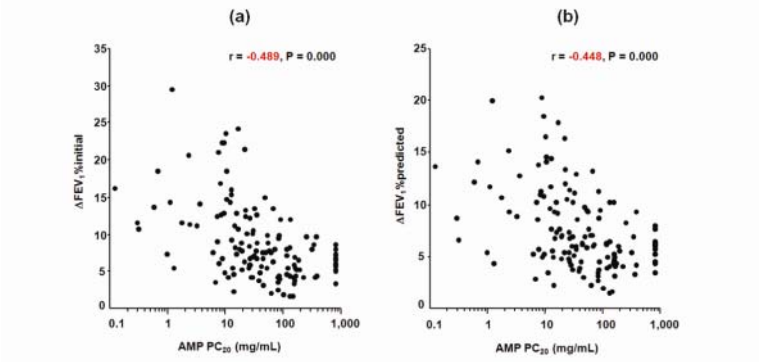
Fig. 3. Scatter plots of the change in FEV_1, expressed as a percentage increase over the initial value (ΔFEV_1%initial), against adenosine 5'-monophosphate (AMP) provocative concentration causing a 20% decline in FEV_1 (PC_{20}) (Fig. 3a). Scatter plots of the change in FEV_1, expressed as a percentage increase over the predicted value (ΔFEV_1%predicted), against AMP PC_{20} (Fig. 3b).



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