

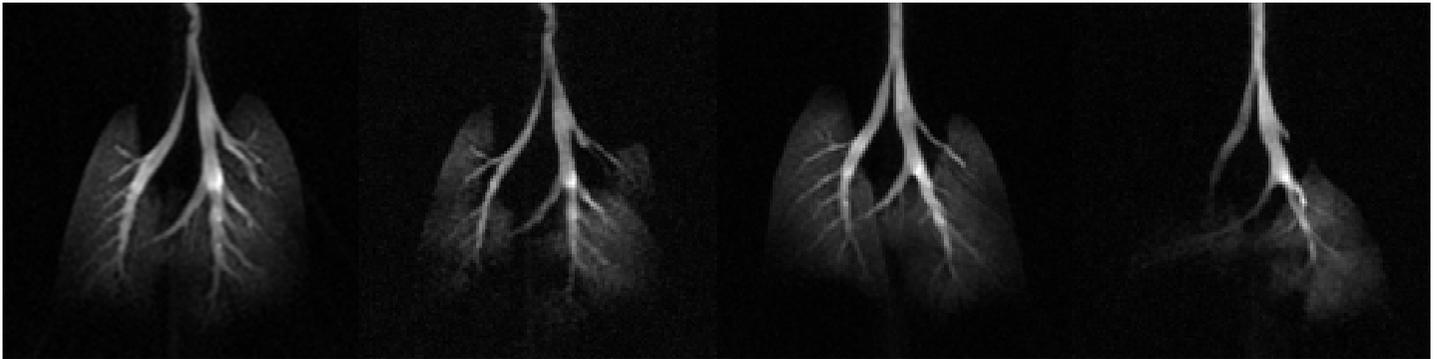
## <sup>3</sup>He Imaging of Methacholine Challenge in Mouse Models of Asthma

B. Driehuys<sup>1</sup>, G. P. Cofer<sup>1</sup>, J. Pollaro<sup>1</sup>, J. K. Walker<sup>2</sup>, D. A. Schwartz<sup>3</sup>, G. A. Johnson<sup>1</sup>

<sup>1</sup>Center for in vivo Microscopy, Duke University, Durham, NC, United States, <sup>2</sup>Department of Pulmonary and Critical Care Medicine, Duke University, Durham, NC, United States, <sup>3</sup>National Institute for Environmental Health Sciences, Research Triangle Park, NC, United States

**Introduction:** The study of asthma increasingly relies on transgenic and knock-out mouse models to elucidate the fundamental underpinnings of this disease. However, methods for *in vivo* assessment of lung function have failed to keep pace with the increasingly sophisticated genetic manipulations. Currently, global measurements of airway pressure before and after methacholine challenge are the primary tool available [1,2], leaving unanswered questions regarding the degree of small versus large airway involvement, whether the site of broncho-constriction is reproducible, and the degree to which mouse models reproduce human disease. High resolution <sup>3</sup>He imaging which can answer these questions, was recently demonstrated in mice, but required a 25minute scan [3]. Here, we present the first observation of methacholine challenge in mice using a 5 minute scan consuming 100ml of <sup>3</sup>He.

**Methods:** In accordance with an approved Duke IACUC protocol BALB/c mice (Jackson Labs, Bar Harbor, ME) were sensitized to ovalbumin on days 0 and 14 with an intraperitoneal injection of 10.0 $\mu$ g of OVA (Sigma, St. Louis, MO) and were challenged with 1% OVA aerosol on days 21, 22 and 23. For imaging on day 24, mice were anesthetized using an ip injection of Nembutal (75mg/kg) and maintained with iv maintenance doses of 20mg/kg every 30min. A 2F gauge jugular catheter was inserted for delivery of methacholine. Mice were intubated using a low dead-volume tracheal tube and ventilated on an MR compatible constant volume ventilator at 100 breaths per minute with 0.2 ml tidal volume [3]. <sup>3</sup>He imaging was performed using a 64.8 MHz dual tuned bird cage coil ( $L=5.5$ cm,  $\phi=3.5$ cm) in a 2.0T horizontal 15cm clear bore magnet (Oxford Instruments, Oxford, UK) with shielded gradients (18G/cm), controlled by a GE Excite 11 console (GE Healthcare, Milwaukee, WI). <sup>3</sup>He (Spectra Gases, Alpha, NJ) was polarized to 30% in batches of 1.2 liter using a prototype commercial polarizer (IGI.9600.He, MITI, Durham, NC). High-resolution 3D images were acquired using 3D projection encoding with a matrix of 256 $\times$ 256 $\times$ 16, FOV=3.2cm in the coronal plane and 1.6cm sagittal direction. K-space was filled with 11,520 radial views acquired 20 views per breath, TR=5ms, variable flip angle with  $\alpha_{20}=90^\circ$ , a bandwidth=62.5kHz. Once in the magnet, Doxacurium Chloride (0.25 mg/kg) paralytic was administered to minimize respiratory efforts against the ventilator in response to methacholine. A baseline <sup>3</sup>He image was acquired, then methacholine was administered (250 $\mu$ g/kg) and a second 3D image was acquired.



**Figure 1** MIP of 3D <sup>3</sup>He ventilation images (125 $\times$ 125 $\times$ 1000 $\mu$ m<sup>3</sup>) in 2 ovalbumin-sensitized mice before and after administration of 250 $\mu$ g/kg methacholine.

**Results and Discussion:** Figure 1 shows a maximum intensity projection (MIP) of 3D <sup>3</sup>He images acquired before and after methacholine injection. Focal bronchoconstriction is clearly apparent and lasts for at least the 5 minutes required to acquire the full 3D scan. An unexpected result is the clear involvement of several of the larger airways. Control animals that had not been sensitized did not show a response to the challenge on the 5 minute imaging time scale, for 3D imaging, although preliminary results using lower resolution, faster 2D scanning do reveal the challenge.

**Conclusion:** The ability to image methacholine response with high spatial and temporal resolution in mice opens up exciting possibilities for the quantitative study of asthma by allowing the effects of genetic manipulations to be assessed at the level of the individual airways rather than the lung as a whole. Combined with <sup>3</sup>He imaging of methacholine challenge in humans [4], hyperpolarized <sup>3</sup>He MRI becomes a truly translational tool in asthma research.

### References

1. R. C. Levitt and W. Mitzner, *Faseb J.* **2** (10), 2605-2608 (1988).
2. E. Hamelmann, J. Schwarze, K. Takeda et al., *American Journal Of Respiratory And Critical Care Medicine* **156** (3), 766-775 (1997).
3. B. T. Chen, A.T. Yordanov, and G. A. Johnson, *Magn. Reson. Med.* **53** (1), 69-75 (2005).
4. S. Samee, T. Altes, P. L. Powers et al., *Journal of Allergy and Clinical Immunology* **107** (2), 757 (2001).

### Acknowledgments

NCRR P41 05959, NHLBI 2R01HL55348