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Pollen starch granules in bronchial inflammation

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ARTICLE INFO

Article history:

Received for publication April 17, 2012.

Received in revised form June 5, 2012.

Accepted for publication June 24, 2012.

ABSTRACT

Background: Pollen grains with a diameter of more than 10 μm preferentially deposit in the upper airways. Their contribution to lower airway inflammation is unclear. One hypothesis is that lower airway inflammation is mainly caused by allergen containing pollen starch granules, which are released from the pollen grains and can easily enter the peripheral airways because of their smaller size.

Objective: To investigate the differential effect of pollen grains and pollen starch granules on nasal symptoms and lower airway inflammation.

Methods: In a 2-period crossover design, 30 patients with allergic rhinitis and mild intermittent asthma underwent 2 allergen challenges on consecutive days in an environmental challenge chamber with either a mixture of pollen grains plus starch granules or starch granules only. End points were the total nasal symptom score (TNSS), nasal secretion weight, nasal flow, spirometry, and exhaled nitric oxide (eNO).

Results: The presence of pollen grains had a significant and considerable effect on increase in TNSS and secretion weight and on decrease in nasal flow. Starch granules alone only had minimal effects on nasal symptoms. Challenges with starch granules significantly increased eNO. Pollen had no effect on eNO.

Conclusion: Pollen grains cause nasal symptoms but do not augment lower airway inflammation, whereas starch granules trigger lower airway inflammation but hardly induce nasal symptoms.

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Introduction

Allergic asthma is a serious global health problem that affects people of all ages throughout the world.¹ Airborne allergens such as pollen are one of the main causes of allergic asthma during the flowering season.^{2–4} However, it is not clear how pollen grains are able to cause lower airway inflammation and therefore asthma because pollen grains with a diameter of more than 10 μm preferentially deposit in the upper airways.^{5,6}

One hypothesis is that lower airway inflammation is not caused by whole pollen grains but by pollen starch granules, which are contained in large numbers within the pollen grain. They have a size of no more than 5 μm and therefore deposit preferentially in the lower airways. Starch granules contain several major pollen allergens,^{7,8} and several studies have found immunologic reactivity of starch granules in allergic patients.^{3,9,10} In addition to starch

granules, other allergen-containing pauci-micronic particles have been suggested to induce lower airway symptoms.^{11,12}

Starch granules are expelled from the pollen grains after coming into contact with a hypotonic liquid, such as rainwater or even morning dew.^{13–15} They then form an inhalable aerosol, which might be the actual trigger for asthma. This is consistent with the epidemiologic observation of a significant increase of asthma exacerbations after thunderstorms and heavy rainfalls.^{16–20}

Until now no study has directly compared the effect of starch granules and pollen grains on nasal symptoms and bronchial inflammation in allergic patients. Our aim was to do so by using an environmental challenge chamber (ECC). ECCs are used throughout the world to expose allergic patients to relevant allergens such as pollen and to test antiallergic treatments.^{21–29} The advantage of an ECC lies in the ability to control environmental conditions, such as temperature, humidity, and allergen concentration. This reduces the variability of symptoms that is unavoidable under natural conditions and thus provides powerful results with small numbers of patients.

In the ECC at the Fraunhofer Institute in Hannover, Germany, 2 different atmospheres of either a mixture of pollen grains (20–40 μm) plus starch granules (<5 μm) or starch granules alone were established. Although it is technically not possible to create an

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Disclosures: Authors have nothing to disclose.

Funding Sources: The study was funded by the Fraunhofer Institute for Toxicology and Experimental Medicine and GlaxoSmithKline.

atmosphere of pollen only, it nevertheless allows investigation and comparison of the effect of these 2 atmospheres on nasal symptoms and bronchial inflammation. Our hypothesis is that starch granules but not pollen grains induce lower airway inflammation and therefore are of major importance for the induction and augmentation of allergic asthma.

Methods

Patients

Thirty adults (20–51 years of age) were enrolled in the study. Inclusion criteria were as follows: history of respiratory physician-diagnosed intermittent allergic asthma due to grass pollen, which is treated with short-acting β_2 -agonists only, allergic rhinitis due to grass pollen, positive skin prick test result to *Dactylis glomerata* within 12 months of enrolment, forced expiratory volume in 1 second (FEV₁) more than 80% predicted, and smoking or nonsmoking with a history of less than 10 pack-years. Exclusion criteria were as follows: respiratory tract infection or exacerbation of asthma within 4 weeks of study entry, history of persistent asthma, pregnancy, breastfeeding, immunotherapy, and participation in another clinical trial within 30 days of study enrollment.

The washout periods for drugs before screening were 4 weeks for inhaled and 8 weeks for oral, intravenous, or dermal steroids and 1 week for cromolyn, antihistamines, antirhinitis, antiasthma (other than short-acting β_2 -agonists), or any other over-the-counter medication used for treatment of hay fever.

The study was conducted at the Fraunhofer ECC in Hannover, Germany.³⁰ Each patient gave written informed consent before study participation. The study was approved by the ethics committee of the Medical School of Hannover. It was conducted in accordance with the Declaration of Helsinki (Somerset West Amendment, 1996) and the International Conference on Harmonisation Guideline on Good Clinical Practice (Note for Guidance on Good Clinical Practice [CPMP/ICH/135/95], January 17, 1997).

Study design

We performed a randomized, double-blind, 2-way crossover study in which 30 adult patients with seasonal allergic rhinitis and intermittent allergic asthma underwent 2 periods of two 4-hour challenges on 2 consecutive days with either a mixture of pollen plus starch granules or starch granules alone (*D glomerata*, Biopol Laboratory Inc, Spokane, Washington). The 2 periods were separated by at least 19 days. (A graph of the study design is shown in eFigure 1.) The order of challenge with the 2 different atmospheres was randomized with only the aerosol technician knowing the predefined randomization code. The study was performed out of the pollen season.

End points of this study were the differences in nasal symptoms, nasal flow, nasal secretion, lung function (FEV₁), and exhaled nitric oxide (eNO) between the 2 allergen atmospheres. Physical and nasal examinations, clinical laboratory tests, vital signs, and pregnancy tests for female patients were conducted at the screening visit. Vital signs measurements were also conducted before each allergen challenge. At each visit, patients were asked by the study physician whether they had experienced any adverse events or deterioration in their well-being or medical condition since the start of the study. Any use of concomitant medication was recorded.

Generation and monitoring of pollen grains and starch granules

The *D glomerata* pollen grains were obtained from Biopol Laboratory Inc. The pollen grains are manufactured according to the guidelines of good manufacturing practice, and Biopol is regularly inspected by the Food and Drug Administration.

The mixture of pollen grains and starch granules were generated using a method previously described.³⁰ The method is based on dispersing native pollen grains using a Ventury-type system. The resulting aerosol is bimodal, consisting of a pollen grain mode, 30 μ m in size, and a debris and/or starch granule mode of particulates with diameters smaller than 5 μ m. The smaller particles are partly present in the original powder but may also be generated in the high-pressure dispersion nozzle. This aerosol is fed into the exposure room via 4 tubes at the ceiling.

For the starch granules only atmosphere, pollen grains are separated from the aerosol by placing an impaction unit directly beneath the tubes. The impaction system is a flat cup filled with an organic liquid of extremely low vapor pressure (di-ethyle-hexyls-sebacate, Sigma-Aldrich, St Louis, Missouri; vapor pressure, <1 Pa at 20°C). The pollen grains are immediately wettened on impaction on the liquid surface and are subsequently irreversibly incorporated in the liquid.

The ratio of total starch counts to pollen counts was 1.67:0.15. These data are based on measurements taken during a period of 1 hour before and after the challenge session on the days when pollen and starch granules were used simultaneously. (In eFigure 2 a representative number size distribution of the aerosol particles in the exposure room is shown.) During the sessions, the measurement of the small particle mode is impaired because of the presence of particles released by the patients (eg, particles that are liberated from the patients' clothing and skin), but the concentration of the pollen grains was closely monitored by the method previously described.³⁰ The mean (SD) concentrations were 4,760 (429) m³ for the pollen grains and 7,814 (660) m³ for the starch granule (determined from the pre-session and post-session measurements at all exposure days).

Study assessments

Nasal symptoms. Nasal symptoms (obstruction, rhinorrhea, sneezing, and itching) were self-assessed before and every 20 minutes during the allergen challenge in the ECC. For each symptom the patients recorded scores on a diary sheet according to the following 4-point severity scale: 0, none; 1, mild; 2, moderate; and 3, severe. The total nasal symptom score (TNSS) was calculated by adding the scores of the 4 individual nasal symptoms.

Nasal flow. The nasal flow rate was measured by rhinomanometry (Viasys Healthcare, Höchberg, Germany) before and every 60 minutes during challenge. Rhinomanometry is an active anterior measuring technique using a face mask. Inspiratory flow rates at 150 Pa were obtained separately for the right and left nostril. The sum of the flow rates of both nostrils was calculated.

Nasal secretion. Nasal secretion was collected using preweighed paper tissues. Used and unused tissues were saved in a plastic bag by each patient and collected every 60 minutes. The amount of nasal secretion was determined by weighing these tissues. The patients were provided with a new package of tissues for each hour in challenge.

Lung function (FEV₁). Lung function (FEV₁) was measured using a portable electronic asthma monitor (AM-1; Viasys Healthcare) at the screening visit and before and every 60 minutes during allergen challenge. The patients continued to measure their lung function at home every 2 hours until 10 hours after challenge to register any late-phase reaction. They received a salbutamol aerosol as rescue medication in case of a decrease in FEV₁ greater than 20%.

Exhaled nitric oxide. The eNO was measured as a surrogate marker for allergic asthma. It was measured before the first of the 2 consecutive challenges, before the second one, and 24 hours after the second challenge, using the Niox (Aerocrine, Stockholm, Sweden) device according to the manufacturer's instructions.

Table 1
Characteristics of the study patients

Characteristic	Finding (N = 30)
Sex, No. (%)	
Male	10 (33.3)
Female	20 (66.7)
Age, y	
Mean (SD)	27 (7)
Range	20–51
Body weight, kg	
Mean (SD)	72 (15)
Range	53–117

Statistical analysis

The end points in this 2-treatment, 2-period crossover design was the change of nasal symptoms and lower airway inflammation between baseline and the 24-hour measurement after challenge.

The primary one-sided null hypotheses to be tested were HoA: $\mu_A \leq 0$ and HoB: $\mu_B \leq 0$, where μ_A and μ_B denote the expected means of the changes (for the challenge with [a] pollen plus starch granules and [b] starch granules alone, respectively).

A linear mixed model was applied with the type of challenge and the period number as fixed effects and the patient as random effect. Carryover could be excluded because of nonsignificant test results ($P > .10$).

The hypotheses were tested as linear contrasts within the mixed model. The significance level was $\alpha = .05$. No α correction was performed for the analysis of the 2 separate hypotheses.

Comparisons of the changes μ_A and μ_B after challenge were performed within the framework of the crossover design as stated. In addition, a previous test of no interaction effects between type of challenge and period (carryover effects) was performed to justify the general comparison of the challenge effects μ_A and μ_B . Thirty patients were used in this explorative study because of feasibility reasons.

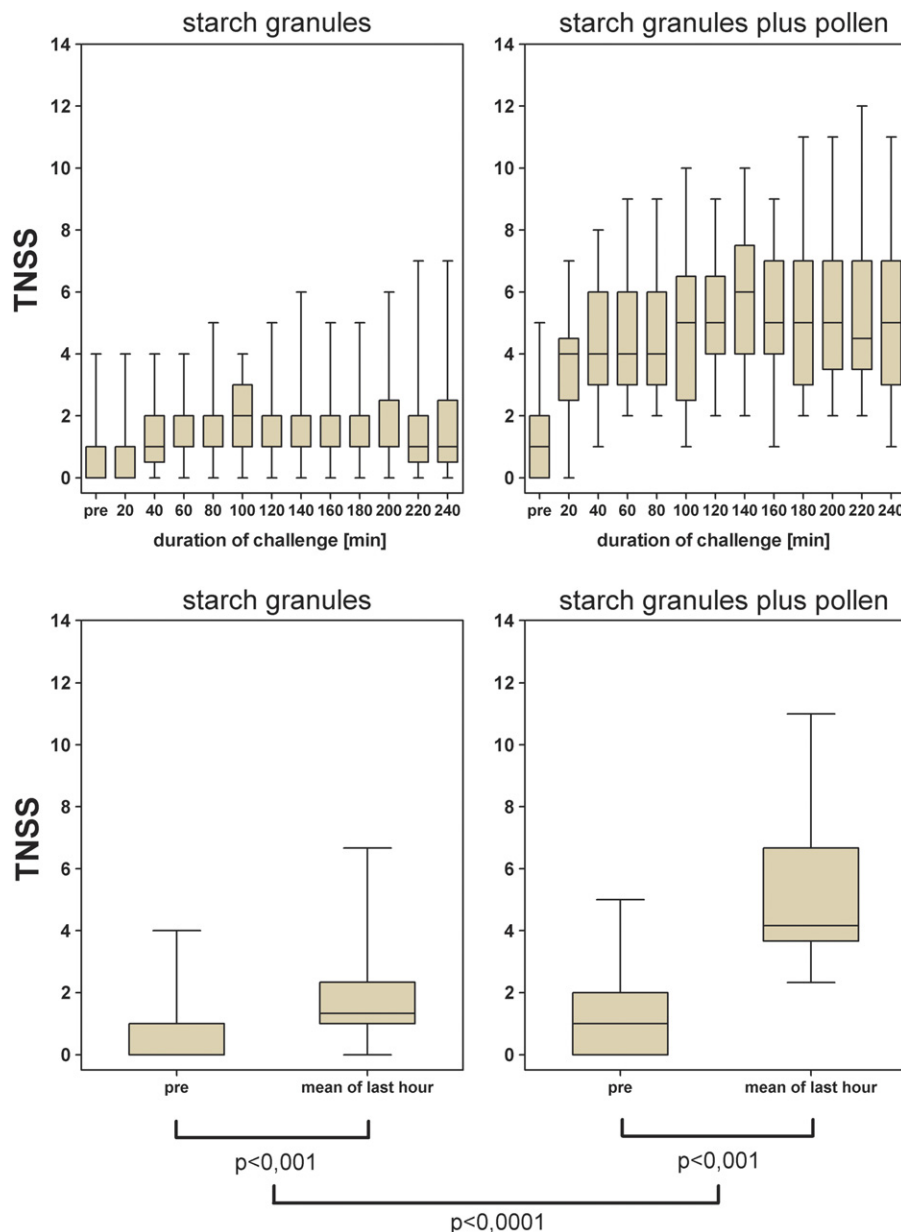


Fig. 1. Top, Total nasal symptom score (TNSS) during 4 hours of challenge with either starch granules (left) or starch granules plus pollen (right). Bottom, The prechallenge TNSS and the mean TNSS during the last hour of challenge. Data are displayed in a box-and-whiskers graph. The box extends from the 25th percentile to the 75th percentile, with a line at the median. The whiskers extend to the highest and lowest value.

Results

Study patients

Thirty patients with mild intermittent asthma and seasonal allergic rhinitis were randomized (see Table 1 for patient characteristics). During the study, 29 adverse events were observed in 13 patients. The most frequent was headache (12/29). The events resolved by the end of the study. No serious adverse events occurred. Two participants dropped out of the study: 1 because of a respiratory tract infection and 1 withdrew his consent. The remaining 28 patients completed the study per protocol. The data of the intent-to-treat group were analyzed.

Period effect and carryover effect

No period and no carryover effect were observed. The order of challenge with the 2 different atmospheres did not influence the study results, indicating that the washout period of 19 days between the 2 periods was sufficient.

Efficacy results

Because the efficacy results for nasal symptoms, nasal flow, nasal secretion, and FEV₁ of the first and second challenges of each period are almost identical, only the results of the second challenge days are presented. The results of the first challenge are presented in eFigures 3 through 6. The results for NO are presented for both challenge days.

Nasal symptoms

Challenges with starch granules alone and starch granules plus pollen both increased nasal symptoms significantly (Fig 1, $P < .001$). However, in the presence of pollen grains, nasal symptoms were significantly higher than with starch granules alone (Fig 1, $P < .001$).

Nasal flow

Challenges with starch granules alone and starch granules plus pollen both reduced nasal flow significantly (Fig 2, $P = .03$ and $P < .001$).

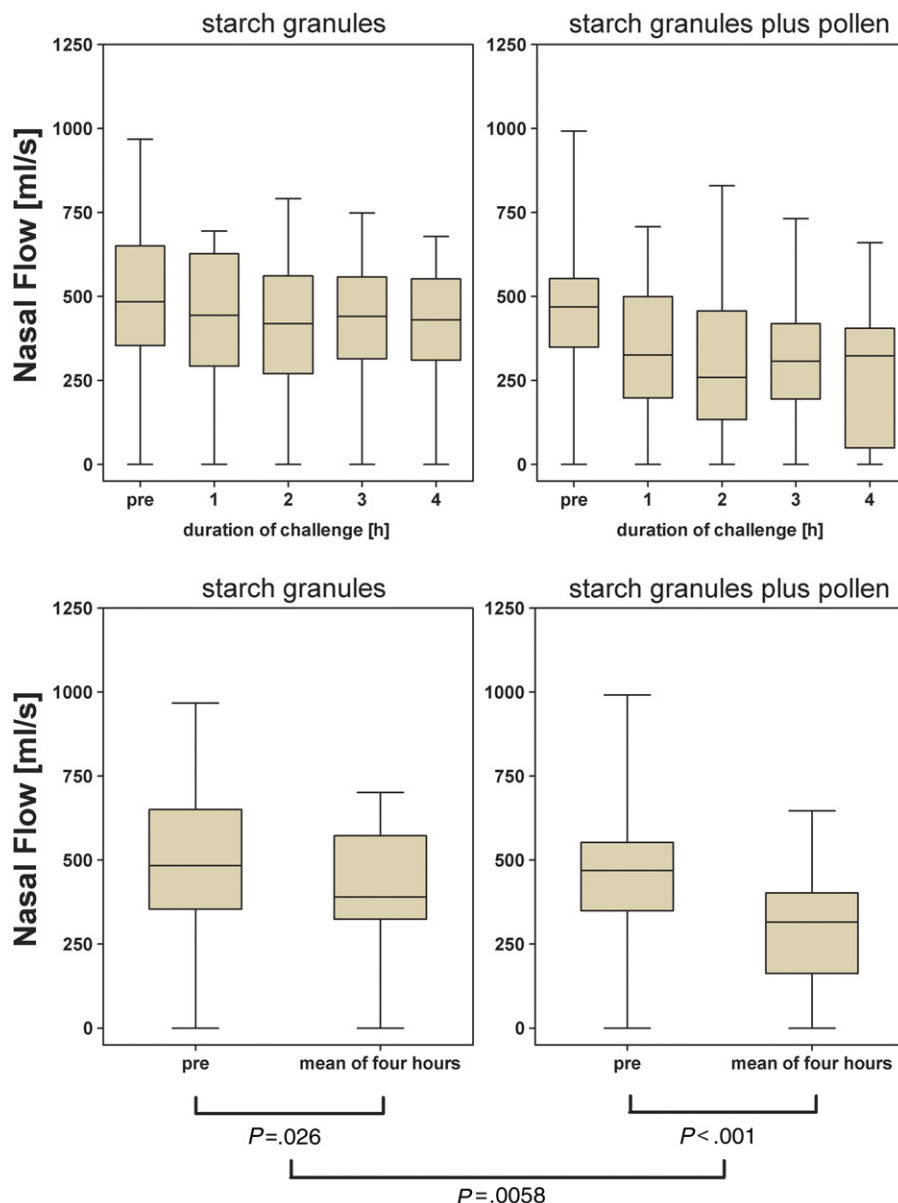


Fig. 2. Top, The nasal flow during 4 hours of challenge with either starch granules (left) or starch granules plus pollen (right). Bottom, The prechallenge nasal flow and the mean nasal flow during the entire challenge. Data are displayed in a box-and-whiskers graph (see the legend to Figure 1).

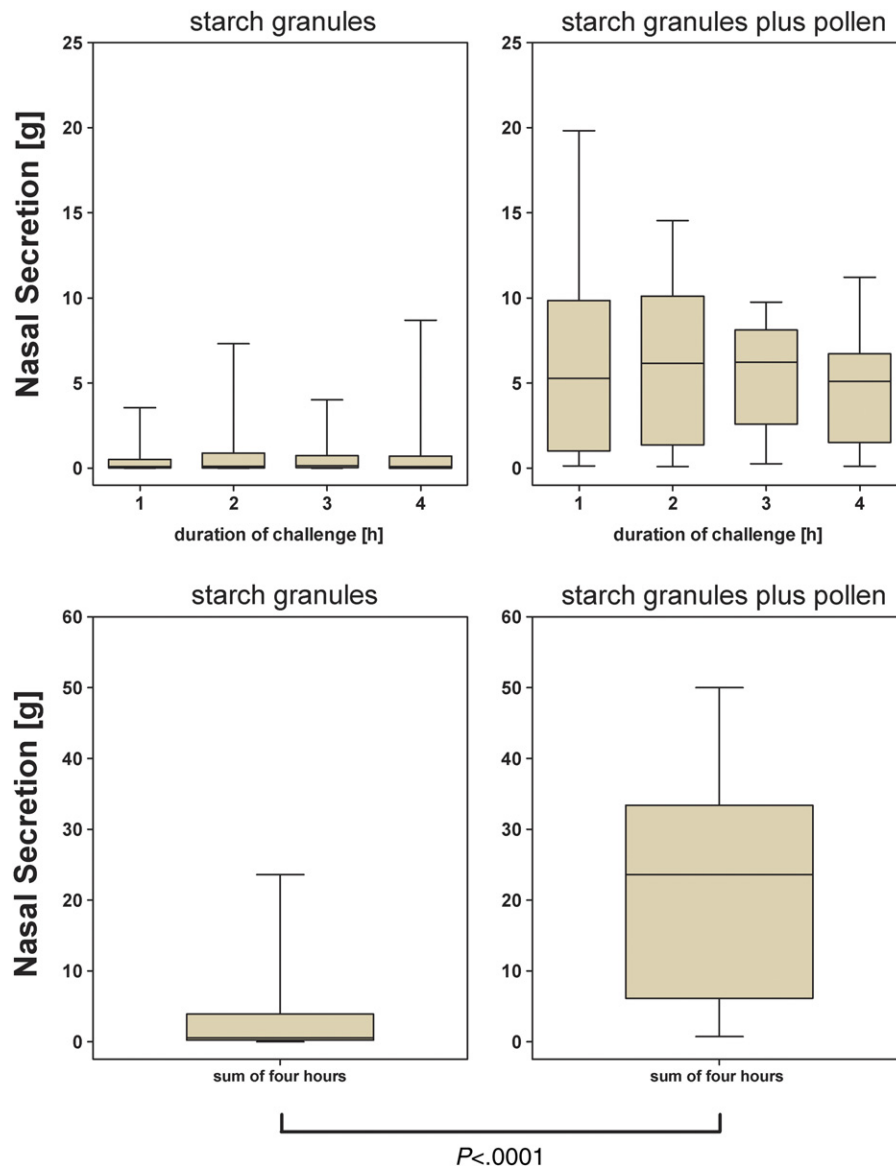


Fig. 3. Top, The amount of nasal secretion for each of the 4 hours of challenge with either starch granules (left) or starch granules plus pollen (right). Bottom, The total amount of nasal secretion during the challenge. Data are displayed in a box-and-whiskers graph (see the legend to Figure 1).

.001, respectively). However, in the presence of pollen grains decrease in nasal flow was significantly higher than with starch granules alone (Fig 2, $P = .006$).

Nasal secretion

Challenges with starch granules plus pollen caused significantly more nasal secretion than challenges with starch granules alone (Fig 3, $P < .001$).

Lung function (FEV_1)

The analysis of FEV_1 measurements did not reveal a clinically significant decrease during or after challenges with starch granules alone or with starch granules plus pollen or an overall difference between the 2 types of challenges (Fig 4, $P > .05$).

Exhaled nitric oxide

The results of both challenge days are presented for eNO. eNO was elevated after the first and the second challenges compared with the value before the first challenge in challenges with starch granules alone and challenges with starch granules plus pollen

(Fig 5, $P < .01$ each). No difference was found in the increase of eNO between the 2 types of challenges after neither the first nor the second challenge day (Fig 5, $P > .05$ each).

Discussion

The aim of this study was to compare the effect of pollen grains and pollen starch granules on nasal symptoms and bronchial inflammation in patients with seasonal allergic rhinitis and mild intermittent asthma during challenges in an ECC. The results clearly indicate that nasal symptoms are considerably higher in the presence of pollen grains. This is true for subjective symptoms, such as the TNSS, and for objective measurements like nasal flow and the amount of nasal secretion.

Starch granules alone do not have a clinically significant effect on nasal symptoms even though they are well able to induce an allergic reaction.^{3,9,10} The slight increase in symptoms they do cause, although statistically significant, is not higher than the reaction that can be seen in the Fraunhofer ECC when patients are exposed to clean air.³⁰ Such a sham exposure has been done in the validation study of the Fraunhofer ECC. Patients were then exposed

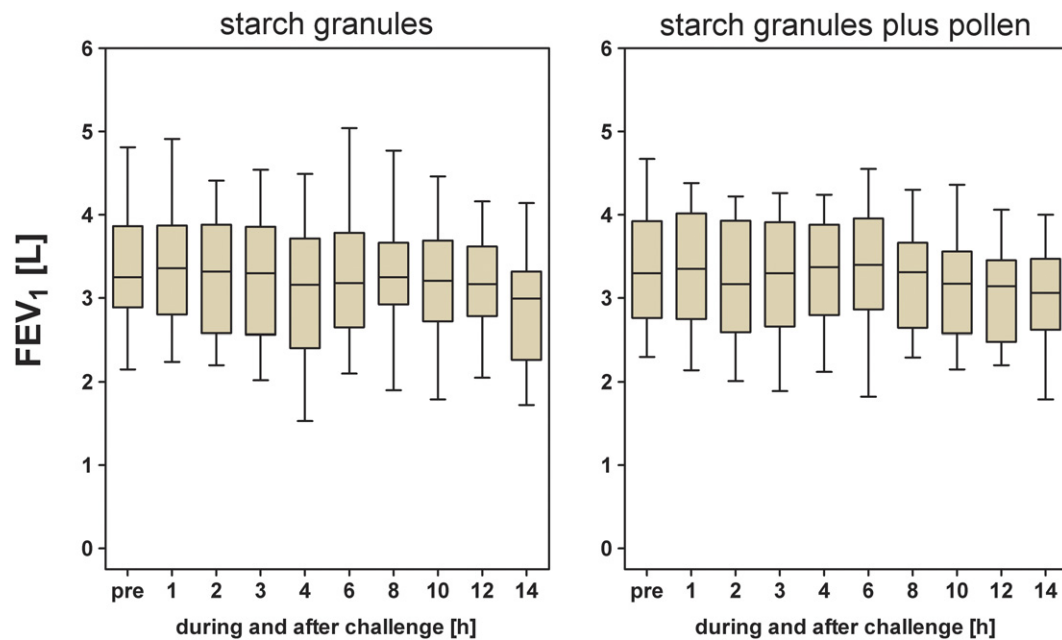


Fig. 4. Forced expiratory volume in 1 second (FEV₁) during 4 hours of challenge and up to 10 hours after challenge with either starch granules (*left*) or starch granules plus pollen (*right*). Data are displayed in a box-and-whiskers graph (see the legend to Figure 1).

to different concentrations of pollen and also clean air in a double-blind design. Even when exposed to clean air the patients developed a mean TNSS of up to 2 points, while showing no nasal secretions. The increase in subjective nasal symptoms in the pres-

ence of starch granules alone is in the range of this observed placebo effect.

eNO increased significantly after challenges. In contrast to nasal symptoms, the increase of eNO is not different in the presence of

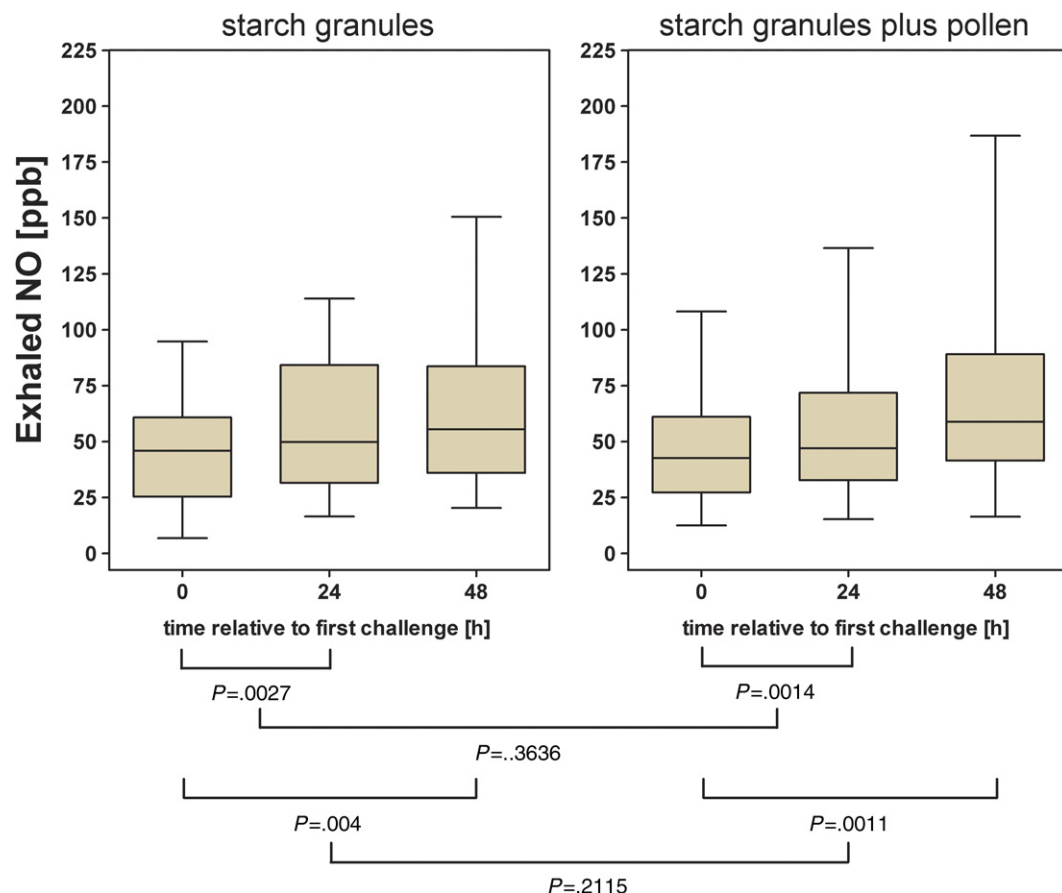


Fig. 5. Exhaled nitric oxide (NO) measured before challenges and 24 hours after challenge 2. Data are displayed in a box-and-whiskers graph (see the legend to Figure 1).

pollen compared with starch granules alone. This finding leads to the conclusion that bronchial inflammation is indeed triggered by starch granules and that the addition of pollen grains to the atmosphere of the ECC does not further enhance the lower airway inflammation.

The FEV₁ during and 10 hours after the challenges did not decrease, and no significant difference was found between the 2 types of challenges. As with eNO, the presence of pollen did not alter this lower airway response. A study by de Kluivijver et al.³¹ demonstrated that repeated low-dose allergen challenges increased NO and other inflammatory markers without an effect on symptoms or lung function. This higher sensitivity of NO compared with FEV₁ is also seen in our study.

Unfortunately, it was not possible to assess the sole effect of pollen grains because up to now it is technically not possible to create an atmosphere containing pollen grains without starch granules. However, by adding pollen grains to an atmosphere of starch granules, we were able to assess the additional effect pollen grains had on upper and lower airway symptoms. By this we were still able to discern the effect of starch granules and pollen grains.

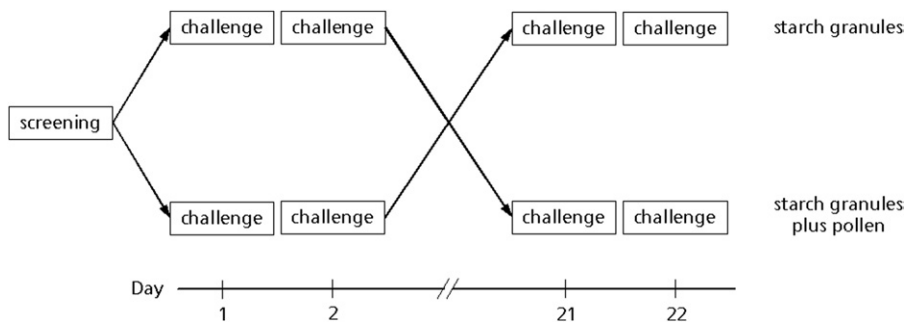
In this study 2 challenges with the same atmosphere were performed on 2 consecutive days. We initially believed that this might cause a priming effect that further enhances the patient's reaction and lead to more distinct results. Other studies^{32–34} conducted in ECCs have shown that there is a priming effect in nasal symptoms and inflammatory markers when challenges are conducted close to each other. However, we did not observe a priming effect in this study because there was no change in measures between the 2 challenge days. If a priming effect is of importance in future studies, more than 2 consecutive challenges are probably needed.

Although the effect on nasal symptoms was assessed by various measures in this study, bronchial inflammation was evaluated by eNO only. To see a more impressive lower airway effect, for example, a marked reaction in FEV₁, future studies could be performed with patients with moderate to severe asthma. However, if patients with moderate to severe asthma are challenged, safety considerations have to be taken into account. Additional tests, such as airway hyperresponsiveness or the assessment of biomarkers in nasal secretion, bronchoalveolar lavage, or induced sputum, could be included to assess nasal and bronchial inflammation. This could give further evidence that lower airway symptoms are triggered by starch granules only and that pollen grains do not enhance them.

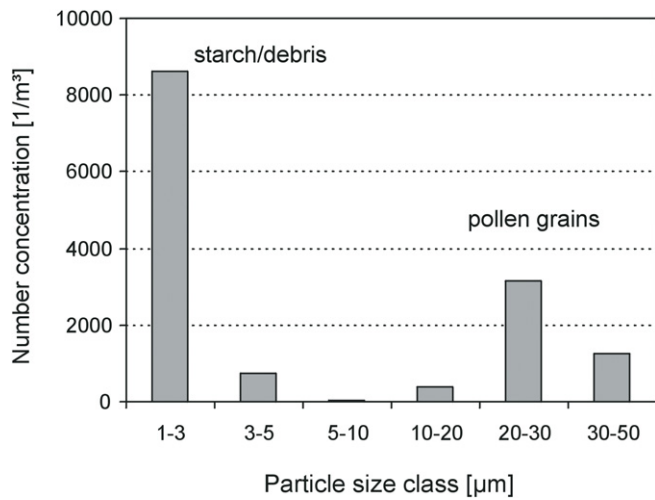
In conclusion, this study demonstrated that starch granules trigger lower airway inflammation in allergic asthma, whereas pollen grains predominantly cause nasal symptoms. This difference is probably due to the different sizes of pollen and starch granules that determines their deposition in the airways.

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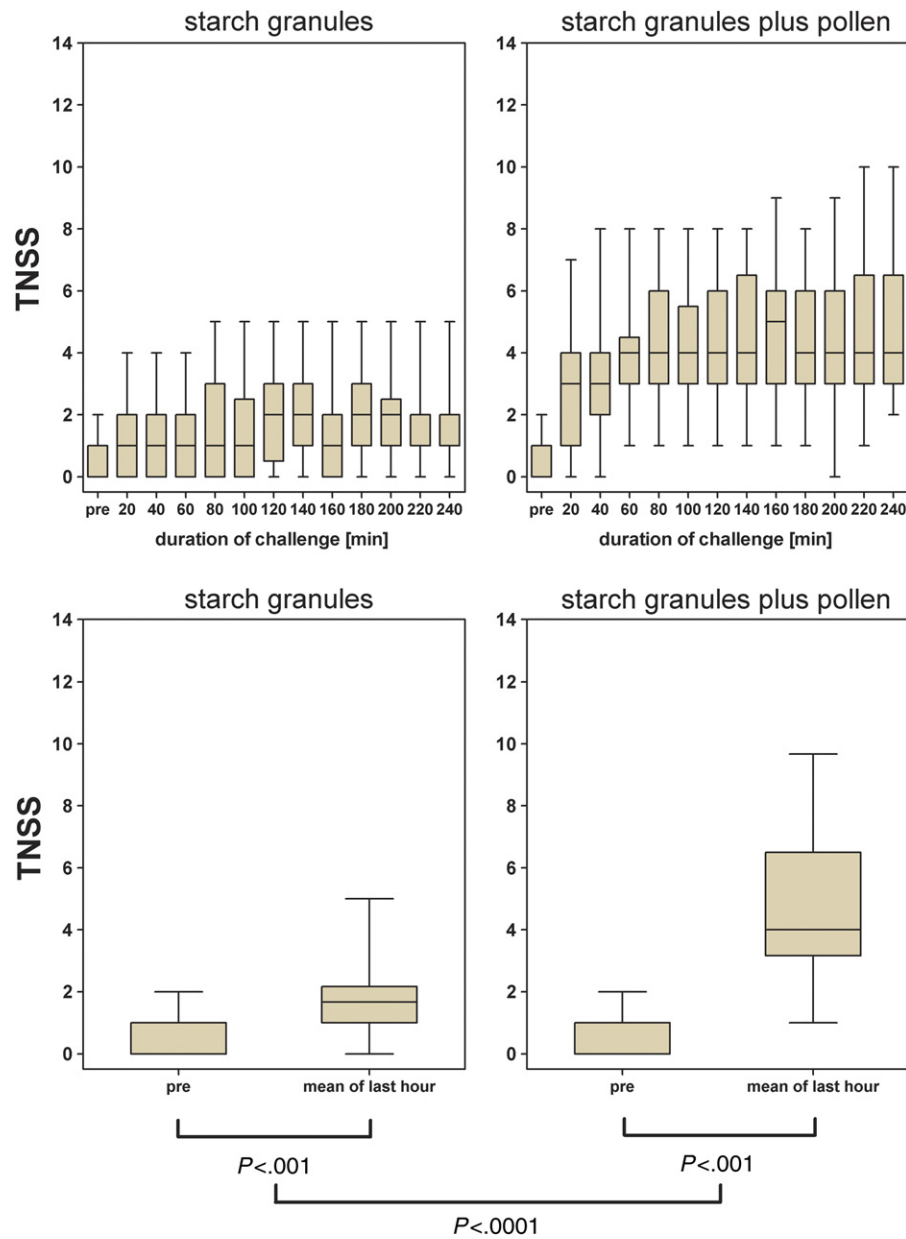
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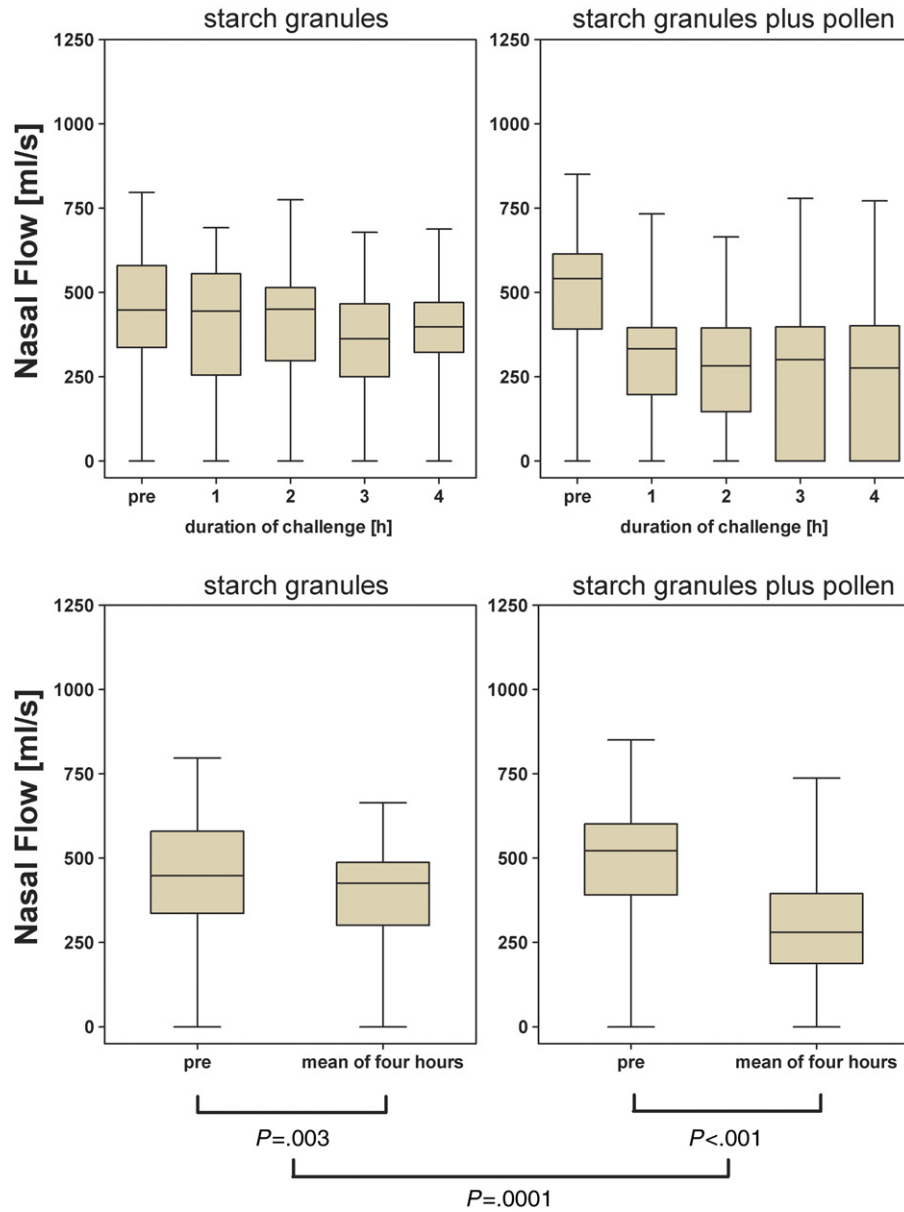
eFig. 1. Study design.



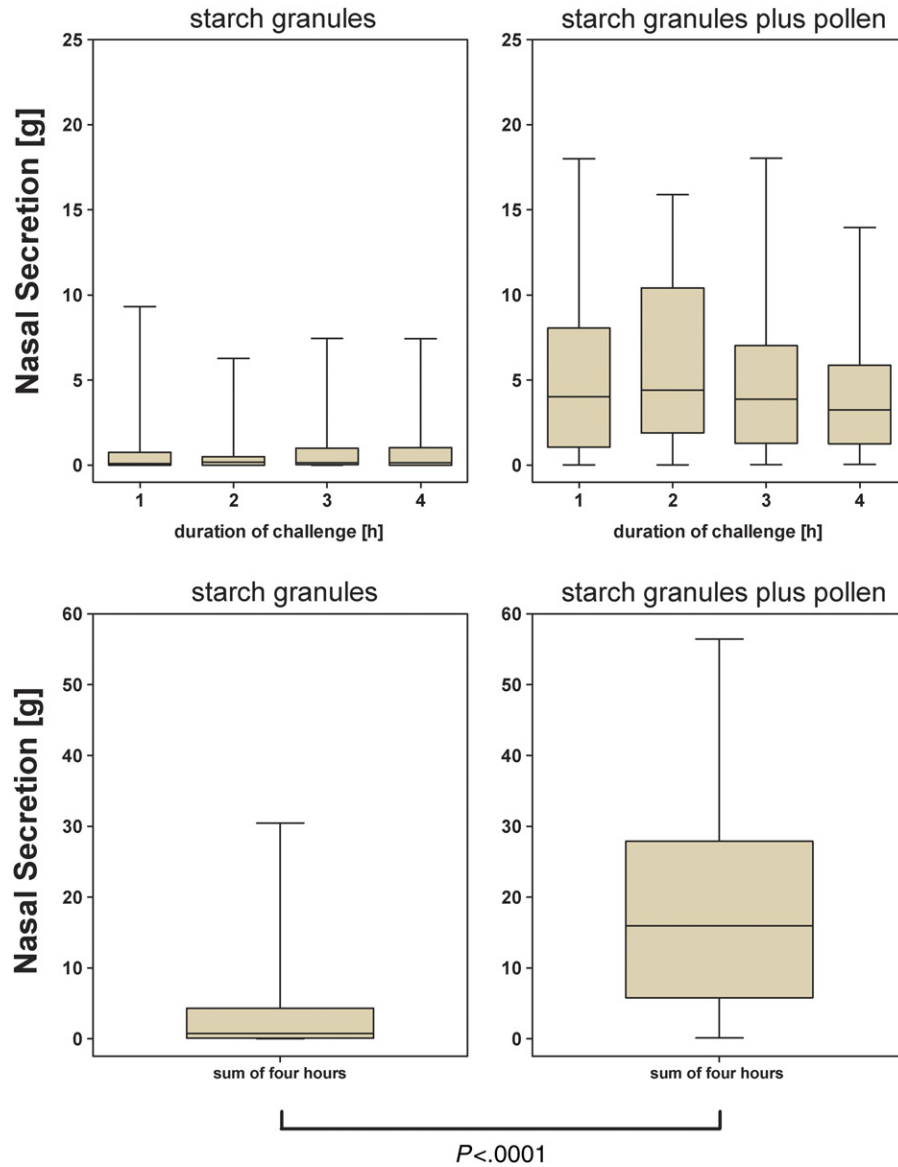
eFig. 2. The number size distribution of the challenge aerosol as measured with an optical particle counter (LASAIR Type 550, PMT, Karlsruhe, Germany) clearly shows a bimodal structure.



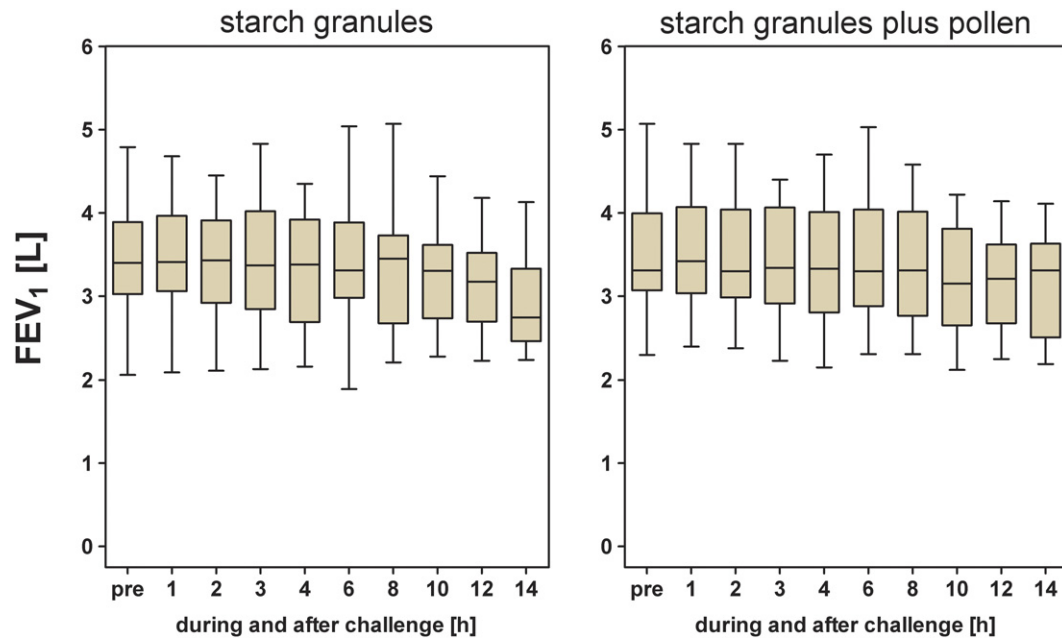
eFig. 3. Top, Total nasal symptom score (TNSS) during 4 hours of challenge with either starch granules (left) or starch granules plus pollen (right). Bottom, The prechallenge TNSS and the mean TNSS during the last hour of challenge. Data are displayed in a box-and-whiskers graph. The box extends from the 25th percentile to the 75th percentile, with a line at the median. The whiskers extend to the highest and lowest value.



eFig. 4. Top, The nasal flow during 4 hours of challenge with either starch granules (left) or starch granules plus pollen (right). Bottom, The prechallenge nasal flow and the mean nasal flow during the entire challenge. Data are displayed in a box-and-whiskers graph (see the legend to Figure 1).



eFig. 5. *Top*, The amount of nasal secretion for each of the 4 hours of challenge with either starch granules (*left*) or starch granules plus pollen (*right*). *Bottom*, The total amount of nasal secretion during the challenge. Data are displayed in a box-and-whiskers graph (see the legend to Figure 1).



eFig. 6. Forced expiratory volume in 1 second (FEV₁) during 4 hours of challenge and up to 10 hours after challenge with either starch granules (*left*) or starch granules plus pollen (*right*). Data are displayed in a box-and-whiskers graph (see the legend to Figure 1).