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Bronchial reactivity to cigarette smoke in smokers: repeatability, relationship to methacholine reactivity, smoking and atopy

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Bronchial reactivity to cigarette smoke in smokers: repeatability, relationship to methacholine reactivity, smoking and atopy. E.J. Jensen, R. Dahl, F. Steffensen. ©ERS Journals Ltd 1998.

ABSTRACT: Bronchial reactivity to cigarette smoke (CBR) in a cross-section of 98 smokers has been investigated.

All participants were subjects to skin-prick tests to common allergens, lung function measurements and bronchial challenges with methacholine and cigarette smoke. In 38 participants a rechallenge with cigarettes was performed 1 h after the first cigarette challenge. Lung function indices analysed were: forced expiratory volume in one second (FEV_1); maximal expiratory flow at 75% of the forced vital capacity ($MEF_{75\%}$); and forced mid-expiratory flow between 25 and 75% of the forced vital capacity ($FEF_{25-75\%}$). All participants were tested for asthma and allergy, and were required to provide information regarding respiratory symptoms, first degree relatives with asthma and allergy and smoking habits.

A substantial decrease was seen in all lung function indices after 12 cigarette-smoke inhalations, but only FEV_1 was related to other variables. The maximal mean percentage fall in FEV_1 was 10%, which was directly related to the number of inhalations ($p<0.05$). In multiple regression analyses the percentage fall in FEV_1 was directly related to: FEV_1/vital capacity (VC) ($p<0.01$); to the asthmatic/bronchitic status ($p<0.05$); and to the accumulated and standardized cigarette consumption ($p<0.05$). The percentage fall in FEV_1 bore no relationship to methacholine bronchial reactivity, sex or age and had a continuous distribution. The repeat challenge showed a smaller fall in FEV_1 compared to the first challenge after 12 cigarette smoke inhalations ($p<0.05$). The percentage fall in FEV_1 correlated after the first and the repeat challenge ($p<0.05$). Repeatability of the challenge could not be determined in this study because of tachyphylaxis.

Bronchial reactivity to cigarette smoke is a tobacco smoke-specific bronchial response. All participants responded and the response showed a continuous distribution. Bronchial reactivity to cigarette smoke may be of importance for symptoms and prognosis in chronic bronchitis and chronic obstructive pulmonary disease and should be studied in relation to the degree of accelerated lung function loss in smokers and other cigarette induced lung abnormalities.

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Cigarette smoking is the greatest single preventable cause of premature disability and death [1]. Disabilities include the loss of ventilatory capacity due to chronic obstructive bronchitis and emphysema [2, 3]. Personal risk factors for the development of lung disease in smokers are virtually unknown except for a few well-defined deficiency syndromes.

Chronic bronchitis, emphysema and asthma have been suggested to be different manifestations of the same disease process and to some extent inherited [4]. This theory gained support as levels of serum(s) immunoglobulin (Ig) E were found to be elevated in smokers compared to nonsmokers [4–6], although a specific allergy to constituents of tobacco has never been shown [7]. Studies have suggested an increased annual loss of lung function in persons with increased bronchial reactivity to histamine/methacholine [8–12] but the influence of baseline forced expiratory volume in one second (FEV_1) on bronchial reactivity may explain this association [13]. Although comparisons of bronchial reactivity to histamine/methacholine in smokers and nonsmokers have shown inconsistent results, a consensus for an increased bronchial reactivity in smokers has emerged [10, 12–18]. Presence of atopy in smokers may also add to the bronchial reactivity [14, 19, 20].

Bronchial reactivity to cigarette smoke (CBR) has been tested in several studies [21–29]. However, the presence and magnitude of a bronchoconstriction as a result of inhaled cigarette smoke has been inconsistent. This may be because only a small number of subjects have been investigated to date, thus, not allowing conclusions about associations between CBR and gender, age, asthma, bronchial reactivity to histamine/methacholine, atopy, smoking history, baseline lung function, or heredity for atopy and asthma to be drawn. None of the studies has demanded tobacco abstinence before the challenge and tachyphylaxis to cigarette smoke may have influenced the response.

The purpose of the present study was to assess the magnitude of the bronchial response to inhaled cigarette smoke measured with different lung function indices and to relate the response to the above-mentioned variables. In addition the validity of the response was tested and tachyphylaxis observed by repeating the challenge after 1 hr.

Subjects and methods

Subjects

A total of 98 smokers were randomly selected among 198 persons who participated in a smoking cessation programme. The presence of asthma, type 1 allergy, chronic bronchitis or chronic obstructive pulmonary disease (COPD) did not exclude subjects from recruitment to the study. Subjects were otherwise healthy and had experienced no airway infections within the last 2 weeks prior to commencement of the study. Demographic data for the total population and those enrolled in this study are listed in table 1. The groups were compatible with respect to all variables.

Methacholine bronchial challenge

If participants had an FEV_1>1 L a bronchial challenge with methacholine bromide was performed. A Wright nebulizer (Aerosol Products, Colchester, UK), driven by compressed air at 0.13 kPa and a flow of 5 $L \cdot min^{-1}$ and with a mean output of 0.14 $mL \cdot min^{-1}$ was used for inhalations. After an initial saline inhalation participants inhaled unbuffered methacholine in doubling doses from 0.03–16 $mg \cdot mL^{-1}$. The inhalation was performed with tidal breathing for 2 min and intervals of 5 min. FEV_1 was measured 30 and 90 s after the inhalation, and the highest value selected. The challenge was stopped if FEV_1 decreased 20% or more from baseline or if the maximal dose of 16 $mg \cdot mL^{-1}$ was reached. The slope of the regression line through all datapoints (SAP) was determined for each participant and used as an expression of the bronchial response to methacholine, as this expression has been shown to be continuous and suitable for regression analyses [11]. By natural logarithmic transformation, the expression was normally distributed and to avoid a zero value a small constant (0.2) was added to the SAP before transformation. The response was the percentage change in SAP per milligram per millilitre methacholine.

Participants abstained from methylxanthines and oral β_2-agonists for 48 h and inhaled β_2-agonists and smoking from the evening prior to the challenge.

Table 1. – Demographic data for the total population and the study population

	Total population (n=198)	Study population (n=98)
Age yrs	49 (21–74)	50 (21–70)
Sex M/F	97/101	53/45
Height cm	172 (150–193)	172 (153–193)
FEV_1 L	2.6 (0.6–5.0)	2.4 (0.6–4.8)
VC L	3.8 (1.4–7.4)	3.8 (1.7–6.8)
FEV1/VC	0.67 (0.33–0.95)	0.63 (0.33–0.88)
Smoking duration yrs	28 (6–55)	30 (6–55)
Daily cigarette consumption*	32 (6–41)	33 (15–40)
Pack-years consumption*	43.4 (7–78.4)	43.7 (8.4–76)
Asthma/bronchitis/ healthy	24/111/63	13/58/27

Data are presented as mean with range in parenthesis. There were no significant differences between total and study populations. M: male; F: female; FEV_1: forced expiratory volume in one second; VC: vital capacity.

Cigarette-smoke bronchial challenge

An unfiltered commercial and popular brand of cigarettes with a nicotine content of 1.4 mg and a tar content of 15 mg was used. Participants were asked to smoke and inhale as usual. Participants were requested to avoid smoking 24 h prior to the challenge. Partial and maximal flow-volume curves were obtained: before the challenge; 30 s after 3, 6 and 12 inhalations; and 5, 10, 15 and 30 min after the last inhalation. The average of three measurements with less than 5% variation in FEV_1 was chosen as the baseline value. During challenge only one lung function manoeuvre was possible at each measuring point. All had practised the lung function manoeuvres and obtained a reproducible technique. One hour after the last inhalation a second cigarette-smoke bronchial challenge was performed in 38 persons, randomly selected among the smokers, to determine repeatability and the possible development of tachyphylaxis.

Allergological examinations

All had an allergological examination with skin-prick tests (Soluprick; ALK-Laboratories, Copenhagen, Denmark) and allergen-specific IgE analyses (Phadebas RAST; Pharmacia Diagnostics, Uppsala, Sweden). The 14 allergens included detect more than 95% of allergens in Denmark. Participants were considered allergic if they presented a wheal of at least half the size of the histamine response or had a specific radioallergosorbent test (RAST) Š0.35 $kU \cdot L^{-1}$.

Tobacco abstinence

Tobacco abstinence was controlled by measurement of carbon monoxide (CO) in expired air (Ecolyzer CO-monitor, Hawthorne, NY, USA) [30]. Cigarette smoke bronchial challenge was not performed in participants with a CO concentration in expired air greater than 3 parts per million (ppm).

Lung function measurements

Lung function measurements were performed on a Jaeger Transfer screen II (Erich Jaeger GmbH, Würzburg, Germany and included: total lung volume; FEV_1; vital capacity (VC); forced vital capacity (FVC); peak expiratory flow (PEF); and transfer factor of the lung for carbon

monoxide ($T_{L,CO}$). Normal values were obtained from the European working party on lung function measurements [31]. The mean standard residual values of FEV1 were normally distributed and independent of height, age and gender [32].

Participants presenting an abnormal FEV1 curve were given four inhalations of an aerosol containing terbutaline 0.5 mg·dose^{-1}. FEV1 was measured 30 min after the inhalation.

In methacholine bronchial challenge FEV1 was measured using a dry wedge spirometer (Vitalograph, Buckingham, UK). A rolling seal spirometer (Morgan, Gillingham, UK) was used for partial and maximal flow-volume curves during the cigarette smoke bronchial challenge. The lung function indices analysed were FEV1, maximal expiratory flow at 75% of FVC (MEF75%) and forced mid-expiratory flow between 25 and 75% of FVC (FEF25–75%). The average of three measurements of each index with a variation of less than 5% in FEV1 was chosen as baseline value. FEV1 values measured with the three different de-vices were identical.

Questionnaires

An investigator filled in a questionnaire regarding: the individuals use of medicine; symptoms of asthma; chronic bronchitis; hayfever; urticaria; and if their physician had given them the diagnosis asthma, chronic bronchitis or asthmatic bronchitis. They were asked if any first degree relatives had asthma, hayfever, eczema or urticaria. Presence of airway symptoms such as daily cough, wheeze, and shortness of breath were also enquired after. Our questionnaire was a modification of the questionnaire recommended by the British Committee on Research into Chronic Bronchitis.

Classification of participants

Asthma was considered present if a physician had given a diagnosis of asthma or asthmatic bronchitis, and a ≥20% increase in FEV1 could be obtained after terbutaline inhalation. Bronchitis was considered present if a physician had given a diagnosis of bronchitis, asthmatic bronchitis or asthma, a 20% increase in FEV1 could not be obtained after terbutaline inhalation and/or subjects presented with airway symptoms such as daily cough and/or expectoration in at least 3 months of a year.

They were considered to have good respiratory health if they did not meet the criteria for asthma or bronchitis and presented with a normal lung function.

Smoking history

The participants were asked about duration of former and actual consumption of light filter cigarettes, ordinary filter cigarettes, unfiltered cigarettes, small cigars, cigars and pipe tobacco. Different forms of tobacco products often contain different concentrations of active constituents which may serve as irritants of the airways. Therefore, tobacco consumption was standardized according to nicotine content and the content of tar since the nicotine and tar contents of tobacco products are usually related.

Each product was transformed into "standard" cigarettes: one light filter cigarette (nicotine content = 0.6–1.0 mg) = two/three standard cigarettes; one ordinary filter cigarette (nicotine content = 1.0–1.6 mg) = one standard cigarette; one unfiltered cigarette (nicotine content = 1.6–2.4 mg) = one and one third standard cigarettes; one small cigar = three standard cigarettes; one cigar = five standard cigarettes; and 1 g of pipe tobacco = one standard cigarette. The standardization of cigars and small cigars was arbitrary, although, very few of the participants used these products and they were thus rendered unimportant in the analyses.

Daily cigarette consumption was: the sum of all the tobacco forms smoked at present transformed into standard cigarettes. Pack-years consumption was: the transformed sum of each of the tobacco forms smoked over the years multiplied by the number of years the specific tobacco form had been smoked, divided by 20.

Ethics

All participants gave their informed consent to participate. The study was approved by the Scientific Ethics Committee of Aarhus county.

Statistics

The BMDP statistical package (BMDP Statistical Software, Cork Technology Park, Cork, Ireland) [33] was used in all calculations. Natural log transformed values of the percentage fall from baseline in a specific lung function index were used to quantify the magnitude of the bronchial response to cigarette smoke, as these numbers were normally distributed. Values are given as geometric mean (Gmean)±1SEM. Bivariate and multivariate linear regression analyses were used to evaluate the relationship bet-ween the bronchial response to inhaled cigarette smoke and other variables.

$$\text{The coefficient of repeatability} = 2 \times \text{Ð}(\text{E}\,(x_1 - x_2)^2/n)$$

where: Ð is the square root; E: is the sum of the squared differences; n is the number of participants and x_1 and x_2 are the percentage fall in FEV1 after 12 cigarette smoke-inhalations in the first and second challenge, respectively. This test assumes that the mean difference between the measurements is zero [34].

Measurement error was also calculated as the "reliability", which was the correlation between the two pairs of measurements of the percentage fall in FEV1. The correlation coefficient was calculated in a two-way analysis of variance [35].

A p-value less than 0.05 was considered significant and a p-value greater than 0.05 and less than 0.1 were considered to represent a trend.

Results

Values for VC, FEV1, FEV1/VC, SAP, age, sex, height, pack-years consumption, allergy, asthma, and asthma and allergy in the family for the total study population, for participants classified as having asthma, bronchitis, and those classified as healthy (without signs of asthma or bronchitis) are given in table 2.

Table 2. – Demographic data according to asthmatic/bronchitic status

	Overall (n=98)	Asthma (n=13)	Bronchitis (n=58)	Healthy (n=27)	p-value
Sex M/F	53/45	8/6	27/30	18/9	NS
Age yrs	49 (21–70)	36 (21–65)	52 (24–70)	50 (32–65)	<0.0001
Height cm	172 (153–193)	175 (160–190)	171 (160–193)	174 (159–192)	NS
VC L	3.8 (1.7–6.8)	4.4 (2.8–5.9)	3.5 (1.7–6.8)	4.0 (2.4–6.4)	<0.05
FEV_1 L	2.4 (0.6–4.7)	3.0 (2.1–4.5)	2.1 (0.6–4.4)	2.9 (2.3–4.7)	<0.0001
FEV_1/VC	0.63 (0.3–0.88)	0.68 (0.60–83)	0.58 (0.3–0.88)	0.71 (0.6–0.86)	<0.0001
SAP $\%^{-1}\cdot mg^{-1}\cdot mL^{-1}$	11.3 (8.6)	39.6 (16.4)	42.6 (9.4)	0.2 (0.5)	<0.0001
Pack-years consumption*	43.7 (8.4–76)	30.8 (8–49)	47.4 (13–76)	42.3 (20.3–63)	<0.0001
Allergy present	7	5	1	1	<0.0001
Allergy in family	35	7	23	5	<0.05
Asthma in family	25	7	14	4	<0.05

Values are presented as absolute number, mean with range in parenthesis and for the slope of regression through all data points (SAP) mean with SEM in parenthesis. For further definitions see table 1. *: see text for definition.

Most of these variables were different between asthmatic, bronchitic and healthy subjects, but height and sex were equally distributed.

FEV_1 as the measured variable

Gmean±SEM values of the percentage fall in FEV_1 ($\Delta FEV\%$) after 3, 6 and 12 inhalations and 5, 10, 15 and 30 min after the last inhalation for all 98 participants are shown in figure 1. The maximum percentage fall in FEV_1 was 10.2±1.3% and occurred immediately (30 s) after the last inhalation. Thereafter, FEV_1 improved but did not reach baseline values within 30 min of the challenge.

FEV_1 measured after six inhalations was lower than after three inhalations ($p<0.05$) and FEV_1 measured after 12 inhalations was lower than after 6 inhalations ($p<0.0001$) (fig. 1). Bivariate linear regression analyses showed statistically significant relationships between $\Delta FEV\%$ and baseline FEV_1 ($p<0.005$), FEV_1/VC ($p<0.0001$), $T_{L,CO}$ ($p<0.0005$), PEF ($p<0.001$), SAP ($p<0.005$), pack-years consumption ($p<0.05$), asthmatic/bronchitic status ($p<0.01$) and asthma in family ($0.05<p<0.1$), but not between Δ FEV% and VC, age, sex, height, daily cigarette consumption or allergy in the family (table 3). The number of participants with an allergy was too small to be evaluated.

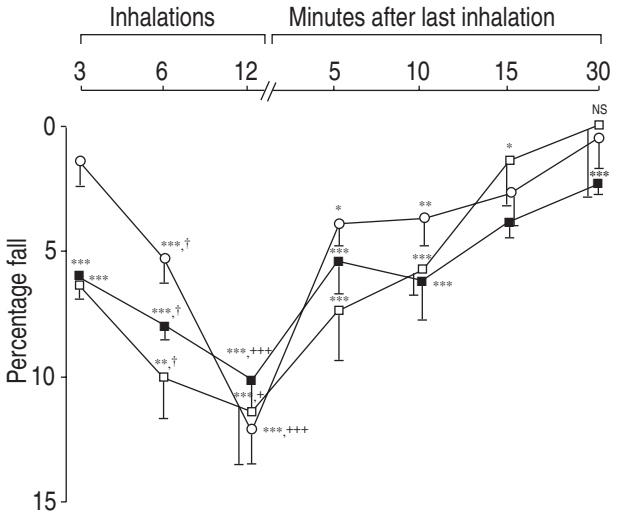

Fig. 1. – The percentage fall in forced expiratory volume in one second (FEV_1) (■), maximal expiratory flow at 75% of the forced vital capacity ($MEF_{75\%}$) (❑) and maximal forced expiratory flow between 25 and 75% of the forced vital capacity (○) from baseline values after 3,6 and 12 cigarette smoke inhalations and 5, 10, 15 and 30 min after the last inhalation. Each point on the curves represents a geometric mean and the vertical lines represent 1SEM. *,**,***: $p<0.05$, $p<0.01$, $p<0.001$, compared to baseline values. †: $p<0.05$, compared to three inhalations. +,+++: $p<0.05$, $p<0.001$, compared to six inhalations.

As the number of participants in the study was rather small it was necessary to limit the number of variables in the multivariate analyses. FEV_1/VC had the highest significance in the bivariate analysis and was chosen as the representative lung function index. In addition, variables with $r>0.1$ in the bivariate analyses were included in the analyses as independent variables. $\Delta FEV\%$ was the dependent variable.

The multivariate linear regression analysis showed statistically significant relationships between $\Delta FEV\%$ and FEV_1/VC ($p<0.01$), asthmatic/bronchitic status ($0.05< p< 0.1$), and pack-years consumption ($p<0.05$). There was no relationship between $\Delta FEV\%$ and age, sex, SAP or asthma in the family (table 4).

Asthma in the family was not an obvious confounder to any of the other variables and a multivariate analysis without this variate analysis without this variable showed similar results except for the variable asthmatic/bronchitic status, which became significant (table 5).

A total of 38 smokers participated in a second cigarette smoke bronchial challenge 1 h after the first challenge. Baseline FEV_1 in the first and second challenge were similar. After six inhalations in the first and second challenge, respectively Gmean $\Delta FEV\%$ was 5.3±1.0 *versus* 3.4±0.9% ($p<0.05$) and after 12 inhalations 7.2±1.4 versus 4.5±0.8% ($p<0.05$).

Measurement error could not be calculated, as the mean of the differences of $\Delta FEV\%$ readings between the first and the second challenge after 12 inhalations was significantly different from zero.

By using the two-way analysis of variance a significant correlation between $\Delta FEV\%$ after 12 inhalations of the first and second challenge could be shown ($r=0.33$; $p< 0.05$).

$MEF_{75\%}$ as the measured variable

Gmean $\Delta MEF_{75\%}$ was the percentage fall from baseline in $MEF_{75\%}$. Gmean $\Delta MEF_{75\%}$±1SEM 30 s after 3, 6 and 12 inhalations 5, 10, 15 and 30 min after the last inhalation shown in figure 1. The maximal fall of 14.5±1.7%

Table 3. – Cigarette smoke bronchial challenge. Bivariate regression analyses with percentage fall in FEV_1 as the dependent variable

Independent variables	Regression coefficient	Intercept	Correlation coefficient	p-value
FEV_1	-0.3±0.08	3.0	0.36	<0.0005
FEV_1/VC	-1.9±0.55	3.5	0.36	<0.0001
VC	-1.2±0.007	2.7	0.17	NS
FVC	-0.02±0.006	3.6	0.36	<0.0005
PEF	$(-0.13\pm0.04)\cdot10^{-2}$	3.0	0.36	<0.001
$T_{L,CO}$	-0.005±0.001	2.5	0.40	<0.001
SAP	0.10±0.03	2.0	0.33	<0.005
Pack-years consumption*	0.03±0.01	1.9	0.23	<0.05
Daily cigarette consumption*	0.03±0.05	1.2	0.06	NS
Asthmatic/bronchitic status*	-0.37±0.13	2.7	0.3	<0.01
Age	-0.006±0.006	1.8	0.11	NS
Sex	-0.04±0.01	2.1	0.02	NS
Asthma in family	-0.3±0.18	2.7	0.19	0.05<p<0.01
Allergy in family	-0.1±0.2	2.4	0.005	NS

Values are presented as geometric mean±1SEM. FVC: forced vital capacity. PEF: peak expiratory flow; $T_{L,CO}$: transfer factor of lung for carbon monoxide. *: see text for definition. For further definition refer to tables 1 and 2.

Table 4. – Multiple linear regression analysis with percentage fall in forced expiratory volume in one second (FEV_1) as the dependent variable (n=98)

Independent variable	Multiple r^2	Δr^2	p-value
FEV_1/VC	0.13	0.13	<0.01
Asthmatic/bronchitic status	0.17	0.04	0.05<p<0.01
Pack-years consumption*	0.21	0.04	<0.05
Asthma in family	0.22	0.01	NS
Age	0.23	0.01	NS
SAP*	0.23	0.005	NS

Δ: change in. For further definition refer to tables 1 and 2. *: see text for definitions.

Table 5. – Multiple linear regression analysis with percentage fall in forced expiratory volume in one second (FEV_1) as the dependent variable (n=98)

Independent variable	Multiple r^2	Δr^2	p-value
FEV_1/VC	0.13	0.13	<0.01
Asthmatic/bronchitic status	0.17	0.04	<0.05
Pack-years consumption*	0.21	0.04	<0.05
Age	0.21	0.005	NS
SAP*	0.22	0.005	NS

Δ: change in. For further definition refer to tables 1 and 2. *: see text for definitions.

was seen after 12 inhalations. Gmean $\Delta MEF_{75\%}$ was higher after six compared to after three inhalations ($p<0.05$), and after 12 compared to six inhalations ($p<0.05$). This measure was independent of baseline FEV_1, smoking history, gender, asthmatic or allergic disease of familial disposition to asthmatic or allergic disease. In persons aged ð50 yrs and aged >50 yrs Gmean $\Delta MEF_{75\%}$ was 11.8± 14.6% and 16.4±13.8%, respectively ($p<0.05$).

$FEF_{25-75\%}$ as the measured variable

Gmean $\Delta FEF_{25-75\%}$ 30 s after 3, 6 and 12 inhalations and 5, 10, 15 and 30 min following the last inhalation is shown in figure 1. The maximal decrease in $FEF_{25-75\%}$ of 12.1±1.0% took place after 12 inhalations.

In all analyses $\Delta FEF_{25-75\%}$ had similar relations as $\Delta MEF_{75\%}$.

Discussion

Inhalation of cigarette smoked results in an immediate fall in FEV_1. The mean decrease in FEV_1 after 12 inhalations was about 10% and the response showed a clear dose-dependent relationship to the number of inhalations. The response was inversely related to FEV_1/VC and directly related to the accumulated and standardized life-long consumption of cigarettes. Participants with asthma or bronchitis had a higher response than those in good respiratory health. The relationship between bronchial reactivity to methacholine and CBR was very weak and CBR bore no relation to height, age, sex or asthma or allergy in the family.

During the cigarette smoke bronchial challenge it was not possible to perform more than one lung function measurement at each time point. All participants, however, were very carefully instructed in the manoeuvres and could present curves with less than 5% variation in FEV_1 and VC. We regarded the measurements as reliable.

The asthma diagnosis was obtained by use of the general practitioner's diagnosis supplemented with a test for reversibility in FEV_1 and participants were classified as asthmatics only if a positive test was obtained. This test was valid, as all participants who were classified as asthmatics had a baseline FEV_1 >2 L. We also had a substantial knowledge of the clinical history of all participants, as they visited the clinic several times during the year. The classification was supported by the observation that persons classified as having "good respiratory health" had a much lower methacholine bronchial reactivity compared to the two others groups (table 2).

The number of participants with bronchitis was high and the provocation sample was probably not representative of all smokers. Bronchitic smokers in the sample who were overweight, however, had no influence on the results of the multiple regression analyses. Bronchitic/asthmatic

status was controlled in these analyses by including a group of respiratorily healthy smokers in the analyses.

Our tests for type I allergy were extensive and included allergens covering 95% of the sensitizations in Denmark. The risk of misclassification of participants with respect to type I allergy was, therefore, very small. The number of participants with type I allergy, however, was very small and we were, thus, unable to test for this factor in our analyses.

To ensure that participants had abstained from smoking for 24 h prior to cigarette bronchial challenge, the content of CO in expired air was measured. Participants presenting with a CO concentration greater than 3 ppm were denied challenge. This ensured that only those who had abstained from tobacco consumption were included in the challenge.

Lung function measurement showed substantial decreases for all indices after inhalation of cigarette smoke. Although the maximal decrease in FEV_1 was less than the maximal decrease in the other lung function indices tested, only FEV_1 had a significant relationship with other variables. FEV_1 was therefore superior to the other lung function indices as an expression of CBR. The discussion therefore will be exclusively related to FEV_1 as the measured variable.

The bronchial response was significantly lower in the second challenge compared to the first, which we regard as an expression of tachyphylaxis to inhalation of cigarette smoke. This implies that a relatively prolonged period of smoking abstinence is necessary for a reliable estimate of the bronchial sensitivity to cigarette smoke. The tachyphylaxis may also explain why many smokers cough only after the first cigarette of the day. The correlation between CBR after the first and the second challenge shows some consistency with this measure. A final decision relating to the question of reproducibility of the CBR, however, requires repeated challenges on separate days.

Earlier studies on cigarette smoke bronchial reactivity [23, 29, 30] have not been able to show any consistent changes in FEV_1 during and after the challenge. The reasons may be that these studies included too few participants and allowed smoking until 1 or 2 h before the challenge. This latter factor may be the most important, since our study showed a clear attenuation of the bronchial response to cigarette smoke if the challenge was repeated after 1 h. As none of the published studies controlled for smoking before the challenge some of the participants may even have engaged in smoking immediately prior to challenge.

Some studies on cigarette smoke bronchial reactivity have avoided FEV_1 as an estimate of the bronchial response [26–28] because the maximal inspiratory manoeuvre may diminish the measured bronchial response [36]. The average decrease in FEV_1 after 12 inhalations in this study was 10% in spite of the possible attenuation of the response caused by a previous deep inspiration.

The literature is sparse concerning dose-response relationships between cigarette smoke inhalation and cigarette smoke bronchial reactivity. Conflicting results had been reported concerning the influence of inhalation through filters [25, 27], the amount of cigarette smoked inhaled [26, 28, 31], different patterns of inhalation [29] and the amount of tar in the cigarettes [29]. No further bronchoconstriction after three inhalations has been observed [23, 28–31]. This may be a result of study populations being too small and an insufficient period of time for abstinence from smoking, prior to the challenge. In our study we showed a clear dose-response relationship between the dose of cigarette smoke inhaled and the magnitude of the bronchial response. This further strengthens our view that the response is consistent.

CBR was related to pack-years consumption independent of FEV_1/VC and age. This implies that smoking may induce changes in the bronchial wall, preceding the narrowing of the bronchi, which is a known symptom of smoking. A study of these changes would be helpful in exploring reasons concerning lung function impairment, following smoking.

Earlier studies reported the combined effects of smoking and atopy on methacholine bronchial sensitivity in smokers [16, 20, 21, 37, 38]. No such correlation with regard to CBR could be made in the present study, as the fraction with type I allergy was too small to draw any conclusions regarding allergy and bronchial reactivity. However, we found no indications that allergic predispositions influenced CBR.

Our method of estimating methacholine reactivity using SAP was continuous to a limited degree. All participants without asthma or bronchitis showed no reactivity and hence the value of the slope was zero. This was in contrast to the findings for CBR, as all participants showed some bronchial reaction to inhalation of cigarette smoke and, thus CBR had a continuous distribution and was, therefore, very suitable for analysis. These results, as well as the very weak relationship found between CBR and bronchial reactivity to methacholine, also suggest that cigarette smoke and methacholine have partly different mechanisms of action: methacholine is a direct smooth muscle stimulant and cigarette smoke works through indirect pathways.

The continuous distribution and consistency of cigarette smoke bronchial reactivity suggests that this measure might be valuable in the prediction and investigations of reasons for increased loss of lung function in smokers. In addition bronchial reactivity to cigarette smoke is an objective response and a powerful tool in demonstrating the adverse effects of tobacco smoke. This can be used as a tool, along with other arguments in the urging of smokers to quit. Further studies, however, are needed to evaluate the pathogenetic mechanisms leading to the acute obstruction.

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