In vivo high-resolution magic angle spinning proton NMR spectroscopy of *Drosophila melanogaster* flies as a model system to investigate mitochondrial dysfunction in trauma

V. Righi[1,2], G. Apidianakis [3], N. Psychogios[1,2], L. G. Rahme[3], R. G. Tompkins[4], and A. A. Tzika[1,2]

[1]Department of Surgery, NMR Surgical Laboratory, MGH and Shriners Burn Institute, Harvard Medical School, Boston, MA, United States, [2]Department of Radiology, Athinoula A. Martinos Center of Biomedical Imaging, Boston, MA, United States, [3]Department of Surgery, Molecular Surgery Laboratory, MGH and Shriners Burn Institute, Harvard Medical School, Boston, MA, United States, [4]Department of Surgery, MGH and Shriners Burn Institute, Harvard Medical School, Boston, MA, United States

Introduction– *Drosophila melanogaster* glutathione S-transferase DmGSTS1-1 (earlier designated as GST-2) is related to sigma class GSTs and was previously described as an indirect flight muscle-associated protein with no known catalytic properties. State-of-the art, *in vivo* NMR techniques are used to elucidate metabolic patterns in *Drosophila melanogaster* as a model organism of interest due to the remarkable parallels between metabolism in *Drosophila* and mammals (1,2). Indeed, study of *Drosophila* metabolism is an emerging field that can potentially elucidate conserved metabolic mechanisms. Furthermore, powerful genetic tools available in *Drosophila* research make the fruit fly a particularly tractable model organism in which to probe metabolic pathways and lead to a better understanding of human metabolic disorders. Here, we created mutant flies that under-express the GST-2 gene in skeletal muscle. We examined the feasibility of a novel, *in vivo* high-resolution magic angle spinning proton NMR spectroscopy (HRMAS ^{1}H NMR) approach towards the investigation of the metabolic derangements in these flies and compared them to isogenic control flies..

Materials and Methods– We used male wild-type (wt) and mutant *GST2 Drosophila* flies (n=6 per group). The flies were placed in the spectrometer and special care was taken to avoid 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 HRMAS 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) HRMAS probe (Bruker). The temperature was kept at (or adjusted to) 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. Eight µl D_2O (deuterium lock reference) containing 50 mM TSP (trimethylsilyl propionic-2,2,3,3-d_4 acid, M_w=172, δ=0ppm, external chemical shift reference) were added to the rotor with the sample. To avoid contact between flies and D_2O, a small piece of parafilm was inserted on top of the insert. The MAS rotation frequency was stabilized at 2.0 ± 0.001 kHz by a MAS speed controller. One-dimensional ^{1}H HRMAS NMR single-fly spectra were acquired on all samples using a rotor synchronized Carr-Purcell-Meiboom-Gill (CPMG) spin echo pulse sequence, [90°-(τ-180°-τ)n], which works as a T2 filter to remove the spectral broadening. The inter-pulse delay (τ = 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 3.0 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. Peak heights were scaled with respect to TSP.

Results– Representative *in vivo* 1D HRMAS ^{1}H CPMG spectra of *Drosophila* flies are shown in figure. 1. Note that the metabolic profile of GST2 (A) and WT flies (B) exhibit differences characterized by a substantial increase in both $(CH_2)_n$ lipids at 1.33 ppm (**2**) and CH_2C-CO lipids at 1.58 ppm (**3**), as well as increases in other lipids. Quantitative analysis showed a significant increase in (C**H**$_2$)n lipids and CH_2C-CO at 1.58 ppm, (figure 2). at 1.58 ppm of GST fly respect to wt (figure 2).(Y axis represents the amount of lipids resonance)

Discussion– The main finding of this study was an increase in $(CH_2)_n$ lipids at 1.33 ppm which is an insulin resistance biomarker in *skeletal muscle of Drosophila* (3). We thus provide evidence for the hypothesis that *GST2 mutation* is linked to insulin signaling. This link may explain the mitochondrial dysfunction that accompanies

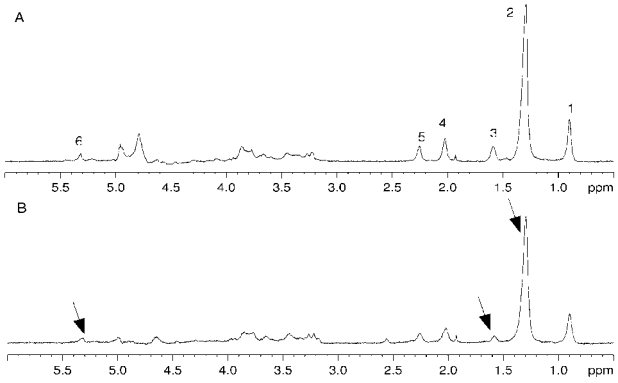

Figure 1. *In vivo 1D HRMAS ^{1}H CPMG spectra of:* ***A)*** *GST2 and* ***B)*** *WT Lipid components: 1, C**H**$_3$ (0.89 ppm); 2, (C**H**$_2$)n (1.33 ppm);3, C**H**$_2$C-CO (1.58ppm); , 4, C**H**$_2$C=C (2.02 ppm); 5, C**H**$_2$C=O (2.24 ppm); 6, C**H**=C**H** (5.33 ppm).*

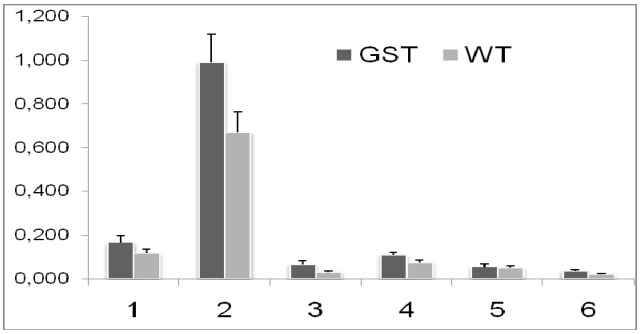

Figure 2. *GST2 and WT lipid amount: 1, C**H**$_3$ (0.89 ppm); 2, (C**H**$_2$)n (1.33 ppm);3, C**H**$_2$C-CO (1.58ppm); , 4, C**H**$_2$C=C (2.02 ppm); 5, C**H**$_2$C=O (2.24 ppm); 6, C**H**=C**H** (5.33 ppm).*

insulin resistance and muscle wasting that occurs in *GST2 mutants* (3) and burn patients (who exhibit reduced expression of *GSTA4* gene, the mammalian ortholog of *GST2* gene) in muscle, (preliminary data analysis not shown here) and may lead to higher susceptibility to infection. Our approach advances the development of novel *in vivo* non-destructive research approaches in *Drosophila*, suggests biomarkers for investigation of biomedical paradigms, and thus may contribute to novel therapeutic development.

References: 1. Baker, K. D., Thummel, C. S. Cell Metab. 6:257, 2007; **2.** Leopold, P., Perrimon, N. Nature 450:186, 2007. **3.** Thompson, LH, Kim, HT, Ma, Y, Kokorina, NA, Messina, J. Mol Med 14:715, 2007; **4.** Apidianakis et al PLoS ONE 2007