RESPIRATORY MEDICINE

Bronchial reactivity to cigarette smoke; relation to lung function, respiratory symptoms, serum-immunoglobulin E and blood eosinophil and leukocyte counts

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Study objectives: The aim of the study was to investigate the relationship between the immediate bronchial response to inhaled cigarette smoke [cigarette smoke bronchial reactivity (CBR)] and lung function, respiratory symptoms and markers of allergy and inflammation.

Design, participants and measurements: This cross-sectional study included 98 smokers. Their lung function and reversibility to inhaled terbutaline was measured. Their clinical history was obtained, an allergological examination was done, and bronchial reactivity to methacholine and inhaled cigarette smoke was measured. Questionnaires about respiratory symptoms, smoking history and drug usage were completed and a blood sample was obtained. Participants were divided into three groups: with asthma, chronic bronchitis and persons without asthma or chronic bronchitis (the respiratory healthy).

Results: Forced expiratory volume in 1sec (FEV_1) residuals were independently related to the % fall in FEV_1 after 12 cigarette smoke inhalations (DFEV%) in all participants ($P < 0.01$), in asthmatic smokers ($P < 0.01$) and in smokers with chronic bronchitis ($P < 0.05$). In smokers with asthma and chronic bronchitis FEV_1 residuals explained 51% and 13% of the variation in DFEV%, respectively, but only 8% ($P < 0.05$) and 1% (N.S.) of the variation in the methacholine bronchial reactivity. In the total population the presence of wheeze ($P < 0.01$), attacks of breathlessness ($P < 0.05$) and daily expectoration ($P < 0.001$) were related to higher DFEV% readings. Serum immunonoglobulin (ES-IgE) was independently related to DFEV% in all participants ($P < 0.01$), in smokers with chronic bronchitis ($P < 0.01$) and in the respiratory healthy ($0.05 < P < 0.1$). The eosinophil blood count was, in similar analyses, related to DFEV% in all participants ($P < 0.05$) and in persons with chronic bronchitis ($0.05 < P < 0.1$).

Conclusion: Cigarette smoke bronchial reactivity was strongly associated to actual FEV_1 in smokers with asthma and bronchitis, overall to most respiratory symptoms and in smokers without asthma to S-IgE. Cigarette smoke bronchial reactivity might be suitable to test further how cigarette smoke influences the pathophysiology of the bronchial wall, especially in smokers with asthma.

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Introduction

Cigarette smoking is a major cause of morbidity and death (1). Smoking is the most important cause of development of chronic bronchitis and emphysema (2,3).

The reasons for the susceptibility to the development airflow of limitation are virtually unknown, and both asthmatics (4) and non-asthmatics (5) may develop respiratory insufficiency even after minimal cigarette consumption.

Whether smoking causes increased bronchial reactivity [bronchial hyper reactivity (BHR)] in histamine/methacholine remains controversial (6–12), and it is unclear if atopy contributes to the development of BHR in smokers (6,10, 12). Studies have suggested that BHR in smokers is related to an increased loss of lung function (5,13–16). However, studies on BHR in smokers have not given an explanation for the accelerated lung function loss in some smokers.

In a previous publication we described the relationship between the immediate bronchial response to inhaled cigarette smoke (CBR), BHR and smoking (17).

The present study was performed to investigate relationships between CBR and lung function, respiratory symptoms and objective markers of atopy and inflammation.

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Population and methods

Ninety-eight smokers were randomly selected from among 198 persons who participated in a smoking cessation programme. Presence of asthma, type 1 allergy, chronic bronchitis, or chronic obstructive airway disease were not exclusion criterion, but subjects were otherwise healthy and had had no airway infections within the 2 weeks before entering this study. Demographic data for those enrolled in this study are listed in Table 1.

METHACHOLINE BRONCHIAL CHALLENGE

Participants with $FEV_1 > 1$ had a bronchial challenge with methacholine bromide. A Wright nebulizer driven by compressed air at 1·3 bar and a flow of 5 l min^{-1}, and with a mean output of 0·14 ml min^{-1}, was used for inhalations. After an initial saline inhalation, participants inhaled unbuffered methacholine in doubling doses from 0·03 mg ml^{-1} to 16 mg^{-1} ml. The inhalation was performed with tidal breathing for 2 min intersparsed with 5 min intervals. FEV_1 was measured 30 and 90 sec after the inhalation and the highest value selected. The challenge was stopped if FEV_1 decreased 20% or more from baseline or if the maximum dose of 16 mg 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 is continuous and suitable for regression analyses (11). By natural logarithmic transformation the expression was normally distributed, and to avoid zero a small constant (0·2) was added to SAP before transformation. SAP was the percent change in FEV_1 per mg ml^{-1} methacholine.

Participants abstained from methylxanthines and oral β_2-agonists for 48 h and from inhaled β_2-agonists and smoking from the evening before the challenge. Inhaled long acting β_2-agonists were not used.

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 for 24 h before the challenge. Partial and maximum flow volume curves were obtained before the challenge, and 30 sec after three, six, and 12 inhalations, and 5, 10, 15 and 30 min after the last inhalation. The avarage 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 subjects had practised the lung function manoeuvres and obtained a reproduceable technique.

ALLERGOLOGICAL EXAMINATIONS

All had an allergological examination with a skin prick test (Soluprick, ALK-Laboratories, Denmark) and allergen-specific IgE analyses (phadebas RAST, Pharmacia Diagnostics, Sweden). The 14 allergens included detect more than 95% of allergies in Denmark. Participants were considered allergic if they presented a wheal at least half the size of the histamine response or had a specific radioallergosorbent test (RAST) greater or equal to 0·35 kU l^{-1}.

BLOOD SAMPLES

Total blood white cell counts were measured on a Coulter counter S (Coulter Electronics, Hialeah, FL, U.S.A.) Serum

TABLE 1. Demographic data according to asthma and chronic bronchitis [means (range)]

	Overall	Asthma	Bronchitis	Healthy	P
N	98	13	58	27	
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)	<0·001
FEV$_1$/VC	0·63 (0·3–0·88)	0·68 (0·60–0·83)	0·58 (0·3–0·88)	0·7 (0·6–0·86)	<0·0001
SAP(SE % mg^{-1} ml^{-1})	11·3 (8·6)	39·6 (16·4)	42·6 (9·4)	0·2 (0·5)	<0·0001
Age (years)	49 (21–70)	36 (21–65)	52 (24–70)	50 (32–65)	<0·0001
Sex (m/f)	53/45	7/6	27/31	18/9	N.S.
Height (cm)	172 (153–193)	175 (160–190)	171 (160–193)	173 (159–192)	N.S.
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

samples were stored at $-20°C$ until analysed. S-IgE was determined using ELISA. The number of blood eosinophils were counted in a Fuchs–Rosenthal chamber.

TOBACCO ABSTINENCE

Tobacco abstinence was controlled by carbon monoxide (CO) in expired air (Ecolyzer CO-monitor, Hawthorne, New York, U.S.A.) (18). Cigarette smoke bronchial challenge was not performed in participants with a CO concentration in expired air greater than 3 ppm.

LUNG FUNCTION MEASUREMENTS

Lung function measurements were performed on a Jaeger Transfer screen II and included total lung volume, forced expiratory volume in 1 s (FEV_1), vital capacity (VC), forced vital capacity (FVC), peak expiratory flow (PEF) and CO transfer capacity (T_LCO). Normal values were obtained from (19). By means of standard residuals, FEV_1 was normally distributed and independent of height, age and gender (20).

Participants presenting an abnormal spirometry were given four inhalations of an aerosol containing terbutaline 0·5 mg per dose and asked to perform spirometry after 30 min. During methacholine bronchial challenge FEV_1 was measured on a dry wedge spirometer (Vitalograph, Buckingham, U.K.).

A rolling seal spirometer (Morgan, Gillingham, U.K.) was used for partial and maximum flow volume curves during the cigarette smoke bronchial challenge. The lung function indices analysed were FEV_1, MEF_{75}, and $FEF_{25–75}$. The average of three measurements of each index with variations less than 5% in FEV_1 was chosen as baseline value.

FEV_1 measured with the three different devices were identical.

QUESTIONNAIRES

An investigator completed in a questionnaire about the individuals' use of medicine, symptoms of asthma, chronic bronchitis, hay fever, urticaria, and if their physician had given them the diagnosis, asthma, chronic bronchitis or asthmatic bronchitis. Presence of airway symptoms, such as daily cough, wheeze and shortness of breath were sought for to clarify the questionnaire recommended by the British Committee on Research into Chronic Bronchitis.

CLASSIFICATION OF PARTICIPANTS

Asthma was considered present if a physician had given the diagnosis asthma or asthmatic bronchitis and a 20% or more increase in FEV_1 was obtained after terbutaline inhalation.

Chronic bronchitis was considered present if a physician had given the diagnosis bronchitis, asthmatic bronchitis, or asthma and a 20% increase in FEV_1 was not obtained after terbutaline inhalation, and/or they presented airway symptoms such as daily cough and/or expectoration for at least 3 months of a year.

They were considered as respiratory healthy if they did not meet the criteria for asthma or chronic bronchitis and presented 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 that may serve as irritants of the airways. Tobacco consumption was standardized according to nicotine content and, therefore,

TABLE 2. Bivariate regression analyses with DFEV% as the dependent variable and independent variables as indicated. Analyses were done in the total population and in subgroups with asthma, chronic bronchitis and in the respiratory healthy. DFEV% is the percent fall in FEV_1 after 12 cigarette smoke inhalations. SE = standard error

Population sample	Independent variable	Regression coefficient (SE)	Intercept	P
All (n=98)	FEV_1 residuals	−0·2 (0·005)	1·8	<0·0001
	pack–years consumption	0·01 (0·005)	1·9	<0·05
	FEV_1/VC	−0·02 (0·005)	3·6	<0·0001
Asthma (n=13)	FEV_1 residuals	−0·4 (0·1)	1·4	<0·005
	pack–years consumption	0·01 (0·01)	2·0	N.S.
	FEV_1/VC	−0·02 (0·01)	3·8	N.S.
Chronic bronchitis (n=72)	FEV_1 residuals	−0·2 (0·1)	1·9	<0·005
	pack–years consumption	0·01 (0·006)	1·9	<0·05
	FEV_1/VC	−0·02 (0·007)	3·5	<0·01
Respiratory healthy (n=13)	FEV_1 residuals	0·2 (0·2)	1·7	N.S.
	pack–years consumption	0·001 (0·01)	1·6	N.S.
	FEV_1/VC	0·1 (0·1)	−3·8	N.S.

also to tar content. The nicotine and tar content of tobacco products are usually related.

Each product was transformed into 'standard' cigarettes: one light filter cigarette (nicotine content=0·6–1·0 mg)=2/3 standard cigarette; one ordinary filter cigarette (nicotine content=1·0–1·6 mg)=1 standard cigarette; one unfiltered cigarette (nicotine content=1·6–2·4 mg) =1$\frac{1}{3}$ standard cigarette; one small cigar=3 standard cigarettes; one cigar=5 standard cigarettes; one gram of pipe tobacco=1 standard cigarette.

Daily cigarette consumption (DC) was the sum of all the tobacco forms smoked at present, transformed into standard cigarettes. Pack–years consumption (PY) 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 and 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 (21) was used in all calculations. Ln-transformed values of the % fall from baseline in a specific lung function index was used to quantify the magnitude of the bronchial response to cigarette smoke, as these numbers were normally distributed. Values are given as geometric means ± 1 standard error (SE). Bivariate and multivariate linear regression analyses were used to evaluate relationships between the bronchial response to inhaled cigarette smoke and other variables.

P values less than 0·05 were considered significant, and P values greater than 0·05 and less than 0·1 were considered to represent a trend.

Results

DFEV% was the mean % fall in FEV_1 from baseline after 12 inhalations of an unfiltered cigarette. In the total population DFEV% was 10·2$\pm$1·3%

CIGARETTE SMOKE AND METHACHOLINE BRONCHIAL REACTIVITY, LUNG FUNCTION, AND RESPIRATORY SYMPTOMS

The multivariate regression analyses included DFEV% as the dependent variable and FEV_1 residuals, pack–years consumption and the FEV_1/VC ratio as the independent variables. The variables FEV_1/VC and FEV_1 residuals were not independent. Because of this, in the multivariate regression analyses involving both FEV_1/VC and FEV_1 residuals as independent variables, FEV_1/VC was not used

as a continous variable but was stratified into three strata; FEV_1/VC $\leq$ 0·6, 0·6$\leq FEV_1$/VC$\leq$0·8 and FEV_1/VC$>$0·8.

Bivariate relationships between DFEV% and FEV_1 residuals, FEV_1/VC, and pack–years consumption are shown in Table 3.

The multivariate regression analyses showed an independent relationship between FEV_1 residuals and DFEV% in the total population ($P<0·01$), in smokers with asthma ($P<0·01$) and in smokers with chronic bronchitis, ($P<0·05$) (Table 3). FEV_1 residuals independently explained 51% and 13% of the variation in DFEV% in smokers with asthma and chronic bronchitis, respectively (Table 3). The significant relationship between FEV_1 residuals and cigarette smoke bronchial reactivity, persisting after SAP from methacholine bronchial reactivity, was added to the list of independent variables in the total population, in asthmatics and in smokers with chronic bronchitis (Table 4).

When SAP was used as the dependent variable and the above independent variables were maintained, the multivariate regression analyses showed a significant and independent relationship between methacholine SAP and FEV_1 residuals in the total population ($P<0·01$) and in smokers with asthma ($P<0·05$). In the multivariate analyses FEV_1 residuals independently explained 28%, 8% and 1% of the variation of methacholine SAP in the total population of smokers, in smokers with asthma and in smokers with chronic bronchitis, respectively. We found no independent relationship between FEV_1 residuals and DFEV% or methacholine SAP in smokers considered respiratory healthy.

In the total population, persons with attacks of wheeze ($P<0·01$), attacks of breathlessness ($P<0·05$) and chronic sputum ($P<0·001$) had higher DFEV% readings compared to persons without these symptoms (Table 5).

RELATIONSHIPS BETWEEN MARKERS OF ATOPY AND INFLAMMATION AND DFEV%

Bivariate regression analyses, including the total population, showed that DFEV% was significantly related to the FEV_1/VC ratio, pack–years consumption, asthmatic/ bronchitic status, blood eosinophil count and S-IgE. DFEV% was not related to the total blood leucocyte count or allergic status (Table 6).

In the total population, multivariate regression analyses, including the variables mentioned above and with DFEV% as the dependent variable, showed that both S-IgE ($P<0·01$) and the blood eosinophil count ($P<0·05$) were independently related to DFEV% (Table 7). S-IgE and the total blood eosinophil count explained 5% and 4%, respectively, of the variation in DFEV% (Table 7).

The multivariate regression analyses were repeated in the groups of asthmatics, persons with chronic bronchitis and persons considered respiratory healthy. S-IgE was significantly related to DFEV% in persons with chronic bronchitis ($P<0·01$) and tended to be related to DFEV% in persons considered respiratory healthy (0·05$<P<$0·1)

TABLE 3. Multivariate regression analyses with DFEV% or SAP as the dependent variable and independent variables as indicated. Analyses were performed in the total population and in subgroups of smokers as indicated. R^2 is the square of the correlation coefficient from the multivariate regression analyses and P the significance of the individual variable at the 0.05% level. NA: not applicable

Population	Independent variables	DFEV%			SAP		
		R^2	Contribution to R^2	P	R^2	Contribution to R^2	P
All ($n=98$)	FEV$_1$ residuals	0·18	0·18	$P<0·01$	0·28	0·28	$<0·01$
	FEV$_1$/VC	0·19	0·01	N.S.	0·33	0·05	$<0·05$
	pack–years consumption	0·22	0·03	$0·05<P<0·1$	0·34	0·01	N.S.
Smokers with asthma ($n=13$)	FEV$_1$ residuals	0·51	0·51	$<0·01$	0·08	0·08	$<0·05$
	FEV$_1$/VC	0·56	0·05	N.S.	0·46	0·38	$<0·05$
	pack–years consumption	0·60	0·04	N.S.	0·48	0·02	N.S.
Smokers with chronic bronchitis ($n=72$)	FEV$_1$ residuals	0·13	0·13	$P<0·05$	0·01	0·01	N.S.
	FEV$_1$/VC	0·16	0·03	N.S.	0·32	0·31	N.S.
	pack–years consumption	0·22	0·06	$<0·05$	0·34	0·02	N.S.
Smokers considered respiratory healthy ($n=13$)	FEV$_1$ residuals	0·08	0·08	N.S.	NA	NA	NA
	FEV$_1$/VC	0·18	0·10	N.S.	NA	NA	NA
	pack–years consumption	0·21	0·03	N.S.	NA	NA	NA

TABLE 4. Multivariate regression analyses with either DFEV% or SAP as the dependent variables and independent variables as indicated. Analyses were performed in the total population, and in subgroups of smokers with asthma or chronic bronchitis. P is the significance of the individual independent variable at the 0·05% level.

Sample	Independent variable	Dependent variable	
		DFEV%	SAP
		P	P
Total population ($n=98$)	FEV$_1$ resdiuals	$<0·05$	$<0·05$
	pack–years consumption	N.S.	N.S.
	FEV$_1$/VC	N.S.	$<0·05$
	DFEV%	—	N.S.
	SAP	N.S.	—
Smokers with asthma ($n=13$)	FEV$_1$ residuals	$<0·01$	$0·05<P<0·1$
	pack–years consumption	N.S.	N.S.
	FEV$_1$/VC	N.S.	$<0·05$
	DFEV%	—	N.S.
	SAP	N.S.	—
Smokers with chronic bronchitis ($n=72$)	FEV$_1$ residuals	$0·05<P<0·1$	N.S.
	pack–years consumption	N.S.	N.S.
	FEV$_1$/VC	N.S.	$<0·0001$
	DFEV%	—	N.S.
	SAP	N.S.	—

TABLE 5. DFEV% in 98 smokers with and without respiratory symptoms controlled for FEV_1/VC. $P1$ is the difference in DFEV% between persons with and without the symptom and $P2$ the difference in DFEV% between persons with FEV_1/VC <0·7 and FEV_1/VC>0·7. Geometric means (1 standard error)

	Symptom	Present	Symptom	Absent				$P1$	$P2$
Symptom	$FEV_1/VC \leqslant 0·7$		$FEV_1/VC > 0·7$		$FEV_1/VC \leqslant 0·7$		$FEV_1/VC > 0·7$		
	n	DFEV%	n	DFEV%	n	DFEV%	n	DFEV%		
Attacks of wheeze	33	15·5 (1·2)	12	7·8 (2·8)	30	9·0 (1·9)	23	5·7<1·7)	<0·01	<0·005
Attacks of breathlessness	34	15·5 (1·2)	17	7·8 (2·8)	24	10·1 (2·0)	18	57 (1·0)	<0·05	<0·005
Chronic expectoration	47	14·8 (1·1)	21	6·4 (1·3)	16	6·0 (1·3)	14	5·8 (2·7)	<0·001	<0·05
Dry cough	36	12·6 (1·9)	14	7·3 (1·2)	27	12·3 (2·1)	21	7·3 (1·2)	N.S.	<0·005

TABLE 6. Relationship between DFEV% and serum immunoglobulin E, blood eosinophil count, total leucocyte blood count, pack–years consumption, FEV_1/VC, allergic status, and presence of asthma/bronchitis. Bivariate regression analyses with DFEV% as the dependent variable and independent variables as indicated. SE is 1 standard error

Independent variable	Regression coefficients (SE)	Intercept	P
FEV_1/VC	6.47·4 (21)	1·5	<0·05
Pack–years consumption	0·03(0·02)	1·9	<0·05
Serum IgE	$-0·7*10^{-3}$ ($0·3*10^{-3}$)	2·3	<0·05
Blood eosinophil count	$-0·9*10^{-3}$ ($0·4*10^{-3}$)	2·3	<0·05
Blood leukocyte count	$-0·6*10^{-3}$ ($0·5*10^{-3}$)	2·2	N.S.
Allergic status (yes/no)	0·3 (0·3)	1·7	N.S.
Asthma/chronic bronchitis	$-0·3$ (0·1)	1·9	$0·05 < P < 0·1$

TABLE 7. Relationship between DFEV% and serum immunoglobulin E, blood eosinophil count, and total blood leucocyte count. Multivariate regression analyses with DFEV% as the dependent variable and independent variables as indicated. Analyses were carried out in the total population ($n=98$). r^2 is the square of the correlation coefficient

Independent variable	r^2	increase r^2	P
FEV_1/VC	0·05	0·05	<0·05
Pack–years consumption	0·08	0·03	<0·05
Serum IgE	0·13	0·05	<0·01
Blood eosinophil count	0·17	0·04	<0·05
Blood leucocyte count	0·17	0·00	N.S.
Allergic status (yes/no)	0·18	0·01	N.S.
Asthma/chornic bronchitis	0·28	0·10	<0·0001

(Table 8). The blood eosinophil count tended to be related to DFEV% in persons with chronic bronchitis ($0·05 < P < 0·1$) (Table 8).

There were no relationships between S-IgE and pack–years consumption in the total population, in asthmatics, or in the respiratory healthy, but in smokers with chronic bronchitis S-IgE was related to pack–years consumption ($r=0·27$, $P<0·05$). There was no relationship between blood eosinophil count, pack–years consumption and S-IgE.

Discussion

This study was performed to investigate the acute bronchial response to cigarette smoke and methacholine inhalation, and the relationship to baseline lung function in a population of smokers. In addition the relationship between cigarette smoke bronchial reactivity and respiratory symptoms, S-IgE, blood eosinophil count and the total blood leucocyte count was studied. The analyses were performed in the total population and in subgroups

TABLE 8. Relationship between DFEV% and serum immunoglobulin E, blood eosinophil count, and blood leucocyte count. Multivariate regression analyses with DFEV% as the dependent variable and independent variables as indicated. Analyses were performed in subgroups of persons with asthma, chronic bronchitis and persons regarded respiratory healthy. r^2 is the square of the correlation coefficient

Group	Independent variables	r^2	increase r^2	P
Persons with asthma ($n=13$)	FEV_1/VC	0·01	0·01	N.S.
	Pack–years consumption	0·08	0·07	N.S.
	Serum IgE	0·27	0·19	N.S.
	Blood eosinophil count	0·28	0·01	N.S.
	Leucocyte blood count	0·30	0·02	N.S.
	Allergic status (yes/no)	0·31	0·01	N.S.
Persons with chronic bronchitis ($n=72$)	FEV_1/VC	0·14	0·14	$<0·01$
	Pack–years consumption	0·17	0·03	$<0·05$
	Serum IgE	0·29	0·12	$<0·01$
	Blood eosinophil count	0·32	0·03	$0·05<P<0·1$
	Blood leucocyte count	0·33	0·01	N.S.
	Allergic status (yes/no)	0·33	0·00	N.S.
Respirtory healthy persons ($n=13$)	FEV_1/VC	0·04	0·04	N.S.
	Pack–years consumption	0·04	0·00	N.S.
	Serum IgE	0·45	0·41	$0·05<P<0·1$
	Blood eosinophil count	0·45	0·00	N.S.
	Blood leucocyte count	0·49	0·04	N.S.
	Allergic status (yes/no)	0·49	0·00	N.S.

according to the presence of asthma or chronic bronchitis.

The main results of the study showed that DFEV% was independently related to baseline lung function and to most respiratory symptoms. S-IgE was related to DFEV% in the total population, in persons with chronic bronchitis and in the respiratory healthy. The blood eosinophil count tended to be related to DFEV% in smokers with chronic bronchitis. The population was unevenly composed with respect to asthmatics, persons with chronic bronchitis and the respiratory healthy. The group with chronic bronchitis was of a suitable size for performing multivariate analyses but the groups, with asthma and good respiratory health were small. This probably limited the possibility of showing relationships between some of the variables in the multivariate analyses. Relationships between DFEV% and S-IgE, blood eosinophil count and total blood leucocyte count, therefore, could not be elucidated clearly in this study regarding asthmatics and persons considered respiratory healthy.

Classification, with respect to asthma, was performed according to the information provided by the participants, supplemented with a test for reversibility in FEV_1. The classification was supported by a higher bronchial sensitivity to methacholine in smokers with asthma and chronic bronchitis, compared to persons considered respiratory healthy. The respiratory healthy all had negative methacholine challenges. Asthmatics had higher lung function than smokers classified as having chronic bronchitis. The risk of mis-classification of persons with type 1 allergy was very low as the allergens used for testing covered 95% of known inhaled allergens in Denmark.

The cigarette smoke bronchial challenge was not standardized. Results with less variability could probably be achieved by monitoring plasma nicotine or carbon monoxide in expired air during the challenge. In an earlier report (17) we showed a good correlation between two cigarette smoke bronchial challenges performed with an interval of 1 h.

The immediate bronchial response to cigarette smoke has been tested in several studies (22–29). However, its relation to lung function, respiratory symptoms, or markers of atopy and inflammation has not been investigated.

The bronchial response to methacholine/histamine in earlier studies has explained 0–35% of the variation in FEV_1(30–33). This is consistent with the results of this study, as the percentage of variation in the bronchial reactivity to methacholine explained by FEV_1 residuals in the total population was within this range. The lower percentage of variation in FEV_1 residuals explained by the methacholine bronchial reactivity in this study, compared to an earlier study in asthmatics (33), may be due to different analysing methods. The earlier study did not control for the FEV_1/VC ratio and this might have influenced results significantly. Controlling for the FEV_1/VC ratio in studies analysing bronchial reactivity and baseline lung function is an important issue. This was clearly shown in this study as we found no independent relationship between bronchial reactivity to methacholine and lung function, after controlling for FEV_1/VC in smokers with chronic bronchitis. The bronchial reactivity to cigarette smoke had a closer relationship to baseline lung function in smokers with chronic bronchitis and especially in smokers with asthma compared to the methacholine

bronchial reactivity. Reasons for this may be a more continous distribution of bronchial cigarette smoke reactivity and, possibly, different modes of action on the bronchial wall. Cigarette smoke probably mainly acts through indirect mechanisms which may accelerate loss of lung function. The different mode of action of cigarette smoke and methacholine on the bronchial wall was supported by the observation that both bronchial reactions kept their significance after the addition of one of these variables as an independent variable in the multivariate analyses, in which the other was the dependent variable.

Not all asthmatic smokers develop chronic airflow obstruction. The cigarette smoke bronchial reactivity may aid in identifying those asthmatic smokers at risk of developing airflow obstruction. To further study the nature and possible importance of the bronhial response to cigarette smoke, the inflammatory response, investigated by means of studying the cellular and other changes in broncho-alveolar lavage fluid after the smoking of a cigarette, should be looked into and compared to the responses from other compounds with an indirect mode of action on the bronchial wall such as adenosin, allergens and others.

The relationship between DFEV% and the presence of respiratory symptoms indicated that the bronchial sensitivity to cigarette smoke might also have importance with respect to development of airway symptoms from smoking.

The significant relationhip between S-IgE and DFEV% was seen in non-asthmatic smokers. The relationship persisted after controlling for other variables that might influence DFEV% and therefore S-IgE had an independent relationship to the bronchial sensitivity to cigarette smoke. The reason why we found no such relationship in asthmatic smokers was probably the greater variation in the S-IgE in asthmatics and the small population of asthmatic smokers.

The relationship between S-IgE and smoking history was in accordance with an earlier report (34) and indicates that smoking affects the level of S-IgE *per se*. Whether this indicates the development of an atopic predisposition caused smoking in some smokers is not known, and the mechanisms by which S-IgE influences the bronchial reactivity to cigarette smoke is also unknown. DFEV% was only weakly related to the blood eosinophil count and the mechanism by which the blood eosinophil count influences the bronchial sensitivity to cigarette smoke is unknown. The lack of association between S-IgE and the blood eosinophil count indicated that S-IgE and the blood eosinophil count might influence the bronchial reactivity to cigarette smoke differently.

The independency between DFEV% and the total blood leucocyte count might be caused by a large variation in blood counts. It also might indicate that the bronchial reactivity to inhaled cigarette smoke was independent of the inflammation present in the airways in most smokers.

The relationship between DFEV% and baseline lung function, respiratory symptoms, S-IgE and the blood eosinophil count, indicated that the bronchial reactivity to inhaled cigarette smoke might be useful in the investigation of how smoking affects the bronchial mucosa in smokers in general and especially in smokers with asthma.

References

1. Fielding JE. Smoking: health effects and control. *N Engl J Med* 1985; **313:** 491–498.
2. Petty TL, Ryan SF, Mitchell RS. Cigarette smoking and the lungs: relation to postmortem evidence of emphysema,chronic bronchitis, and black lung pigmentation. *Arch Environ Health* 1967; **14:** 172–177.
3. Auerbach O, Hammond EC, Garfinkel L, Benante C. Relation of smoking and age to emphysema: whole lung section study. *N Engl J Med* 1972; **286:** 853–857.
4. Peat JK, Woolcock AJ, Cullen K. Rate of decline of lung function in subjects with asthma. *Eur J Respir Dis* 1987; **70:** 171–179.
5. Barter CE, Cambell AH. Relationship of constitutional factors and cigarette smoking to decrease in 1-second forced expiratory volume. *Am Rev Respir Dis* 1976; **113:** 305–314.
6. O'Connor GT, Sparrow D, Segal MR, Weiss ST. Smoking, atopy, and methacholine airway responsiveness among middle-aged and elderly men. *Am Rev Respir Dis* 1989; **140:** 1520–1526.
7. Burney PGJ, Britton JR, Chinn S, *et al.* Descriptive epidemiology of bronchial reactivity in an adult population: results from a community study. *Thorax* 1987; **42:** 38–44.
8. Cerveri I, Bruschi C, Zoia MC, *et al.* Smoking habit and bronchial reactivity in normal subjects. *Am Rev Respir Dis* 1989; **140:** 191–196.
9. Woolcock A, Peat JK, Salome CM, *et al.* Prevalence of asthma in a rural population. *Thorax* 1987; **42:** 361–368.
10. Buczko GB, Zamel N. Combined effect of smoking and allergic rhinitis on airway responsiveness to inhaled methacholine. *Am Rev Respir Dis* 1984; **129:** 15–19.
11. Rijken B, Schouten JP, Mensinga TT, *et al.* The distribution of bronchial responsiveness to histamine in symptomatic and in asymptomatic subjects. *Am Rev Respir Dis* 1989; **140:** 615–623.
12. Kennedy SM, Burrows B, Vedal S, Enarson A, Chan Yeung M. Methacholine responsi-veness among working populations. *Am Rev Respir Dis* 1990; **142:** 1377–1383.
13. Kanner RE. The relationship between airways responsiveness and chronic airflow limitation. *Chest* 1984; **86:** 54–57.
14. Rijken B, Schouten JP, Weiss ST, Speizer FE, Van der Lende R. The relationship between airway responsiveness to histamine and pulmonary function level in a random population sample. *Am Rev Respir Dis* 1988; **137:** 826–832.
15. Parker D., O'Connor GT, Sparrow D, Segal MR, Weiss ST. The relationship of non-specific airway responsiveness and atopy to the rate of decline of lung function. *Am Rev Respir Dis* 1990; **141:** 589–594.
16. Postma D, De Vries K, Koeter GH, Sluiter HJ. Independent influence of reversibility of air-flow obstruction and non-specific hyperreactivity on the long term course of lung function in chronic air-flow obstruction. *Am Rev Respir Dis* 1986; **134:** 276–280.

17. Jensen EJ, Dahl R. Cigarette smoke bronchial reactivity; repeatability, relation to methacholine bronchial reactivity , smoking history, asthma, and disposition to asthma and allergy. *Eur Respir J* 1998; **11:** 670–676.

18. Jarvis MJ, Russell MAH, Saluoje Y. Expired air carbon monoxid: A simple breath test of tobacco smoke intake. *Br Med J* 1980; **281:** 484–485.

19. Quanjer P, ed. Standardised lung function testing (report working party 'Standardization of lung function tests'). *Bull Eur Physiopathol Respir* 1983; **19**(Suppl. 5): 45–51.

20. Miller MR, Pincock AC. Predicted values: How should we use them. *Thorax* 1985; **43:** 265–267.

21. BMDP statistical software. University of California press. Los Angeles, 1990.

22. Hamosh P, Da Silva AMT. The effect on expiratory flow rates of smoking three cigarettes in rapid succession. *Chest* 1977; **72:** 610–613.

23. Higenbottam T, Hamilton D, Feyerabend C, Clark TJH. Acute effects of smoking a single cigarette on the airway resistance and the maximal and partial forced expiratory flow volume curves. *Br J Dis Chest* 1980; **74:** 37–46.

24. Melville N, Kumar M, Ismail S, Mills JL. Response of the central and peripheral airways to cigarette smoking in humans and rats. *Respiration* 1982; **43:** 29–34.

25. Da Silva AMT, Hamosh P.The immediate effect on lung function of smoking filtered and unfiltered cigarettes. *Am Rev Respir Dis* 1980; **122:** 792–797.

26. Costello JF, Douglas NJ, Sudlow MF, Flenley DC. Acute effects of smoking tobacco and a tobacco substitute on lung function in man. *Lancet* 1975; **2:** 678–681.

27. Rees PJ, Chowienczyk PJ, Clark TJH. Immediate response to cigarette smoke. *Thorax* 1982; **37:** 417–422.

28. Higenbottam T, Feyerabend C, Clark TJH. Cigarette smoke inhalation and the acute airway response. *Thorax* 1980; **35:** 246–254.

29. Da Silva AMT, Hamosh P. Airways response to inhaled tobacco smoke: Time course, dose dependence and effect of volume history. *Respiration* 1982; **41:** 96–105.

30. Bahous HA, Cartier A, Ouimet G, Pineau L, Malo J-L. Non-allergic bronchial hyper-excitability in chronic bronchitis. *Am Rev Respir Dis* 1984; **121:** 389–413.

31. Rubenfeld AR, Pain CF. Relationship between bronchial reactivity, airway caliber, and severity of asthma. *Am Rev Respir Dis* 1977; **115:** 3831–3837.

32. Cockcroft DW, Killian DN, Mellon JJA, Hargreave FE. Bronchial reactivity to inhaled histamine: A method and clinical survey. *Clin Allergy* 1977; **7:** 235–243.

33. Ulrik CS. Bronchial response to inhaled histamine in both adults with intrinsic and extrinsic asthma: The importance of prechallenge forced expiratory volume in one second. *J Allergy Clin Immunol* 1993; **91:** 120–126.

34. Jensen EJ, Pedersen B, Schmidt E, Dahl R. Serum IgE in nonatopic smokers, non-smokers, and recent ex-smokers:Relation to lung function, airway symptoms, and atopic predisposition. *J Allergy Clin Immunol* 1992; **90:** 224–229.