

Bronchial Challenge Tests in Patients with Asthma Sensitized to Cats

The Importance of Large Particles in the Immediate Response

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Our aim was to compare bronchial responses to major cat allergen (Fel d 1) in individuals with intermittent asthma sensitized to cats (19 subjects) according to the droplet particle size. We used three nebulizers, which delivered particles with mass median aerodynamic diameters of 1.4, 4.8, and 10.3 μm . A dosimeter nebulizer was used. The cat allergen was diluted to obtain the same amount of Fel d 1 per puff with each nebulizer. Each patient underwent three methacholine bronchial challenge tests (BCT), each followed 24 hours later by a cat allergen BCT, each performed with a different nebulizer (randomly selected each time, with patient and tester always blinded). Subjects did not differ for methacholine responsiveness, FEV₁, mean forced expiratory flow during the middle half of the FVC (FEF₂₅₋₇₅), PEF, or dyspnea (Borg scale) before any of the three cat BCTs. Cat allergen PD₂₀ was 271 ng of Fel d 1 with the 1.4 μm nebulizer, 46 ng with the 4.8 μm nebulizer, and 13.5 ng with the 10.3 μm nebulizer ($p = 0.00001$). Inhalation of small particles (1.4 μm) resulted in significantly lower FEF₂₅₋₇₅ 24 hours after provocation than large particles did. In conclusion, immediate bronchial response appears to be localized in large airways, and the use of large particles is more appropriate for cat allergen BCTs.

Keywords: bronchial challenge test; particle size; cat allergen; asthma

The particle size of droplets used during bronchial challenge tests (BCT) with allergen has not been substantiated since Tiffeneau (1), and current guidelines on BCT with allergen offer no recommendations concerning particle size (2, 3).

Patients appear to recognize the relation between exposure and symptoms more readily for cat than for mite allergens. It has been suggested that the rapidity of bronchial symptoms to cat allergen may be due to its high proportion of small particles (40% <5 μm), which can enter deeply into the lung (4). Moreover, recent studies show that bronchial inflammation is more severe in small than in large airways (5).

On the other hand, the dose of cat allergen required to induce symptoms during a standard BCT (with a nebulizer releasing particles ranging from 3 to 5 μm) has been reported to be 5 to 300 times higher than the doses needed to induce symptoms at home (6–8). One possible explanation among several hypotheses (e.g., during BCT, aqueous concentrated allergen extract is delivered, inhalation is only oral, and cofactors are absent) is the absence of large particles (10 μm or larger) during a standard BCT. In contrast, during domestic

exposure, cat allergen is inhaled on a wide range of particle sizes—39% on particles measuring 6 to 20 μm (9, 10).

To assess the bronchial effect of cat allergen according to the size of the particles nebulized, we used three different aerosols with mass median aerodynamic diameters of 1.4, 4.8, and 10.3 μm to compare bronchial response in individuals with intermittent asthma sensitized to cats.

METHODS

Measurement of Particle Size

Laser particle size analysis (MasterSizer X; Malvern Instruments Ltd, Worcestershire, UK) (11) was used to help select three air-driven nebulizers of different sizes. Cat extract (Stallergenes Laboratories, Antony, France) was nebulized, and particle size distribution was measured with a cascade impactor (Andersen 2000 Inc., Schaefer, Germany) (12). The cat major allergen Fel d 1 was measured with a two-site monoclonal antibody ELISA (Indoor Biotechnology, Charlottesville, USA) (10).

Patients

Inclusion criteria were a history of cat-allergic intermittent asthma with a positive skin prick test to cat allergen extract and specific IgE to cat allergen higher than Class 2 (13). None had cats at home or any contact with cats. Airborne Fel d 1 levels were measured at their homes as described previously (10, 14).

The Study Design

It was approved by the local ethics committee and involved a randomized, crossover, double-blinded protocol. Each patient underwent a cat BCT on three separate occasions, each with a different randomly selected nebulizer (Figure 1). Twenty-four hours before each cat BCT and 24 hours after each negative cat BCT, the patient underwent a methacholine BCT. The patients remained under medical supervision for 6 hours after each of the cat BCTs, which were performed 4 weeks apart. Results for the Borg scale for dyspnea and the pulmonary function tests were recorded before each cat BCT, at the end of BCT, and 3, 6, and 24 hours later. PEF was recorded twice a day for 1 week before and after each cat BCT.

Methacholine BCTs

They were performed with a nebulizer (Mediprom FDC 88, Paris, France) that administered doubling doses of a 1% methacholine solution, from 25 to 3,200 μg (cumulative), as described previously (13).

Cat BCTs

They were performed with an automatic inhalation-synchronized dosimeter jet-nebulizer (Spira Elektro 2, Hameenlinna, Finland), as described previously (13) with two modifications: the nebulization time was set at 0.8 seconds and each cat allergen (Stallergenes Laboratories, batch no. 90430; 100 Indice de Réactivité (IR); 8.2 μg Fel d 1/ml) was diluted to obtain the same amount of Fel d 1 per puff with each nebulizer. A separate flow meter controlled the patient's inspiratory flow rate. After baseline measurement of FEV₁, the subject inhaled, from residual volume (RV) to total lung capacity (2), a saline solution and then, every 15 minutes thereafter, a cat allergen solution, taking a 2-second breath hold after each inhaled puff. The solution contained

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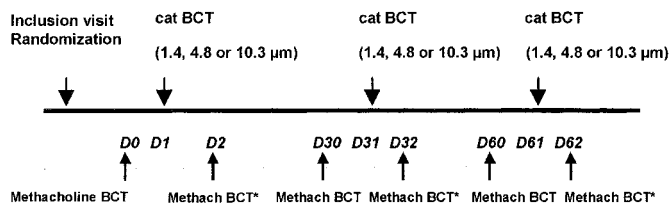


Figure 1. Study design. * A second methacholine bronchial challenge test (BCT) was performed 24 hours only after a negative cat BCT.

a progressively increasing allergen dilution (0.1 to 100 IR for 1.4 and 4.8 μm nebulizers and 0.05 to 50 IR for the 10.3 μm nebulizer because the puff weight was twice as heavy with it); the number of inhalations (2, 4, 8, 16) doubled, and the cumulative doses were increased (0.4 to a maximum of 666.6 ng of Fel d 1). The challenge stopped when the FEV₁ dropped by 20% (provocative dose for a 20% fall in FEV₁ [PD₂₀]) or more or when the full dose was administered without such a drop. At the end of the test, subjects inhaled β₂-agonists as necessary.

Statistical Analysis

STATsoft 5.1 was used. The pulmonary function test results for smokers and nonsmokers, as well as methacholine responsiveness before and after negative cat BCT, were compared with Student *t* tests. Univariate analysis of variance compared basal FEV₁, mean forced expiratory flow during the middle half of the FVC (FEF₂₅₋₇₅), PEF, dyspnea, and methacholine responsiveness before the three cat BCTs; it also compared FEV₁, FEF₂₅₋₇₅, and dyspnea results at different times and with each particle size (with Bonferroni corrections) (15). To analyze the PD₂₀ Fel d 1 data according to particle size, we performed an analysis of covariance on repeated values, after adjustment for prechallenge FEV₁. Analysis of variance compared differences in FEF₂₅₋₇₅ and dyspnea between two moments according to particle size. Multivariate analysis of variance for repeated values compared PEF before and after cat BCT with each particle size. The significance level was set at 5%. To estimate the cat allergen deposited in the respiratory tract at the end of each cat BCT, we used "DEVORE" software on the basis of a semi-empirical deposition model that has been validated for adults (16, 17).

RESULTS

Measurement of Particle Size

The particle actually delivered by each of the three air-driven nebulizers chosen (Microcirus and Cirrus; Intersurgical Laboratories, Fontenay-sous-bois, France and Pari Tia; Pari Aerosol Research Institute, Munich, Germany) was measured repeatedly by laser particle size analysis and with the cascade impactor (Table 1) (18). There was almost no overlap between the three size distributions. The mass median aerodynamic diameters ob-

tained with the cascade impactor were comparable with those measured with the laser particle size analysis.

Patients

Nineteen patients (11 women and 8 men, mean age 27 years) participated in the study, after giving written informed consent (Table 2). No detectable levels of airborne Fel d 1 were found in the homes of 15 patients, and the levels were close to the detection limit in the other 4. The median time from exposure to onset of cat-related symptoms at home was 30 minutes. Those sensitized to tree and grass pollen were studied out of pollen season. Smokers and nonsmokers did not differ significantly for any of the parameters measured (methacholine responsiveness, PEF, FEV₁, FEF₂₅₋₇₅, or dyspnea Borg scale); we note that none of the smokers smoked more than 5 cigarettes per day.

Dyspnea (Borg Scale), Pulmonary Functions, and Methacholine Challenge Test before the Cat BCTs

Table 2 reports preinclusion FEV₁ and FEF₂₅₋₇₅. The patients did not differ significantly in their dyspnea (Borg scale), FEV₁, FEF₂₅₋₇₅, or PEF before the three cat BCTs (*p* = 0.21, *p* = 0.55, *p* = 0.19, and *p* = 0.78, respectively). The methacholine PD₂₀ did not differ significantly before any of the three cat BCTs (*p* = 0.127) (Table 3).

Effect of Particle Size on Fel d 1 PD₂₀

PD₂₀ for cat allergen decreased from a geometric mean value of 271 ng of Fel d 1 with the 1.4 μm nebulizer to 46.0 ng with the 4.8 μm nebulizer and 13.5 ng with the 10.3 μm nebulizer (*p* = 0.00001; after adjustment for prechallenge FEV₁) (Table 3). This result was obtained for all patients, except Patient 5, whose PD₂₀ for Fel d 1 was the same with the 4.8 and 10.3 μm nebulizers but dramatically different with the smallest droplets (1.4 μm). All patients had a positive bronchial response to the 4.8 and 10.3 μm particles but six had a negative cat BCT with the 1.4 μm aerosol.

Effect of Particle Size on Estimated Deposition of Fel d 1 after Cat BCT

The 1.4 μm aerosol Fel d 1 mass was predominantly deposited in the alveolar airway (21.6%) (Table 4); this corresponds to 30 times more Fel d 1 there than with the 10.3 μm aerosol (58.5 vs. 1.7 ng Fel d 1). The mass of Fel d 1 deposited in the tracheo-bronchial airways to obtain a 20% drop in the FEV₁ was comparable with the three aerosols (i.e., 3.0 ng with the 1.4 μm, 4.4 ng with the 4.8 μm, and 1.5 ng with the 10.3 μm aerosol).

TABLE 1. PARTICLE SIZE OF DROPLETS PRODUCED BY NEBULIZERS, ACCORDING TO LASER PARTICLE SIZE ANALYSIS, AND CASCADE IMPACTOR ANALYSIS

Nebulizer	n	Laser Particle Size Analysis		n	Nine-Stage Cascade Impactor	
		MMAD (μm)	GSD		MMAD dry (μm)	MMAD Hydrated* (μm)
Microcirus	50	1.41	1.766	4	0.62	1.15
Cirrus	50	4.77	1.985	4	2.65	4.90
Pari Tia	50	10.26	2.072	4	7.2	13.3

Definition of abbreviations: GSD = geometric standard deviation; MMAD = mass median aerodynamic diameter.

The MMAD and GSD were measured on 10 separate occasions for each of 5 nebulizers from the same batch.

* Because the particle size measured by the cascade impactor was the aerodynamic diameter of dried particles, we used Knoch's formula (MMAD primary/MMAD dry = C primary^{-1/3}) to obtain the aerodynamic diameter of hydrated particles (18). The airflow pressure was adjusted to obtain the chosen MMAD (2.5 bars for Microcirus nebulizer and 1.2 bars for two others).

TABLE 2. CHARACTERISTICS OF STUDY SUBJECTS

No	Ages (yr)	Sex	Smoking Habit	Positive Skin Prick Tests to Common Aeroallergens	Specific IgE to Cat Allergen	Time until Onset of Symptoms Related to Cat (min)	Airborne Fel d 1 at Home (ng/m ³)	Preinclusion FEV ₁ (% of the predicted value)	Preinclusion FEF ₂₅₋₇₅ (% of the predicted value)
1	30	F	No	Dpt; Df; cat; dog	6	15	< 0.21*	92	52.5
2	38	M	No	Dpt; Df; cat; dog	3	30	< 0.21	98.1	76.3
3	27	M	No	Cat; dog	3	360	0.22	89.7	86.2
4	25	F	Five a day	Cat; dog	4	720	0.28	89.3	38.5
5	33	F	Five a day	Dpt; Df; cat; dog; grass pollens	3	30	< 0.21	102.6	78.1
6	49	F	No	Cat; tree pollens	3	30	< 0.21	105	87.9
7	22	M	No	Dpt; Df; cat; dog; grass pollens	2	30	0.31	114	95.9
8	21	F	No	Dpt; Df; cat; grass pollens	3	300	0.33	107.6	102.6
9	24	M	Five a day	Cat; grass and tree pollens	2	30	< 0.21	89.6	76.5
10	23	F	No	Dpt; Df; cat; dog; grass pollens	2	180	< 0.21	93.4	58.7
11	24	M	No	Dpt; Df; cat; grass and tree pollens	3	30	< 0.21	113.3	100
12	21	M	No	Dpt; Df; cat; dog; grass and tree pollens	3	45	< 0.21	102.7	76.4
13	21	F	No	Dpt; Df; cat; dog; grass and tree pollens	3	120	< 0.21	97.9	85.6
14	27	F	Five a day	Df; cat; grass and tree pollens	5	15	< 0.21	107.9	97.3
15	21	F	No	Dpt; Df; cat; dog; grass and tree pollens	3	30	< 0.21	106.9	97.7
16	27	F	No	Dpt; Df; cat; dog; grass and tree pollens	3	15	< 0.21	100.3	75.5
17	20	M	Five a day	Dpt; cat; dog; grass and tree pollens	3	20	< 0.21	106.8	52.2
18	23	F	No	Cat; dog; grass and tree pollens	3	60	< 0.21	97.9	96.1
19	36	M	No	Dpt; Df; cat	3	30	< 0.21	74	40.2
Mean	27				3	110	0.14	96.25	77.6
Median	24				3	30	< 0.21	98.5	78.1

Definition of abbreviations: Df = *Dermatophagoides farinae*; Dpt = *Dermatophagoides pteronyssinus*; F = female; M = male.

* 0.21 ng/m³ was the detection limit for airborne Fel d 1 concentrations, obtained for an air sample of 8 h at an airflow at 2 L/min. The mean and the median values were calculated considering the nondetectable values to be half of the detection limit value.

Effect of Particle Size on Immediate Bronchial Response to Cat Allergen

Because 6 of the 19 patients had no immediate bronchial response to the 1.4 μm particles, the FEV₁ and FEF₂₅₋₇₅ were closer to their predicted value at the end of the cat BCT for the 1.4 μm particles than for the other particle sizes ($p = 0.019$ for FEV₁ and $p = 0.0009$ for FEF₂₅₋₇₅) (Figures 2A and 2B); this was also the case for dyspnea ($p = 0.013$) (Figure 2C).

Effect of Particle Size on Late Bronchial Response to Cat Allergen

At 6 hours, the frequency of a late 15% drop in FEV₁ did not differ significantly for any of the three particle sizes (i.e., 2 after 1.4 μm , 4 after 4.8 μm , and 1 after 10.3 μm aerosol). FEV₁ values did not differ significantly by particle size at 3, 6, or 24 hours after the end of the cat BCT (Figure 2A). FEF₂₅₋₇₅ did not differ according to particle size at 6 hours ($p = 0.919$); after 24 hours, however, it was significantly lower with the 1.4 μm particles ($p = 0.006$) (Figure 2B). In addition, its difference between before and 24 hours after the cat BCT was significantly greater with the 1.4 μm aerosol than with the others (means of 8.6% [95% confidence interval: 3.7; 13.7]; -1.2% [-5.4 ; 3] and -3.4% [-7.9 ; 1.1] with 1.4, 4.8, and 10.3 μm particles, respectively; $p = 0.003$) (Figure 2B). Six hours after the end of the BCT, Borg

scale values for dyspnea were significantly higher with the 1.4 μm aerosol than with the other two ($p = 0.02$) (Figure 2C). Moreover, the difference between these Borg values before and 6 hours after cat BCT also differed according to particle size (means of 0.3 cm [95% confidence interval: 0; 0.6]; 0.15 cm [-0.22 ; 0.52] and -0.6 cm [-1.06 ; -0.14] with 1.4, 4.8, and 10.3 μm particles, respectively; $p = 0.007$). The 6 patients with negative cat BCTs (with the 1.4 μm aerosol) underwent methacholine challenge tests the next day: methacholine responsiveness did not differ from that measured the day before the cat BCT ($p = 0.25$; Student's *t* test). The mean PEF values measured during the week after the cat BCT with all three nebulizers did not differ significantly from those recorded the week before. Moreover, the aerosol particle size had no effect on the PEF response during the week after the BCT ($p = 0.46$).

DISCUSSION

Our study has demonstrated that individuals with intermittent asthma sensitized to cats developed an immediate bronchial response with dramatically smaller amounts of major cat allergen (Fel d 1) when it was inhaled on large (10.3 μm mass median aerodynamic diameter) compared with smaller (1.4 and 4.8 μm mass median aerodynamic diameter) particles.

To ensure that particle size was the only difference between

TABLE 3. METHACHOLINE PD₂₀ AND FEL D 1 PD₂₀ FOR THE 19 PATIENTS OBTAINED 24 HOURS BEFORE AND DURING EACH BRONCHIAL CHALLENGE TESTS WITH CAT ALLERGEN IN 19 PATIENTS

Subject No.	Methacholine before 1.4 μ m Cat BCT	1.4 μ m Cat BCT	Methacholine before 4.8 μ m Cat BCT	4.8 μ m Cat BCT	Methacholine before 10.3 μ m Cat BCT	10.3 μ m Cat BCT
1	25	52.5	Nd	Nd	25	1.55
2	300	605.2	600	74	400	34.2
3	100	666.6*	200	419.6	300	117
4	25	450	400	88	25	6.4
5	300	240	300	15.36	1,400	26.6
6	150	666.6	600	92.2	300	33.32
7	300	666	100	34	100	18
8	25	666.6*	25	104	25	37.5
9	200	54.56	600	18.88	1,200	9.36
10	400	666.6*	100	62	100	18
11	400	666.6*	1,600	61.52	2,000	19
12	25	666.6*	25	261.2	25	37
13	75	499.6	300	53.52	800	23.25
14	200	666	200	64.4	200	15.76
15	50	105.2	50	15.92	25	5.58
16	75	666.6*	50	62.16	50	13.68
17	400	192	25	31.44	100	5.68
18	100	179.6	400	15.92	100	4.4
19	25	1.98	25	1.89	25	1.625
GM	106	271 [†]	152	46 [‡]	133	13.5
95% CI	43–169	152–390	0–329	0–93	0–387	1.5–25.5

Definition of abbreviations: GM = geometric mean; CI = confidence interval; Nd = not done; PD₂₀ = provocative dose for a 20% fall in FEV₁.

* The cat BCT was negative for immediate bronchial response; we considered the highest value of Fel d 1 dose given as the theoretical value for a negative BCT.

[†] The cat allergen PD₂₀ differed with the 1.4 μ m aerosol compared with the 4.8 μ m aerosol ($p < 0.00001$) and with the 10.3 μ m aerosol ($p < 0.00001$).

[‡] The cat allergen PD₂₀ differed with the 4.8 μ m aerosol compared with the 10.3 μ m aerosol ($p < 0.006$).

the three BCTs, we used two different methods described in the European Norm project for nebulizing systems (19)—cascade impaction and laser diffraction—to check the particle size of the nebulizers. The mass median aerodynamic diameters were comparable with both methods, and there was limited overlap between the three particle size distributions. Accordingly, we obtained three different patterns of deposition in the respiratory tract.

We also verified that the inhalation method was reproducible. We performed BCT with an inhalation dosimeter, using a quantitative method, so that we could measure the PD (3). Because the breathing method is also known to be important for bronchial particle deposition (20), we trained our patients to standardize their breathing method to avoid any such bias: they inhaled cat

allergen from RV to total lung capacity, with a 2-second breath hold after each inhaled puff. The inhalation dosimeter had a control system for inspiration flow (set at 0.5 l/second) and for puff duration (0.8 seconds). Before the cat BCTs, all the patients were comparable for methacholine PD₂₀, dyspnea (Borg scale), FEV₁, FEF_{25–75}, and PEF.

We showed that the dose of Fel d 1 provoking immediate bronchial symptoms was 20 times smaller when the allergen was carried on 10.3 μ m particles than on 1.4 μ m particles. With the DEVORE software, we estimated that 12.4% of the 10.3 μ m aerosol Fel d 1 mass was deposited in the distal airways. The small airways do not, however, seem to be the major site for immediate bronchial response to cat allergen: 30 times more

TABLE 4. ESTIMATED DEPOSITION OF THE MASS OF MAJOR CAT ALLERGEN (FEL D 1) INHALED SOLUTION WITH THE THREE NEBULIZERS IN DIFFERENT PORTIONS OF THE LUNG, ACCORDING TO THE “DEVORE” SOFTWARE, WHICH IS BASED ON THE STAHLHOFEN MODEL (16, 17)

	Small Droplets		Medium Droplets		Large Droplets	
Nebulizer MMAD, μ m	1.4		4.8		10.3	
Total mass inhalation of Fel d 1, ng*	271		46		13.5	
	(%)	(ng)	(%)	(ng)	(%)	(ng)
Total mass deposition of Fel d 1	24.5	66.4	60.3	27.7	79.0	10.7
Larynx deposition	1.8	4.9	25.9	11.9	55.1	7.4
Tracheobronchial deposition	1.1	3.0	9.5	4.4	11.5	1.5
Alveolar deposition	21.6	58.5	24.9	11.4	12.4	1.7

Definition of abbreviation: MMAD = mass median aerodynamic diameter.

This estimation was performed for 1 mg of inhaled aerosol, with a V_T of 500 ml and a respiratory frequency of 14 breaths per minute.

* The total mass of Fel d 1 inhaled corresponds to the geometric mean of the Fel d 1 PD₂₀ (ng).

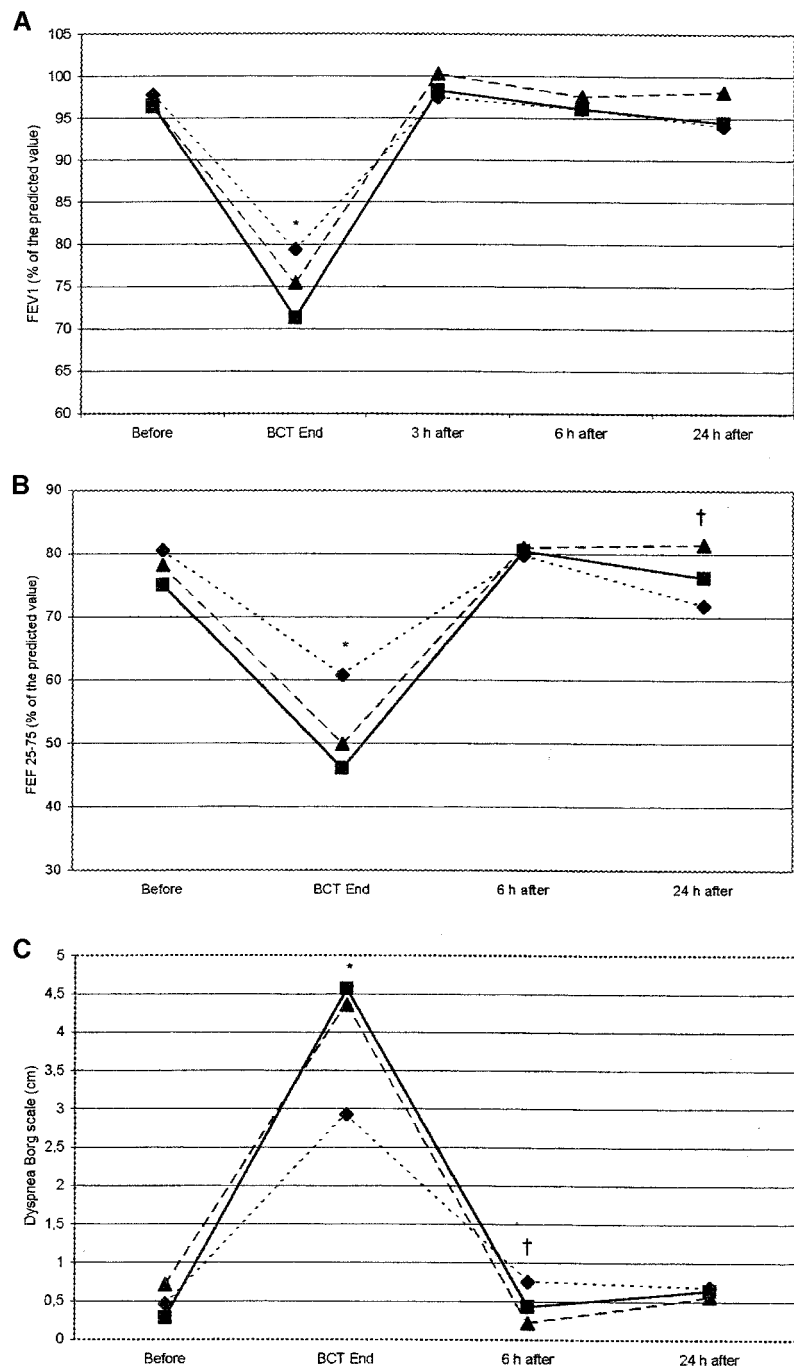


Figure 2. Pulmonary function and dyspnea (Borg scale) results according to particle sizes of 1.4 μm (diamonds, dotted lines), 4.8 μm (squares, solid lines), and 10.3 μm (triangles, dashed lines). (A) FEV₁ results before each cat BCT, at the end of the test, at 3, 6, and 24 hours. *At the end of the test, FEV₁ values differed according to particle size ($p = 0.019$). At 3, 6, and 24 hours after cat BCT, FEV₁ values did not differ significantly according to particle size ($p = 0.13$, $p = 0.45$, and $p = 0.07$, respectively). Mean within-subject coefficient of variation (CV) for FEV₁ was 2.58% (confidence interval 1.98–3.18, range 1–6%). (B) FEF₂₅₋₇₅ results before each cat BCT, at the end of the test, at 6, and 24 hours. *At the end of the test, FEF₂₅₋₇₅ values differed according to particle size ($p = 0.0009$). Six hours after cat BCT, FEF₂₅₋₇₅ did not differ according to particle size (means of 79.8% with 1.4 μm , 80.5% with 4.8 μm , and 81.0% with 10.3 μm aerosol; $p = 0.919$). †Twenty-four hours later, FEF₂₅₋₇₅ decreased significantly more with small particles (means of 71.9% with 1.4 μm , 76.3% with 4.8 μm , and 81.5% with 10.3 μm aerosol; $p = 0.006$). Mean within-subject CV for FEF₂₅₋₇₅ was 6% (confidence interval 4.52–6.68, range 2.7–12%). (C) Dyspnea (Borg scale) results before each cat BCT, at the end of the test, at 6, and 24 hours. At the end of the test, dyspnea was less important with the 1.4 μm aerosol (2.9 cm with 1.4 μm , 4.5 cm with 4.8 μm , and 4.4 cm with 10.3 μm aerosol; $p = 0.013$). †Six hours after cat BCT, dyspnea differed according to particle size (0.8 cm with 1.4 μm , 0.4 cm with 4.8 μm , and 0.1 cm with 10.3 μm aerosol; $p = 0.02$). Twenty-four hours later, it no longer differed by particle size (0.68 cm with 1 μm , 0.63 cm with 5 μm , and 0.56 cm with 10 μm aerosol; $p = 0.861$). Mean within-subject CV for dyspnea (Borg scale) was 6.65% (confidence interval 1.63–11.7, range 0–33%).

Fel d 1 was deposited there with the 1.4 μm aerosol than with the 10.3 μm aerosol at the end of cat BCT, and six patients had no significant immediate bronchial response to the 1.4 μm aerosol. In contrast, all patients responded immediately to the 4.8 and 10.3 μm doses. Accordingly, we considered that the dose that induced an immediate bronchial response in our patients appears to be the dose deposited in the proximal airways. According to DEVORE software, the dose of Fel d 1 deposited in proximal airways necessary to induce an immediate bronchial reaction varied from 1.5 to 4.4 ng according to particle size. The role of central airways in the reversal of bronchoconstriction was also found by Usmani and colleagues (21): inhalation of salbutamol, the reference treatment for reversing immediate bronchial response to specific and nonspecific agents, was more

effective with the larger particles (3 μm), producing greater clinical response and improvement in FEV₁ than did the small particles (1.5 μm).

Moreover, the Fel d 1 PD₂₀ of the cat BCT with the large particles seemed closest to the levels of airborne Fel d 1 that induce symptoms at home. Mean airborne exposure to cat allergen in 50 homes with a cat in Strasbourg has been measured at 14 ng Fel d 1/m³ (22), and among our 19 subjects, the median time to onset of developing bronchial symptoms was 30 minutes in the presence of a cat: the PD inducing asthma in real life was 4.2 ng of Fel d 1. This differed dramatically from previous results in which the dose of cat allergen inducing symptoms during BCT with 5 μm particles was 5 to 300 times higher (6, 7). It thus appears to us that large particles are very important in the occur-

rence of immediate bronchial response. BCT with a 10.4 μm aerosol carrying cat allergen corresponds better to exposure in daily life than BCT with smaller particles.

Late asthmatic responses assessed with FEV_1 were not more frequent with any particle size and no isolated late asthmatic response was observed. The results of the Borg scale for dyspnea at 6 hours and of FEF_{25-75} at 24 hours indicate, however, that small particles did induce late symptoms and decreased peripheral airway caliber. The six patients with a negative BCT with the 1.4 μm aerosol did not have any change in FEV_1 at 6 and 24 hours, and all had decreased FEF_{25-75} at 24 hours (compared with baseline). Although the increase in the Borg scale for dyspnea at 6 hours was relatively slight (7%) with small particles, it may be clinically relevant: the coefficient of variation of the visual analog scale measurement of dyspnea has been reported to be 6% (23, 24), close to our coefficient of variation on dyspnea scale. Because late asthmatic response is known to be mediated by eosinophils, our results are consistent with those of Hamid and coworkers, who reported more activated eosinophils in the small than in the large airways (5); these eosinophils may induce a late inflammatory process in small airways. Moreover, inhalation challenge of guinea pigs (25) with a small aerosol (1.6 μm) of diphenyl-methane-4,4'-diisocyanate provoked greater recruitment of eosinophilic granulocytes in bronchial tissue taken 24 hours after the challenge than did a similar challenge with large-sized (5.1 μm) diphenyl-methane-4,4'-diisocyanate aerosol.

In conclusion, our results demonstrated that, although inhalation of aqueous allergen extract for a short period of time during BCT is artificial compared with natural exposure, particle size seems to be important: the Fel d 1 PD_{20} obtained in BCT with a 10.3 μm aerosol carrying cat allergens approaches most closely the PD inducing asthma in real life. This suggests that immediate bronchial response to cat allergen is localized in the large airways. Consequently, immediate bronchial reaction in patients allergic to cats may be studied most usefully with BCT with large particles.

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References

1. Tiffeneau R. Les aérosols pharmacodynamiques. *Progr Med (Paris)* 1946; 19:1-12.
2. Sterck PJ, Fabbri LM, Quanjer PH, Cockcroft DW, O'Byrne PM, Anderson SD, Juniper EF, Malo JL. Airway responsiveness: standardized challenge testing with pharmacological, physical and sensitizing stimuli in adults. *Eur Respir J* 1993;6:53-83.
3. Melillo G, Bonini S, Cocco G, Davies RJ, de Monchy J, Frolund L, Pelikan Z. Provocation tests with allergens. *Allergy* 1997;52:5-36.
4. Platts-Mills T, Heymann P, Longbottom J, Wilkins S. Airborne allergens associated with asthma: particles sizes carrying dust mite and rat allergens measured with a cascade impactor. *J Allergy Clin Immunol* 1986; 77:850-857.
5. Hamid Q, Song Y, Kotsimbos T, Minshall E, Bai T, Hegele R, Hogg J. Respiratory pathophysiologic responses: inflammation of small airways in asthma. *J Allergy Clin Immunol* 1997;100:44-51.
6. Van Metre T, Marsh D, Adkinson N, Fish J, Kagey-Sobotka A, Norman P, Radden E, Rosenberg G. Dose of cat (*Felis domesticus*) allergen 1 (*Fel d 1*) that induces asthma. *J Allergy Clin Immunol* 1986;78:62-75.
7. Lewis J, Wentz P, Swanson M, Reed C. Immunochemical assay of airborne cat allergen: value in assessing variability of allergen shedding and evaluating human bronchial response. *Aerobiologia* 1990;6:193-196.
8. de Blay F, Kopferschmitt MC, Griess P, Frey F, Linder J, Mahr L, Vérot A, Pauli G. Comparison between *Fel d 1* levels provoking respiratory symptoms at home and during bronchial challenge tests. *J Allergy Clin Immunol* 1995;95:A262.
9. Luczynska CM, Li Y, Chapman MD, Platts-Mills T. Airborne concentrations and particle size distribution of allergen derived from domestic cats (*Felis domesticus*). *Am Rev Respir Dis* 1990;141:361-367.
10. de Blay F, Chapman M, Platts-Mills T. Airborne cat allergen (*Fel d 1*): environmental control with the cat *in situ*. *Am Rev Respir Dis* 1991; 143:1334-1339.
11. Merkus HG, Marijnissen J, Jansma EHL, Scarlett B. Droplet size distribution measurements for medical nebulizers by the forward light scattering technique ("Laser diffraction"). *J Aerosol Sci* 1994;25:S319-S320.
12. Vaughan NP. The Andersen impactor: calibration, wall losses and numerical simulation. *J Aerosol Sci* 1989;20:67-90.
13. de Blay F, Krieger P, Spirlet F, Moreau L, Duvernelle C, Kassel O, Kopferschmitt MC, Gasser B, Demangeat C, Pauli G, et al. Repeated inhalation of low doses of cat allergen that do not induce clinical symptoms increases bronchial hyperresponsiveness and eosinophil cationic protein levels. *Int Arch Allergy Immunol* 1999;120:158-165.
14. de Blay F, Sanchez J, Hedelin G, Perez-Infante A, Vérot A, Chapman M, Pauli G. Dust and airborne exposure to allergens derived from cockroach (*Blattella germanica*) in low-cost public housing in Strasbourg (France). *J Allergy Clin Immunol* 1997;99:107-112.
15. Senn S, Stevens L, Chaturvedi N. Repeated measures in clinical trials: simple strategies for analysis using summary measures. *Stat Med* 2000;19:861-877.
16. Fabrière JF. Calculation of the physical characteristics of deposited particles in the respiratory airways. *J Aerosol Med* 1993;6:A22.
17. Heyder J, Gebhart J, Rudolf G, Schiller F, Stahlhofen W. Deposition of particles in the human respiratory tract in the size range 0.005-15 μm . *J Aerosol Sci* 1986;17:811-825.
18. Knoch M, Wunderlich E, Roth C, Gebhart J. Output characterization of medical nebulizers. *J Aerosol Sci* 1994;25:S439-S440.
19. European Committee for Standardization. Respiratory therapy equipment. Part 1: nebulizing systems and their components. Lisbon: European Committee for Standardization; 1999. Pr EN 13544-1.
20. Ryan G, Dolovich M, Obminski G, Cockcroft D, Juniper E, Hargreave FE, Newhouse MT. Standardization of inhalation provocation tests: influence of nebulizer output, particle size, and method of inhalation. *J Allergy Clin Immunol* 1981;67:156-161.
21. Usmani OS, Barnes P, Biddiscombe M. Effects of beta2-agonists as a function of particle size. *J Aerosol Med* 2001;14:382.
22. Casel S, Lieutier F, Spirlet F, Gries P, Ott M, Pauli G, de Blay F. Comparison of airborne *Fel d 1* levels in dwellings of atopic, non-atopic and cat-allergic patients with cats and in houses without cats. *J Allergy Clin Immunol* 1999;103:A30s.
23. Mador J, Kufel T. Reproducibility of visual analog scale measurements of dyspnea in patients with chronic obstructive pulmonary disease. *Am Rev Respir Dis* 1992;146:82-87.
24. Mador J, Rodis A, Magalang U. Reproducibility of Borg scale measurements of dyspnea during exercise in patients with COPD. *Chest* 1995; 107:1590-1597.
25. Pauluhn J, Thiel A, Emura M, Mohr U. Respiratory sensitization to diphenyl-methane-4,4'-diisocyanate (MDI) in guinea pigs: impact of particle size on induction and elicitation of response. *Toxicol Sci* 2000; 56:105-113.