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Analysis of bronchial reactivity in epidemiological studies

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Abstract

The measurement of bronchial reactivity in epidemiological studies has the advantage of quantifying an objective physiological feature of asthma. Bronchial reactivity was developed in a clinical setting and has been conventionally expressed as the dose of agonist producing a 20% fall in FEV_1(PD_{20}). As PD_{20} can be estimated for less than 20% of subjects in general community surveys with the doses of agonist that are usually given, data from most subjects must be censored. Thus PD_{20} alone is a poor index of bronchial reactivity for epidemiological studies. Data from 809 aluminium smelter workers were used to evaluate alternative methods of analysing bronchial reactivity. Dose-response relationships were analysed by four methods: (1) PD_{20} by the conventional method of interpolating the dose on a logarithmic scale between the last two measurements of FEV_1; (2) PD_{20} (with allowance for extrapolation), estimated by fitting an exponential curve to the dose-response data; (3) the linear regression slope between dose and FEV_1 when significant; (4) the dose-response slope obtained in all subjects as the % change in FEV_1 from baseline in response to total dose. When each of these measures was related to symptoms, diagnosis, and treatment of asthma, all differentiated between "asthmatic" and "non-asthmatic" subjects. The dose-response slope (method 4) had the advantages of simplicity and no censored data, and was shown to be clinically relevant. It is suggested that the dose-response slope should be used for the analysis of bronchial reactivity in epidemiological studies.

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Bronchial reactivity or responsiveness is both an important physiological outcome and a quantifiable feature of asthma. Epidemiological studies have investigated its distribution in general community surveys conducted in Australia,[1-3] New Zealand,[4 5] Papua New Guinea,[6] the United Kingdom,[7 8] and many other countries. For the laboratory diagnosis of asthma bronchial reactivity has conventionally been expressed as the dose of agonist producing a 20% fall in FEV_1(PD_{20}). Most healthy subjects, however, do not show a 20% fall in FEV_1 even with high doses of agonist. Because PD_{20} can be estimated in only a minority of subjects in community surveys, it is not an ideal measure of bronchial reactivity for epidemiological research. To describe the distribution of bronchial reactivity in a healthy population completely requires a continuously distributed index that summarises the dose-response data for all individuals.

The slope of the dose-response line has been investigated in laboratory studies as an alternative index of bronchial reactivity.[9-11] These studies have all included substantial numbers of asthmatic subjects, however, and have not considered the value of slope coefficients in epidemiological studies. Empirical evidence[9 12] favours the use of a linear rather than a logarithmic dose scale. In the present study of aluminium workers two methods for deriving PD_{20} were compared and alternative indices of bronchial reactivity evaluated.

Methods

The subjects were participants in a longitudinal study of respiratory symptoms and lung function in aluminium smelter workers. After giving informed consent, 809 (96%) of the 843 male employees completed a methacholine challenge, in which they inhaled increasing doses up to 6·14 μmol from hand held glass nebulisers according to a rapid method protocol.[13] FEV_1 was measured from maximal flow-volume loops performed to American Thoracic Society standards[14] on a water sealed spirometer interfaced with a microcomputer (Gould 2400 system). All subjects wore nose clips and were tested in the sitting position. The test was stopped when FEV_1 had fallen by 20% or the highest dose of histamine had been given. Twelve subjects were not challenged because of impaired baseline lung function (FEV_1 below 65% predicted for age and height); 22 declined to participate. No appreciable side effects were experienced; one subject complained of flushing and a few developed symptoms of hyperventilation. Salbutamol 5 mg was administered by nebuliser when FEV_1 fell by 10% or more, and bronchoconstriction was reversed promptly in all cases. For assessment of reproducibility 727 (90%) of the workers were rechallenged 12 months later, the same protocol being used.

Dose-response relationships were analysed by four methods:

1 PD_{20} was estimated by the conventional method of interpolating between the last two

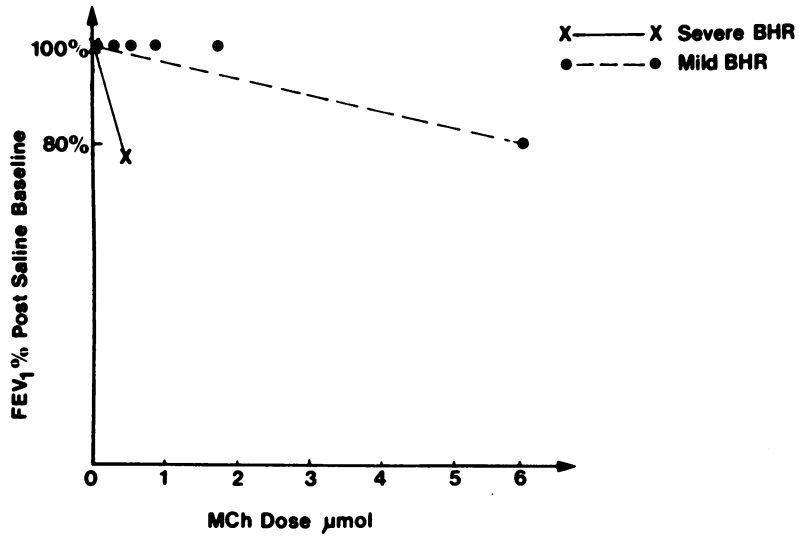

Figure 1 Dose-response slope (DRS) representing a line joining the baseline with the final measurement of FEV_1. Dose-response slope is calculated as % change in FEV_1/total dose methacholine (µmol).

measurements on an FEV_1-log dose methacholine plot.[15]

2 A Fortran program[16] was used to fit the exponential function $FEV_1 = c - \exp(a + b * \log \text{dose (µmol methacholine)})$. The parameter c was initially set to post-saline FEV_1. Ordinary least squares regression was used to estimate the parameters a and b. A new value of c was calculated to minimise the residual sum of squares and the regression repeated. Iteration continued until convergence was achieved or the procedure had been performed 50 times. If the algorithm converged, an "exponential" estimate of PD_{20} was extrapolated up to 12·28 µmol. Otherwise "exponential" PD_{20} was censored at 12·28 µmol.

3 Ordinary least squares regressions were performed on the data for each subject to fit the dose-response line: $FEV_1 = a + b * \text{dose}$ methacholine (µmol). Given a significant linear relationship ($p < 0{\cdot}05$), the slope parameter (b) was considered as an index of bronchial reactivity. This parameter showed a severely skewed distribution. As the negative reciprocal of slope ($-1/b$) was still skewed to the right other power and log transformations of this coefficient were explored in an effort to find the most normally distributed index.

4 The dose-response slope was calculated for each subject as % decline in FEV_1 from post-saline value/cumulative methacholine dose.[11] This parameter represented a line joining the origin of the the dose-response curve with the final measurement (fig 1). Before it was expressed on a logarithmic scale 1·5%/µmol was added to eliminate negative and zero values.

Descriptive statistics, transformations, correlations, analyses of variance, linear regressions, and associated diagnostics were performed in Minitab.[17] The correlation of a variable with normal scores for all subjects was used to estimate the Shapiro Wilk coefficient.[17] The closer the correlation was to unity the more normal the distribution; the significance of deviations from unity was tested. Correlation between continuous indices was assessed by the Pearson's product-moment correlation coefficient.[18] Reproducibility of indices was assessed by the intraclass correlation coefficient.[19]

Clinical information on any history of asthma, symptoms such as wheeze, and use of bronchodilator medication was obtained from a modified Medical Research Council questionnaire,[20] completed by all subjects before lung function testing. Differences in two means were assessed by Student's t test and differences in three or more means by analysis of variance.[21] Separation of symptom groups was assessed by the index D/s, which was the difference between means divided by the standard deviation for the entire sample.[22]

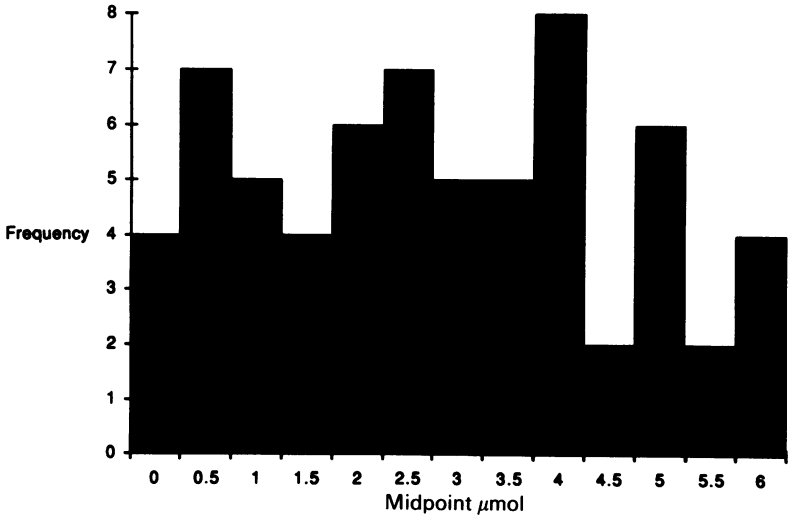

Figure 2 Distribution of PD_{20} interpolated in the usual manner between the last two measurements of FEV_1 on a log dose scale (n = 65).

Results

ESTIMATES OF PD_{20}

After the administration of 6·14 µmol methacholine FEV_1 increased from baseline in 123 (15%) subjects, showed no change in 21 (3%), a decrease of less than 10% in 509 (63%), and a decrease of 10–20% in 91 (11%) subjects. Only 65 (8%) subjects had a decline in FEV_1 of more than 20%. For these 65 cases PD_{20} was inter-

*Table 1 Descriptive statistics and tests of normality for different indices of bronchial reactivity (PD_{20})**

Estimate	*n*	*Mean (SD)*	*Minimum*	*Maximum*	*Shapiro-Wilk coefficient*	*p*
Interpolated PD_{20}	65	2·8 (1·7)	0·06	6·0	0·984	NS
Exponential PD_{20}	132	5·6 (3·4)	0·10	12·1	0·981	<0·05
Inverse cube root of slope	353	2·69 (0·83)	0·54	5·21	0·997	NS
Log dose-response slope	809	0·36 (0·29)	−0·73	2·48	0·902	<0·01

*See under "Methods" for derivation of the parameters.

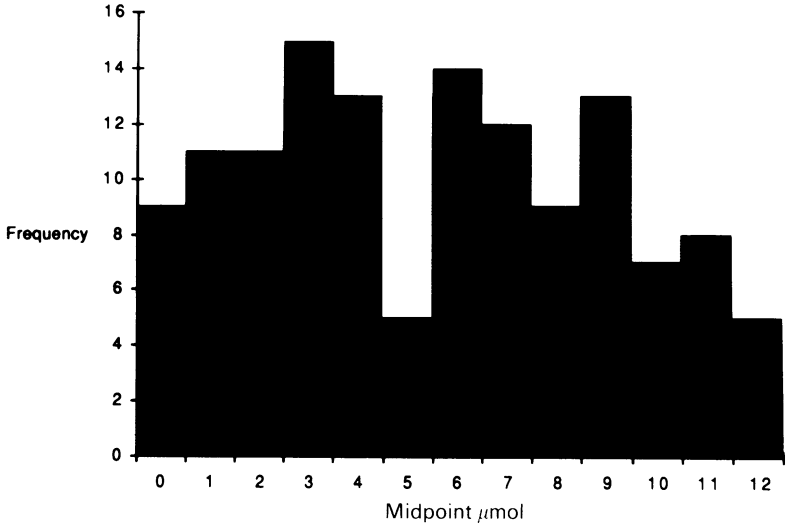

Figure 3 Distribution of PD_{20} extrapolated up to 12·28 μmol by an exponential algorithm—see text (n = 132).

polated (see table 1 for descriptive statistics). The distribution of the interpolated PD_{20} (fig 2) did not deviate significantly from a normal distribution.

The exponential function (method 2) successfully extrapolated a PD_{20} value for 132 (16%) subjects (see table 1 for descriptive statistics). The distribution of this estimate of PD_{20} (fig 3) deviated significantly from a normal distribution. This deviation was worsened by log transformation (Shapiro-Wilk coefficient = 0·926). One subject's value was censored for being too reactive (a greater than 20% drop after the first dose) and 676 "non-reactive" subjects were given the censored value 12·28 μmol.

Complete data for interpolated and exponential estimates of PD_{20} were available for 63 cases. The correlation between the two estimates was 0·94 ($p < 0·0001$).

SLOPE PARAMETERS

The slope of the line of best fit between FEV_1 and methacholine dose on a linear scale (method 3) provided an index of bronchial reactivity for a much larger proportion of subjects. Linear regressions of FEV_1 against dose yielded a slope coefficient (b) that was significantly negative in 353 (44%) of cases. The most normally distributed transformation proved to be the inverse cube root of the slope ($b^{-\frac{1}{3}}$) (fig 4). The descriptive statistics and normality tests for this index are summarised in table 1.

A dose-response slope could be estimated for all 809 subjects. The distribution of the dose-response slope was severely skewed with two outlying values from extremely reactive individuals. Log transformation yielded a symmetric though significantly non-normally distributed index (fig 5). Descriptive statistics and normality tests for the log dose-response slope are summarised in table 1.

RELATIONSHIPS BETWEEN PD_{20} AND SLOPE PARAMETERS

There was substantial agreement between the ability to obtain a PD_{20} from the exponential algorithm (method 2) and the presence of a significant negative slope (b) to the dose response line (method 3)—see table 2. Subjects with an exponential PD_{20} below 12·28 μmol are almost entirely within the group with a significant slope. Iterative exponential curve fitting provided an index of bronchial reactivity in six further cases where simple linear regression failed. The mean inverse cube root of the slope of the 126 subjects with both a significant slope and a PD_{20} below 12·28 μmol was significantly less than the mean inverse cube root of the slope for the 227 subjects with a PD_{20} censored to 12·28 μmol (table 3). In other words, subjects with an extrapolated exponential PD_{20} have a steeper dose-response line than those without a PD_{20} value. The correlation between the inverse cube root of the slope and PD_{20} was very strong ($r = 0·96$, $p < 0·0001$).

As the dose-response slope is a summary measure of the slope of the the dose-response line, it is not surprising that there is an extremely strong correlation between this measurement and the slope parameter (b) derived from linear regression ($r = -0·97$, $p < 0·0001$). The strong negative correlation between log dose-response slope and log PD_{20} ($r = -0·98$, $p < 0·0001$). The mean log dose-response slope for the 132 subjects with an exponential PD_{20} below 12·28 μmol was significantly greater than the mean log dose-response slope for the 227 subjects with a significant negative slope but without a PD_{20}, and this in turn was greater than the mean log dose-response slope for the remaining 450 "non-reactive" subjects without a significant negative slope (table 3).

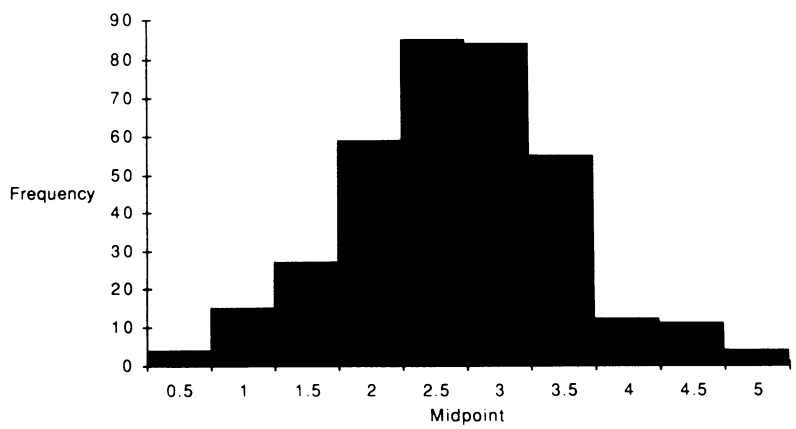

Figure 4 Distribution of the inverse cube root of the slope from significant dose-response lines by linear regression (n = 353). The quantities are dimensionless.

Table 2 Cross tabulation between non-censored and censored "exponential" PD_{20} values and the presence or absence of a significant negative slope coefficient on linear regression of dose on FEV_1 data

PD_{20}	*Slope present*	*Slope absent*	
Non-censored "exponential"	126	6	132
Censored (12·28 μmol)	227	450	677
Total	353	480	809

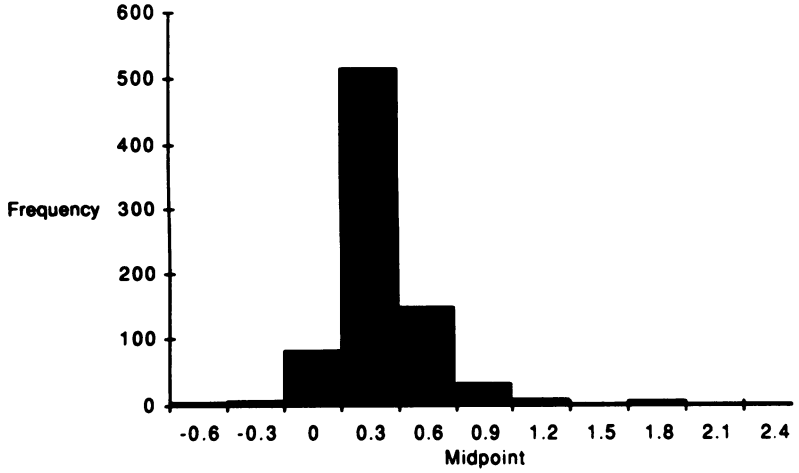

Figure 5 Distribution of log transformed dose-response slope—see figure 1 (n = 809). The quantities are dimensionless.

REPRODUCIBILITY

The reproducibility or repeatability of bronchial reactivity indices were assessed by measurement of agreement between indices for the same subjects on rechallenge. The intraclass correlation coefficients are listed for interpolated and exponential PD_{20}, inverse cube root of the slope, and log dose-response slope in table 4. The first three indices could be remeasured for only about 60% of the subjects with non-censored initial values. Adding 1·5%/μmol before log transformation failed to eliminate one negative value from the second measurement of dose-response slope. There was a statistically significant agreement between measurements for all indices. The inverse cube root of the slope and log dose-response slope were highly reproducible, exponential PD_{20} was moderately reproducible, and interpolated PD_{20} was less reproducible.

CLINICAL VALIDITY

The clinical validity of the various indices of bronchial reactivity was assessed by comparing subjects who reported features of asthma with those who did not report such features (table 5). Means values of interpolated PD_{20} were significantly lower in subjects with a history of asthma and in those who used salbutamol. Mean values of "exponential" PD_{20} and the inverse cube root of the slope were also significantly lower in subjects who wheezed; mean log dose-response slope was significantly higher in subjects with any of these features. The indices of separation (D/s) indicated that log dose-response slope best separated asthmatic from non-asthmatic individuals; the inverse cube root of the slope was less efficient, and there was greatest overlap for PD_{20}.

Table 3 Comparison of mean inverse cube root of the slope and log dose-response slope in patients according to their reactivity categories (exponential PD_{20} < 12·28 μmol, significant negative slope on linear regression but censored PD_{20}, and non-reactivity according to both indices)

	PD_{20} < 12·28 μmol (n = 132)	Negative slope (n = 227)	Non-reactive (n = 450)	Test used	p
Inverse cube root of the slope	1·89	3·14	—	*t* test (*t* = 20·6)	<0·0001
Log dose-response slope	0·826	0·387	0·217	Analysis of variance (F = 484)	<0·0001

Table 4 Reproducibility of bronchial reactivity indices

Index	n	Intraclass correlation	p
Interpolated PD_{20}	36	0·28	<0·05
Exponential PD_{20}	84	0·52	<0·0001
Inverse cube root of the slope	206	0·74	<0·0001
Log dose-response slope	726	0·73	<0·0001

Discussion

These results support the use of the dose-response slope[11] for measuring bronchial reactivity in epidemiological studies. In view of the widespread use and clinical understanding of interpolated PD_{20} we could argue that this index should also be reported. Interpolated PD_{20} was of little value in the present study, however, because it applied to only 8% of subjects. Other attempts to reduce censored data, such as the use of exponential PD_{20} and the inverse cube root of the slope, do not appear to have a major role.

The apparent improvement in FEV_1 with increasing doses of methacholine, seen in 15% of our subjects, has been reported previously.[16] It might be an effect of practice or a consequence of slight stiffening of the central airways with agonist administration moving the equal pressure (choke) point downstream. It is also possible that a decline in FEV_1 with increasing doses of methacholine could occur from fatigue rather than bronchoconstriction. Subjects had at least two minutes, however, to recover between blows. The reproducibility of bronchial reactivity makes it very unlikely that fatigue is causing a dose related reduction in FEV_1.

The usual clinical method of estimating PD_{20} from interpolation between the last two measurements fails to obtain data from most normal subjects. It provided an index of bronchial reactivity for only 8% of this occupational sample. The use of a higher dose of agonist might have provided more subjects with an interpolated PD_{20} as well as information on a plateau response;[23] but as subjects had to return to work after being tested we did not think it appropriate to explore maximal responses to methacholine.

The exponential algorithm yields an estimate for PD_{20} in less than half the number of cases for which simple linear regression yields a significant slope. It has the disadvantage of estimating three parameters from five data points. Extrapolation beyond the dose administered is potentially hazardous for a non-linear function with no estimate of error variance. The results agree well, however, with the conventional interpolated estimate of PD_{20}. The Fortran program operates rapidly and efficiently on a computer.

In summary, the exponential algorithm uses

Table 5 Comparison of bronchial reactivity indices between subjects with and without features of asthma

	History of asthma	*No history of asthma*	*D/s*	*p*
Mean interpolated PD_{20}	1·76	3·18	0·81	<0·005
Mean exponential PD_{20}	3·84	6·08	0·65	<0·005
Mean inverse cube root of the slope	1·91	2·79	1·07	<0·0001
Mean log dose-response slope	0·864	0·335	1·80	<0·0001
	Wheeze	*No wheeze*	*D/s*	*p*
Mean interpolated PD_{20}	2·40	3·08	0·39	NS
Mean exponential PD_{20}	4·10	6·31	0·64	<0·0005
Mean inverse cube root of the slope	2·26	2·82	0·67	<0·0001
Mean log dose-response slope	0·556	0·327	0·78	<0·0001
	Salbutamol used	*Salbutamol not used*	*D/s*	*p*
Mean interpolated PD_{20}	1·51	2·99	0·85	<0·05
Mean exponential PD_{20}	2·41	5·89	1·02	<0·001
Mean inverse cube root of the slope	1·71	2·73	1·24	<0·0001
Mean log dose-response slope	1·054	0·350	2·39	<0·0005

*Differences in means were assessed by the *t* test and the index of separation (D/s) was calculated as difference in means/pooled standard deviation.[22]

all data and does not assume linearity. It provided an estimate of PD_{20} in a greater proportion of this occupational sample than interpolation, was reproducible and valid, and has been used in other population studies.[1 24] In studies including many asthmatic subjects the results have often been expressed as log PD_{20}. As log transformation did not normalise the distribution of the exponential estimate of PD_{20} in our occupational population the original scale in μmol was retained for analysis. In any case a normal distribution might not be expected in the "hyperreactive" tail of bronchial reactivity measurements. A censored index such as PD_{20}, which can be derived for only a small proportion of the general population, represents only this tail of the distribution.

The transformed slope coefficient is a continuous index of bronchial reactivity applicable to a larger proportion of the population than PD_{20}. The inverse cube root of the slope was estimated for 44% of subjects in this aluminium smelter who showed a significant linear dose-response relationship. There are some concerns about its use. Slopes from regression lines that did not significantly differ from zero were excluded, so that 56% of subjects had censored values. Very small slopes became large outlying values after reciprocal transformation. Although these outlying values could be reduced by the addition of an increment (similar to the one added to the dose-response slope before log transformation), none of our conclusions was altered by including slopes from non-significant regression lines. We thought that this effort added little useful information to the dose-response slope, which was much simpler to calculate.

Another theoretical problem was the undue influence of outlying points on each regression line. In a minimally reactive subject the highest dose given (6·14 μmol) was four times the previous dose (1·54 μmol). Statistical testing did not, however, identify excessive influence on the line of best fit from this outlying point. In any case, such influence could be considered biologically appropriate as most of the response occurred at higher doses.

The inverse cube root of the slope had the desirable biological and statistical property of being normally distributed, was reproducible, and had clinical validity. The inverse cube root scale is unusual, but not entirely without parallel in respiratory epidemiology. For instance, the latent period for the development of respiratory cancer appears to be related to the inverse cube root of asbestos dose.[25] The inverse cube root of the slope is interpreted in the same manner as PD_{20} in that the larger the value the less reactive the subject. There was excellent agreement between PD_{20} and the cube root of the slope measurements, and subjects lacking an estimate of PD_{20} had significantly greater values for the inverse cube root of the slope. Subjects with slope alone have measurable reactivity, but of a lesser degree than those with both slope and PD_{20}. This was confirmed by the differences in mean log dose-response slope.

The most widely applicable index of bronchial reactivity is the dose-response slope, which could be calculated for all subjects. The single parameter is simple to calculate and agrees well with the slope from linear regression, as has been previously found.[11] This model does, however, assume that the first and last measurements of FEV_1 were made without error, which is unlikely, and the absence of any test of statistical significance means that all subjects contribute a dose-response slope and this will include random noise. Subjects showing an increase in FEV_1 from its post-saline baseline to the final dose of methacholine are certainly less reactive than those who show a decrease, but they may differ little from those whose FEV_1 shows little or not change. Thus assignment of a number to their lack of reactivity is hard to justify. Despite the incorporation of data from such subjects log dose-response slope was highly reproducible.

Although the distribution of log dose-response slope was not normal, log transformation allowed bronchial reactivity to be expressed on a familiar logarithmic scale. The increment for eliminating negative and zero values before log transformation requires standardisation. The previously suggested 0·3%/μmol[11] would have been insufficient in the present study. Alternatively, untransformed dose-response slope values could be analysed by non-parametric statistical tests, which are sufficiently robust not to be unduly influenced by the severely skewed distribution.

In conclusion, measuring the dose-response slope has the advantages of simplicity, reproducibility, clinical validity, and absence of censored data. Neither exponential PD_{20} nor the inverse cube root of the slope combines these desirable features in a single index. We would recommend using the dose-response slope for analysis of bronchial reactivity in future epidemiological studies.

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