



DR. AARIF EIFAN (Orcid ID : 0000-0003-4098-4211)

PROF. STEPHEN R. DURHAM (Orcid ID : 0000-0001-5264-6207)

Article type : Letter to the Editor

Title: Comparison of nasal allergen challenges with dissolved Timothy Grass pollen tablets and aqueous extract

Word Count: 1141

To the Editor:

We and others have used nasal allergen challenge (NAC) to investigate the clinical and immunological effects of allergen exposure (1, 2, 3) and the efficacy of immunotherapy (4). The procedure is safe and well tolerated (5). For NAC to be a valid tool, it is essential that the allergen extracts used are standardised. The availability of these extracts is limited in some areas. We have previously used an aqueous extract of Timothy Grass (*Phleum pratense*) pollen, Aquagen® (ALK-Abello, Denmark), supplied as a dry powder, to be reconstituted prior to use by dissolving in an albumin-based diluent, to 100,000 SQ-U/ml. Aqueous extract of Timothy Grass (*Phleum pratense*) pollen production has been discontinued, but the identical allergen is available as Timothy Grass lyophilisate tablets, Grazax® (ALK-Abello), 75,000 SQ-T, an approved, widely available and licensed product for treatment of severe seasonal allergic rhinitis. Timothy Grass lyophilisate tablets consists of purified, freeze-dried Timothy Grass pollen extract, plus three

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1111/ALL.14590](#)

This article is protected by copyright. All rights reserved

excipients, (fish) gelatine, mannitol and sodium hydroxide. We anticipated that the clinical effect when dissolved and used for NAC would be equivalent to the use of aqueous extract of Timothy Grass (*Phleum pratense*) pollen for a given allergen concentration. We therefore conducted a randomised-order, cross-over trial of NAC with the two allergen extracts.

Grass pollen allergic adults (18-65 years) and non-atopic controls were recruited from the Allergy Clinic at Royal Brompton Hospital, London. For inclusion/exclusion criteria see *online supplement*. Following screening, participants underwent two NACs, at least 4 weeks apart, between September 2019 and January 2020. Participants were randomised to have either NAC using aqueous extract first, dissolved tablet second, or vice versa. Non-atopics underwent a single NAC with the dissolved tablet. Participants gave written, informed consent; the study was approved by regional ethics committee (ref.19/LO/1346) and registered with clinicaltrials.gov (NCT04078009). NACs were performed with aqueous extract/dissolved tablets diluted in 0.9% normal saline to 16,667 SQ-U/mL or 16,667 SQ-T/ml (equivalent to 5,000 BU/mL or approximately 1 mcg major allergen (Phl p 5)/mL, *see supplement for further details*). Total nasal symptom scores (TNSS, 0-12) and peak nasal inspiratory flow (PNIF) were recorded before NAC and at 5, 15, 30 and 60 minutes. Peak expiratory flow (PEF) was recorded before and at 60 minutes. The primary endpoint was the area under the curve for TNSS 0-60 minutes (TNSS-AUC). The primary comparison was between NAC with aqueous extract and NAC with dissolved tablet. Secondary comparisons were between the first NAC and second NAC visit, and by first and second visit according to challenge extract.

Twenty-two volunteers were screened, of which 20 were included (Supplementary Table 1). All 20 participants completed both challenges except one who did not undergo Visit 2 due to an upper respiratory tract infection. Ten non-atopic individuals underwent dissolved tablet NAC only. Mean TNSSs were similar during NACs with each extract at baseline, 5, 15, 30 and 60 minutes (Fig.1A). Mean TNSS AUC was no different between the two ($p=0.19$, paired t-test; Fig.1B). Mean change from baseline PNIFs were also similar at 5, 15, 30 and 60 minutes (Fig.1C) and AUC was not different ($p=0.99$; Fig.1D). Pearson's coefficient showed correlation between TNSS AUC following each challenge ($r=0.65$, $p=0.002$; Fig.1E). Bland-Altman plot of the difference between the TNSS AUC showed 18 of 19 (94.7%) paired results lying within ± 1.96 standard

deviations of the mean (Fig.1F). NAC in non-atopic individuals had no effect on TNSS or PNIF (Fig.1A, C). Individual TNSS following nasal challenge with aqueous extract of Timothy Grass (*Phleum pratense*) pollen and Timothy Grass lyophilisate tablets are depicted in *supp* Fig 3.

Whilst peak (5 minute) TNSS was equivalent at Visits 1 and 2, mean scores at 15, 30 and 60 minutes were lower at Visit 2 than Visit 1, without reaching statistical significance (Fig.2A). Mean TNSS AUC was greater at Visit 1 than Visit 2 ($p=0.03$; paired t-test, Fig.2B). A similar trend was seen for Δ PNIF (Fig.2C, D). This effect appeared to be predominantly driven by participants who received aqueous extract NAC first (Supplementary Fig.1 A, B), rather than those who received challenges in the opposite order (Supplementary Fig.2 A, B).

There were no serious or unexpected adverse events. One participant with seasonal asthma developed cough but no wheeze or breathlessness at 30 mins following both challenges, with falls in PEFR of 17% after dissolved tablet and 14% after aqueous extract that responded promptly to 200mcg inhaled Salbutamol.

Responses to nasal allergen challenge with Timothy grass pollen allergen derived from two sources, aqueous extract and dissolved tablets, diluted to the same concentration in saline, were similar both in terms of pattern and magnitude of response. Comparison by two methods, Pearson's correlation coefficient and Bland-Altman plot, show good correlation and acceptable concordance. Nasal challenge with dissolved tablets produced no response in non-atopic individuals. Nasal challenge with either material was safe, with no severe or unexpected adverse events. Overall, NAC using dissolved tablets appears to be a suitable alternative to aqueous extract.

This study provides validation for allergy researchers for the use of an alternative, readily available allergen source for grass pollen nasal allergen challenges. The study was exploratory, without a pre-specified power calculation, and was not specifically designed to test for non-inferiority. Nonetheless, we feel it provides good evidence for future use of dissolved tablets in NAC studies. We did not include a diluent control to exclude underlying nasal hyperreactivity. However, the primary aim here was to compare two allergen preparations within the same

individuals with the order of challenge randomised. It is therefore unlikely that variation in non-specific nasal reactivity over time had an impact on results. Moreover, we have undertaken previous studies including diluent challenges with no evidence of a non-specific effect (2).

The possibility of an order effect between challenges is interesting. We chose a gap of 4 weeks between the two NACs in the expectation that this would be long enough to allow inflammation to have resolved and thereby prevent priming at the second challenge, an effect seen with repeat nasal allergen challenges at shorter intervals (6). In fact, we found the opposite, a degree of tolerance to the second challenge. This effect seems to be driven by the participants who had aqueous extract NAC first, dissolved tablet NAC second, and not vice versa. Other than suggesting a subtle difference in relative 'strength' of the two materials in nasal challenge, it is difficult to come up with a biological explanation for this (we have not seen an effect with NAC using the albumin diluent alone in previous studies (2)). Determining whether there is a degree of clinical (and presumably immunological) tolerance with repeat challenges at 4 weeks would require a larger study that includes immune biomarkers.

We have found NAC to be a very useful endpoint for clinical trials of allergen immunotherapy. These current data allow us to continue this work, and, we hope, enable others to do the same.

References

1. Scadding GW, Calderon MA, Bellido V, Koed GK, Nielsen NC, Lund K et al. Optimisation of grass pollen nasal allergen challenge for assessment of clinical and immunological outcomes. *J Immunol Methods*. 2012;31;384(1-2):25-32.
2. Scadding GW, Eifan A, Penagos M, Dumitru A, Switzer A, McMahon O et al. Local and systemic effects of cat allergen nasal provocation. *Clin Exp Allergy*. 2015;45(3):613-23
3. Scadding GW, Eifan AO, Lao-Araya M, Penagos M, Poon SY, Steveling E et al. Effect of grass pollen immunotherapy on clinical and local immune response to nasal allergen challenge. *Allergy*. 2015;70(6):689-96.

4. Scadding GW, Calderon MA, Shamji MH, Eifan AO, Penagos M, Dumitru F et al. Effect of 2 Years of Treatment with Sublingual Grass Pollen Immunotherapy on Nasal Response to Allergen Challenge at 3 Years Among Patients With Moderate to Severe Seasonal Allergic Rhinitis: The GRASS Randomized Clinical Trial. JAMA. 2017;317(6):615-625
5. Eguiluz-Gracia I, Testera-Montes A, González-Visiedo M, Pérez N, Ariza-Veguillas A, Salas M et al. Safety and reproducibility of Nasal Allergen Challenge. Allergy. 2019;74(6):1125-1134
6. Bacon JR, McLean JA, Mathews KP, Banas JM. Priming of the Nasal Mucosa by Ragweed Extract or by an Irritant (Ammonia). J Allergy Clin Immunol. 1981;67(2):111-6.

Author names and affiliations:

Aarif O Eifan^{1,2}, Alexandra Fedina¹, Stephen R Durham^{1,2} and Guy w Scadding^{1,2*}

¹Allergy and Clinical Immunology, National Heart and Lung Institute, Imperial College, London

²Allergy Department, Royal Brompton and Harefield Hospitals NHS Trust, London

Key word: Allergy, aqueous extract of Timothy Grass (*Phleum pratense*) pollen, Timothy Grass lyophilisate tablets , Immunotherapy, Nasal Allergen Challenge, Rhinitis

***Corresponding Author:**

Dr Guy Scadding

Allergy Department, Royal Brompton and Harefield Hospitals NHS Trust,

Allergy and Clinical Immunology, National Heart and Lung Institute,

Imperial College, London

g.scadding@imperial.ac.uk

Conflict of Interest:

Dr. Eifan has nothing to disclose.

Ms Fedina has nothing to disclose.

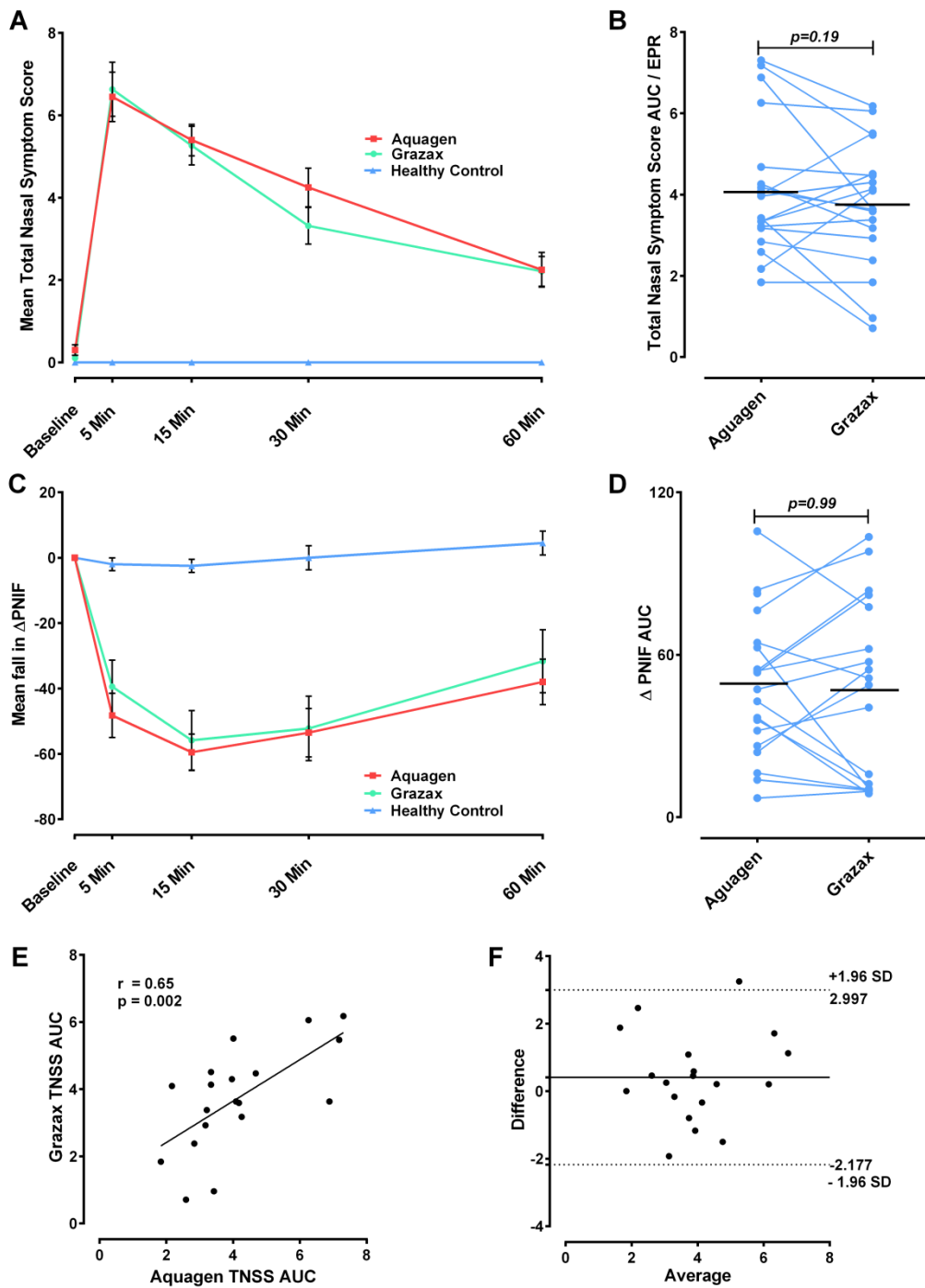
Prof Durham has nothing to disclose.

Dr Scadding has nothing to disclose.

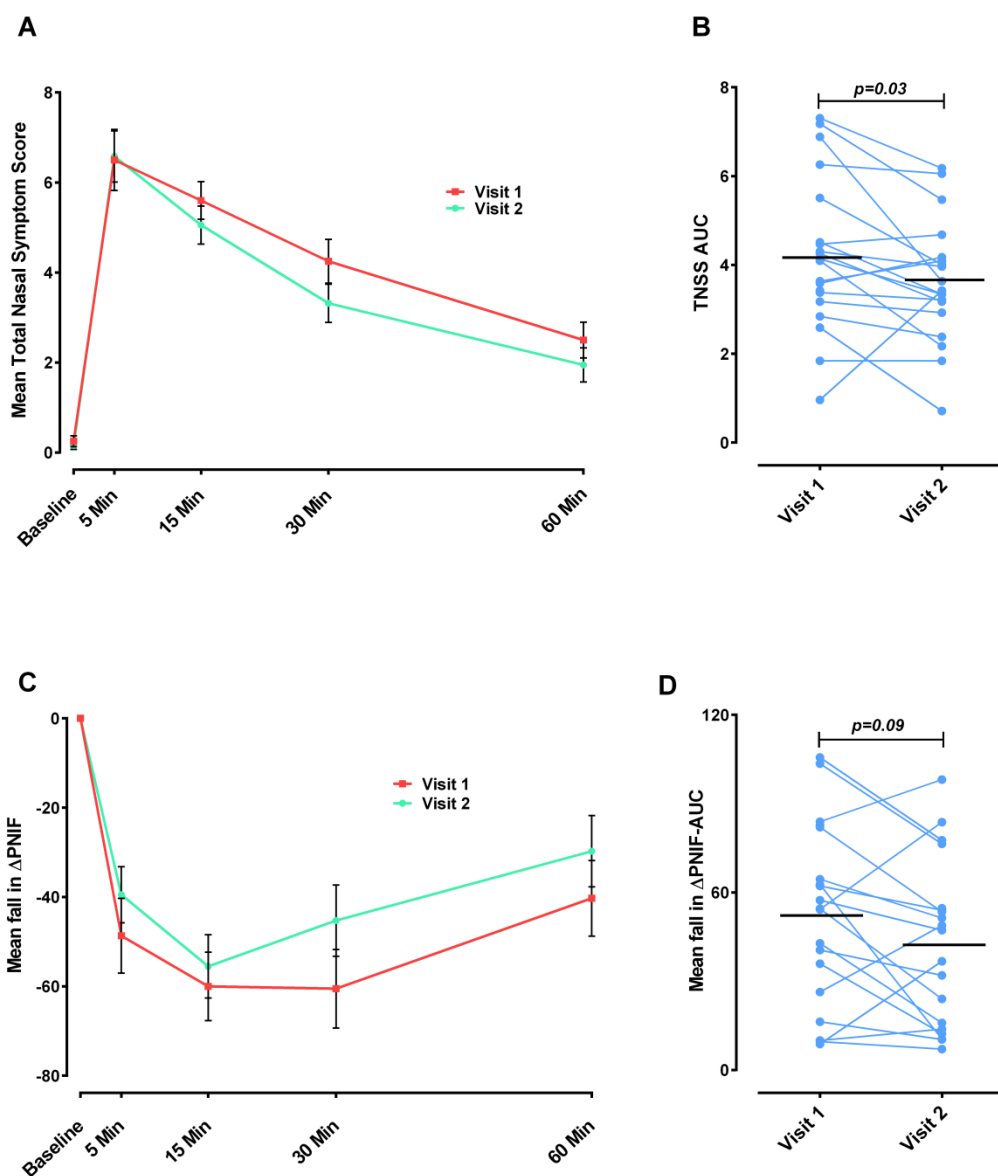
Figure legends:

Figure 1: **A**, mean ($\pm$ s.d.) of total nasal symptom scores (TNSS) after NAC with aqueous extract of Timothy Grass (*Phleum pratense*) pollen and Timothy Grass lyophilisate tablets; **B**, mean TNSS AUC, individual values, paired t-test; **C**, change from baseline PNIF (Δ PNIF); **D**, mean Δ PNIF AUC; **E**, correlation by Pearson's coefficient of TNSS AUC with aqueous extract of Timothy Grass (*Phleum pratense*) pollen and Timothy Grass lyophilisate tablets; **F**, Bland-Altman plot of TNSS AUC with aqueous extract of Timothy Grass (*Phleum pratense*) pollen and Timothy Grass lyophilisate tablets.

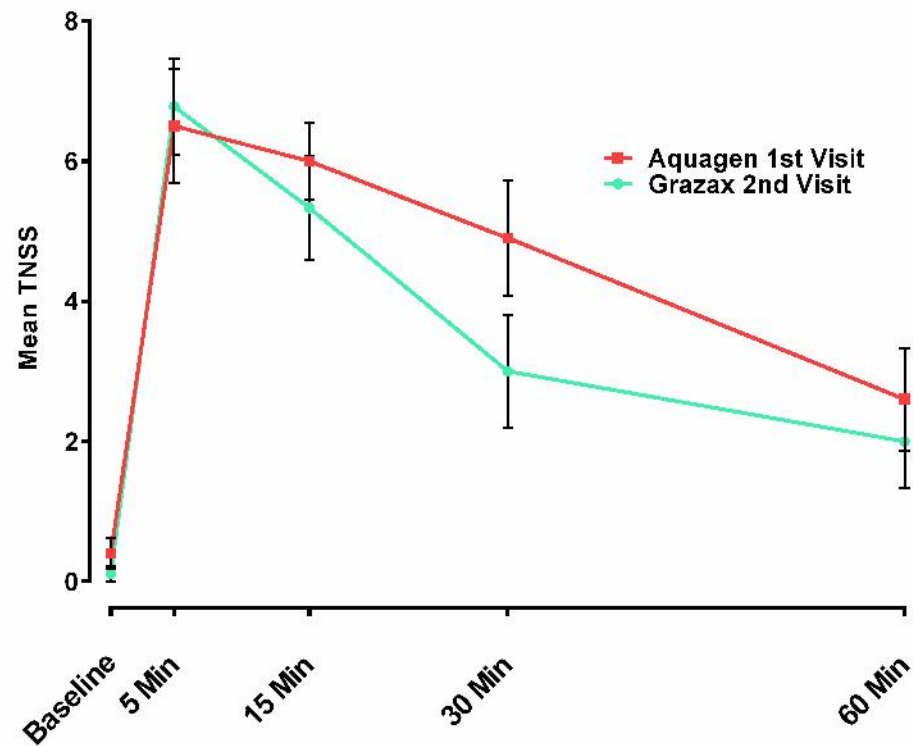
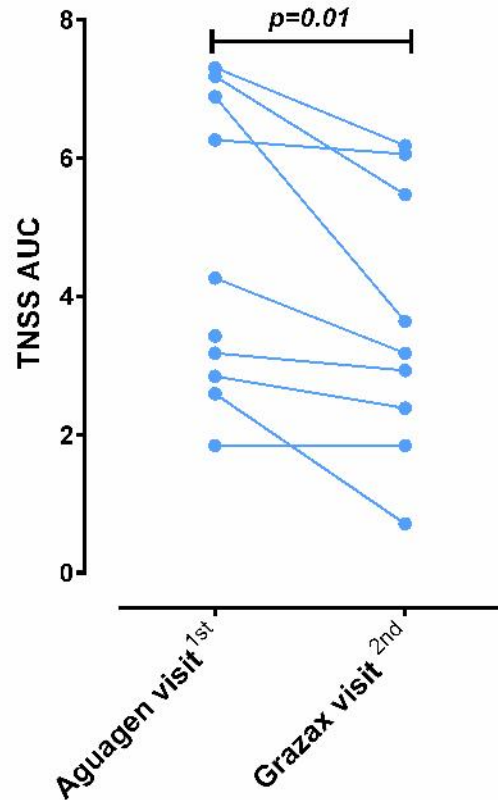
Figure 2: **A**, mean ($\pm$ s.d.) TNSS at visits 1 and 2; **B**, TNSS AUC at visits 1 and 2; **C**, change from baseline PNIF (Δ PNIF) at visits 1 and 2; **D**, Δ PNIF AUC at visits 1 and 2.

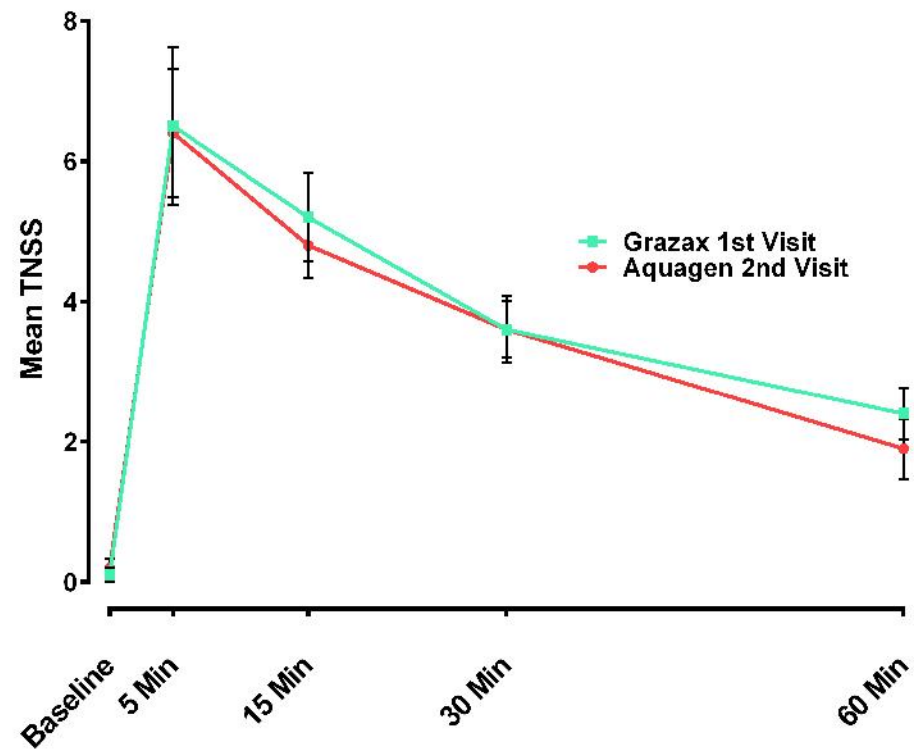
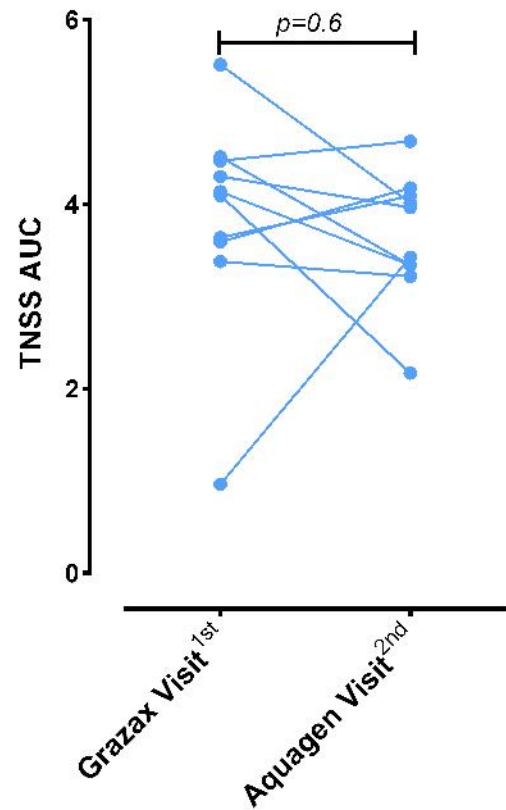


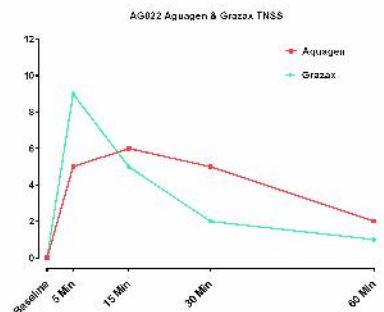
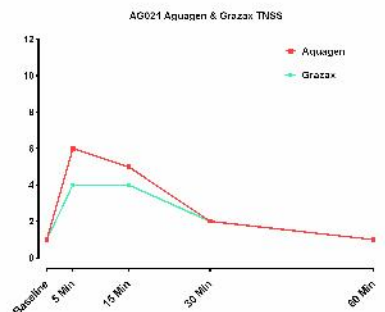
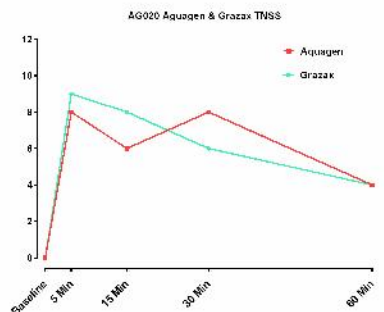
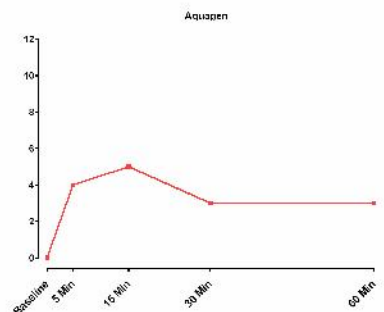
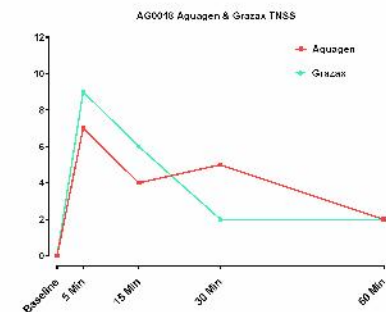
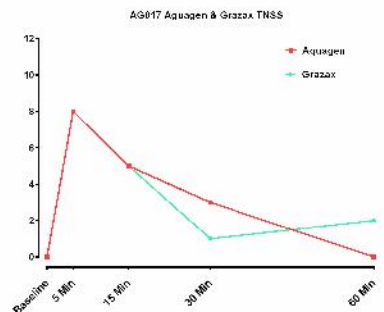
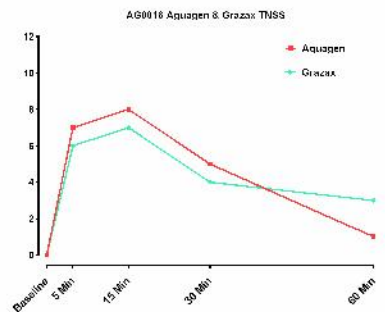
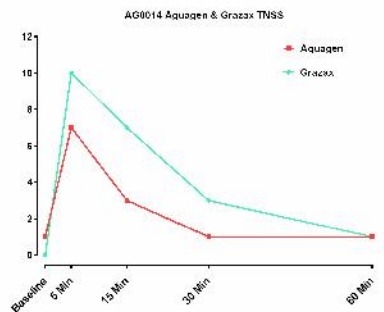
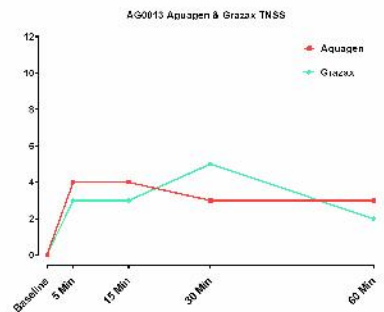
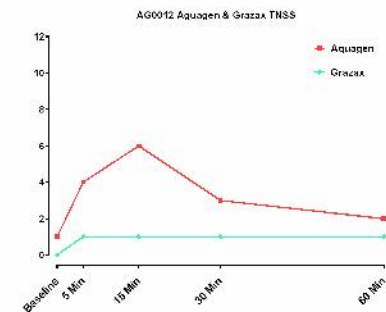
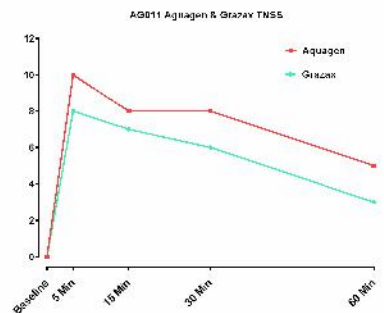
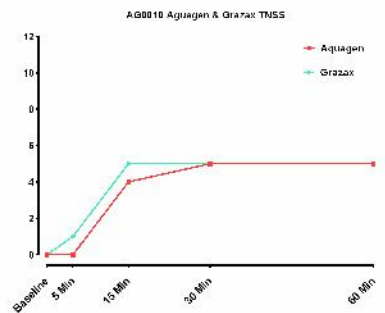
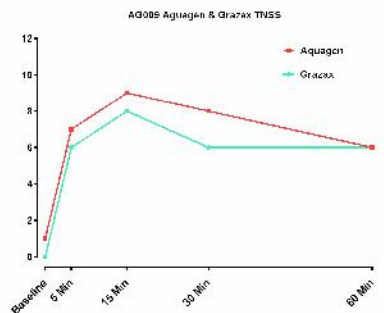
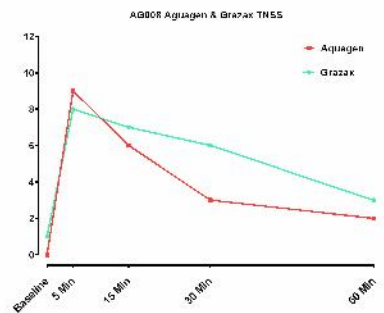
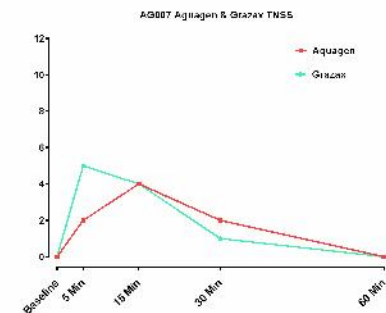
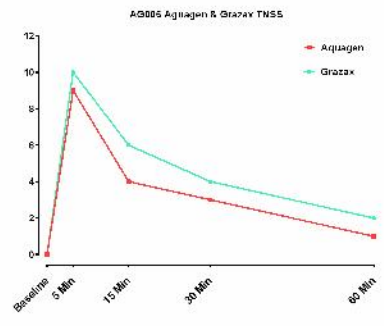
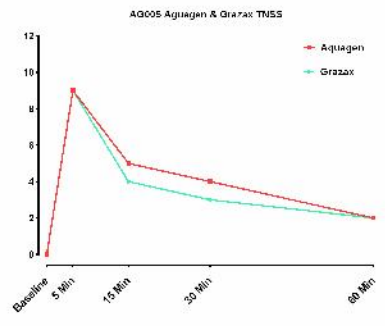
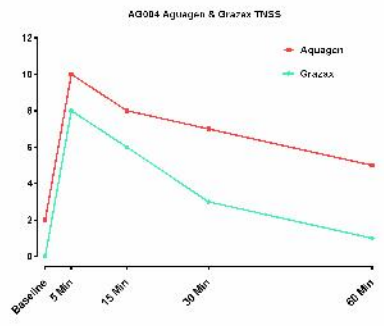
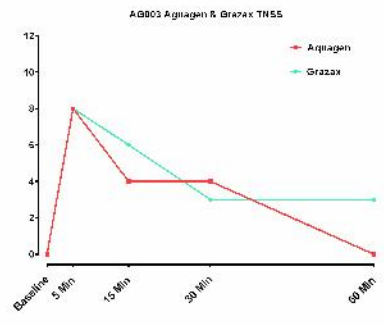
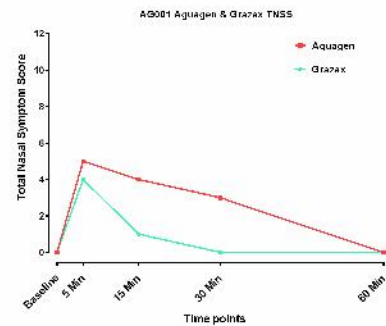
all_14590_f1.tif



all_14590_f2.tif

A**B**

A**B**



Supplement

Inclusion and exclusion criteria:

Inclusion criteria for allergic participants were: age 18-65, a history of at least two years of seasonal allergic rhinitis between May and July, skin prick test to Timothy grass pollen allergen (Soluprick, *Phleum p.* ALK-Abello, Denmark) $\geq 5\text{mm}$, and a negative pregnancy test for women of childbearing age; exclusion criteria included previous grass pollen allergen immunotherapy, clinical history of allergic rhinitis induced by allergen other than grass pollen, perennial asthma requiring regular treatment with inhaled corticosteroids or $\text{FEV}_1 < 70\%$ predicted or an asthma exacerbation requiring hospital attendance in the last 12 months, current acute or chronic rhinosinusitis, current smokers, and a history of fish allergy confirmed by positive skin test/specific IgE. Non-atopic controls were aged 18-65, with no history of atopic disease and negative skin tests to Timothy grass pollen and seven other common aeroallergens; exclusion criteria were as for the allergic group.

Preparation of extracts for NACs:

Preparation of solution for NAC with Timothy Grass lyophilisate tablets:

Two 75,000 SQ-T tablets of purified *Phleum pratense* lyophilisate were placed into a sterile specimen container; 1.5mL of sterile 0.9% saline was added, dropwise, using a 1mL syringe, onto the tablets, ensuring that the tablets completely dissolved; the solution was then shaken gently to aid mixing. This gave a stock solution at a concentration of 100,000 SQ-T/mL

(approximately 20mcg of major allergen, Phl p5 per millilitre). 0.5mL of stock solution was then withdrawn in a sterile 1mL syringe and added to 1mL of saline in a second sterile specimen container and mixed gently. This gave a solution of 33,333 SQ-T/ml. A further 0.5mL of this solution was added to 0.5mL saline in a third specimen container and mixed, giving a concentration of 16,667 SQ-T/mL of *Phleum pratense* extract.

New stock solution and serial dilutions were made on a daily basis. Within 1 hour of each challenge beginning, 230mcL of the 16,667 SQ-T/mL solution was added to a nasal applicator device (Bidose, Aptar Pharma/Pfeiffer, Germany), designed to provide two 100µL sprays.

Preparation of solution for NAC with aqueous extract of Timothy Grass (*Phleum pratense*) pollen:

4.5mL of sterile albumin-based diluent (ALK-Abello) was added into a vial containing aqueous extract Timothy grass pollen, *Phleum pratense*, freeze-dried extract (ALK-Abello) and mixed gently, giving a stock solution with a concentration of 100,000 SQ-U/mL (approximately 20mcg of major allergen, Phl p5 per millilitre). 0.5mL of stock solution was then withdrawn in a sterile 1mL syringe and added to 1mL of saline in a second sterile specimen container and mixed gently. This created a solution of 33,333 SQ-U/ml. A further 0.5mL of this solution was added to 0.5mL saline in a third specimen container and mixed, giving a concentration of 16,667 SQ-U/mL of *Phleum pratense* extract.

New stock solution and serial dilutions were made on a daily basis. Within 1 hour of each challenge, 230mcl of the 16,667 SQ-U/mL solution was added to a nasal applicator device (Bidose, Aptar Pharma/Pfeiffer, Germany), designed to provide two 100µL sprays.

Recording of peak nasal inspiratory flow (PNIF):

Recorded using a PNIF meter (Clement-Clark, UK) plus disposable anaesthetic face mask. The investigator reset the meter to zero, the participant sat up-right in a chair, breathed out fully, then placed the mask attached to the meter over the mouth and nose to form a good seal. The participant then inhaled through the nose as fast as possible whilst keeping the mouth closed, a good seal on the mask, and the meter horizontal. The meter and mask were then passed to the examiner to read and record the result. The best of 3 attempts was recorded at each time point.

Supplementary Table 1: participant characteristics

	Allergic participants	Non-atopic participants
Age in years (+/- s.d.)	38.5 ($\pm$ 11.2)	36.8 $\pm$ 7.3
Male : female	8 : 12	3 : 7
Mono : polysensitised	7 : 13	NA
Grass pollen skin test (mm)	8.8 ($\pm$ 3.9)	0.0 ($\pm$ 0.0)

Supplementary Figure legends

Supplementary Figure 1: A, mean ($\pm$ s.d.) of total nasal symptom scores (TNSS) at baseline and following nasal challenge with aqueous extract of Timothy Grass (*Phleum pratense*) pollen NAC at Visit 1 and Timothy Grass lyophilisate tablets NAC at Visit 2. B, Area under the curve for TNSS from baseline to 60 minutes, comparison by paired t-test.

Supplementary Figure 2: A, mean ($\pm$ s.d.) of total nasal symptom scores (TNSS) at baseline and following nasal challenge with Timothy Grass lyophilisate tablets NAC at Visit 1 and aqueous extract of Timothy Grass (*Phleum pratense*) pollen NAC at Visit 2. B, Area under the curve for TNSS from baseline to 60 minutes, comparison by paired t-test.

Supplementary Figure 3: total nasal symptom score (TNSS) following nasal challenge with aqueous extract of Timothy Grass (*Phleum pratense*) pollen and Timothy Grass lyophilisate tablets in each participant.