Extrapolation of methacholine log-dose response curves with a Cumulative Gaussian Distribution function

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ABSTRACT: Methacholine provocation tests are aimed at determining bronchial responsiveness. Recent investigations stress the importance of considering the entire log-dose response curve, yielding not only the provocative dose producing a 20% change in forced expiratory volume in one second (FEV_1) (PC_{20}), but also the plateau value and the steepest slope of the curve (reactivity).

In three control subjects and seven patients with mild to moderate asthma, we have obtained methacholine log-dose response curves in which a plateau was reached. A new model, the Cumulative Gaussian Distribution (CGD) function was fitted to the whole curve. The upper part of the curve was also analysed with the Hofstee equation, which has been used in a number of other investigations and aimed at plateau estimation. The plateau values obtained by the fits were compared with the values actually measured (average response of last 3 points with a variation coefficient <5% of the mean value) by using the coefficient of determination (R^2) (Applied Statistics. Sachs).

If all data points were considered, both fits yielded a plateau which slightly overestimated the measured plateau values. R^2 for the CGD fit ranged from 0.93–0.99, indicating a highly significant correlation between actual and fitted data points. If the curves were truncated, such that the last four provocative doses were omitted from the analysis, the CGD fit still yielded plateau values; the mean difference from the measured plateaux, in % of the measured plateau values, was -2.6% (SD 18.2). In only three out of the 10 cases did the Hofstee equation yield plateau values with a deviation from 'measured' <47% of the measured value.

We conclude that the CGD function is a promising model for the fit of methacholine log-dose response curves in mild to moderate asthma.
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Bronchoconstrictor stimuli, such as histamine and methacholine, are widely used for the generation of log-dose response curves in asthmatic patients. In clinical and epidemiological settings, the forced expiratory volume in one second (FEV_1) is usually the response variable of choice, and is preferred for its high reproducibility and repeatability [1].

The provocative concentration producing a fall of 20% in the FEV_1 (PC_{20}) is called the sensitivity, which is lowered in asthmatics and is associated with a leftward shift of the log-dose response curve.

Recent investigations have focused on the importance of other parameters of the sigmoid-shaped log-dose response curve, *i.e.* the steepest rise in the middle part (reactivity) and the maximal obtainable response (plateau value). Although reactivity is certainly coupled to pharmacodynamic mechanisms, its interpretation is still unclear [2–4]. However, the plateau value has been considered to be a variable which is related to mechanical properties of small airways [5–9].

For the interpretation of the entire log-dose response curve a model fit to the experimental data may be necessary. Firstly, a mathematical model enables a smoothing of the curve, thus minimizing fluctuations, *e.g.* due to varying patient co-operation or other causes. Secondly, the model parameters, if obtained from a part of the curve, may enable the extrapolation of the whole curve. It has been shown that direct estimates of the plateau value are possible in the majority of normal subjects and mild asthmatics, but not in moderate or severe cases of asthma [7, 8, 10]. Because the reaching of a plateau can be associated with a large drop in FEV_1, extrapolation may be necessary.

Models used until now are the Hofstee equation [11], a double reciprocal plot [7], and a sigmoid equation [9]. These models have drawbacks, as will be discussed later.

The aim of our investigation was to test a new mathematical model, describing the shape of methacholine log-dose response curves in normal individuals, as well as in asthmatic patients. In addition, we tested the accuracy of

extrapolated plateau values, after truncation of the curves, compared to directly measured plateaux. The new model was compared with the extrapolation by the Hofstee equation [11], which has been applied in a number of earlier studies [12–14].

Methods and material

Subjects

Three normal volunteers (2 males and 1 female; mean age 41 yrs, range 40–42 yrs), and seven patients with mild to moderate asthma (2 males and 5 females; mean age 40 yrs, range 28–52 yrs) volunteered to participate in the study (table 1). In all subjects, a plateau in the methacholine log-dose response curve could be obtained experimentally. Asthma was diagnosed by a positive history of episodic dyspnoea and wheezing, and mildly to moderately increased airway responsiveness. As determined from a standardized method (see below) the geometric mean provocative concentration of methacholine causing a fall of FEV_1 of 20% (PC_{20}) was 5.6 mg·ml^{-1}; range 0.7–9.9 mg·ml^{-1}. In the normal subjects, no PC_{20} could be detected. Mean FEV_1 in the normal subjects was 110% of reference (range 106–117%), and in the asthmatics 86% of reference (range 63–107%). Reference values were according to Quanjer et al. [15].

The study was approved by the Ethics Committee of the Dijkzigt hospital and informed consent was given by the volunteers.

Inhalation test

A methacholine challenge test was performed using a standardized tidal breathing method [16]. Dose-response curves were obtained after inhalation of doubling concentrations of acetyl-β-methylcholinebromide (0.04–314 mg·ml^{-1}) in normal saline. The aerosols were generated by a De Vilbiss 646 nebulizer (output 0.13 ml·min^{-1}) and inhaled by tidal breathing for 2 min. The response was measured as a change in FEV_1, expressed as percentage of the initial value and related to ^{2}log dose. A test was terminated if the FEV_1 fell by more than 60%, or if unpleasant side-effects or dyspnoea compelled the volunteer to stop.

In all cases, a plateau was reached according to the criterion that the last three response values showed a variation coefficient of less than 5% of the mean value.

Mathematical models for log-dose response curves

Before a mathematical model can be applied, it must fulfil certain criteria. Firstly, the model should describe the entire sigmoid-shaped curve. Secondly, the plateau value and reactivity should be obtainable from the fitted model parameters. See the Appendix for more details.

The Cumulative Gaussian Distribution (CGD) function. The CGD model is explained in fig 1. A continuous Gaussian distribution (fig. 1a) is defined by three parameters, a mean value $\bar{x}$, a standard deviation σ and a normalizing factor equal to the area under the distribution α. In figure 1b the CGD function is denoted, being equal to the cumulative area (A) under the curve as function of the abscissa. The CGD function was chosen because it reflects the pattern of log-dose response curves qualitatively, x denoting the response and Y_c the plateau.

The Hofstee equation. According to this equation, the curve is considered as a rectangular hyperbole. The response is plotted against the response divided by the log dose [11]. By linear least squares regression analysis, the maximal response was found as the intercept with the Y axis. This model is only aimed at extrapolation of the plateau value. Therefore, only plateau estimates were compared with both experimentally determined values and with fits as obtained with the CGD model.

CGD fit procedure. Because the equation describing the CGD function is nonlinear, we used a nonlinear regression technique, as first described by Marquard [17], and later adapted by Press et al. [18] in a volume on

Table 1. – Anthropometric data and FEV_1, VC and PC_{20} values of the three normal volunteers and seven patients with bronchial asthma

Subject No.	Sex	Age yrs	Height cm	Weight kg	FEV_1 *l*	FEV_1 % ref	VC *l*	VC % ref	PC_{20} mg·ml^{-1}
1	M	40	178	65	4.33	108	6.10	120	*
2	M	42	175	78	4.06	106	5.76	119	*
3	F	40	158	53	3.10	117	3.92	125	*
4	F	31	165	60	2.71	86	3.44	94	5.0
5	F	45	167	58	2.42	84	3.29	99	2.8
6	M	52	170	82	2.08	63	3.58	88	0.7
7	F	26	165	57	3.50	107	4.27	114	9.9
8	M	51	168	75	3.34	103	4.59	115	7.5
9	F	46	164	63	2.60	94	3.38	106	7.7
10	F	28	160	55	2.74	90	3.49	100	7.1

Subjects No. 1–3 were normal volunteers. Subjects No. 4–10 had mild to moderate asthma. FEV_1: forced expiratory volume in one second; VC: vital capacity; PC_{20}: provocative concentration producing a 20% fall in FEV_1.
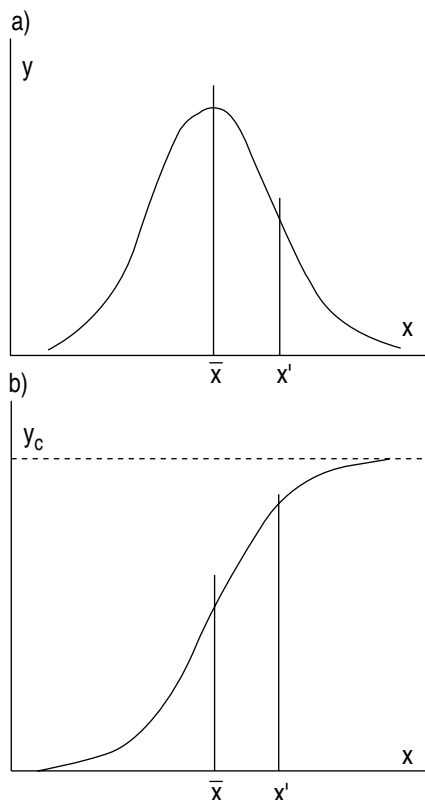
Fig. 1. – Example of Gaussian distribution (a) and the sigmoid shaped cumulative distribution function (b). At the mean of the distribution (x) the slope in b gives the reactivity. The ordinate value A(x') in 1b is equal to the area under the curve (A) up to x' in 1a. For further explanation see text.

numerical recipes. The software for the Marquard algorithm described by PRESS *et al.* [18] is available, together with the volume, from Cambridge University Press. Data from the methacholine log-dose response (^{2}log concentration and ΔFEV_1 (%)) were imported off-line. The fit procedure yielded estimates for plateau and reactivity, defined as (ΔFEV_1(%)/^{2}log dose) in the steepest part of the curve. The goodness of fit was expressed by the coefficient of determination R^2, being the fractional decrease of the initial variance of the experimental response data by fitting the model. The root of this index yields the correlation coefficient for the correlation of experimental and fitted data. Furthermore, the root mean square (RMS) deviation between experimental and fitted response values was determined and expressed as percentage of the plateau value.

The randomness of subsequent residuals after fitting the CGD-function was evaluated using the runs-test [19].

Truncation of the curves. The reliability of the extrapolation of the log-dose responses curves in order to estimate a plateau value was also studied. Therefore, up to four last data points were left out. The fitted plateau estimates were compared with the measured values, being the average response of 3 data points, with a variation coefficent <5% of the mean value, at the highest methacholine concentrations [12].

Results

Examples of the log-dose response curves of the asthma patients and normal volunteers are shown in figure 2a and b, together with the CGD fit. The numerical data concerning the fits are presented in table 2. By one-way analysis of variance, a significant difference was found between normal subjects and asthmatic patients in both the plateau value (p=0.005) and the reactivity estimates (p=0.01).

The R^2 values, shown in the table, range from 0.93–0.99, indicating a highly significant correlation between experimental and fitted response data. As judged by the "runs" test, the probability of a lower number of runs ranged
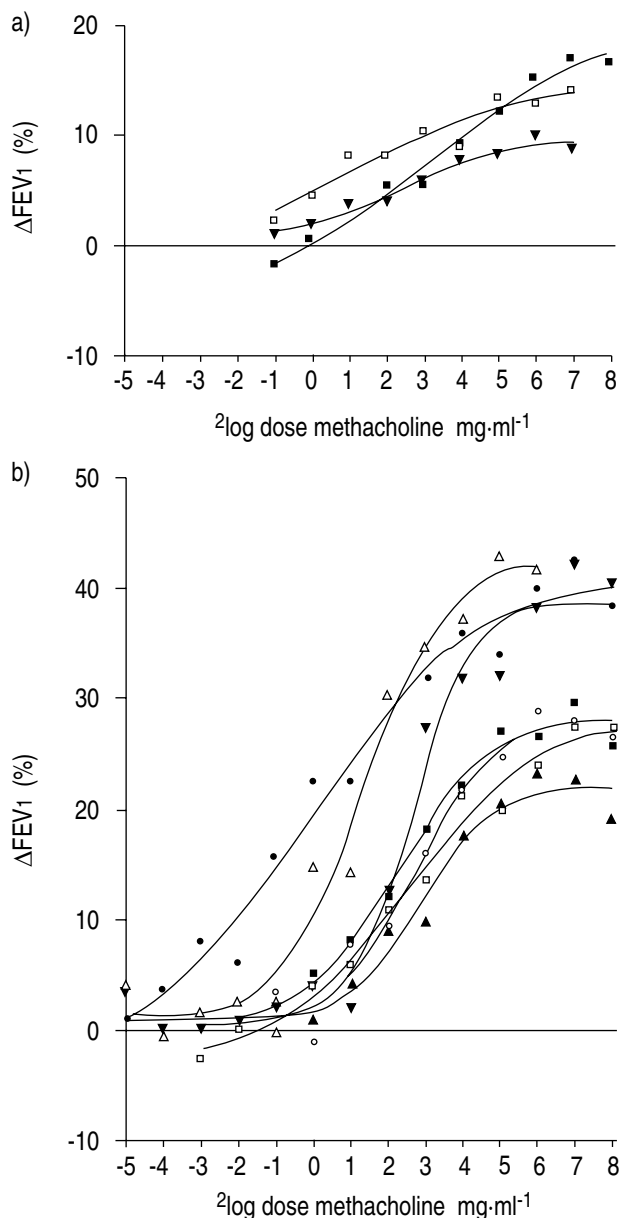

Fig. 2. – a) Experimental data and Cumulative Gaussian Distribution (CGD) model fits for the normal volunteers: No. 1 (▼); No. 2 (▫); No. 3 (■). b) Experimental data and CGD model fits for the asthmatic patients No. 4 (▼); No. 5 (△); No. 6 (●); No. 7 (▲); No. 8 (○); No. 9 (▫); No. 10 (■). FEV$_1$ forced expiratory volume in one second.

Table 2. – Measured plateau values, last provocative dose and plateau estimates by the CGD and hyperbolic fit using all available data

Subject No.	Plateau % meas.	Last dose mg·ml⁻¹	Hyperbolic model		CGD model				
			Plateau % fit	Rel. dev.	Plateau % fit	Rel. dev.	React. %FEV₁/²log-dose	RMS/plateau %	R²
1	8.5	157	9.4	0.11	9.0	0.06	1.6	5.2	0.98
2	13.1	157	13.8	0.05	13.9	0.06	1.7	7.5	0.93
3	15.8	314	18.3	0.16	18.8	0.19	2.7	4.3	0.99
4	39.9	314	41.5	0.04	39.2	-0.02	10.2	5.9	0.98
5	40.3	78	44.7	0.11	41.9	0.04	9.1	5.7	0.98
6	39.9	314	39.3	-0.02	40.3	0.01	4.8	5.1	0.98
7	21.1	314	23.2	0.10	21.7	0.03	5.2	7.2	0.97
8	26.8	314	30.0	0.12	27.7	0.03	6.2	5.8	0.98
9	26.0	314	27.3	0.05	27.8	0.07	4.3	4.3	0.99
10	27.0	314	29.1	0.08	27.7	0.03	5.4	4.0	0.99
Mean				0.07		0.05		5.5	
SD				(0.04)		(0.06)		(1.2)	

Subjects No. 1–3 were normal volunteers. Subjects No. 4–10 had mild to moderate asthma. CGD: Cumulative Gaussian Distribution. The root mean square (RMS) deviations, reactivity estimates and coefficients of determination (R^2) for the CGD fit *versus* the measured data are also presented. Rel. dev: relative deviation of fitted *versus* measured plateau value.
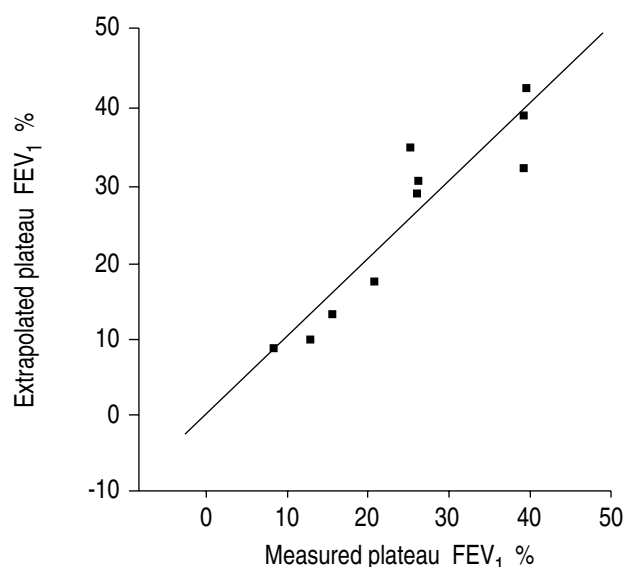

Fig. 3. – Relationship between the Cumulative Gaussian Distribution (CGD) extrapolations of the plateaux and the measured plateaux in case of omitting data from the last four provocative doses. FEV_1: forced expiratory volume in one second.

from 0.50–1.00 (mean 0.84, SD 0.21). This means a highly significant random behaviour of the residuals [19]. If the fit of all data points is considered, both the CGD model and the hyperbolic fit show a slight overestimation of the experimentally determined plateaux, the difference between the two approaches being nonsignificant (table 2). Truncation of the curves, by omitting the last four data points, enabled a plateau extrapolation for all subjects with the CGD model. Figure 3 shows the extrapolated plateaux *versus* the experimentally determined plateaux. The regression line (ΔFEV_1 (%, extrapolated))= $-0.06+0.99 \times (\Delta FEV_1$(% measured)) was nearly identical with the identity line, the linear correlation coefficient

(R=0.93) indicating a highly significant relationship (p<0.001). The mean deviation of the fitted plateau in percentage of the measured plateau was -2.6% (SD 18.2%).

For the hyperbolic fit procedure, in three cases insufficient data were available for the fit procedure, in three cases the absolute deviation between fitted and measured plateau was less than 47%, and in the remaining four cases this deviation was markedly larger than 47%.

Discussion

Methacholine log-dose response curves were obtained in 3 normal volunteers and 7 patients with mild to moderate asthma in a standard manner, in order to evaluate the fit ability of a new model, the Cumulative Gaussian distribution (CGD) function. In all curves, an experimental plateau could be reached.

To our knowledge, the three models used up to now are the Hofstee equation [11–14], the double reciprocal plot [7] and a sigmoid equation [9]. The Hofstee equation is based on regarding the curve as a rectangular hyperbola in the upper part, while the reciprocal plot uses the straightening of a sigmoid curve if the reciprocals of response and log-dose are related. As MICHOUD *et al.* [7] stated these fits are aimed at the expression of the well-recognized sigmoid shape of the curves, but both need a plateau or an extended part of the log-dose response curve in order to extrapolate the plateau with reasonable accuracy. The sigmoid equation, as described by WOOLCOCK *et al.* [9], fits only two parameters and does not incorporate the plateau as a fit parameter. For an accurate fit, this parameter had to be estimated by trial and error. Values of 100% had to be chosen as a limit in a number of patients with moderate or severe asthma [9]. In our opinion, this is a drawback of this model, where a plateau value as fit parameter has to be preferred. We have compared our CGD model with the Hofstee approach. Because

the Hofstee equation is aimed at a plateau estimation, only this variable is incorporated in the comparison. The whole CGD function is estimated, yielding the plateau and the reactivity.

The highly significant correlation between experimental and fitted response data, as derived from the R^2 values, showed a good fit ability of the CGD function in our group of volunteers. Also, the runs-test showed a random behaviour of the residuals. Both the hyperbolic and the CGD fit showed a slight overestimation of the measured plateaux if all data points were used, the accuracy between both approaches not differing significantly. With respect to extrapolation of truncated curves, the CGD fit proved to be superior to the hyperbolic fit. Even when data from the last four provocation doses were left out, a reasonably accurate plateau estimation proved to be possible (fig. 3). The hyperbolic fit, however, failed in 7 out of the 10 cases, either because a limited number of data was available or because the relative errors exceeded 47% of the measured plateau.

The reactivity can be derived from the CGD function as the slope in the steepest part of the curve, *i.e.* halfway plateau value, this slope definition also being used by CHUNG *et al.* [2]. Some studies determined the slope by linear regression analysis of the data from the threshold (PC_{20}) upward and considered this slope as reactivity [3, 4]. This last procedure becomes inaccurate when the log-dose response curve is extended, such that a curvelinear part is reached at higher doses. We think that a slope should be defined as the steepest part of an experimental curve or, as in our case, as model parameter which enables comparison between different studies.

We found the mean reactivity of the normal volunteers to be significantly lower than in the asthmatics. This confirmed the relationship found previously between PC_{20}, expressed as log-dose, and reactivity [4]. Also, the experimentally determined plateaux were significantly lower in the normal subjects.

We conclude that the CGD function may be promising in describing the whole methacholine log-dose response curve in normal volunteers as well as in patients with mild to moderate asthma. The fit appears to be superior to the hyperbolic fit with respect to the plateau extrapolation in truncated curves.

Although the numerical mathematical analysis is complex, the software is commercially available [18], and can be implemented on a standard personal computer.

Appendix

The Cumulative Gaussian Distribution function

A continuous non-normalized Gaussian distribution is described by the equation:

$$f(x) = \frac{\alpha}{\sigma\sqrt{2\pi}}e^{-1/2((x-\bar{x})/\sigma)^2} \tag{1}$$

with $\bar{x}$=mean value; σ=standard deviation; and α=normalizing factor.

The cumulative distribution is derived from Equation (1) as:

$$A(x')= \frac{\alpha}{\sigma\sqrt{2\pi}} \int_{-\infty}^{x'} e^{-1/2((x-\bar{x})/\sigma)^2}dx \tag{2}$$

In which $A(x')$ is the area under the curve up to the value x' (fig. 1).

To fit the model to the experimental data a least squares criterion, minimizing:

$$\sum_{i-1}^{N} (A(x_i)-y_i)^2 \tag{3}$$

is used, the y_i values representing the experimentally determined ΔFEV_1 (%) values.

The Hofstee equation

This equation was derived in order to describe the relationship between reaction rate and substrate concentration in an enzymatic reaction. The similarity with the stimulus-response relation, obtained with the methacholine log-dose response curves, gives:

$$Plateau=\Delta FEV_1 - \alpha \frac{(\Delta FEV_1)}{\log \text{ dose}} \tag{4}$$

ΔFEV_1 data as % initial are taken from the inflection point upward in the log-dose response curves. The vertical and horizontal axis of the hyperbole are given by log-dose=α ($\Delta FEV_1 \rightarrow \infty$) and ΔFEV_1=plateau value (log-dose $\rightarrow \infty$). Linear regression analysis is applied to Equation (4) with ΔFEV_1 as Y and ΔFEV_1/log-dose as X-axis, so that the plateau is found as the intercept with the Y-axis.

References

1. Eiser NM, Kerrebijn KF, Quanjer PhH (eds). Guidelines for standardisation of bronchial challenges with (non-specific) bronchoconstricting agents. *Bull Eur Physiopathol Respir* 1983; 19: 495–514.
2. Chung KF, Morgan B, Keyes SJ, Snashall PD. Histamine dose-response relationships in normal and asthmatic subjects. *Am Rev Respir Dis* 1982; 126: 849–854.
3. Cockroft DW, Berscheid BA. Slope of the dose response curve: usefulness in assessing bronchial responses to inhaled histamine. *Thorax* 1983; 38: 55–61.
4. Malo JL, Cartier A, Pineau L, Gagnon G, Martin RR. Slope of the dose response curve to inhaled histamine and methacholine in subjects with symptoms of airway hyperexcitability and in normal subjects. *Am Rev Respir Dis* 1985; 132: 644–647.
5. Bel EHB, Veen H van der, Kramps JA, Dijkman JH, Sterk PJ. Maximal airway narrowing to inhaled leukotriene D_4 in normal subjects. *Am Rev Respir Dis* 1987; 136: 979–984.
6. Bel EHB, Veen H van der, Dijkman JH, Sterk PJ. The effect of inhaled budesonide on the maximal degree of

airway narrowing to leukotriene D_4 and methacholine in normal subjects *in vivo*. *Am Rev Respir Dis* 1989; 139: 427–431.

7. Michoud MC, Lelorier J, Amyot R. Factors modulating the interindividual variability of airway responsiveness to histamine. The influence of H1 and H2 receptors. *Bull Eur Physiopathol Respir* 1981; 17: 807–821.

8. Moreno RH, Hogg JC, Paré PD. Mechanics of airway narrowing. *Am Rev Respir Dis* 1986; 133: 1171–1180.

9. Woolcock AJ, Salome CM, Yan K. The shape of the dose-response curve to histamine in asthmatic and normal subjects. *Am Rev Respir Dis* 1984; 130: 71–75.

10. Foresi A, Chetta A, Corbo GM, Cuomo A, Olivieri D. Provocative dose and dose response curve to inhaled propanolol in asthmatic patients with bronchial hyper-responsiveness to methacholine. *Chest* 1987; 92 (3): 455–460.

11. Hofstee BHJ. Graphical analysis of single enzyme systems. *Enzymologia* 1956; 17: 273–278.

12. Sterk PJ, Daniel EE, Zamel N, Hargreave FE. Limited bronchoconstriction to methacholine using partial flow-volume curves in nonasthmatic subjects. *Am Rev Respir Dis* 1985; 132: 272–277.

13. Sterk PJ, Timmers MC, Dijkman JH. Maximal airway narrowing in humans *in vivo*. *Am Rev Respir Dis* 1986; 134: 714–718.

14. Sterk PJ, Daniel EE, Zamel N, Hargreave FE. Limited maximal airway narrowing in nonasthmatic subjects. *Am Rev Respir Dis* 1985; 132: 865–870.

15. Quanjer PhH, (ed.) Standardized lung function testing. *Bull Eur Physiopathol Respir* 1983; (Suppl. 5): 1–95.

16. Hargreave FE, Sterk PJ, Ramsdale EH, Dolovich J, Zamel N. Inhalation challenge tests and airway responsiveness in man. *Chest* 1985; 87 (Suppl.): 202–206

17. Marquard DW. An algorithm for least squares estimation of nonlinear parameters. *J Soc Ind Appl Math* 1963; 11: 431–441.

18. Press Wh, Flannery BP, Teukolsky SA, Vetterling WT. *In*: Numerical Recipes in Pascal. Cambridge University Press, 1989.

19. Sachs L. *In*: Applied statistics. New York, Springer Verslag, 1982.