Measurement of bronchial hyperreactivity: comparison of three Nordic dosimetric methods

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ABSTRACT
Clinical testing of bronchial hyperreactivity (BHR) provides valuable information in asthma diagnostics. Nevertheless, the test results depend to a great extent on the testing procedure: test substance, apparatus and protocol. In Nordic countries, three protocols predominate in the testing field: Per Malmberg, Nieminen and Sovijärvi methods. However, knowledge of their equivalence is limited. We aimed to find equivalent provocative doses (PD) to obtain similar bronchoconstrictive responses for the three protocols. We recruited 31 patients with suspected asthma and health care workers and performed BHR testing with methacholine according to Malmberg and Nieminen methods, and with histamine according to Sovijärvi. We obtained the individual response-dose slopes for each method and predicted equivalent PD values. Applying a mixed-model, we found significant differences in the mean (standard error of mean) response-dose (forced expiratory volume in one second (FEV1)%/mg): Sovijärvi 7.2 (1.5), Nieminen 13.8 (4.2) and Malmberg 26 (7.3). We found that the earlier reported cut-point values for moderate BHR and marked BHR between the Sovijärvi (PD15) and Nieminen (PD20) methods were similar, but with the Malmberg method a significant bronchoconstrictive reaction was measured with lower PD20 values. We obtained a relationship between slope values and PD (mg) between different methods, useful in epidemiological research and clinical practice.

Abbreviations: ANOVA: analysis of variance; BHR: bronchial hyperreactivity; CI: confidence interval; $F_{ENO}$: fractional exhaled nitric oxide; FEV1: forced expiratory volume in one second; IQR: interquartile range; MMAD: mass median aerodynamic diameter; NTP: normal temperature and pressure; PD: provocative dose; SEM: standard error of the mean

Introduction
Bronchial challenge testing with inhaled methacholine (chloride or bromide) and histamine diphosphate may provoke bronchoconstriction. This transient alteration of pulmonary function is expressed as a reduction of the forced expiratory volume in one second (FEV1). An absent bronchoconstrictive reaction to these substances usually indicates an absence of current asthma in test subjects [1]. Inversely, a positive bronchial reaction concomitant with current symptoms can confirm an asthma diagnosis. Decades of bronchial hyperreactivity (BHR) testing with histamine or methacholine, acting directly on the bronchial histamine or cholinergic wall receptors, came hand in hand with different testing protocols and equipment. In two Nordic countries, three dosimetric protocols employed in clinical settings possess noteworthy methodological differences: methacholine testing with a nebuliser and a drying device developed by Per Malmberg et al. [2], methacholine testing with a jet dosimeter according to Nieminen et al. [3] and histamine testing with the same dosimeter according to Sovijärvi et al. [4]. Previous researchers have compared the Sovijärvi and Nieminen methods in an epidemiological setting [5], but the lack of further quantitative comparisons has resulted in limited knowledge of their equivalence, as well as a missing comparison with the Malmberg method. Through early studies, similarities between histamine and methacholine as provocative agents are well known [6]. Nevertheless, comparison of the aforementioned protocols is a challenging task, considering that the endpoint level (provocative dose) varies between protocols and there are inherent differences in the testing substances, such as molecular mass and pharmacodynamics. A direct translation between provocative doses may yield erroneous interpretations. Therefore, a comparison study was needed.

The aim of this study was to find equivalent dose-response slopes and provocative dose values between the methods, for the same levels of bronchial response.
Methods

We recruited prospectively 31 volunteers, either patients with a referral for BHR testing or healthcare workers and included fractional exhaled nitric oxide (FENO) values from medical records if available.

We assessed BHR on the volunteers, employing two protocols with methacholine (Malmberg and Nieminen) and one protocol with histamine (Sovijärvi), all methods commonly employed in Finland, Sweden and Estonia. The challenge tests were performed in the Helsinki University Central Hospital area, Finland including the Skin and Allergy Hospital and the Laboratory of Clinical Physiology at the Meilahti Hospital, Finland. For each participant, the three challenge tests were scheduled within a two weeks period, with a minimum time interval of three days. The order of the challenge tests was based on the availability of the appointment times, with a fixed testing hour during the morning. Prior to BHR testing, the participants discontinued sympathomimetic and antimuscarinic medication according to the ATS technical standard [7], and withheld antihistamines for at least five days. We followed the recent safety and quality standards [8]. Additionally, the participants refrained from ingesting coffee, tea and cola for 4 h, alcohol for 24 h, and heavy meals or smoking 2 h before the challenge, in agreement with our laboratory guidelines. Furthermore, BHR was tested at least one month after any respiratory infections. The testing phase was during fall and winter seasons.

All participants signed a written consent agreement and the Helsinki University Hospital Ethical Committee approved our project (HUS 1417/2016). We followed the ethical principles stated in the Declaration of Helsinki [9].

We determined a minimum of 30 participants in our study to achieve a statistical power of 80% for detecting an effect size of 0.25 (Cohen’s d) and an α error of 5% if considering repeated measurements with three methods and testing the difference between methods with an analysis of variance (ANOVA) test (G*Power 3.1.9.4 software)[10].

Bronchial hyperreactivity protocols

Per Malmberg methacholine test

The device (Figure 1) developed by Malmberg et al. [2], employs a drying device, a polymethyl methacrylate tower (Nova Plast, Karlskoga, Sweden) with 3.4 l volume and a Sidestream Durable jet nebuliser (Medic Aid Ltd, West Sussex, UK). The nebuliser chamber’s aerosol is driven by pressurised medical air (AGA, Espoo, Finland), supplied from a gas cylinder adjusted at 1.6 bar pressure, providing a primary flow towards the nebuliser of 8.7 l/min (20°C, NTP). The Venturi-flow of 15.31/min (20°C, NTP) is adjusted by a Fischer and Porter rotameter (ABB Limited, Cambridgeshire, UK). These settings give a total flow in the drying device of 24 l/min and deliver the aerosol to the mouthpiece at the top of the tower. The mass output was determined gravimetrically, filling 3 ml of Aqua and nebulisation during 1 min, and set to 0.5–0.6 ml/min (20°C, NTP). Reported mass median aerodynamic diameter (MMAD) for this setting is 1.7 μm [2,11]. Further on, the mass output was corrected with the efficiency coefficient 0.75, previously defined by Eklund et al. [11].

Before the methacholine challenge (5 min), FEV₁ was determined by spirometry (Masterscreen, Jaeger GmbH, Würzburg, Germany). The dose delivered during 1 min was controlled through 15 tidal inspirations and expirations, each lasting 2 s and synchronised by a metronome, equivalent to 0.5 min total inhalation time and 0.81 volume per inhalation [12]. The delivered dose was determined by the product of the mass output (ml/min), the delivery time (0.5 min) and the methacholine concentration of the solution (mg/ml). The challenge started with 3 ml isotonic sodium chloride solution and the methacholine concentration was increased in doubling steps: 0.5, 1, 2, 4, 8, 16 and 32 mg/ml, respectively. The non-cumulated doses inhaled (after correction with the efficiency coefficient) at each step were: 0.1125, 0.225, 0.45, 0.9, 1.8, 3.6 and 7.2 mg, respectively. Accordingly, the cumulated doses were: 0.1125, 0.3375, 0.7875, 1.6875, 3.4875, 7.0875 and 14.2875 mg, respectively.
FEV₁ was acquired 4 min after starting the inhalation of each dose. The time between doubling concentrations was 6 min. The challenge was performed until achieving a minimum reduction of 20% in FEV₁ or until reaching the highest concentration of 32 mg/ml. The methacholine chloride solution employed (2-acetyloxypropyl(trimethyl)azanium; chloride) was prepared by the Helsinki University Hospital’s pharmacy.

Nieminen methacholine test

The protocol developed by Nieminen et al. [3] employs a dosimetric Spira Elektro 2 automatic jet nebuliser device (Respiratory Care Centre, Hämeenlinna, Finland; Figure 2), driven by pressurised medical air at 2 bar and providing a primary flow towards the nebuliser of 7.5 l/min (20°C, NTP). It produces a MMAD of 1.6 μm [13]. According to this protocol, the inhalation-synchronised-dosimeter delivers methacholine over 0.5 s triggered by tidal breath, controlled during nebulisation with an optimal inspiratory peak flow-rate of 0.5 l/s and employs an inspiratory volume of at least 0.4 l. The optimal inspiratory flow level is visually controlled. In a five-step concentration increment, this setting produces, in fourfold increasing concentration increments, non-cumulative doses of 0.025, 0.1, 0.4 and 1.6 mg, respectively. The four steps are: one inhalation at 4 mg/ml, one inhalation at 16 mg/ml, four inhalations at 16 mg/ml and 16 inhalations at 16 mg/ml. The interval between steps is 5 min. The mass output was determined by weight [4].

Prior to histamine challenge testing (5 min), FEV₁ was obtained by spirometry (Masterscreen). FEV₁ was measured after each dose, and the test continued until the onset of a 15% reduction in FEV₁ or until the highest dose of 1.6 mg was given.

The Helsinki University Hospital’s pharmacy delivered the buffered histamine phosphate solution (2-(1 H-imidazol-5-yl)ethanamine; phosphoric acid).

Provocative dose

The provocative doses (PD) for each positive test were calculated (i.e. 15% FEV₁ reduction for histamine (PD₁₅) and 20% FEV₁ reduction for methacholine (PD₂₀)). We performed a two-point logarithmic interpolation of PD₂₀ with the Equation (1):

\[
PD_{20} = e^{\ln(D_1) + \frac{\ln(D_2) - \ln(D_1)}{\ln(R_2/R_1)}}
\]

with \(D_1\) being the second to last dose (one step before \(D_2\)), \(D_2\) the last dose provoking at least a 20% reduction in FEV₁, \(R_1\) the FEV₁ change in % measured after \(D_1\) and \(R_2\) the FEV₁ change in % measured after \(D_2\) [8].

For the histamine challenge test, the provocative dose causing a 15% reduction in FEV₁ (PD₁₅) was calculated with Equation (1), by substituting 20 with 15.

The provocative dose was not calculated for subjects not achieving a significant reduction in FEV₁ (i.e. 20% with methacholine and 15% with histamine). Nevertheless, we obtained response-dose slopes for all subjects (see statistics below).

The doses for the methacholine BHR tests are cumulative, since methacholine is regarded as partially having a cumulative effect [15–17] during the testing time of 5–6 min. Additionally, the original protocols describe cumulative doses

Figure 2. Spira Elektro 2 dosimeter employed in the Sovijärvi and Nieminen testing protocols.
for methacholine. Histamine is rapidly metabolised [15,17], showing no significant cumulative effect and the doses delivered are regarded in the model as non-cumulative. Nevertheless, since the ERS technical standard on bronchial challenge testing recommends a non-cumulative approach on PD [8], non-cumulative PD were also calculated.

**Statistics**

Using the R language [18] and Rstudio frontend version 1.1.383 (Boston, MA), we analysed the three protocols applying a mixed-effect model with crossed and nested effects (mathematical notation of the model is specified in the Supplementary Appendix A). The doses given for each subject are nested within each protocol and our study design specifies a crossed model, (i.e. every subject is tested with each protocol). The model defines random slopes for individual subjects, expressing the changes in FEV1 in percent per dose of the substance in milligrams. The intercepts were suppressed from the model, since physiologically the changes in FEV1 for each subject started from zero (a baseline FEV1 value of 100%). The mixed model was analysed with the lmer function from the lme4 package [19], and we obtained the restricted maximum likelihood (REML) estimates. The p values for the fixed effects were calculated with the Satterthwaite’s method from the lmerTest package [20].

We extracted the slopes (FEV1%/mg) for each subject and protocol and compared the differences in the log-transformed slopes between protocols with an ANOVA, passing the assumptions with a Shapiro-Wilk test, a Bartlett test and a Fligner-Killeen test. The untransformed slopes were tested for differences with a Kruskal-Wallis Rank Sum test. Furthermore, we fitted the slopes to a non-linear model with the obtained PD15 or PD20 as dependent variables. We specified a non-linear model for each protocol and applied the non-linear models to predict equivalent PD values between protocols, based on the slopes. We analysed the non-linear models with the Levenberg-Marquardt nonlinear least-squares algorithm nlsLM from the minipack.lm package [21]. We regarded $\alpha = 0.05$ as a significant level.

**Results**

The 28 participants completed the three challenges, except three participants, who omitted one methacholine test (Nieminen). No severe adverse reactions were observed. The characteristics of this population, including spirometric values are summarised in Table 1. The standard error of the mean (SEM) response in 31 subjects to histamine was a 7.2% (1.5) reduction in FEV1 per milligram. A 1mg dose of methacholine caused a mean 13.8% (4.2) reduction in FEV1 with the Nieminen method in 28 subjects and a 26% (7.3) reduction in FEV1 with the Malmberg method in 31 subjects. These fixed effects are visualised in Figure 3. The mixed model (see Supplementary Appendix A) had an $R^2$ of 0.99, i.e. explaining 99% of the variance in our population. The observed power of the model for the fixed effects was 97%.

The slopes (FEV1%/mg) were extracted from the model for each subject and separately for each method, and the individual slopes from the model and the individual response-dose measurements were plotted and arranged according to their steepness and can be found in the Supplementary Appendix A (Figures A1–A3). We found in

**Figure 3.** Dose (mg) provoking FEV1 changes from baseline (%) for each substance. Lines depict the fixed effects from the mixed model and shaded area the 95% confidence interval.

| Table 1. Study participants’ demographics, spirometric, anthropometric data, medication and fractional expiratory nitric oxide ($F_{ENO}$) values. |
|---|---|---|---|---|---|---|
| | n | Median (IQR) | Spirometry | n | Median (IQR) | Range |
| Age (years) | 31 | 42 (29) | FVC (l) | 31 | 4.1 (2.3) | 2.6–7.0 |
| Height (cm) | 31 | 171 (14) | FVC (%)a | 31 | 95 (18) | 73–125 |
| Weight (kg) | 31 | 72 (20) | FEV1 (l) | 31 | 2.9 (1.4) | 1.9–5.6 |
| Female/Male | 18/13 | 58%/42% | FEV1 (%)a | 31 | 90 (21) | 71–117 |
| Asthma | 4 | 13% | FEV1/FVC ratio | 31 | 0.78 (0.5) | 0.66–0.91 |
| Allergic rhinitis | 12 | 39% | FEV1/FVC (%)a | 31 | 94 (7) | 84–106 |
| Smoking | 4 | 13% | $F_{ENO}$ (ppb)b | 25 | 19 (11) | 5–63 |
| Salbutamol | 5 | 16% | | | |
| Antihistamines | 4 | 13% | | | |
| Budesonide | 1 | 3% | | | |

Data presented as median (interquartile range) or percentage of total case number.

aPercentage of predicted value [45].

bMeasured at expiratory flow rate 50 ml/s.
testing with the non-parametric test between methods that, the differences in slopes (within-subjects) were not statistically significant \((p = .22)\). The ANOVA of the log-transformed slopes produced similar results \((p = .3)\). The distribution of all measured FEV\(_1\) responses for the three dosimetric methods is plotted in Figure 4.

Fitting non-linear models to the PD and the extracted slopes resulted in the following regression equations:

\[
\text{Methacholine Malmberg } \quad PD_{20} = e^{3.0897 \times \text{slope}^{-1.05721}} \\
\text{Methacholine Nieminen } \quad PD_{20} = e^{3.6279 \times \text{slope}^{-1.3257}} \\
\text{Histamine Sovijärvi } \quad PD_{15} = e^{3.4906 \times \text{slope}^{-1.3773}}
\]

where slope is the individual slope denoting the changes in FEV\(_1\) in percent per mg of inhaled substance, for the specific protocol and method. The estimates for the three models were statistically significant on the \(p < .001\) level.

Figure 5 exemplifies the model for methacholine and the Malmberg method (Equation (2)). The PD values employed for obtaining the models were the calculated PD values for the participants. These PD values were positive (i.e. 15\% FEV\(_1\) reduction for histamine (PD\(_{15}\)) and 20\% FEV\(_1\) reduction for methacholine (PD\(_{20}\)) with the highest dose) in 77\% of the subjects with the Malmberg method, in 36\% with the Nieminen method and 26\% with the Sovijärvi method.

Table 2. Provocative doses (PD) classified according to the studied methods in our study population \((n = 31)\).

| Method                  | No reaction | Mild | Moderate | Marked reaction |
|-------------------------|-------------|------|----------|-----------------|
| Methacholine Malmberg   | 7 (23\%)    | 14 (45\%) | 10 (32\%) |                  |
| Methacholine Nieminen   | 18 (64\%)   | 6 (21\%) | 3 (11\%)  | 1 (4\%)          |
| Histamine Sovijärvi     | 23 (74\%)   | 7 (23\%) | 1 (3\%)   | 0               |

Table 3. Predicted equivalent provocative doses (mg) and corresponding slope values.

| Response categories | Methacholine PD\(_{20}\) (mg) | Methacholine PD\(_{15}\) (mg) | Histamine PD\(_{15}\) (mg) |
|--------------------|-------------------------------|-------------------------------|----------------------------|
| 7.5                | 2.6                           | 2.6                           | 2.1                        |
| 9.0                | 2.2                           | 2.0                           | 1.6                        |
| 23.5               | 0.8                           | 0.6                           | 0.4                        |
| 30.5               | 0.6                           | 0.4                           | 0.3                        |
| 65.0               | 0.3                           | 0.15                          | 0.1                        |

Table 2 shows the percentages of PD values with or without significant FEV\(_1\) decrease.

We predicted a series of PD\(_{15}\) and PD\(_{20}\) values with equal slopes utilising the Equations (2)–(4). These equivalent predicted provocative dose values are presented in Table 3 (selected cut-point values) and extensively in Table A1 in Supplementary Appendix A.

Non-cumulative results for PD and tables can be found in Supplementary Appendix B.

Discussion

Mean reduction in FEV\(_1\) in the three dosimetric methods

We found that the response-dose relationship in the assessment of BHR was different between the methods when analysing the fixed effects (i.e. the mean FEV\(_1\) changes per milligram of test substance independent from the individual responses). The Malmberg method produced the greatest reduction in FEV\(_1\) per milligram, followed by the Nieminen method, both employing methacholine as the test substance. The Sovijärvi method with histamine produced the least changes per milligram. Preceding studies argue that when considering the molar masses of histamine and methacholine, their effect is roughly equivalent when employing the same protocol and device [6]. Our results also indicate similarities when applying molar masses instead of mass (mg) to the Sovijärvi and Nieminen protocols, which utilise an identical device and considering a small disagreement probably originating from the characteristics of the substances used, since, as earlier reported, methacholine may partially have a cumulative effect and histamine not. Analysing the distribution of the FEV\(_1\) responses reveals similar curves for the Sovijärvi and Nieminen methods, but the Malmberg protocol produced a higher frequency of FEV\(_1\) responses over 20
percent from the baseline. Malmberg et al. [2] developed the drying tower to improve the efficacy of the device and reported a 1.6-fold greater response in FEV$_1$ changes than without the drying tower. This partly supports our findings concerning the differences between the methacholine protocols. It may be speculated that the afore-mentioned overall differences for the Malmberg methodology may result from an indirect stimulus caused by dry-air. This indirectly acting bronchoconstrictive effect of mucosal dehydration [22] may sum up with the direct effect of methacholine on airway smooth muscle.

We employed mass (mg) and the accumulation as in the original protocols, despite their differences, because our main aim was to find corresponding PD in milligram, as near as the clinically employed protocols. For informational purposes, additional guideline-accurate non-cumulative PD’s was provided, with corresponding response-dose slopes. These non-cumulative values are very near to the cumulative values, supporting the fact, that the cumulative effect of methacholine is very limited within the testing times [8]. Furthermore, the assumption of delivered doses in all the investigated methods were originally based on gravimetric measurements, which are inaccurate, since gravimetical estimation of the mass output ignores evaporation, resulting in over-estimation of the mass output and the dose also. From this fact originates some degree of discrepancies between methods that is expressed as differences in PD values. The Malmberg protocol includes an adjustment considering evaporation [11]. Evaporation values for the Sovijärvi and Nieminen methods are not currently available. Nevertheless, all the three methods have reported MMAD values, which are almost identical and therefore the issue of different particle sizes affecting the bronchial response could be eliminated.

**Response categories**

**Reported response categories**

For the three different BHR provocation tests, cut-off values were previously determined in different populations and with distinct methods. For the Malmberg method [23], a PD$_{20}$ of 0.6 mg corresponded to an 80% sensitivity in asthmatics (n = 102) and an 85% specificity in healthy subjects (n = 203).

Nieminen et al. [24] retrospectively gathered PD$_{20}$ values in an adult population (n = 791), including patients with asthma, allergic rhinitis, chronic bronchitis and healthy subjects. This study yielded the following cut-off categories of BHR: no reaction, PD$_{20}$ $>$ 2.6 mg; mild, 2.6–0.61 mg; moderate, 0.6–0.15 mg; marked, $<$ 0.15 mg.

Sovijärvi et al. [4] acquired cut-off values for histamine challenge testing with clinical material, including patients with asthma (n = 64), chronic bronchitis (n = 71), chronic rhinitis (n = 41) and healthy subjects (n = 31). The cut-off values were classified as the following: no reaction, PD$_{15}$ $>$ 1.6 mg; mild, 1.6–0.41 mg; moderate, 0.4–0.11 mg; marked, $\leq$ 0.1 mg. The analyses of the asthmatic patients in the study [4], showed a sensitivity of 86% and specificity of 92% with a PD$_{15}$ cut-off level of 0.1 mg and a sensitivity of 66% and specificity of 100% with a PD$_{15}$ cut-off level of 0.4 mg.

**Response-dose slopes and PD**

When examining the individual slopes of the response-dose relationship, the individual slopes were similar between the methods. We assumed equal method-independent slopes, as a measure of individual reactivity and obtained PD for each method with the slopes extracted from the mixed model. These equivalent PD are very near to the clinically significant cut-off values reported by the original studies [4,23,24].

**Moderate and mild hyperreactivity**

The equivalent dose-response slopes are similar for the PD reported by Sovijärvi et al. [4] and Nieminen [24], when observing the cut-off between moderate and mild hyperreactivity (see bold values in Table 3). We can assert that these cut-off values are interchangeable. With the Malmberg method, this equivalent response-dose slope translates into an analogous provocative dose of approximately 0.8 mg.

Ehrs et al. [23] reported a cut-off value for the Malmberg method of 0.6 mg methacholine to distinguish between healthy and asthmatic subjects, which equals provocative doses of 0.4 mg for Nieminen and 0.3 mg histamine for Sovijärvi. This cut-point value lies between moderate and marked hyperreactivity.

**Marked hyperreactivity**

When observing the corresponding cut-off values for marked hyperreactivity, a similarity between the Sovijärvi and Nieminen provocative doses is seen. For the Malmberg method, no provocative dose has previously been reported to define marked hyperreactivity. Our results imply that this provocative dose corresponds to 0.3 mg of methacholine with the Malmberg method.

**No hyperreactivity**

The borders for classifying a normal reaction (no hyperreactivity) were more far away, when analysing the Sovijärvi and Nieminen methods. This may suggest differences in the grading of no hyperreactivity between the Sovijärvi and Nieminen methods.

**Previous studies**

Our results find support from Juusela et al. [5], who found good agreement between the cut-off values for moderate and as well as marked BHR between the dosimetric methods of Sovijärvi and Nieminen but found only a moderate agreement for the cut-off values for a negative reaction. Response-dose slopes have been previously investigated and have been found able to discern between healthy and asthmatic subjects [25]. These slopes were proven useful in population studies [26], with good repeatability [27]. Preceding Malmberg method analyses of the response-dose slopes and PD$_{20}$ have found values in different studies and
populations, that when compared to our dose-response slopes and provocative dose equivalences, show similar values [12,28,29]. However, to the best of our knowledge, there are no earlier comparisons between the Malmberg and other BHR testing methods.

Utility

We provided an exhaustive table with equivalent provocative doses as reference for clinical application. Our reference table provides aid when comparing patient results obtained with different methods. Additionally, it facilitates comparison of epidemiological results. Recent population-based studies and clinical trials utilise one of the aforementioned testing methods each: Sovijärvi [30–37], Malmberg [38–41] or Nieminen [14,42–44]; the reference table may serve the purpose of offering comparisons in these example studies and others, or in future studies. Nowadays, the supply of histamine as a challenge test substance is very limited, therefore, a comparison to other challenge methods was also needed.

Strengths and limitations

The strengths of this study are employing the unmodified equipment and the original protocols for the comparison, except for omitting a step with inhalation of saline solution described in the Nieminen method. This omission is due to practicability. Additionally, we performed carefully the challenge tests with varying test order. Nevertheless, the number of volunteers acquired was limited, due to lack of motivation in potential study candidates to perform three challenge tests, availability of the tests and interruptions in the three-day protocol. Although the number of participants could represent a limiting factor, we calculated a priori a minimum of 30 participants needed for the study and fulfilled this objective. Our results indicate a difference in the methods studied, giving discordant quantities of positive results. However, we showed that evaluating the FEV₁ reduction and dose-response slopes resulted in similarities between the methods at the level of PD₁₅ or PD₂₀, when regarding the diagnostic cut-off levels for asthma. This was more evident with the Sovijärvi and Nieminen methods.

Conclusion

In conclusion, we provided equivalent provocative doses for the Per Malmberg, Nieminen, and Sovijärvi methods of BHR testing and determined that the available cut-off values for a moderate reaction and a marked reaction between the Sovijärvi and Nieminen methods are similar. Additionally, we deduced cut-off values for the Malmberg method. The response-dose slopes can be used when comparing BHR methods in further physiological research.

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Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent

Informed consent was obtained from all individual participants included in the study.

Author contributions

AS, PLK, and PP conceived and designed the research. PM and PP supervised the experiments. BMS contributed with experimental tools. PLK analysed the data and wrote the manuscript, under the tutelage of PP, AS, BMS, PM and PP reviewed the manuscript. All authors read and approved the manuscript.

Disclosure statement

The authors declare that they have no conflict of interest.

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