In-vivo high-resolution magic angle spinning proton MR spectroscopy of Drosophila melanogaster flies as a model system to investigate trauma, innate immunity and aging

V. Righi[1,2], D. Mintzopoulos[1,2], Y. Apidianakis[1,3], O. C. Andronesi[1,2], L. G. Rahme[1,3], and A. A. Tzika[1,2]

[1]NMR Surgical Laboratory, MGH & Shriners Hospitals, Harvard Medical School, Boston, MA, United States, [2]Radiology, Athinoula A. Martinos Center for Biomedical Imaging, Boston, MA, United States, [3]Molecular Surgery Laboratory, MGH & Shriners Hospitals, Harvard Medical School, Boston, MA, United States

Introduction– *Drosophila melanogaster* has emerged as an ideal model organism to study the genetic control of immune recognition, physiology and aging, because of the high degree of conservation between the fly and mammalian signaling pathways involved in these processes (1), and its genetic tractability and simplicity. *Drosophila* expresses antimicrobial peptides upon injury and/or infection via the evolutionarily conserved innate immunity signaling pathways Toll and Imd (2, 3). Here, we examined the feasibility of an *in vivo* high-resolution magic angle spinning proton MR spectroscopy (HRMAS ^{1}H MRS) approach and suggested its biological significance towards the investigation of the metabolic effects of trauma on *Drosophila* innate immunity and aging.

Materials and Methods– We used male *Drosophila* wild type (wt) and innate imunity mutant (imd) flies, with n=7 for young (5-6 days old) fly group and n=4 for old flies (30 days old). Experiments were performed on: (a) control healthy, un-injured flies; and (b) traumatized flies, injured with thoracic non-lethal, needle puncture. All flies were placed in the spectrometer 24 hours after trauma and special care was taken to avoid further injury during moving in and out of the rotor. Prior to fly insertion in the spectrometer, flies were immobilized by placing them on ice for less than 1 min and were kept at 4°C while in the spectrometer. ^{1}H HR-MAS experiments were performed on a Bruker Bio-Spin Avance NMR spectrometer (600.13 MHz) using a 4mm triple resonance (^{1}H, ^{13}C, ^{2}H) HR-MAS probe (Bruker). The temperature was controlled at 4°C by a BTO-2000 unit in combination with a MAS pneumatic unit (Bruker). The flies were placed into 4mm zirconium oxide (Zirconia, Bruker) rotors with spherical inserts. 10 µl D_2O (deuterium lock reference) containing 10 mM TSP (trimethylsilyl propionic-2,2,3,3-d_4 acid, M_w=172, δ=0ppm, external chemical shift reference) was added to the rotor with the sample. To avoid contact between flies and D_2O parafilm was inserted at the bottom of the rotor. The MAS rotation frequency was stabilized at 2.0 ± 0.001 kHz by a MAS speed controller. We have performed one and two dimensional experiment. One-dimensional ^{1}H HR-MAS NMR single-fly spectra were acquired on all samples using a rotor synchronized Carr-Purcell-Meiboom-Gill (CPMG) spin echo pulse sequence, [90°-(⎮-180°-⎮)n], which works as a T2 filter to remove the spectral broadening. The inter-pulse delay (⎮ = 500µs) was synchronized to the MAS rotation frequency. The number of transients was 256 with 32,768 (32k) data points. The 1D fully-relaxed spectra were also obtained using a single pulse sequence with water pre-saturation signal. A line-broadening apodization function of 1.0 Hz was applied to all HRMAS ^{1}H FIDs prior to Fourier transformation. Spectra were referenced with respect to TSP, manually phased, and a Whittaker baseline estimator was applied to subtract the broad components of the baseline prior to peak area calculations (using MestReC software, Mestrelab Research). Peak heights were scaled with respect to TSP for each acquired spectrum. Two-dimensional (2D) ^{1}H-^{1}H HRMAS NMR single-fly spectra were acquired on all samples using a TOBSY sequence with adiabatic pulses (4). Acquisition parameters were: 2k data points direct dimension (11ppm spectral width), 1 sec water pre-saturation during relaxation delay, 8 scans per increments, 128 increments, 2 sec total repetition time, 45 ms mixing time and total acquisition time 29 min. We had 0 mortality.

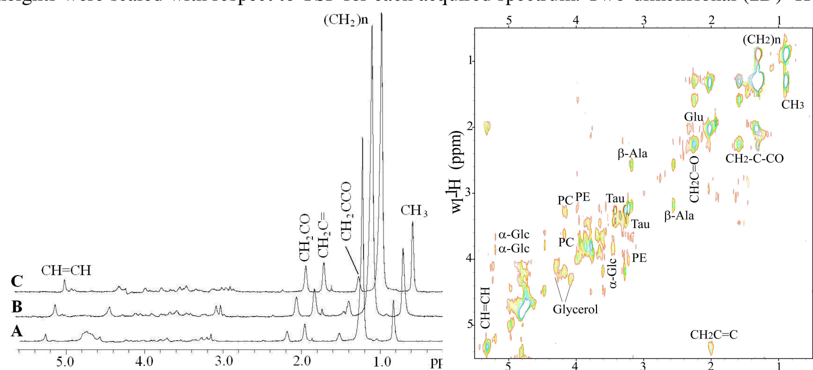

Figure 1. *In vivo* HR-MAS ^{1}H spectra: A) wt-injured young flies, B) wt-injured old flies and C) imd-injured young flies.

Figure 2. Representative *in vivo* TOBSY spectrum of a wild-type (control) fly.

Results– Figure 1 presents summed *in vivo* CPMG ^{1}H HRMAS MR spectra from *Drosophila*. Lipid peaks are labeled to emphasize their accumulation in old and *imd* flies as compared to wt young flies. Lipid components and other metabolites are assigned using TOBSY spectra (Fig. 2). We identified the following resonances of fatty acids chains: *terminal methyl-* CH_3, *acyl chain methylene-* $(CH_2)n$, *β-methylene-* CH_2-C-CO, *α-methylene=* CH_2C=C, *α-methylene-* CH_2CO, *vinyl proton-* CH=CH (i.e. ceramide) and small metabolites such as: Tau , taurine; α- and β-Glc, α- and β-glucose; β-Ala, β-alanine; PC, phosphocholine; PE, phosphor-ethanolamine; and Glu, glutamate and Glycerol. Signals at 2.02 ppm are assigned to methylene protons of the CH_2-CH=CH moiety of mono-unsaturated fatty acids (i.e. palmitoleic and oleic acid). Interestingly, we did not detect poly-unsaturated fatty acids (linoleic and/or linolenic acid), and thus the signal at 2.78 ppm, attributable to the methylene protons between two double bonds (=C-CH_2-C=) in poly-unsaturated acids, is not present. The unsaturated acids are identified by the signals at 5.33 ppm due to the protons of the -CH=CH- moiety. Figure 3A shows that most lipids are significantly increased in injured as compared to un-injured old wt flies. No such difference was observed upon injury in young wt flies (data not shown). In contrast, both injured and un-injured *imd* young flies show increased levels of these lipids when compared to wt young injured flies (Fig. 3B).

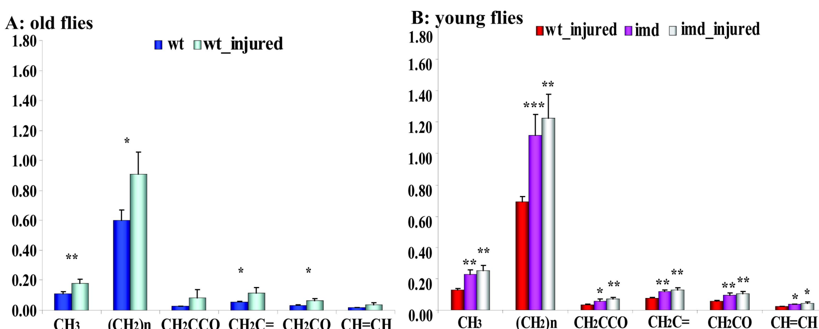

Figure 3. Average scaled ratios of lipid components A) Wild-type (wt, dark blue) and injured (light blue) old flies B) Wild-type injured (wt_injured, red), imd-uninjured (imd, purple) and imd-injured (imd_injured, grey) young flies. The error bars represent standard errors (SE). * P<0.05; ** P<0.005) *** P<0.001

Discussion– Our results show that trauma promotes accumulation of specific lipids only in old flies, and this accumulation is augmented in young but immunocopromised flies, indicating that trauma elicits an age-dependent lipid homeostatic perturbation that may promote immune dysfunction. Moreover, as *Drosophila melanogaster* flies exhibit similar patterns in biomarkers detected by *in vivo* HRMAS ^{1}H MRS to the ones detected in the skeletal muscle of mice post-trauma [5], suggests that this lipid perturbation may be a phylogenetically-conserved response. To this end, also the increase in $(CH_2)n$ signal observed in both injured old and *imd* flies may be indicative of insulin resistance as it is in mammals [5]. Similarly, the one of vinyl signal (CH=CH), which includes protons from ceramide, biomarker of apoptosis, suggests apoptosis in these flies [5]. We propose for the first time *in vivo* HRMAS ^{1}H MRS of live *Drosophila* flies in a 14 tesla spectrometer as a model system to investigate trauma, innate immunity responses and aging.

References

1. Hoffmann, JA, Reichhart, JM. Nat . Immunol . 3:121, 2002.
2. Hetru, C, Troxler, L, Hof fmann, JA. J. Infect . Dis. 187, Suppl. 2, S327, 2003.
3. De Gregorio, E, Spellman, PT, Tzou, P, Rubin, GM, Lemaitre, B. EMBO J. 21, 2568, 2002.
4. Andronesi, O, Mintzopoulos, D, Struppe, J, Black, PM, Tzika, AA. JMR. 193: 251, 2008.
5. Astrakas LG et al. FASEB J. 2005 Sep;19(11):1431-40.