

Matrix Metalloproteinases and Tissue Inhibitors of Metalloproteinases in Nasal Lavage After An Inhalation Challenge with Flour

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Objectives/Hypothesis: The existence of nasal mucosa remodeling in allergic rhinitis is controversial. Few data are available on the dynamics of matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) in nasal fluid after an allergen challenge. We examined whether an immediate allergic reaction that induces nasal congestion and inflammation is able to also induce changes in remodeling parameters in nasal fluid.

Study Design: Controlled experimental study.

Methods: Ten patients with allergic occupational rhinitis due to flour underwent a control and active inhalation challenge with serial monitoring of nasal congestion and nasal symptoms with acoustic rhinometry and a visual analogue scale. Levels of remodeling markers (MMP-2, MMP-7, MMP-9, MMP-13, TIMP-1, TIMP-2) and inflammatory cells in nasal fluid were measured before the challenge and at 30 minutes, 6 hours, and 24 hours following the challenge.

Results: In contrast to the control challenge, the flour challenge induced nasal symptoms and significant decreases in nasal volume in all subjects. After the flour challenge, a significant increase in nasal levels of TIMP-2 and a nonsignificant increase in TIMP-1 levels were observed, whereas no significant changes in nasal levels of MMPs were documented.

Conclusions: This study showed that after an inhalation challenge with an occupational allergen, the nasal mucosa displayed an imbalance in favor of TIMPs enzymes activity as compared to MMPs enzymes activity, represented in an increase in nasal levels of TIMP-2 during the course of the early reaction following the allergen challenge.

Key Words: Rhinitis, occupational rhinitis, occupational diseases, airway remodeling, metalloproteinases.

Level of Evidence: 2c

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INTRODUCTION

It is now widely recognized that the airway wall in asthma shows structural changes that contribute to an increase in airway wall thickness. These changes, which are collectively referred to as airway remodeling, include epithelial damage, mucous gland hypertrophy, goblet cell hyperplasia, increased matrix protein and collagen deposition, smooth muscle hyperplasia and hypertrophy, angiogenesis, and airways myofibroblasts transformation.¹ Interestingly, despite the multiple links between the upper and lower airways, the presence of nasal

mucosa remodeling as a feature of allergic rhinitis is still a subject of controversy. Some studies have reported no epithelial damage in allergic rhinitis, whereas others have found enhanced epithelial shedding and intercellular edema.² However, presently it is believed that nasal remodeling occurs, but to a lesser extent than asthma, perhaps due to protective mechanisms developed by the nasal mucosa to act as a first defense mechanism against noxious agents entering the respiratory tract.³

Overexpression and activation or an imbalance between matrix metalloproteinases (MMPs) and tissue inhibitors of matrix metalloproteinases (TIMPs) can induce extracellular matrix deposition and degradation of airway tissue with subsequent structural and functional changes in the respiratory tract.⁴ In the upper airways, abnormal expression of MMPs and their inhibitors are thought to play a role in allergic rhinitis, chronic rhinosinusitis with and without nasal polyps, and head and neck cancer.⁵ Few studies have examined MMPs and TIMPs expression in nasal mucosa of allergic rhinitis patients, and the results are inconsistent. Kim et al. detected increases in MMP-9 but not in MMP-2, TIMP-1, and TIMP-2 in severe persistent allergic rhinitis compared with healthy nasal mucosa.⁶ In contrast, Shaida et al. showed that TIMP-1 and TIMP-2 mRNA and protein were present in large amounts in the nasal mucosa of patients with allergic rhinitis, whereas only

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small amounts of MMP-1, -2, -3, and -9 mRNA were detected in the same samples.⁷

A nasal challenge with common aeroallergens in sensitized patients with allergic rhinitis induces nasal symptoms and leads to reduction in nasal volume. Also, eosinophilic inflammation is a feature of the allergic reaction to an allergen challenge.⁸ An inhalational challenge (IC) is useful for examining nasal physiologic changes and the associated mechanisms of allergen-induced airways inflammation. Moreover, it is a valid approach to assess airway remodeling.⁹ Few studies have investigated the effect of an allergen challenge on nasal remodeling. Particularly, few data are available on the dynamics of MMPs and TIMPs in nasal fluid as surrogate parameters for nasal remodeling. We examined whether an immediate allergic reaction that induces nasal congestion and inflammation is able to also induce changes in levels of MMPs (-2, -7, -9, -13), and TIMPs (-1 and -2) in nasal lavage (NAL) samples from patients sensitized to flour.

MATERIALS AND METHODS

Subjects

Ten sensitized male patients (mean age, 39.1 years) with a diagnosis of occupational rhinitis (OR) due to flour were recruited to characterize mediators of airways remodeling during IC. In all cases the diagnosis of OR was initially suspected by means of a comprehensive medical and occupational history and by skin prick testing, which was then confirmed by an IC in specialized laboratories. All subjects had been removed from the workplace, and rhinitis symptoms were controlled at the time of investigation. Written consent for participation in the study was obtained from all study participants. The ethics committees of Sacre-Coeur Hospital and Mont-Godinne Hospital approved this study

IC

Subjects underwent two consecutive IC sessions. Briefly, the first day they were exposed to lactose as a control agent, and the next day they were exposed to flour by a realistic method recreating working conditions within an exposure chamber following international recommendations.^{10,11} The assessment of nasal parameters included measurement of total nasal volume and nasal symptoms by acoustic rhinometry and a visual analogue scale (VAS), respectively, before and serially for 6 hours after exposure. Nasal fluid was collected by NAL before the challenge and at 30 minutes, 6 hours, and 24 hours following the challenge.

NAL

NAL was performed using a modified 10F standard silicone Foley catheter (Teleflex Medical GmbH/Ruesch, Tuttlingen, Germany).¹² The entire procedure involved three instillation/aspiration cycles with normal saline solution. The NAL fluid from both nasal cavities was pooled in the same plastic container and kept on ice until processed. Two NALs were performed before the challenge. The first NAL was intended to remove preexisting mediators and the second to obtain baseline mediators levels for comparison with postchallenge values. Within 2 hours the sample was measured and centrifuged at 3,300 rpm for 8 minutes at 4°C, and the supernatant was frozen at -80°C for future analysis. The pellet was resuspended in

0.5 mL phosphate-buffered saline containing 0.1% wt/vol bovine serum albumin. Cytocentrifuge preparations were made by using 100 μ L of the remaining resuspended cell suspension. The preparation was centrifuged at 450 rpm, and slides were stained with Wright-Giemsa.¹³

Quantification of Remodeling and Inflammatory Markers in Nasal Fluid

Six parameters were selected to examine remodeling in NAL supernatants: MMPs (-2, -7, -9, and -13) were quantified using the MultiAnalyte Profiling Fluorokine MAP assay (R&D Systems, Minneapolis, MN) based on the Luminex technology and the Bio-Plex workstation (Bio-Rad, Mississauga, Ontario, Canada), whereas TIMPs (-1 and -2) were quantified using commercial enzyme-linked immunosorbent assay kits (Calbiochem EMD Chem. Inc., San Diego, CA) according to the manufacturers' instructions. Total cell numbers were determined by hemacytometer, and differential cell counts were performed on cytospin preparations stained with Wright-Giemsa.

Acoustic Rhinometry and VAS

Acoustic rhinometry was performed according to a standardized procedure.¹⁴ An acoustic rhinometer (Hoods Laboratories, Pembroke, MA) was used to measure the nasal volume between 2 and 5 cm into the nose (Vol₂₋₅). The Vol₂₋₅ was selected as an end point for this study to better reflect mucosal changes during the challenge.^{14,15} The subject's subjective bilateral perception of nasal congestion was quantified using a 100-mm VAS.

Statistical Analysis

Data are expressed as median and 25th and 75th percentiles. The nonparametric Friedman test was used to test the significance of changes in levels of remodeling and inflammatory parameters after the control and active challenge. All differences within sets of paired data representing before and after challenge measurements were analyzed using the Wilcoxon signed rank test. The analysis was performed using the SPSS 18.0 statistical package (SPSS, Inc., Chicago, IL).

RESULTS

The skin prick test with common aeroallergens and flour were positive in all subjects. Most of them were nonsmokers or former smokers (8/10), with a mean duration of exposure to flour at the workplace of 8.9 years.

Acoustic Rhinometry and VAS

During the control day the mean $\pm$ standard deviation maximum percentage decrease in nasal volume (Vol₂₋₅) as compared to baseline was 15.0% $\pm$ 10.4%. After the challenge with flour, the maximum percentage decrease was 38.7% $\pm$ 8.6%. These changes were observed within the first hour after the challenge in nine patients and at 6 hours in one patient.

On the control day, the median (25th and 75th percentiles) VAS rating at baseline was 0.3 (0.0–2.7), and no increase was observed after the control challenge. On the active challenge day, the median VAS rating at baseline was 0.3 (0.0–2.3), whereas the median highest VAS rating was 3.4 (0.0–4.9) at 10 minutes after the challenge with flour.

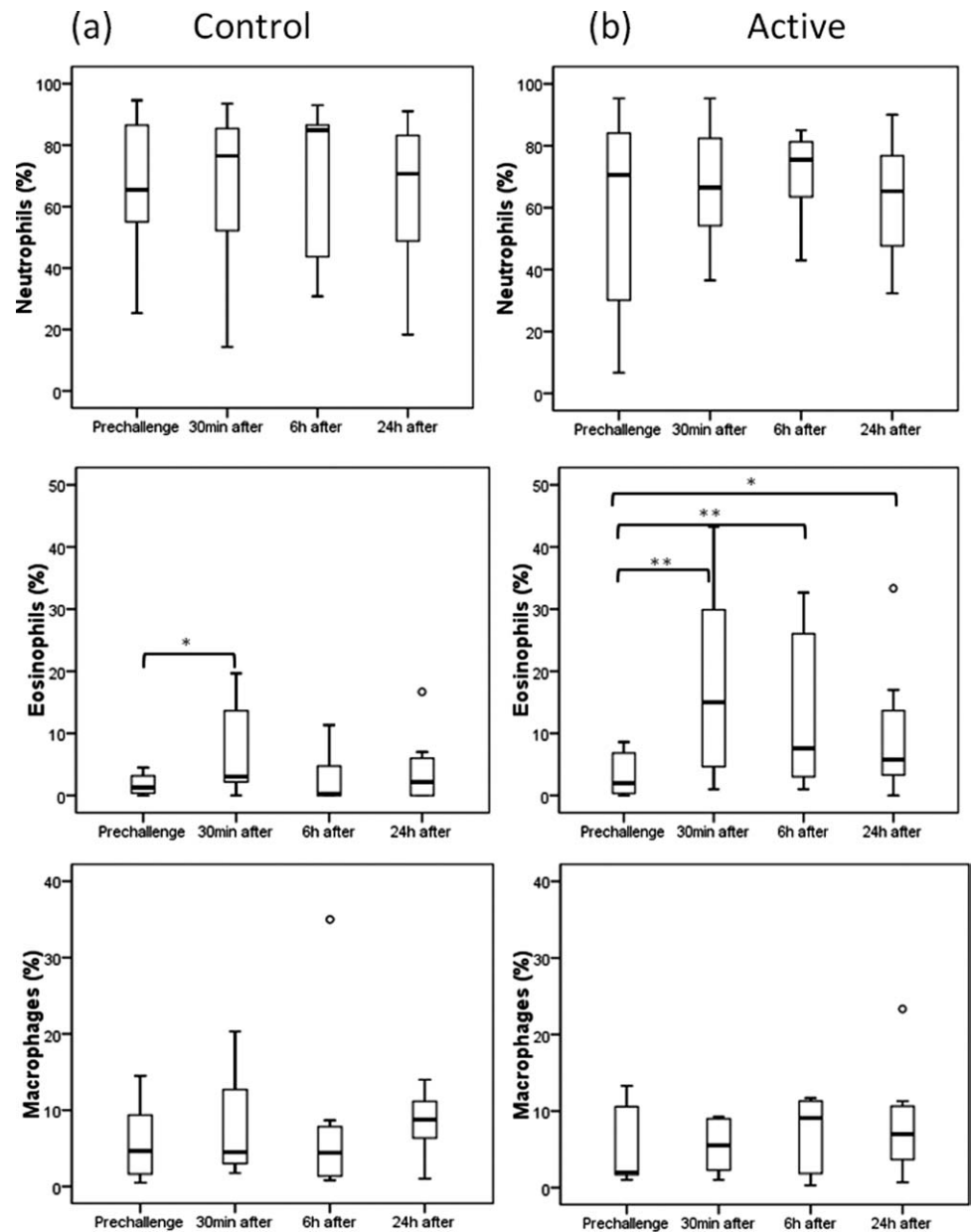


Fig. 1. Levels of inflammatory cells in nasal lavage during control and active challenge days. Data are expressed as percentage of total cell count. (a) Levels measured before and after the challenge with the control agent. (b) Levels measured before and after the challenge with flour. Bars represent medians with 25 and 75 interquartiles. * $P < .05$. ** $P < .01$.

Inflammatory and Remodeling Markers in Nasal Fluid

Figure 1 shows the changes in the percentage of inflammatory cells in nasal fluid after the control and flour challenges. After the control challenge, there was a significant increase in eosinophils in NAL at 30 minutes following the challenge ($P = .02$). After the flour challenge, subjects had a significant increase in eosinophils in nasal fluids at 30 minutes ($P = .008$), 6 hours ($P = .008$), and 24 hours ($P = .03$) following the challenge (Fig. 1). During the control and flour challenge, increases in neutrophils and macrophages were not significant.

During the control and flour challenges, levels of MMPs (-2, -7, -9), and TIMPs (-1 and -2) were detectable in nasal fluid samples from all subjects, except MMP-13, which was detectable in nine of 10 subjects. Figure 2

shows the changes in TIMPs during the control and active (flour) challenge days.

There were no significant changes in nasal levels of TIMPs during the control day. By contrast, a significant increase in TIMP-2 ($P = .05$) and a nonsignificant increase in TIMP-1 levels was observed after flour exposure. After the control challenge, no increases in levels of MMPs were detected. After the flour challenge, there was a nonsignificant increase in levels of MMP-2 and MMP-13 (Fig. 3).

We further compared nasal levels of MMPs and TIMPs measured at 30 minutes and 6 hours after the control challenge with those obtained after the flour challenge. Data from each remodeling factor were analyzed by calculating a ratio that accounted for prechallenge differences in concentrations of the marker (e.g.,

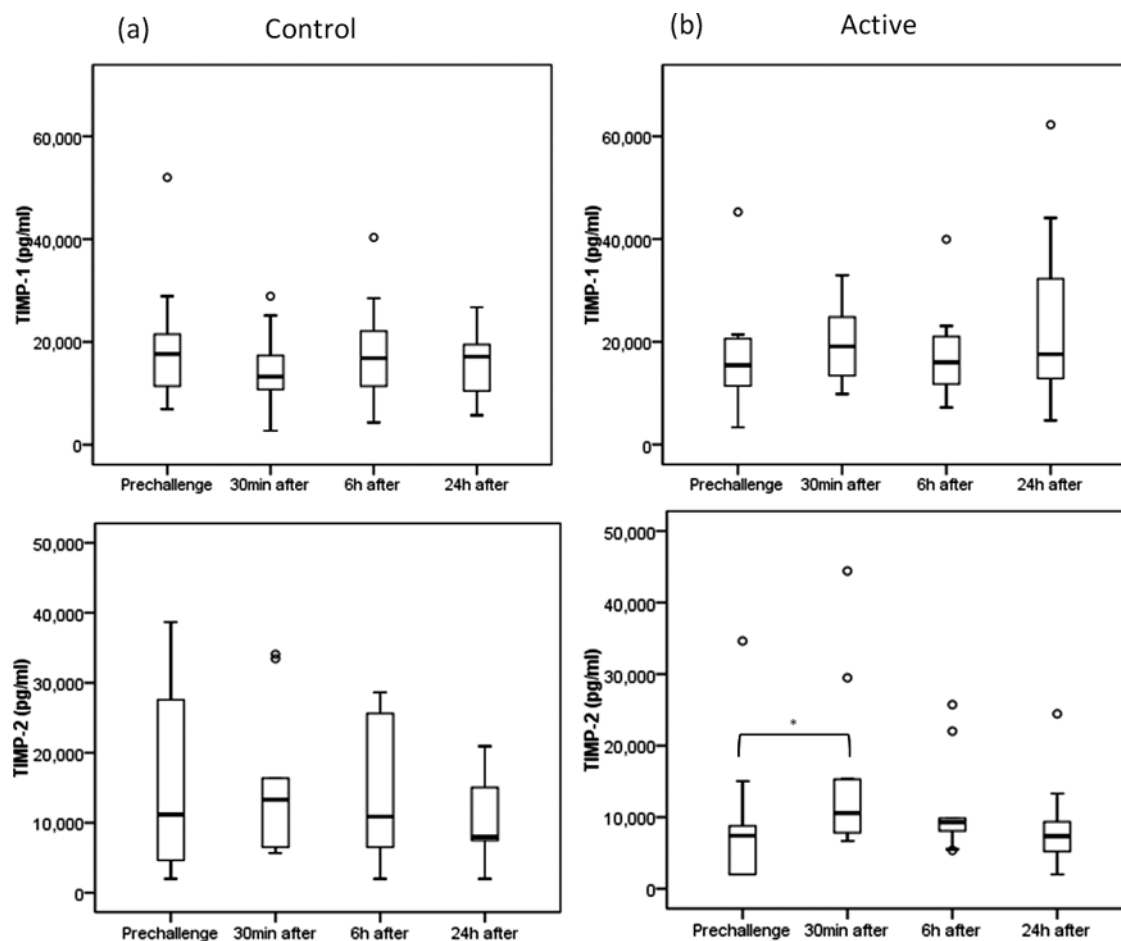


Fig. 2. Concentrations of tissue inhibitors of metalloproteinases (TIMPs) in nasal lavage during control and active challenge days. (a) Concentrations of TIMPs before and after the challenge with the control agent. (b) Concentrations of TIMPs before and after the challenge with flour. Bars represent medians with 25 and 75 interquartiles. * $P = .05$

enzyme level at 30 minutes following the challenge/prechallenge level, enzyme level at 6 hours following the challenge/prechallenge level). The analysis showed a statistically significant difference at 30 minutes following the challenge ($P = .005$) and a marginally significant difference ($P = 0.07$) at 6 hours following the challenge for the MMP-2 ratio. Also, a marginally significant difference ($P = .09$) at 6 hours following the challenge for the TIMP-2 ratio was observed. These results indicate higher levels of enzymes after the flour challenge as compared to the control challenge. Results from other remodeling markers did not yield significant results (data not shown).

Data from remodeling markers showing the greater changes in NAL after the flour challenge were also expressed and analyzed as the molar ratio of enzyme to inhibitor (MMP-9/TIMP-1, MMP-9/TIMP-2, MMP2/TIMP-1, MMP-2/TIMP-2, MMP-7/TIMP-1, and MMP-7/TIMP-2). This analysis showed no significant changes after the flour challenge (data not shown).

DISCUSSION

Airway remodeling can be examined either directly by examination of airway tissues or indirectly by analy-

sis of airway fluids.^{9,16} This study measured markers of remodeling in nasal fluid collected during IC from patients with confirmed allergic OR. As expected, after the flour challenge, patients with allergic OR displayed a significant fall in nasal volume associated with an influx of eosinophils into the nasal mucosa. These physiological and inflammatory changes were related to a significant increase in TIMP-2 levels at 30 minutes following the challenge. These findings suggest a predominant effect of TIMPs in the nasal mucosa during the early allergic reaction following the flour challenge in patients with allergic OR. Shaïda et al. proposed that the presence of larger amounts of TIMPs in the nasal mucosa would minimize MMPs-mediated damage.⁷ They found increased TIMP-1 and TIMP-2 mRNA and protein levels in the nasal mucosa of patients with allergic rhinitis and only small levels of MMP-1, -2, -3, and -9 mRNA in the same samples. Our findings are in accordance with this hypothesis. Thus, this antagonistic effect would ultimately be crucial in maintaining nasal mucosa integrity.

Only two studies have examined MMPs and TIMPs release after an allergen challenge in the nasal mucosa of patients with allergic rhinitis, and the results are also

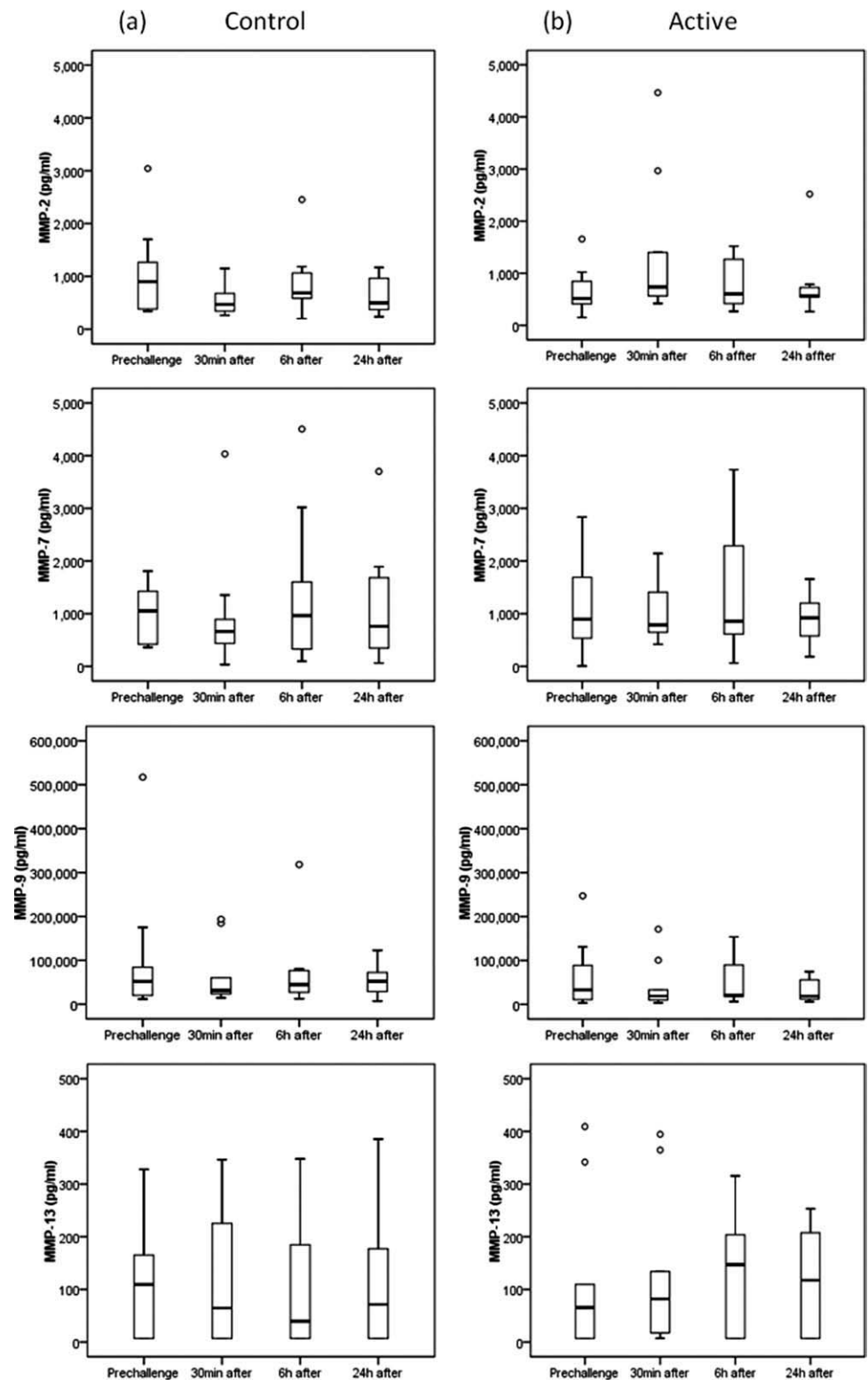


Fig. 3. Concentrations of matrix metalloproteinases (MMPs) in nasal lavage during control and active challenge days. (a) Concentrations of MMPs before and after the challenge with the control agent. (b) Concentrations of MMPs before and after challenge with flour. Bars represent medians with 25 and 75 interquartiles. Increases in MMPs levels were not statistically significant

inconsistent. Van Toorenenbergen et al. showed a parallel increase in MMP-9 and eosinophil cationic protein in nasal fluid collected during the late-phase reaction to an allergen challenge.¹⁷ In our study, MMP-9 was the remodeling marker showing the higher concentrations in nasal fluid at baseline on 2 consecutive days. However,

no increase in nasal levels of MMP-9 was observed after the flour challenge, suggesting a less relevant role of MMP-9 enzyme in nasal mucosa remodeling as compared to its well-established role in asthma.¹⁸ The other study showed high levels of MMP-2, -13, TIMP-1, but not MMP-9 after an allergen challenge.¹⁹ In our study,

we observed the same effects, but the detected changes were not statistically significant. It is likely that despite our patients being a selected group of sensitized patients with confirmed OR, the fact that they were no longer exposed to flour at work contributed to the observed low concentrations and nonsignificant increases in MMP-2 and MMP-13 nasal levels observed after the allergen challenge. Moreover, TIMP-2 levels increased significantly after the flour challenge, thus an inhibitory effect on MMP-2 nasal levels should be expected as well.

The novelty of this study resides in the fact that it measured remodeling markers in nasal fluid during an allergic reaction. However, the study has limitations that should be considered when interpreting the results. First, this study has a small sample size. However, by using a well-defined and homogenous population of subjects with a confirmed diagnosis of OR, as well as by using standardized diagnostic methods, we believe we were able to identify a true effect of flour exposure on nasal levels of MMPs and TIMPs. Second, we assumed that remodeling markers in nasal fluid reflect remodeling processes occurring in the nasal mucosa. We acknowledge that nasal fluid is an indirect marker of any potential remodeling and inflammatory process occurring in the nasal mucosa. However, the utility of these measurements in assessing airway remodeling is recognized.^{9,16}

CONCLUSION

This study showed that during acute allergen exposure, the nasal mucosa displays an imbalance in favor of TIMPs enzymes activity compared to MMPs enzymes activity. Specifically, TIMP-2 increased during the early reaction after the flour challenge. This finding should be further corroborated in larger studies involving challenge testing with different aeroallergens and incorporating histopathological assessments of nasal mucosa.

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