

**Non-invasive allergic sinus congestion and resolution assessments using
micro-computed tomography imaging**

Julia Litvinov^{*1}, Walter C. Spear^{*1}, Igor Patrikeev², Massoud Motamedi², and Bill T. Ameredes¹

*These authors contributed equally to the manuscript

¹ Department of Internal Medicine, Division of Pulmonary, Critical Care, and Sleep Medicine,
University of Texas Medical Branch, Galveston, TX 77555

² Center for Biomedical Engineering, University of Texas Medical Branch, Galveston, TX 77555

Running head: MicroCT imaging of allergic sinus congestion

Author e-mails (in authorline order): julitvin@utmb.edu; wcspear@gmail.com;
igpatrik@utmb.edu; mmotamed@utmb.edu; btamered@utmb.edu

Corresponding Author:

Bill T. Ameredes, M.S., Ph.D.
Professor, Internal Medicine
Division of Pulmonary, Critical Care, and Sleep Medicine
Department of Internal Medicine
University of Texas Medical Branch
301 University Blvd.
Galveston, TX 77555-1083
V: 409-772-8104
F: 409-772-5481
btamered@utmb.edu

ABSTRACT

Sinus congestion resultant of allergic rhinosinusitis is associated with development and worsening of asthma and can result in difficulty breathing, headaches, and missed days of school and work. Quantification of sinus congestion is important in the understanding of allergic rhinosinusitis, and the development of new drugs for its treatment. Non-invasive micro-computed tomography (CT) was investigated in a Guinea pig model of allergic rhinosinusitis to determine its utility to accurately determine the degree of sinus congestion and resolution with anti-inflammatory drug administration. Three-dimensional sinus airspace volume, 2-dimensional sinus width, sinus image airspace area, and sinus image sinus perimeter, were measured in Guinea pigs administered ragweed pollen (RWP), intranasally (i.n.), followed by administration of fluticasone, i.n. To determine their relative accuracy in assessing sinus congestion, the micro-CT image results were compared with the “gold-standard” method of sinus fluid fill-volume (SFFV) measurements. As measured by SFFV method, RWP increased sinus congestion in a RWP concentration-dependent fashion, approaching near-total sinus blockage with concentrations $\geq 22 \mu\text{g}$ RWP. At this level of congestion, fluticasone (25-100 μg) progressively decreased sinus congestion in a concentration-dependent fashion. The non-invasive micro-CT methods were found to accurately determine the amount of sinus congestion and resolution, with patterns of increases and decreases of congestion that were nearly identical to the SFFV method. We conclude that non-invasive micro-CT measurements of allergic sinus congestion can be useful as an investigative tool in the assessment of congestion intensity and the development of new drug therapies for its treatment.

Key Words: allergic sinus inflammation, guinea pig, microCT, ragweed pollen, rhinosinusitis

55

56 **New and Noteworthy**

57 Allergic rhinosinusitis afflicts significant portions of the world population, resulting in
58 loss of work productivity and decreased quality of life. Thus, the development of methodologic
59 approaches, which incorporate accurate and reproducible non-invasive assessments of sinus
60 congestion are desirable. Micro-computed tomography of the guinea pig sinuses offers a non-
61 invasive evaluation tool in an animal model of IgE-dependent allergy similar to that in humans,
62 with potential relevance toward development of therapeutics for human sinus diseases.

INTRODUCTION

Allergic rhinosinusitis is one of the most common diseases of inflammation of the upper airways in humans; oftentimes, allergic rhinosinusitis also co-exists with, and exacerbates, asthma (5, 14, 30). Clinically, allergic rhinosinusitis is characterized by early phase symptoms such as sneezing, rhinorrhea, and lacrimation; whereas, late phase symptoms include sinus congestion and drainage, which can lead to headache, coughing, sore throat, and significant breathing difficulties (16, 50), with the increased airflow resistance due to reduction in sinus airway caliber. Specifically, histamine, stored primarily in mast cells, is known to play a primary role in the late-phase inflammatory reactions of allergic rhinosinusitis, which are IgE-dependent, and associated with asthma (1, 46, 47, 51). Typical therapeutic treatments for allergic rhinosinusitis include antihistamines, inhaled nasal corticosteroids, and, in some cases, regular sinus washings (4, 24, 41). While effective to varying degrees, there are issues related to side-effect profiles, inconvenience, and patient compliance that can limit the effectiveness of those therapeutic approaches. Thus, exploration of new sinus congestion assessment techniques and potential therapeutic agents in the study of allergic rhinosinusitis are desirable (22, 23).

Quantitative assessment of sinus congestion in humans can be challenging due to the structure of the nasal sinuses, and the invasiveness required by a number of methods in obtaining reliable measurements (e.g., nasal endoscopy and biopsy). The development of a reliable animal model with translational relevance to human allergic rhinosinusitis is desirable to facilitate study of new therapeutic agents for potential development in humans. While mouse models of allergic rhinosinusitis have been beneficial, they are associated with limitations of size and, most importantly, have serotonin as the major pre-formed mediator released from mast cells associated with the IgE-dependent allergic response (25, 26, 34). In contrast, Guinea pigs, which

also have been used for many years in studies of allergic airway inflammation (3, 6, 42), can represent a more relevant model of human allergic rhinosinusitis, due to having a primarily mast cell histamine-driven IgE allergic response (31, 49).

Accordingly, the objective of the present study was to compare two non-invasive computed tomography image-based approaches of measuring sinus congestion (2D and 3D micro-CT assessments) with a method that utilizes direct quantitation of sinus congestion, based on a sinus fill fluid volume (SFFV) method adapted from prior published studies using fluid-displacement in the Guinea pig (44). First, we quantified the magnitude and drug-dependent resolution of the sinus allergic response to ragweed pollen extract, with the SFFV method. These results were compared to 2-dimensional and 3-dimensional micro-computed axial tomography (micro-CT) methods, to determine the consistency of allergic and resolution effects, and the comparability of non-invasive imaging methods with the direct SFFV assessment method. Our findings indicated that the Guinea pig was a sensitive model of allergic rhinosinusitis and anti-inflammatory treatment, and that non-invasive digital imaging methods could be utilized reliably, and with accuracy of response characterization, that was similar to the direct quantitation of allergic rhinosinusitis using the SFFV method.

MATERIALS AND METHODS

Animals. Male Hartley Guinea Pigs (9-12 weeks old; weight 600-800 g at time of experiments) were purchased from Charles River and housed in high-efficiency particulate-filtered air cages, with free access to food and water. A total of 42 male guinea pigs were used in the experiments. That includes 16 guinea pigs for RWP challenge dose response experiment, 18

additional guinea pigs for RWP challenge and Fluticasone treatment, and 8 naïve guinea pigs (all groups underwent sinus fill fluid volume determination as the final procedure). Female Guinea pigs were not used in these experiments, to avoid potential issues with estrus hormonal cycling and the inflammatory process being studied. Animals were anesthetized during all experiments. Inhaled isoflurane was utilized for the high-resolution imaging experiments (isoflurane mixed with air to a final concentration of 2% of isoflurane) and sodium pentobarbital (35-40 mg/kg) was utilized during all direct sinus measurement procedures, described below. At the end of all experiments performed, Guinea pigs were humanely euthanized with an overdose of pentobarbital sodium (150 mg/kg; Fatal Plus, MedVet International). All animal care and experimental procedures were in accordance with the National Institutes of Health (NIH) guidelines, and were approved by the University of Texas Medical Branch Institutional Animal Care and Use Committee (IACUC).

Allergic Sensitization and Challenge. Research grade ragweed pollen (RWP) proteins (Giant and Short) were purchased from Greer Labs. Guinea Pigs (GP) were sensitized with an intraperitoneal (i.p.) injection of RWP (100 μ g of each protein) initially on day 0, and with a booster on day 7 (**Figure 1**). On days 15-19, sixteen GPs were given an intranasal challenge of RWP concentrations (0, 6, 12, and 22 μ g/ml), delivered in sterile phosphate buffered saline, (PBS; Sigma). Typically, a total volume of 60 μ L RWP/saline solution was placed into each nostril during each RWP administration. For GPs being used as controls (0 μ g/ml), 60 μ L PBS was given with the same timing as the RWP solution. Preliminary experiments (data not shown) indicated that full 100% sinus occlusion could be obtained with RWP concentrations $\geq$ 25 μ g/ml. Further concentration-response experiments described below, indicated that the optimal RWP

concentration for the induction of a reversible allergic congestion response was 22 µg/ml, which was administered as described above.

(Figure 1 here)

Anti-Inflammatory Drug Treatment. To provide both a test of the ability of the SFFV method to measure changes in RWP-associated sinus congestion, and to determine whether steroid dose-dependent effects on congestion could be observed, fluticasone (as Flonase™; Glaxo-Smith-Kline), a commonly-used anti-inflammatory intranasal steroid drug, was given once per day intranasally, 6-8 hr after each intranasal challenge with RWP, as described above. RWP was given at a highest concentration of 22 µg/ml and fluticasone was given at concentrations 0, 25, 50, 75, and 100 µg to groups of 4-5 GPs. This approach allowed a testing protocol in which treatment for congestion was tested in a time domain occurring after the initial exposure to allergen in a therapeutic fashion, rather than a prophylactic one, in which treatments are given prior to all exposures to allergen. This approach also allowed for establishment of the directly-measured allergic congestion and resolution responses for comparison with the non-invasive sinus congestion assessment methods.

Sinus Fluid Fill Volume Measurements. On day 22, seventy-two hours after the final intranasal instillations described above, sinus congestion was measured directly using an SFFV assessment method, modified after that described by Straszek and Pedersen (44). Decapitation under surgical levels of sodium pentobarbital anesthesia was performed using a guillotine, and the blood was allowed to drain from the head and nasal cavity region. The SFFV was measured as a fluid-volume displacement that was dependent on the measured total sinus airspace volume. Briefly, the head of the Guinea pig was mounted nostrils downward, with tubing inserted into the nostril and sealed in place with cyanoacrylate glue, to form a leak-proof seal. A digital constant-

infusion syringe pump (Harvard Apparatus) was connected to the nostril tube and was utilized to deliver a non-diffusible liquid, perfluorocarbon (PFC; Alcon Research, Ltd.), into the sinus airspaces at a constant rate (**Figure 2A**). A non-diffusible non-compressible liquid was necessary, so that: 1) the fluid would not diffuse across the sinus epithelium and all of the fluid volume delivered could reasonably be assumed to remain within the sinus cavities, , and 2) the fluid would not alter the pressure measurements due to compression as the pump pressure was applied to deliver the fluid into the sinus airspaces. A calibrated pressure transducer connected to an IBM PC-compatible computer was mounted on a side-port of the delivery tubing from the pump, to provide a measurement of the applied pressure within the setup, as pump infusion proceeded. The analog DC pressure signals were acquired with an analog-to-digital card (National Instruments) and converted to digital signals that were visualized, recorded, and analyzed using digital signal analysis software (BioBench, National Instruments). The pressure signal was monitored visually over time, with a sudden pressure drop being identified as the point at which the infused volume reached the rearmost portion of the nasal airways, at the nasopharynx, and the sinus airspaces were completely full (**Figure 2B**). With the known constant pump infusion rate, and the elapsed time from the initiation of delivery to the pressure breakpoint, the resultant timed volume was quantified as the SFFV. All SFFV values obtained were normalized by the body weight of each Guinea pig, to control for variations in sinus sizes and airspace volumes, using animal weight as an acceptable surrogate of body size, and thereby, head and sinus size.

(Figure 2 here)

Micro-Computed Tomography. Micro-computed tomography (microCT) was utilized to acquire two-dimensional (2-D) and three-dimensional (3-D) images to capture stages of allergic congestion and resolution with drug treatment using a Siemens Inveon CT/PET/SPECT small-animal scanner. Prior to this procedure, Guinea pigs were anesthetized with isoflurane diluted with air to 2%. . The sinuses of the Guinea pigs were imaged at 72 hours post final intranasal RWP challenge and fluticasone treatment (day 22). The microCT imaging parameters were set with an exposure time of 1000ms (1 second) per projection, 70kV X-ray source voltage, 500 μ A X-ray source current, 107 μ m isotropic resolution, 360 degrees rotation with 520 projections, 54.85 x 82.27 mm field-of-view at scanning, 512x512x768 voxels of reconstructed image. The software used was Siemens Image Acquisition Workplace (for performing the scan) and Image Research Workplace (for image post-processing). Images collected in this fashion resulted in files that averaged ~400 Mb, typically averaging a total of 768 axial slices, at a resolution of 100 μ m per slice, with no gaps between slices. The radiodensity scale was calibrated with air as -1000 HU, and water as 0 HU. The whole-sinus air volumes were calculated using the free airspace of image densities in the range of -1000 to -200 HU, beyond which, the range of -200 to -50 was considered to be high-density tissue, such as fat, with bone and cartilage considered to be beyond 0HU. Therefore, radiodensity signals greater than -200 HU range were not included in the image and subsequent calculations of airspace.

The 2-D linear distance-based sinus congestion assessment method relied on identification of common anatomical bony landmarks across animals, in which the nasal septum and rear upper molars were used as common landmarks. Three main sinus regions were identified: 1) the proximal sinus cavity (nearest the nares), defined as the cavity space from the tip of the nares to the first incisor tooth, 2) the medial sinus cavity, identified as the cavity space caudal to the

proximal sinus cavity, marked from the first incisor tooth to the most rearward molar tooth, and 3) the distal sinus cavity, identified as the cavity space caudal to the medial sinus space, marked from the most rearward molar tooth to the first appearance of the eye socket. The sinus airspace width measurement was taken as the cumulative length of airspaces across the total length of the linear distance within the proximal sinus cavity region, at a reference image density of -400HU, to insure that bony and cartilaginous structures were accounted for, in the determination and measurement of airspace widths.

We also compared pre-determined singular cross-sectional sinus image slices, taken from the 3-D full sinus image, at specified sinus longitudinal length intervals across animals, within each of the three sinus regions outlined above, to determine whether cross-sectional slice depths across the longitudinal full sinus axis could be reproducibly assessed and compared for similar evidence of sinus inflammation and resolution. Using the static anatomical landmarks of the tip of the nares and the eye socket, the full longitudinal length of the sinus was normalized to represent 100%, with intermediary distances represented as 25%, 50%, and 75% of full longitudinal length. This approach allowed us to account for differences between Guinea pigs in head lengths, and therefore, sinus lengths, to arrive at the same relative depth of the longitudinal sinus axis, across animals.

Lower Airway Allergic Inflammation Assessment. Due to: 1) the invasive nature of the SFFV method, 2) the necessity to maintain an undisturbed sinus for the measurement of allergic sinus congestion and drug-associated resolution, and 3) the fact that sinuses treated with PFC were unsuitable for subsequent sinus wash analyses, we utilized bronchoalveolar lavage in several groups of Guinea pigs to assess the degree of airway leukocyte infiltration, as a further index of allergic airway inflammation associated with intranasal RWP administration. For

comparison of this index of inflammation with other animal models utilizing intranasal administration of allergic agents, we also administered intranasal ovalbumin in several groups of animals, on the same schedule as was used for RWP, outlined above. At the end of those experiments, Guinea pigs were euthanized by pentobarbital sodium injection of 150 mg/kg, and the tracheas were cannulated with polyethylene tubing (#4) connected to a 10cc syringe, into which two administrations of 1 ml of ice-cold sterile phosphate buffered saline (PBS) per 100g body weight was instilled into the lungs, and recovered by gentle aspiration. The collected BAL fluid was centrifuged for 10 min at 4°C at 120×g (at 1000 RPM) to isolate cells, which were then re-suspended, pooled, and spun onto slides using a Cytospin4 cytocentrifuge (Thermo Fisher). The slides were stained with H&E stain (Camco Stain Pak; Fisher Scientific/Cambridge Diagnostics) and eosinophils were counted under standard light microscopy at 40X magnification.

Statistical Analyses. SigmaPlot graphical/statistical software (v. 12 and 13; Systat) was used to calculate descriptive statistics and perform statistical analyses. Independent sample t-tests were performed on BAL leukocyte data, to determine differences between treatment groups, with *P* values less than 0.05 considered to be significant. Normality tests were run on all data tested; if data did not pass the normality tests, then the non-parametric equivalent test was performed, at the same level of significance. In the case of t-tests, the Mann-Whitney U test was performed. The SFFV and image data were analyzed using one-way ANOVA. With acquisition of a significant F-statistic by ANOVA, post-hoc testing using the Student Neuman Keuls method was applied to determine discrete differences between treatment groups. If the sinus data were found to fail the normality test, then the non-parametric Mann-Whitney Rank Sum Test was applied, with significance taken at the level of *P*<0.05.

RESULTS

Induction of Allergic Sinus Congestion. We observed a RWP concentration-dependent increment in sinus congestion (Figure 3), evident as a significant decrement in SFFV values, which reached a maximum of a ~75% average decrement in SFFV, in comparing the control (0 µg/ml RWP) and the highest administered RWP concentration (22 µg/ml). This pattern of concentration-dependent induction indicated that the allergic congestion response was able to be reproducibly modulated to a desired level that could then be studied with imaging, and also could be reversed with administration of an anti-inflammatory drug, as described below.

(Figure 3 here)

Airway inflammation: BAL cell analysis. Leukocyte recruitment to the airways typically increases as part of the allergic airway inflammation response [11, 12,24–26, 40], which provided a measure of the relative level of inflammation in our model of allergic rhinosinusitis. The BAL cell counts and patterns with RWP-induced allergic airway inflammation were comparable with those obtained with administration of ovalbumin (**Figure 4A**), confirming the similarity of allergic inflammation within the airways with application of two different allergens.

With the induction of an allergic response in the RWP-challenged Guinea pigs, the increase in BAL eosinophil count was significantly increased compared to naïve Guinea pigs, as shown in **Figure 4B**. As expected, eosinophil counts in fluticasone-treated RWP-challenged Guinea pigs were significantly decreased compared RWP-challenged Guinea pigs, declining to

counts nearly similar to naïve Guinea pigs, and demonstrating the resolution of allergen-associated airway inflammation with application of an intranasal anti-inflammatory steroid.

(Figure 4 here)

Anti-inflammatory drug effects on sinus congestion. Fluticasone significantly decreased RWP-associated allergic sinus congestion in a concentration-dependent fashion, evident as stepwise increments in SFFV (**Figure 5**). Specifically, fluticasone concentrations of 50-100 µg/ml were effective in progressively increasing SFFV, reaching a point of near-total reversal of the RWP-associated allergic sinus congestion with administration of 100 µg/ml, toward high SFFV values, which was not significantly different from that of naïve Guinea pigs.

(Figure 5 here)

2-D Image Width Analyses of Sinus Congestion and Treatment. Using the linear analysis method outlined above, cumulative linear sinus widths were measured in naïve, RWP, and RWP+fluticasone groups, with representative images in **Figure 6** showing the drawn linear line segment (**Figure 6A**), the image terrain/density analyses, as the density of tissue was followed from left to right along the line segment path in each 2-D image above (**Figure 6B**), and the average linear sinus width measurements (**Figure 6C**). The effect of slight differences in head alignment angles across images was minimized by drawing between bilateral landmarks with line segments that were perpendicular to the central axis of the head. With induction of allergic rhinosinusitis by RWP, linear sinus width was decreased to approximately one-third of that measured in naïve Guinea pigs. With administration of fluticasone, linear sinus width was increased over that observed in the RWP group to an average width that was not statistically different from the Naïve group. The pattern of results with the linear sinus width method (**Figure 6C**) were similar in pattern to those obtained using the SFFV technique, as shown in **Figure 7C**,

suggesting that the linear sinus width method of 2-D image analysis tracked the pattern of changes in congestion with allergic induction and subsequent anti-inflammatory treatment.

(Figure 6 here)

3-D Image Whole-sinus Volume Analyses of Sinus Congestion and Treatment. Using the 3-D image whole-sinus volume analysis method outlined above, the sinus volumes were measured in naïve, RWP, and RWP+fluticasone groups with representative images slices of the 3-D images showing the airspace volume in blue color in each image (**Figure 7A**). As shown in **Figure 7B**, with induction of allergic rhinosinusitis by RWP, 3-D imaged whole-sinus air volume was decreased to approximately one-third to one-quarter of that measured in naïve Guinea pigs. With administration of fluticasone, whole-sinus volume was increased over that observed in the RWP group, to an average volume that approached that of the Naïve group. The results with this method were similar in pattern to those obtained using the SFFV (**Figure 7C**) and the 2-D linear width techniques, (**Figure 6C**), suggesting that the 3-D whole-sinus airway image volume analysis method was accurate in tracking changes in congestion with allergic induction and subsequent anti-inflammatory treatment, as compared with the direct SFFV assessment method. **Figure 7D** shows the linear association of the SFFV and whole-sinus air volume measurements as being along a line of positive slope, in which low SFFV values were correlated with low whole-sinus air volume values with RWP administration, as measured by microCT.

(Figure 7 here)

3-D Image Slice Analyses of Sinus Congestion and Treatment. As a result of calculations and normalization of the full length of the Guinea pig nasal sinuses, based on the 25%, 50%, and 75% length reference points described above, we were able to consistently locate

region-based anatomical structures inside the Guinea pig sinuses with reproducible precision. Accordingly, for each Guinea pig, we back-calculated from the last slice that represented the 100% length, to the slice numbers that represented the 25%, 50%, and 75% of full-sinus length landmarks (moving rostral to caudal), as shown in the representative oblique relief images, in which the full-sinus image (**Figure 8A**) is compared with the single cross-sectional image slices (Figure 8B) from that same full-sinus image, at each specific sinus reference length. We found that this approach allowed us to relocate to the same sinus area within 0-3 slices in either the rostral or caudal direction, making our greatest experimental relocation error less than 1% (6 slice total variance/768 total slices), or a maximum sinus length distance error of $\pm 30 \mu\text{m}$ from the desired reference point. With practice, we found that we were typically either exactly on-target, or no more than one virtual slice ($10 \mu\text{m}$) from our desired target. Thus, this approach allowed us to accurately return to, and thus monitor and assess: 1) the same sinus regions within each Guinea pig, 2) the same relative sinus anatomical area across Guinea pigs, and 3) treatment effects in the same relative anatomical sinus regions, across Guinea pig treatment groups.

(Figure 8 here)

The relative 25%, 50%, and 75% length landmark slice locations corresponding to the proximal, medial, and distal sinus regions, moving from bottom (nostrils) to top (eye socket area) of the image, are shown in **Figure 8C**, illustrating a vertically-oriented sinus image from a typical naïve Guinea pig, with blue coloration indicating whole-sinus airspace volume. For comparison, the resultant cross-sectional images at each length reference point are shown directly to the right, from three separate Guinea pigs representative of each of the respective Naïve, RWP, and RWP+fluticasone treatment groups. In these cross-sectional images, airspace is shown as blue, and can be observed to be decreased at each sinus level with RWP, as compared

to Naïve, and was increased with fluticasone treatment, toward air volume area fills similar to that of the Naïve Guinea pig. While exemplary, further evidence of these differences is shown in a similar comparison of virtual 3-D slice images of three Guinea pigs per treatment group with sections at each length level of the whole-sinus (**Figure 9**). These images are provided to show 1) the repeated similarity of images at each length level within the control and experimental groups, 2) the changes that can be observed repeatedly with RWP-induced allergic congestion, at each length level, and 3) the repeated reversal of those changes at each length level, with fluticasone treatment.

Finally, we measured the both the sinus airspace cumulative area and the sinus surface perimeter within each 3-D slice at the 25%, 50%, and 75% sinus length levels, in Guinea pigs from each of the Naïve, RWP, and RWP+fluticasone groups. As shown in the images of **Figure 9**, the calculated airspace area corresponded to the darker blue color of airspace within each slice (as illustrated in **Figures 9-11**), and the calculated sinus surface perimeter corresponded to a lighter blue highlight of the boundary of each airspace. Visual inspection of the darker blue coloration in those images verifies the relatively open sinus space in the Naïve group, much less darker blue coloration in the RWP group (corresponding to allergic sinus congestion), and increased darker blue coloration in the fluticasone treated group. Using these methods of assessment, we plotted both average sinus airspace area values (**Figure 10A**), and average sinus surface boundary perimeter values (**Figure 10B**), and the ratio of area/ perimeter, for each treatment group (**Figure 10C**), at each sinus slice level. As shown with the other methods of 3-D image assessment, and the “gold-standard” SFFV method, the patterns of sinus area and perimeter changes are consistent with what would be expected with RWP-associated induction of allergic sinusitis and its amelioration with administration of an anti-inflammatory steroid.

However, visual inspection and comparison with the results from the SFFV method and the whole-sinus airspace volume assessments (as shown in Figure 7) suggest that the greatest fidelity of the sinus airspace area, surface perimeter, and area/perimeter ratio, in tracking the allergic sinus congestion and its reversal with anti-inflammatory treatment, occurs within the proximal sinus, at the 25% sinus length level.

DISCUSSION

The main findings of present studies were: 1) 2-dimensional microCT methods utilizing nasal width measurements, 3-D microCT whole-sinus volume measurements, and 3-D microCT sinus image slice analyses provided assessments of allergic sinus congestion that were similar to those obtained with the direct “gold-standard” SFFV method, 2) a normalized 3-D microCT image slice location method allowed a reproducible degree of accuracy in analyses of inflammation and its resolution both across individuals and experimental groups, and 3) microCT 3-D image slice analyses provided assessments of both sinus airspace area and surface perimeter that were likewise consistent with the SFFV, 2-D linear width, and 3-D whole-sinus air volume methods of allergic sinus congestion and resolution measurement.

Guinea Pig Model of Allergic Rhinosinusitis. While investigation of allergic sinus congestion has been undertaken in other animal models, such as mice and rats, the Guinea pig was considered to be superior for the present studies due to its size, and its similarity of allergic responses and symptoms, to those of humans. Consistent with allergic airway inflammation in human asthma, we verified the presence of allergic airway inflammation in our Guinea pig model by quantification of the BAL cell differential and observation of RWP-associated eosinophilia, which was resolved with administration of fluticasone (**Figure 4**). Those findings

in the lower airways were consistent with a prior report, in which eosinophilia and its resolution with fluticasone was observed in the nasal lavage and mucosa of Guinea pigs administered OVA (2) . While it is possible that there could be a lack of association between sinus airway and lung airway inflammation in our model, we note that many animal models of lung airway inflammation are induced through intranasal application of allergens and that the inflammation of both the upper and lower airways often occur together in human asthma. Anecdotally, we often observed enhanced mucus secretion with runny noses and sneezing in our animals that had been given RWP, i.n., similar to that reported previously in Guinea pigs (2, 27) and indicative of the late-phase histamine-associated allergic response (51) and symptoms observed in humans with allergic rhinosinusitis (52).

Sinus Fluid-volume method as a “gold-standard”. There exists a number of methods from which to choose to measure sinus airway congestion; they range from non-invasive, to minimally invasive, and/or fully invasive. One minimally-invasive and non-physiologic method of nasal patency assessment in humans (i.e., no surgical cutting or penetration of skin or other structural barriers) is acoustic sinus rhinometry (28). However, this method requires complex mathematics, subject cooperation with breath-hold maneuvers, ambient temperature and noise control, and is often limited due to variations in structure of nasal cavities besides being based on aspects of subjective assessments (10, 13, 36), some of which require a “standard nose” with which to compare (17), which is clearly unsuitable for investigations in Guinea pigs. Furthermore, due to the tortuosity of sinus structures, the measurement variation obtained with this sound-based method can be significant due to heterogeneity of reflected sound waves when irregular structures within the sinuses are encountered during the measurements. As shown by the 3-D image slices in **Figures 9 and 10**, this would be particularly true for the distal sinus

regions. Indeed, a series of studies comparing acoustic rhinometry to the fluid displacement method in dogs, cats, Guinea pigs, and rats indicated that acoustic rhinometry underestimated sinus volume by an average of approximately 15-30% across those species (44, 45) While non-invasive, for the reasons above, we judged the acoustic rhinometry method to be unacceptable as a “gold-standard” for our purposes, against which to compare result from our non-invasive micro-CT methods.

Another non-invasive technique is magnetic resonance imaging (MRI) assessment, which has advantages similar to micro-CT of being repeatable and non-terminal. However, a study of MRI in comparison with the fluid displacement method in dogs and cats indicated that cross-sectional area of the sinus lumen was limited by the fact that its definition was dependent on subjective choices made by the image assessor, making MRI unsuitable as a “gold-standard” of sinus measurement (45). While we had obtained some interesting and congruent data with the 2-D linear cumulative-width measures of micro-CT images (**Figure 6**), we found this same situation to be true regarding the line segment placement, which is one reason that we favored the whole-sinus 3-D volume assessments.

Two additional more invasive methods for the assessment of sinus congestion are the use of nasal sinus casts (39, 43) and histologic approaches (35), which are more conclusive than acoustic rhinometry, but can be confounded by preparation issues, such as deformation of compliant structures and histological sectioning artifacts and are, likewise, terminal in nature. Thus, we selected the invasive, but direct method, of SFFV measurement as our “gold-standard” method, for these studies.

Reliability of Micro-CT Sinus Imaging Methods. As suggested above, a major advantage of the micro-CT imaging methods was their non-invasive, non-terminal nature. It was

remarkable that each of the CT-imaging techniques we utilized demonstrated a similar sinus congestion and resolution pattern that essentially equated nasal airway width (**Figure 6**), whole sinus airway volume (**Figure 7**), sinus airspace area (**Figure 10A**) and sinus surface perimeter (**Figure 10B**), with direct measurements of SFFV (**Figure 7**). This gave us confidence that non-invasive image-based air volume measurements could be considered equivalent to, direct fluid-volume measurements of sinus congestion.

While we were able to show concordance of allergic sinus congestion and resolution results with the SFFV measurements using the 2-D linear method, the 3-D whole-sinus volume method and the 3-D image slice methods(**Figures 6 and 7**), there were some limitations encountered, as well. For example, the 2-D linear approach for measuring cumulative sinus width was applied only to the proximal sinus area, where relatively unambiguous bony anatomical landmarks were reliably evident and of similar sizes and shapes across Guinea pigs in the age range we studied. The age of the Guinea pig was an important surrogate of Guinea pig size and growth, which can modify apparent width measurements, also adding a level of variance to such measurements. The 2-D linear approach also relied on the subjective skill and experience of the image operator to reliably sample the sinus length and location at a comparable depth across Guinea pigs sampled, adding another potentially confounding variable to the reproducibility of this measurement. Thus, while the 2-D linear method obtained results in the proximal sinus that were consistent with the SFFV method, it had some characteristics that limited its consideration as an optimal image assessment method.

The slice assessment method utilizing normalized sinus length location within the 3-D whole-sinus images was remarkably accurate, from the perspective that the same relative sinus depth from the nostrils to the eye sockets could be reliably located both within a single Guinea

pig, and across multiple Guinea pigs (**Figures 8,-10-11**). This method, likewise required some practice, but was less ambiguous than the 2-D linear width method, due to the reliable mathematical nature of the normalized length location approach, and the consistent image slice thickness for each whole-sinus image. An important aspect of this method was that the sinus surface perimeter, or sinus boundary, was quantifiable and demonstrated changes concordant with the other congestion assessment methods, and particularly the SFFV data (**Figures 7,9, 10**), which could be important in characterization of sinus airway inflammation and disease.

Of the three micro-CT image assessment methods, the 3-D whole-sinus volume measurement approach offered the least ambiguous and most directly comparable results with the direct SFFV method. This is likely because both methods are essentially volume integrations of the whole sinus airspace, which decreases with congestion and increases with resolution of congestion. Furthermore, as suggested by the illustration in **Figure 7**, the 3-D whole sinus volume image also provides the most visually apparent changes in sinus congestion with the induction of allergic response, and its treatment with an anti-inflammatory drug, which is a desirable characteristic of an image-based method of quantification. Thus, while some additional information is obtainable with the other image-based approaches, we judged the 3-D whole-sinus volume method as providing the most reliable overall determinations of sinus congestion consistent with allergic rhinosinusitis.

Other Methodological Considerations.

Micro-CT offers a non-invasive, cost-effective approach allowing imaging of hard structures (e.g., bone and other tissue making up the sinuses), providing high sensitivity and high spatial resolution. This x-ray-based imaging tool can provide good resolution in a time-efficient manner, while facilitating high throughput of analyses (7, 9, 15, 20, 38). However, two potential

limitations of micro-CT are radiation dose and the somewhat low resolution of a number of soft-tissue structures (18, 19, 48). With refinement of the technique, it might be possible to focus attention on some of the resolvable sinus soft tissue changes with allergic rhinosinusitis and its treatment. However, in the present study, soft tissue modifications were not focused upon, and the resolution of the scan of the nasal cavity structures was sufficient to distinguish between bone, cartilage and airspace.

While a necessary concern, the radiation dose would not be considered cumulatively damaging, in the way that we typically applied micro-CT during one pre-terminal sinus imaging session. Micro-CT applied in small animals is generally thought to be harmful only as it relates to sequential and repeated exposures over compressed time intervals, such as days to weeks. Specifically, radiation from repeated micro-CT exposures could ultimately influence animal survival or tumor growth in small animals, such as mice (53), in which the relative radiation dose per total body mass can be high, as compared to much larger Guinea pigs. It has been reported that a lethal dose (LD) for 50% of exposed mice within 30 days ($LD_{50/30}$) can range between 5.0-7.6 Gray (Gy), and that a typical whole-body radiation dose for a 3-D micro-CT can range from 0.017-0.78 (Gy) (7). At the high end of that typical range (0.78 Gy), an estimated cumulative $LD_{50/30}$ of 7.6 Gy would theoretically be reached after approximately 10 closely-timed successive whole-body micro-CT imaging sessions in mice. Furthermore, rodents can repair alterations from sub-lethal doses of radiation in several hours (32) and have been reported to demonstrate no adverse effects within the lower airways during longitudinal micro-CT studies (8).

Presumably, Guinea pigs would be able to tolerate more than 10 sequential micro-CT exposures before reaching $LD_{50/30}$ radiation levels, owing to their larger size. In support of this probability, it has also been reported that the skin of mice and rats can absorb as much 0.045 and

0.028 Gy, respectively, during one micro-CT whole-body scanning session, but that the internal organs receive significantly lower doses, typically averaging <0.035 and <0.024 Gy in mice and rats, respectively (29). Thus, based on body mass and size scaling, with a typical young adult mouse weighing 20-30 g, a typical young adult rat weighing 300-500 g, and our studied young adult Guinea pigs weighing 600-900 g, it would be expected that the skin of Guinea pigs would cumulatively absorb less than 0.028 Gy in one micro-CT exposure, and their internal organs even less than that amount during one micro-CT imaging session.

CT is commonly used in human clinical practice at dosages ranging from 0.005-0.070 Gy for interventional procedures, 0.003-0.020 Gy for most nuclear procedures, and 0.002 Gy for head CT analyses, as compared to the average annual background radiation exposure of approximately 0.3 Gy (Mettler 2008). The acute radiation dosage with a single clinical scan of sinuses is comparatively low, reported to range as low as 0.0005-0.006 Gy (Bang 2016), and 0.0029-0.0316 Gy (Hoxworth 2014); cumulative doses, particularly for a surveillance scan that may be done at most once/year, would be even less. For patients with chronic disorders, such as severe rhinosinusitis, a major issue is induction of cataracts with radiation of the lens of the eye during serial CT imaging of paranasal sinuses, typically considered to occur at levels of 0.250 and 5.0 Gy for pediatric and adult patients, respectively (Bang 2016), but which can be avoided with proper shielding (Hoxworth 2014). Particularly for adults, sinus CT scans are therefore considered to be of minimal risk for the induction of tissue pathology and cancer, especially as compared to the relative benefit of accurate and personalized sinus diagnoses and therapy.

Comparisons with Other Studies of Sinus Congestion. The anatomy and physiological responses of the Guinea pig nasal cavities have been of interest to biomedical scientists as far back as 1949-1950, with the proclamation that the Guinea pig represented the ideal experimental

animal for research work within the nasal cavity (21, 37). Our results obtained with 3-D whole sinus image analyses (**Figures 7, 8**), as well as our 3-D image slices (**Figures 8-10**) compare favorably with a prior study using 2-D and 3-D CT imaging techniques in Guinea pigs (33). Our 3-D image slices also compare favorably with older histological sections of the Guinea pig sinuses (21), suggesting the practicality of the use of microCT as a non-invasive imaging method to assess sinus topography and alterations.

Our findings of significant, and in some cases, near-complete reversal of allergic sinus congestion with i.n. fluticasone administration, as assessed by all of the methods we employed, was strongly consistent with a prior study in Guinea pigs, in which fluticasone was reported to result in complete inhibition of congestion (2). Those data, considered together with the lower airway indices of inflammation and its resolution with fluticasone, further enhanced our confidence in the micro-CT approach, as being valid for testing of drug treatments employed in the treatment of allergic rhinosinusitis.

Summary. Our results indicate that the non-invasive, non-terminal micro-CT sinus airspace volume measurement method can provide allergy-associated sinus congestion data consistent with the more invasive and terminal directly-measured SFFV method. The two methods produced data that was strongly associated when measuring both the degree of allergy-associated congestion induction and its resolution with administration of a classical anti-inflammatory steroid. Furthermore, we found that normalization for relative animal size differences, and the use of consistent anatomical landmarks, could be utilized to insure reproducibility of micro-CT measurements across individuals, and the subsequent identification of treatment effects. Taken in aggregate, the present study provides evidence that the micro-CT

543 method can be used to accurately measure IgE-associated sinus allergic responses, suggesting a
544 potential for utilization of micro-CT as an investigative tool in drug testing and mechanistic
545 studies of allergic rhinosinusitis.

Acknowledgements

The authors would like to thank Spotswood D. Miller for assistance with experiments, and Drs. Sanjiv Sur, William J. Calhoun, and John Yanni, for experiment consultation and advice.

Grants

This research was supported by NIEHS T32ES007254, NIEHS P30ES006676, the Sealy Center for Molecular Medicine (SCMM), the Sealy Center for Environmental Health and Medicine (SCEHM), the UTMB Institute for Translational Sciences (ITS), the Division of Pulmonary, Critical Care, and Sleep Medicine, and the Brown Foundation.

Disclosures

A portion of this work was supported by grants from Alcon Research Ltd. and the Forest Research Institute.

Author Contributions

JL, WCS, and IP contributed with performance of experiments, data reduction, and writing and review of the manuscript; MM and BTA contributed with supervision of experiments and data reduction, and writing and review of the manuscript.

References

1. **Bachert C, Marquardt U, and Korte M.** IgE-positive mast cells play a central role in nasal allergic disease. *American Journal of Rhinology* 4: 215-219, 1990.
2. **Bahekar PC, Shah JH, Ayer UB, Mandhane SN, and Thennati R.** Validation of guinea pig model of allergic rhinitis by oral and topical drugs. *Int Immunopharmacol* 8: 1540-1551, 2008.
3. **Belvisi MG, Birrell MA, Khalid S, Wortley MA, Dockry R, Coote J, Holt K, Dubuis E, Kelsall A, Maher SA, Bonvini S, Woodcock A, and Smith JA.** Neurophenotypes in Airway Diseases. Insights from Translational Cough Studies. *Am J Respir Crit Care Med* 193: 1364-1372, 2016.
4. **Bousquet J, Bachert C, Bernstein J, Canonica GW, Carr W, Dahl R, Demoly P, Devillier P, Hellings P, Fokkens W, Klimek L, Lieberman P, Meltzer E, Price D, Ryan D, and Wahn U.** Advances in pharmacotherapy for the treatment of allergic rhinitis; MP29-02 (a novel formulation of azelastine hydrochloride and fluticasone propionate in an advanced delivery system) fills the gaps. *Expert Opin Pharmacother* 16: 913-928, 2015.
5. **Bousquet J, Khaltayev N, Cruz AA, Denburg J, Fokkens WJ, Togias A, Zuberbier T, Baena-Cagnani CE, Canonica GW, and Van Weel C.** Allergic rhinitis and its impact on asthma (ARIA) 2008. *Allergy* 63: 8-160, 2008.
6. **Buels KS, Jacoby DB, and Fryer AD.** Non-bronchodilating mechanisms of tiotropium prevent airway hyperreactivity in a guinea-pig model of allergic asthma. *Br J Pharmacol* 165: 1501-1514, 2012.
7. **Clark DP, and Badea CT.** Micro-CT of rodents: state-of-the-art and future perspectives. *Phys Med* 30: 619-634, 2014.
8. **Detombe SA, Dunmore-Buyze J, Petrov IE, and Drangova M.** X-ray dose delivered during a longitudinal micro-CT study has no adverse effect on cardiac and pulmonary tissue in C57BL/6 mice. *Acta Radiol* 54: 435-441, 2013.
9. **Dudak J, Zemlicka J, Karch J, Patzelt M, Mrzilkova J, Zach P, Hermanova Z, Kvacek J, and Krejci F.** High-contrast X-ray micro-radiography and micro-CT of ex-vivo soft tissue murine organs utilizing ethanol fixation and large area photon-counting detector. *Sci Rep* 6: 30385, 2016.
10. **Erdogan M, Cingi C, Seren E, Cakli H, Kezban Gurbuz M, Kaya E, Incesulu A, Ozudogru E, and Kecik C.** Evaluation of nasal airway alterations associated with septorhinoplasty by both objective and subjective methods. *Eur Arch Otorhinolaryngol* 270: 99-106, 2013.

11. **Florsheim E, Yu S, Bragatto I, Faustino L, Gomes E, Ramos RN, Barbuto JA, Medzhitov R, and Russo M.** Integrated innate mechanisms involved in airway allergic inflammation to the serine protease subtilisin. *J Immunol* 194: 4621-4630, 2015.
12. **Geering B, Stoeckle C, Conus S, and Simon HU.** Living and dying for inflammation: neutrophils, eosinophils, basophils. *Trends Immunol* 34: 398-409, 2013.
13. **Gershwin ME, and Incaudo G.** *Diseases of the sinuses: a comprehensive textbook of diagnosis and treatment.* Springer Science & Business Media, 2012.
14. **Greiner AN, Hellings PW, Rotiroti G, and Scadding GK.** Allergic rhinitis. *Lancet* 378: 2112-2122, 2011.
15. **Hafeez S, and Huddart R.** Advantages and Limitations. In: *PET/CT in Radiotherapy Planning* Springer, 2017, p. 33-38.
16. **Hellings PW, Fokkens WJ, Akdis C, Bachert C, Cingi C, Dietz de Loos D, Gevaert P, Hox V, Kalogjera L, Lund V, Mullol J, Papadopoulos NG, Passalacqua G, Rondon C, Scadding G, Timmermans M, Toskala E, Zhang N, and Bousquet J.** Uncontrolled allergic rhinitis and chronic rhinosinusitis: where do we stand today? *Allergy* 68: 1-7, 2013.
17. **Hilberg O, and Pedersen OF.** Acoustic rhinometry: recommendations for technical specifications and standard operating procedures. *Rhinol Suppl* 16: 3-17, 2000.
18. **Ho ST, and Hutmacher DW.** A comparison of micro CT with other techniques used in the characterization of scaffolds. *Biomaterials* 27: 1362-1376, 2006.
19. **Holdsworth DW, and Thornton MM.** Micro-CT in small animal and specimen imaging. *Trends in Biotechnology* 20: S34-S39, 2002.
20. **Hupfer M, Nowak T, Brauweiler R, Eisa F, and Kalender WA.** Spectral optimization for micro-CT. *Med Phys* 39: 3229-3239, 2012.
21. **Kelemen G.** Nasal cavity of the guinea pig in experimental work. *AMA Arch Otolaryngol* 52: 579-596, 1950.
22. **Konradsen JR, Fujisawa T, van Hage M, Hedlin G, Hilger C, Kleine-Tebbe J, Matsui EC, Roberts G, Ronmark E, and Platts-Mills TA.** Allergy to furry animals: New insights, diagnostic approaches, and challenges. *J Allergy Clin Immunol* 135: 616-625, 2015.
23. **Matricardi PM, Rosmini F, Riondino S, Fortini M, Ferrigno L, Rapicetta M, and Bonini S.** Exposure to foodborne and orofecal microbes versus airborne viruses in relation to atopy and allergic asthma: epidemiological study. *Bmj* 320: 412-417, 2000.

- 633 24. **Meltzer E, Ratner P, Bachert C, Carr W, Berger W, Canonica GW, Hadley J,**
634 **Lieberman P, Hampel FC, Mullol J, Munzel U, Price D, Scadding G, Virchow JC,**
635 **Wahn U, Murray R, and Bousquet J.** Clinically relevant effect of a new intranasal
636 therapy (MP29-02) in allergic rhinitis assessed by responder analysis. *Int Arch Allergy*
637 *Immunol* 161: 369-377, 2013.
- 638 25. **Meurs H, Gosens R, and Zaagsma J.** Airway hyperresponsiveness in asthma:
639 lessons from in vitro model systems and animal models. *Eur Respir J* 32: 487-502, 2008.
- 640 26. **Moore BD, Hyde DM, Miller LA, Wong EM, and Schelegle ES.** Persistence of
641 serotonergic enhancement of airway response in a model of childhood asthma. *Am J*
642 *Respir Cell Mol Biol* 51: 77-85, 2014.
- 643 27. **Nabe T, Mizutani N, Shimizu K, Takenaka H, and Kohno S.** Development of
644 pollen-induced allergic rhinitis with early and late phase nasal blockage in guinea
645 pigs. *Inflamm Res* 47: 369-374, 1998.
- 646 28. **Nathan RA, Eccles R, Howarth PH, Steinsvag SK, and Togias A.** Objective
647 monitoring of nasal patency and nasal physiology in rhinitis. *J Allergy Clin*
648 *Immunol* 115: S442-459, 2005.
- 649 29. **Obenaus A, and Smith A.** Radiation dose in rodent tissues during micro-CT
650 imaging. *Journal of X-ray Science and Technology* 12: 241-249, 2004.
- 651 30. **Ozdoganoglu T, and Songu M.** The burden of allergic rhinitis and asthma. *Ther*
652 *Adv Respir Dis* 6: 11-23, 2012.
- 653 31. **Palm NW, Rosenstein RK, and Medzhitov R.** Allergic host defences. *Nature* 484:
654 465-472, 2012.
- 655 32. **Parkins CS, and Fowler JF.** A cautionary note on the resolution of paraquat lung
656 damage after radiotherapy. *Br J Radiol* 58: 1137-1140, 1985.
- 657 33. **Phillips JE, Ji L, Rivelli MA, Chapman RW, and Corboz MR.** Three-
658 dimensional analysis of rodent paranasal sinus cavities from X-ray computed
659 tomography (CT) scans. *Can J Vet Res* 73: 205-211, 2009.
- 660 34. **Phillips JE, Peng R, Harris P, Burns L, Renteria L, Lundblad LK, Fine JS,**
661 **Bauer CM, and Stevenson CS.** House dust mite models: will they translate clinically as
662 a superior model of asthma? *J Allergy Clin Immunol* 132: 242-244, 2013.
- 663 35. **Rohrbach S, Olthoff A, Laskawi R, Giefer B, and Gotz W.** Botulinum toxin type
664 A induces apoptosis in nasal glands of guinea pigs. *Ann Otol Rhinol Laryngol* 110:
665 1045-1050, 2001.

- 666 36. **Roopa Manjunatha G, Mahapatra DR, Prakash S, and Rajanna K.** Validation of
667 polyvinylidene fluoride nasal sensor to assess nasal obstruction in comparison with
668 subjective technique. *Am J Otolaryngol* 36: 122-129, 2015.
- 669 37. **Rüedi VL, Furrer W, Escher F, and Lüthy F.** Toxische Wirkungen des
670 Streptomycins. *Acta Oto-Laryngologica* 36: 66-77, 1948.
- 671 38. **Schladitz K.** Quantitative micro-CT. *J Microsc* 243: 111-117, 2011.
- 672 39. **Schreider JP, and Hutchens JO.** Morphology of the guinea pig respiratory
673 tract. *Anat Rec* 196: 313-321, 1980.
- 674 40. **Schuijs MJ, Willart MA, Hammad H, and Lambrecht BN.** Cytokine targets in
675 airway inflammation. *Curr Opin Pharmacol* 13: 351-361, 2013.
- 676 41. **Seidman MD, Gurgel RK, Lin SY, Schwartz SR, Baroody FM, Bonner JR,**
677 **Dawson DE, Dykewicz MS, Hackell JM, Han JK, Ishman SL, Krouse HJ,**
678 **Malekzadeh S, Mims JW, Omole FS, Reddy WD, Wallace DV, Walsh SA, Warren**
679 **BE, Wilson MN, and Nnacheta LC.** Clinical practice guideline: Allergic
680 rhinitis. *Otolaryngol Head Neck Surg* 152: S1-43, 2015.
- 681 42. **Strasser A, Wittmann HJ, Buschauer A, Schneider EH, and Seifert R.** Species-
682 dependent activities of G-protein-coupled receptor ligands: lessons from histamine
683 receptor orthologs. *Trends Pharmacol Sci* 34: 13-32, 2013.
- 684 43. **Straszek SP, McLeod RL, Hey JA, Mosekilde L, and Pedersen OF.** Comparison
685 of feline nasal cavity dimensions measured by acoustic rhinometry and nasal casts. *Am J*
686 *Rhinol* 17: 233-239, 2003.
- 687 44. **Straszek SP, and Pedersen OF.** Nasal cavity dimensions in guinea pig and rat
688 measured by acoustic rhinometry and fluid-displacement method. *J Appl Physiol*
689 (1985) 96: 2109-2114, 2004.
- 690 45. **Straszek SP, Taagehoj F, Graff S, and Pedersen OF.** Acoustic rhinometry in dog
691 and cat compared with a fluid-displacement method and magnetic resonance imaging. *J*
692 *Appl Physiol* (1985) 95: 635-642, 2003.
- 693 46. **Thurmond R.** *Histamine in inflammation.* Springer Science & Business Media,
694 2011.
- 695 47. **Thurmond RL.** Histamine inflammation. Preface. *Adv Exp Med Biol* 709: vii-viii,
696 2010.
- 697 48. **Vasquez SX, Shah N, and Hoberman AM.** Small animal imaging and examination
698 by micro-CT. *Methods Mol Biol* 947: 223-231, 2013.

49. **Wang CY, Walfield AM, Fang X, Hammerberg B, Ye J, Li ML, Shen F, Shen M, Alexander V, and MacGlashan DW.** Synthetic IgE peptide vaccine for immunotherapy of allergy. *Vaccine* 21: 1580-1590, 2003.
50. **Wheatley LM, and Togias A.** Clinical practice. Allergic rhinitis. *N Engl J Med* 372: 456-463, 2015.
51. **White MV.** The role of histamine in allergic diseases. *J Allergy Clin Immunol* 86: 599-605, 1990.
52. **Widdicombe JG.** Nasal pathophysiology. *Respir Med* 84 Suppl A: 3-9; discussion 9-10, 1990.
53. **Willekens I, Bult N, Lahoutte T, Baeyens L, Vanhove C, Caveliers V, Deklerck R, Bossuyt A, and de Mey J.** Evaluation of the radiation dose in micro-CT with optimization of the scan protocol. *Contrast Media Mol Imaging* 5: 201-207, 2010.

Figure Legends

Figure 1. Allergy induction protocol utilized to induce allergic rhinosinusitis in Guinea pigs, using intranasal (i.n.) ragweed pollen (RWP) administration. Guinea pigs progressed toward allergic rhinosinusitis through initial systemic injections of RWP (i.p.), followed by i.n. application of RWP over a 5 day period. In fluticasone-treated groups, the i.n. application of RWP occurred in the early morning, and the fluticasone treatment was applied i.n., in the late afternoon of each day. Bronchoalveolar lavage (BAL), sinus fluid fill volume (SFFV), and microCT (mCT) measurements were taken subsequently 72 hr after the last i.n. RWP administration.

Figure 2. A. Schematic of sinus fill fluid volume measurement setup, incorporating constant-flow infusion pump delivering perfluorocarbon liquid directly into nasal sinuses of Guinea pig, with pressure transducer in delivery line to monitor pressure development and release, with achievement of sinus filling. Amp = DC amplifier feeding DC pressure signal to PC-compatible computer via analog-to-digital conversion board, with digital readout from data display and acquisition program, shown on computer monitor. **B.** Screenshots of typical pressure tracings recorded during the sinus fill fluid volume experiments. Left: Tracing of pressure development and release in Guinea pig with no administration of RWP; elapsed time of pressure development was ~42 s, indicating a long duration of sinus filling, consistent with a high volume in uncongested sinuses. Right: Tracing of pressure development and release in Guinea pig treated with i.n. administration of RWP; elapsed time of pressure development was less than 10 s, indicating a brief duration of sinus filling, consistent with a low volume in congested sinuses. Note the difference in time scale at bottom of each tracing.

Figure 3. Sinus fill fluid volume (SFFV) of Guinea pigs with increasing intranasal ragweed pollen (RWP) administration. SFFV decreased maximally to an average of approximately 25%, indicating an increase in sinus congestion of nearly 75%. Values are means $\pm$ S.E.M.; * $P < 0.05$ as compared to control (no RWP); $^{\vee} P < 0.05$ as compared to next lowest RWP treatment group, $n = 4$ Guinea pigs/bar.

Figure 4. A. Bronchoalveolar lavage (BAL) leukocyte cell differentials in Guinea pigs, either naïve (no sensitization or challenge; $n = 8$), or with intranasal ovalbumin (OVA; 100 mg/ml; $n = 7$), or intranasal ragweed pollen (RWP, 22 μ g; $n = 10$); data are from combined experiments using OVA and RWP. Macs = macrophages, Lymphs = lymphocytes, PMN = polymorphonuclear leukocytes, Eos = eosinophils. Values are means $\pm$ S.E.M.; * $P < 0.05$ vs. naïve, n.s = not significant, 0 = no cells observed. **B.** Total eosinophil counts in naïve ($n = 5$), RWP sensitized and challenged ($n = 5$), and RWP+fluticasone-treated (100 μ g; $n = 5$) Guinea pigs; data are from naïve and RWP-exposed Guinea pigs randomly selected from combined experiments to compare with the RWP+fluticasone experiments. Values are means $\pm$ S.E.M.; * $P < 0.05$ vs. naïve; $^{\vee} P < 0.05$ RWP+fluticasone vs. RWP group.

Figure 5. Sinus fill fluid volume (SFFV) as a function of administered concentration of fluticasone, in Guinea pigs with RWP-associated allergic sinus congestion. For comparison, far left bar indicates average SFFV values in naïve Guinea pigs (no RWP, or drug), which are of

large magnitude, as would be expected with uncongested, untreated sinuses. Similar to **Figure 3**, administration of RWP significantly decreased SFFV by ~75%, indicating significant allergic sinus congestion, as compared to naïve Guinea pigs. Fluticasone treatment significantly increased measured SFFV values in a concentration-dependent fashion, toward values similar to those observed in naïve animals. Values are means $\pm$ S.E.M.; ^v P<0.05 as compared to naïve group; * P<0.05 as compared to RWP alone; ^ P<0.05 as compared to next lowest concentration of fluticasone; n.s. = not significant; respective treatment group n's for Naïve (same as "0" in Figure 3), RWP (same as 22 μ g in Figure 3), RWP+25 μ g fluticasone, RWP+50 μ g fluticasone, RWP+75 μ g fluticasone, and RWP+100 μ g fluticasone were 4, 4, 4, 5, 4, and 5.

Figure 6. Two-dimensional image analyses of sinuses of Naïve, RWP-treated, and RWP+fluticasone treated Guinea pigs, quantifying cumulative linear width of nasal airway passages at a fixed bony landmark, using a high-contrast coloration scheme to allow definitive identification of skull bone structures, cartilage, and air spaces (black), measuring across the linear yellow arrow for image densities of -200HU for soft tissue (cartilage), -800HU for airspace openings, and +1100 to +1500HU for bone. **A.** Representative images indicating linear line segment placement and relative widths observed; note presence of significant mucous accumulation in the proximal and medial sinus spaces of RWP example, shown as strong yellow signal along midline of sinus passage, as compared to heterogeneous yellow and black signals in the distal sinus area, in same image. **B.** Image terrain/density analyses for each of the images shown above in **Panel A**, with lower densities along the ordinate indicating airspaces, and higher densities along the ordinate indicating dense bony and cartilaginous structures of the sinus. Dashed black line indicates -400HU image density reference point, at which cumulative width

measures were made, as shown for Naïve (W1+W2), RWP (W1+W2+W3+W4+W5), and RWP + fluticasone (W1 + W2). **C.** Average cumulative linear widths (mean $\pm$ SE) across groups; *P<0.05 vs. Naïve, ^P<0.05 vs. RWP; Naïve (n=5), RWP (n=4), and RWP+Fluticasone (n=5); n.s. = not significant.

Figure 7. A. Pictures of 3-D whole-sinus images of Guinea pigs from Naïve (n=5), RWP-challenged (n=5), and RWP + Fluticasone (Flutic; 100 μ g; n=4) groups. **B.** Whole-sinus air volume as measured by microCT in same groups as indicated in panel A; statistical significance symbols *P<0.05 as compared to Naïve, ^ P<0.05 RWP + Flutic as compared to RWP, alone. **C.** Sinus fluid-fill volume (SFFV) measurements in same treatment groups as pictured in panel A; significance symbols as in panel B. **D.** Association of sinus volume measured with the SFFV method and the whole-sinus air volume measured by microCT for Guinea Pigs in panels A, B, and C, with linear regression equation ($y = 6.08x$) and correlation coefficient ($r^2=0.989$), as shown; regression was constrained to pass through the origin of the plot; standard error, and lower and upper confidence limits of slope, were 0.38, 4.89 and 7.27, respectively.

Figure 8. A. Oblique-angle view of 3-D naïve Guinea pig full-sinus image, indicating anatomical and sinus length landmarks utilized for microCT image slice analysis, with airspace volume indicated by blue coloration. Nostrils and eye sockets provided the proximal and distal whole-sinus length boundaries, from which the 25%, 50%, and 75% sinus length landmarks were derived. **B.** Same oblique angle view as indicated in panel A, with microCT images of single slices obtained at 25%, 50%, and 75% sinus length intervals, and also with nostril and eye socket

slice images at sinus length extremes, for reference. C. **Left.** Two-dimensional picture of the same naïve Guinea pig full-sinus image with airspace volume, as in **Figure 7**, viewed axially (looking down directly on top of the head), to show the full length, and right-angle-to-longitudinal-axis register of the 25%, 50%, and 75% sinus length slice landmarks, demarcating the proximal, medial, and distal sinus regions. **Right.** Composite of microCT slices taken from 3-D full-sinus image at each length landmark, for representative single Guinea pigs from the Naïve, RWP, and RWP + fluticasone treatment groups, in which dark blue coloration was used to indicate airspaces within the respective slices at each level. Note: 1) the similarity of sinus image topography across sinus landmark levels and Guinea pigs in each group, and 2) the relative lack of dark blue coloration within the image slices of the RWP examples, indicating reduced airspace volume and increased sinus congestion, as compared to the Naïve and RWP + Fluticasone treatment examples.

Figure 9. Three sets of microCT image slices taken from three whole-sinus 3-D microCT images of Guinea pigs from: A.) Naïve group, B.) RWP group, and C.) RWP + fluticasone group, to indicate assessment and comparison of sinus airspace area (darker blue coloration) and sinus perimeter (lighter blue coloration) demarcation. The images from the RWP-treated Guinea pigs consistently demonstrated a decrease in both the darker and lighter blue coloration, as compared to those in the Naïve and RWP + Fluticasone treatment examples.

Figure 10. A. Measurements of sinus airspace area from 25%, 50%, and 75% sinus length landmark slices, as shown in **Figure 6** (areas of darker blue), in Naïve (n=5), RWP (n=5), and RWP + Fluticasone (n=4) treatment groups. Data are means $\pm$ SEM; * $P < 0.05$ as compared

to Naïve; [^] P<0.05 RWP + fluticasone as compared to RWP; n.s. – not significant as compared by ANOVA. **B.** Measurements of sinus surface perimeter from 25%, 50%, and 75% sinus length landmark slices, as shown in **Figure 6** (areas of lighter blue), in same Naïve, RWP, and RWP + Fluticasone treatment groups, as in panel A. Data are means ± SEM; significance symbols as in panel A. **C.** Ratios of sinus airspace area per sinus perimeter, derived from 25%, 50%, and 75% sinus length landmark slices, as shown in **Figure 6** (areas of darker blue and lighter blue), in Naïve, RWP, and RWP + Fluticasone treatment groups, as in panel A. Data are means ± SEM; significance symbols as in panels A and B.

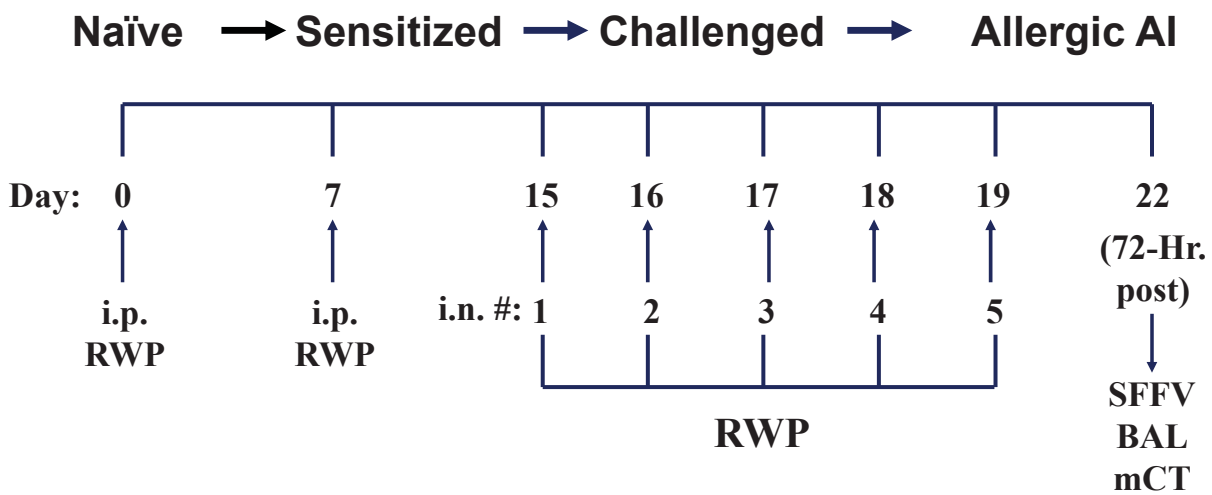
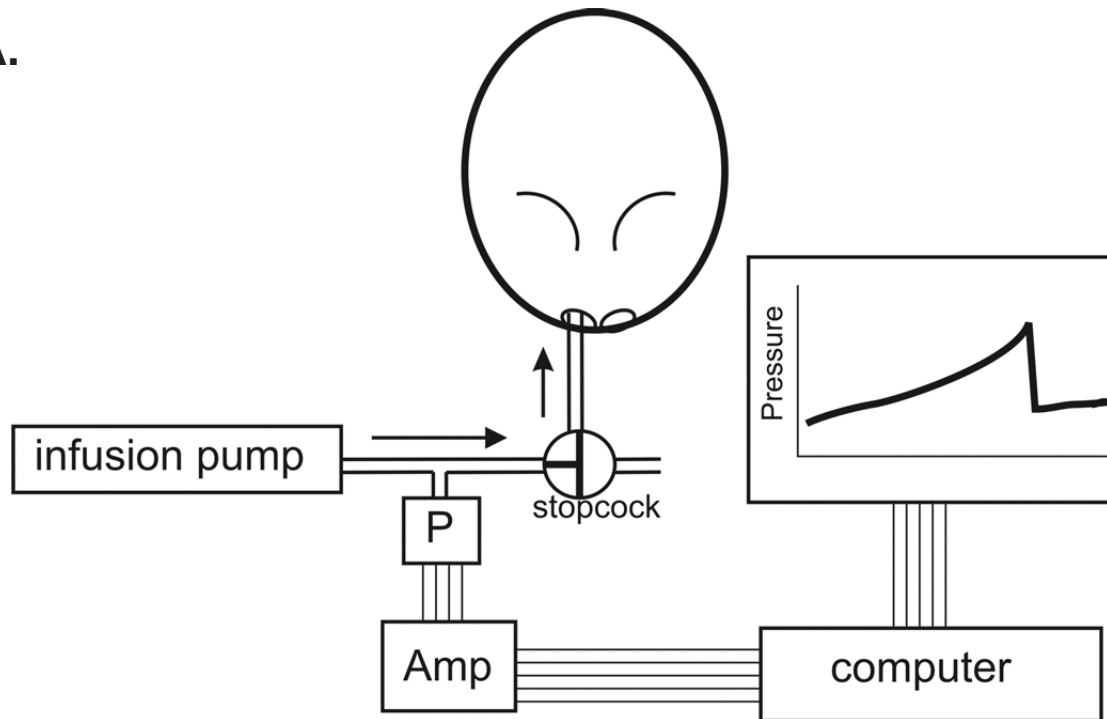


Figure 1

A.



B.

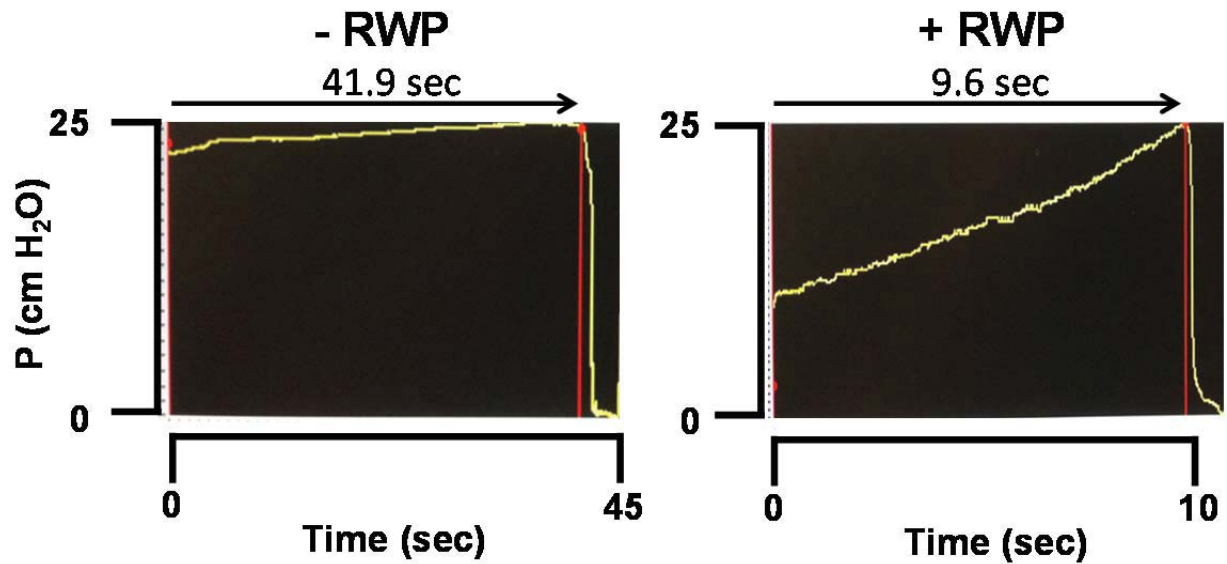


Figure 2

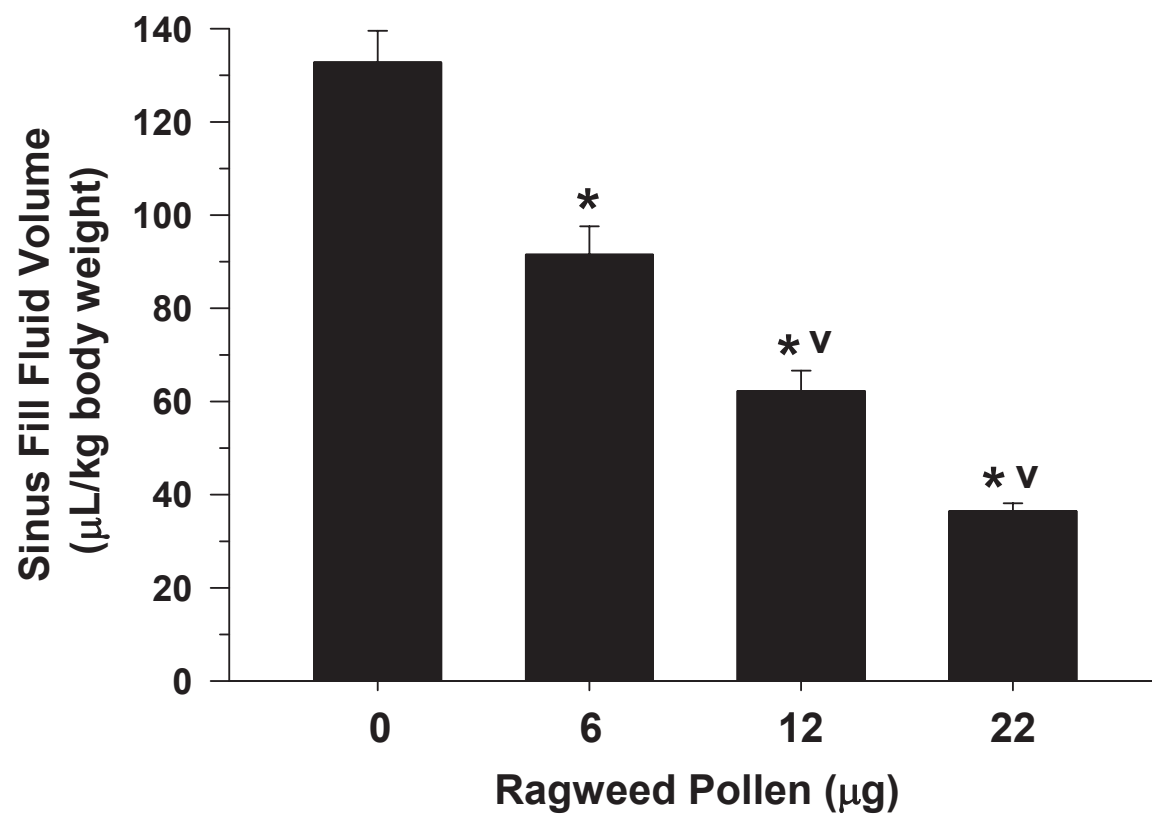


Figure 3

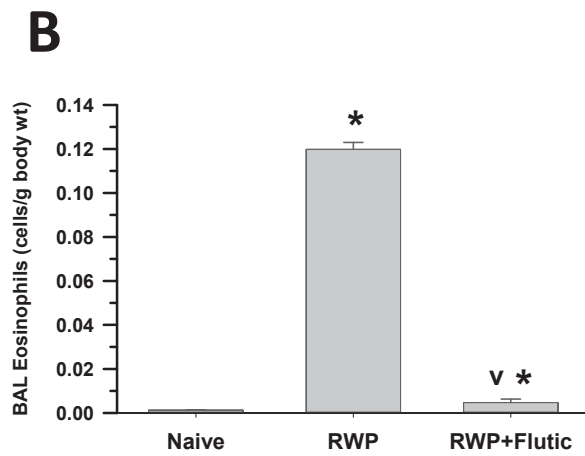
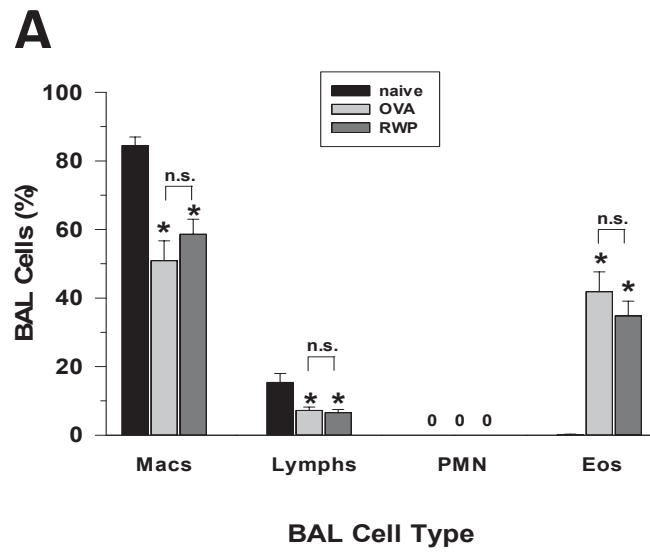


Figure 4

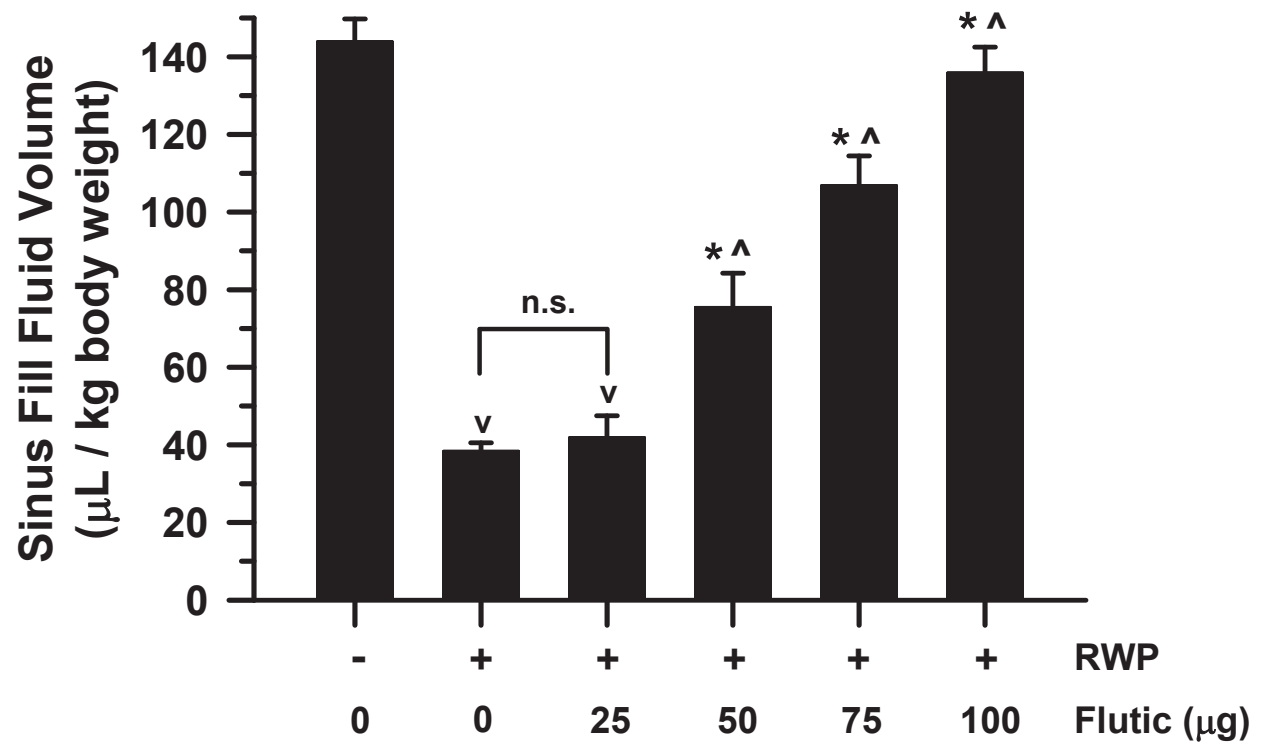
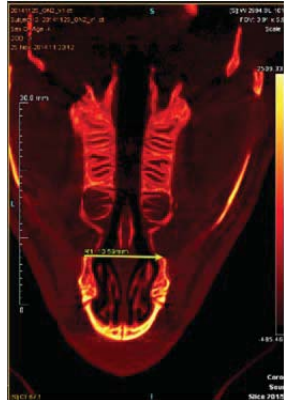
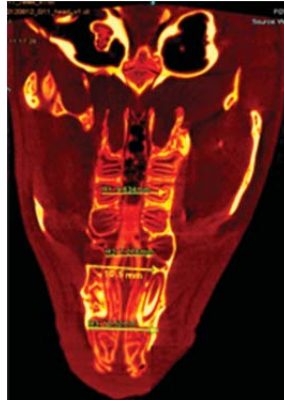


Figure 5

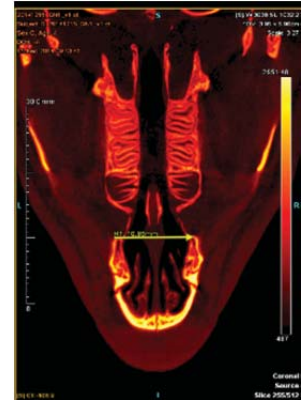
A. Naive



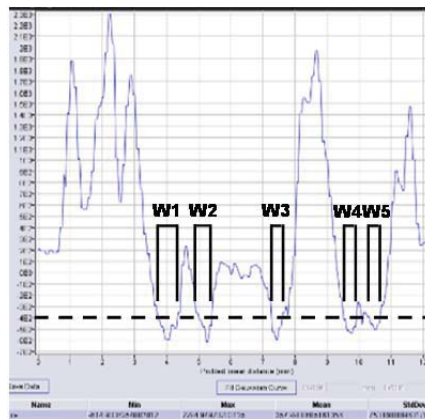
RWP



RWP+Flutic



B.



C.

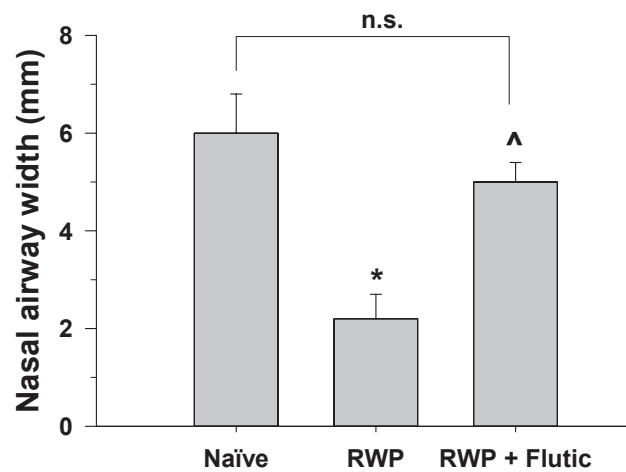


Figure 6

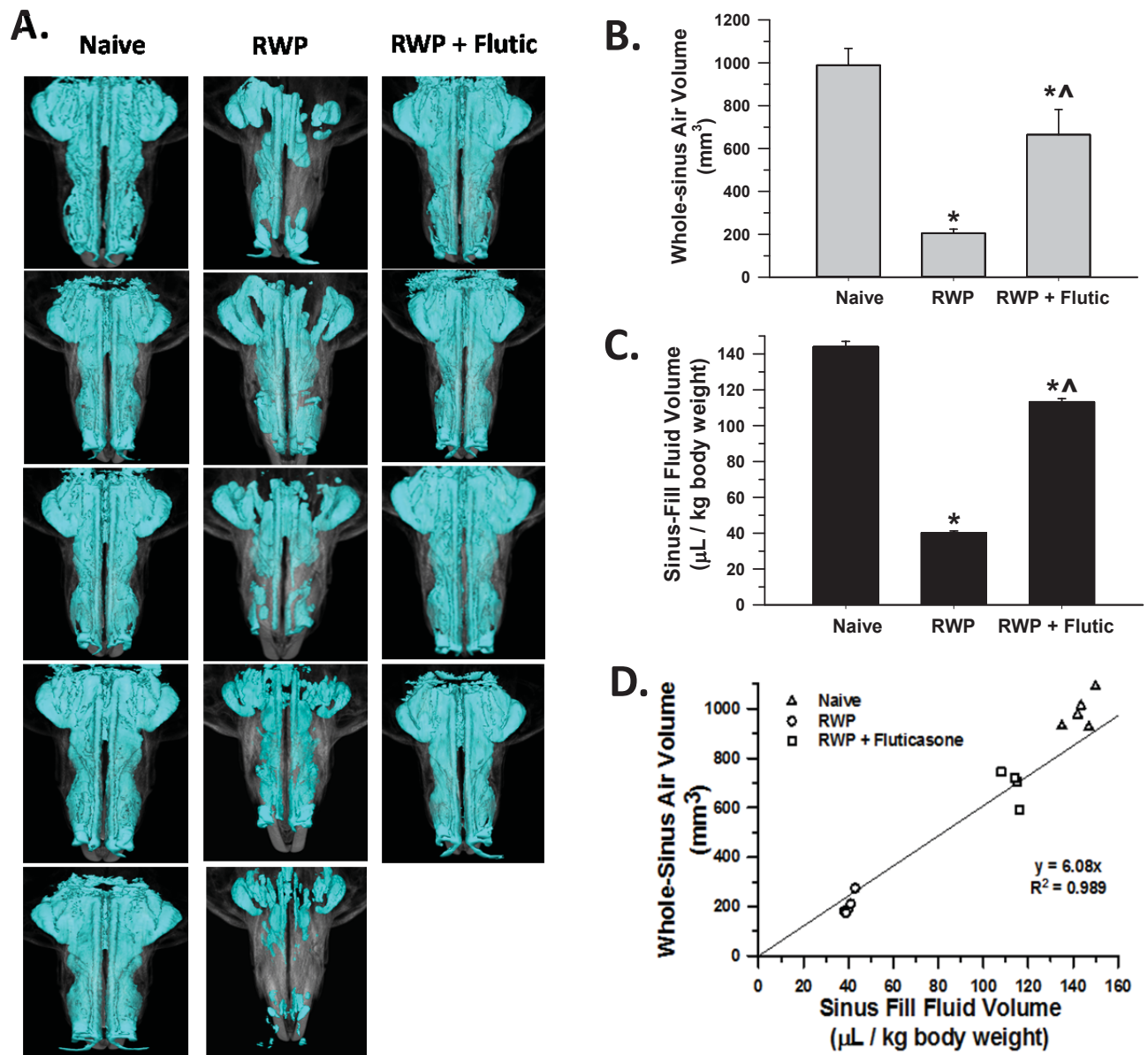
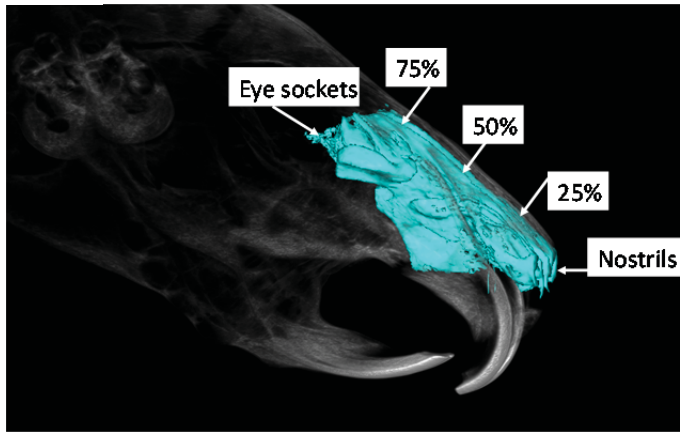
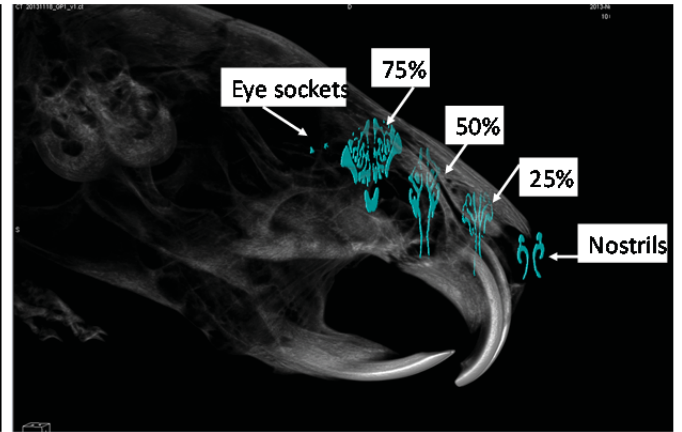


Figure 7

A.



B.



C.

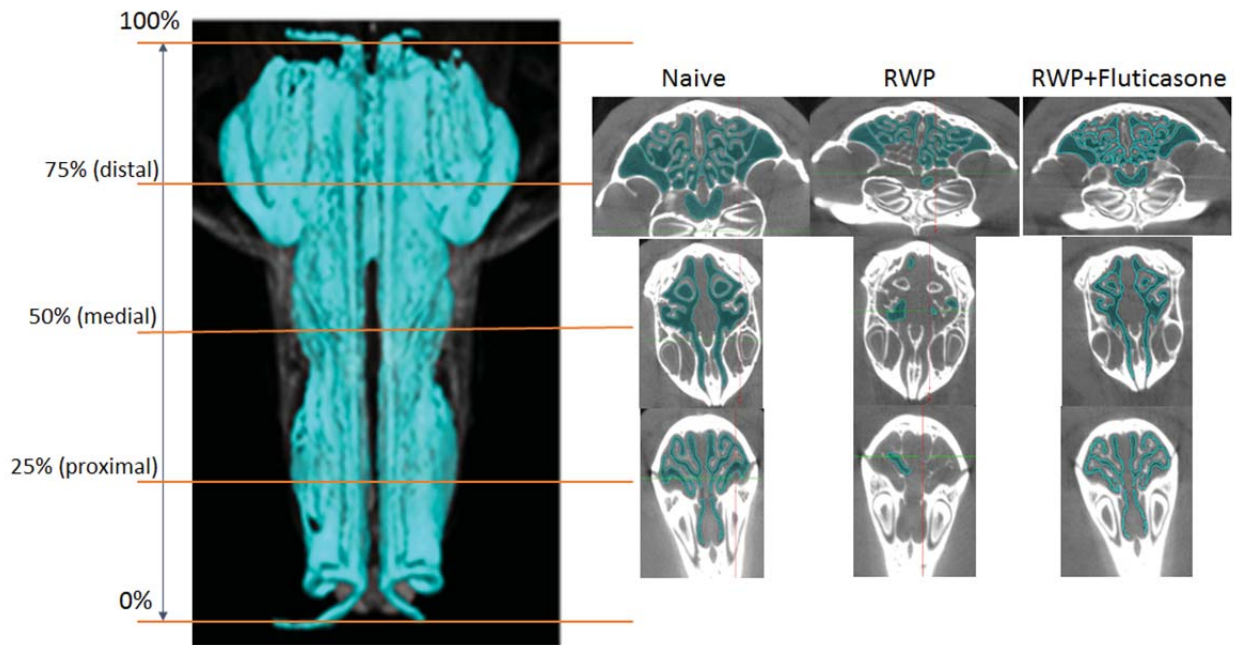


Figure 8

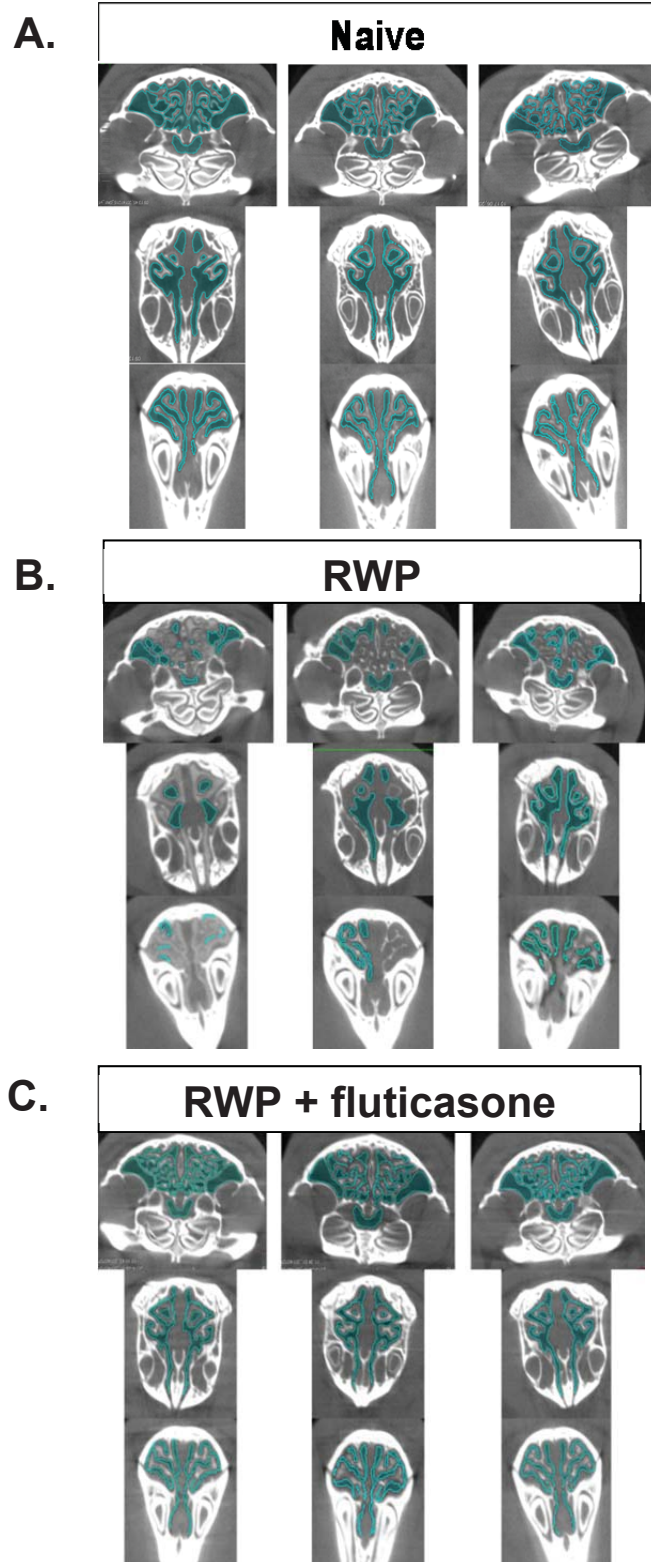


Figure 9

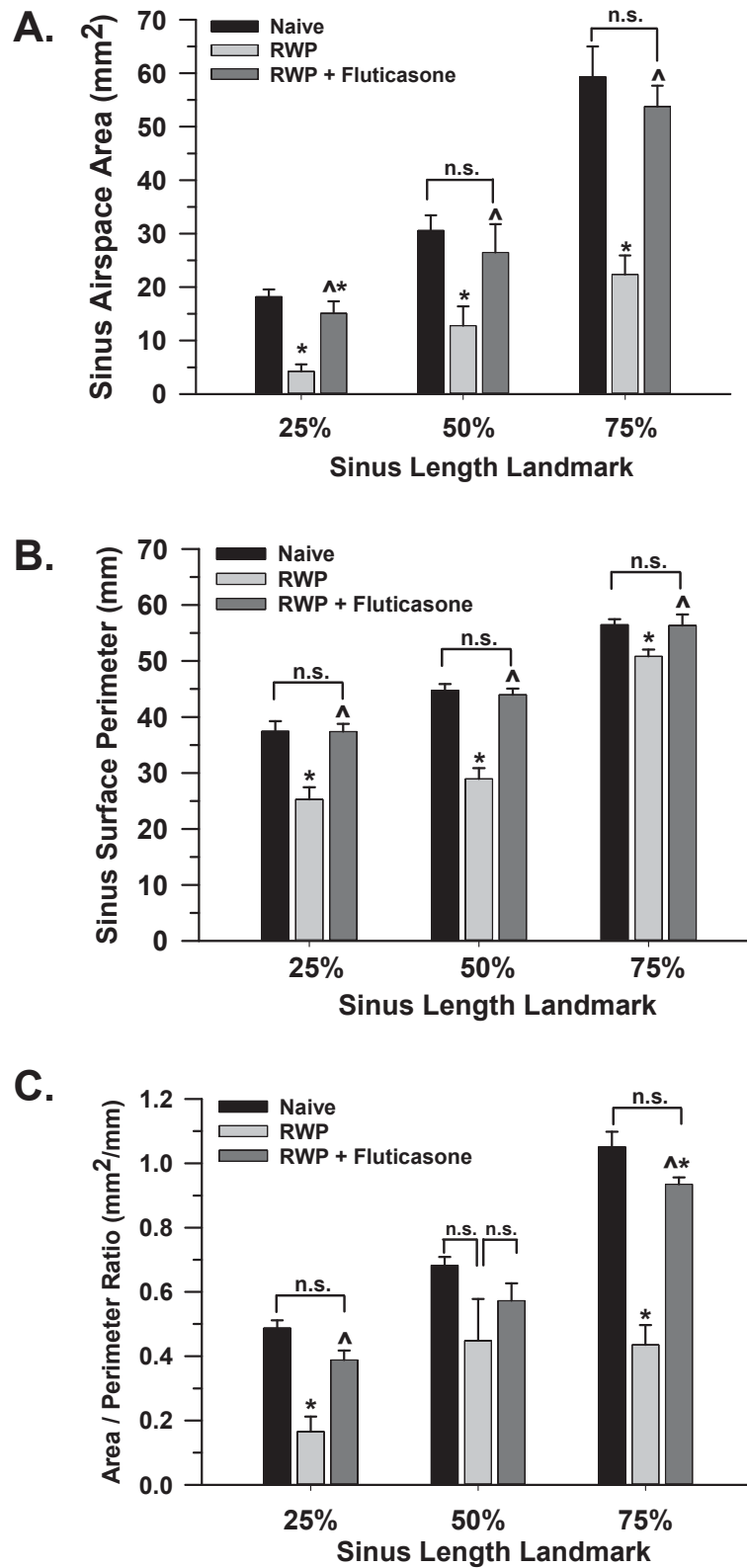


Figure 10