In vivo High-Resolution Magic Angle Spinning Proton MR Spectroscopy of Drosophila melanogaster Flies as a Model System to Investigate Obesity

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Introduction– Recently, the remarkable parallels between metabolism in *Drosophila* and mammals have been reviewed (1,2). Indeed, the study of *Drosophila* metabolism is an emerging field that can potentially elucidate conserved mechanisms that regulate carbohydrate and lipid homeostasis. 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, such as obesity. The adipokinetic hormone (AKH) family of peptides is thought to play a key role in catabolism in a variety of insect species (3). Recently, a Drosophila mutant lacking the adipokinetic hormone receptor (AKHR) of the adipokinetic hormone signaling pathway, an insect lipolytic pathway related to ß-adrenergic signaling in mammals was generated (4). Here, we examined the feasibility of a novel, *in vivo* high-resolution magic angle spinning proton MR spectroscopy (HRMAS ^{1}H MRS) approach towards the investigation of the metabolic derangements in obese *akhr^{null}* mutant flies.

Materials and Methods– We used male *Drosophila* wild type (*akhr^{rev}*) and adipokinetic hormone receptor (*akhr^{null}*) mutant flies, n=7. All 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 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. 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 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. We had zero mortality.

Results– Representative *in vivo* 1D HRMAS ^{1}H CPMG spectra of *akhr^{null}* mutant Drosophila and its isogenic control *akhr^{rev}* are shown in Fig. 1. Note that the metabolic profile of the *akhr^{null}* mutant, which has a phenotype of obesity, showed a substantial increase in both $(CH_2)_n$ lipids at 1.33 ppm and CH_2C-CO lipids at 1.58 ppm, attributed to intra- (IMCL) and extramyocellular (EMCL) respectively, as well as increases in other lipids (Table 1). The *akhr^{null}* mutant flies also showed an increase in the amount of bonded glycerol (signals at 4.10, 4.30 and 5.24 ppm), with respect to the control *akhr^{rev}* flies.

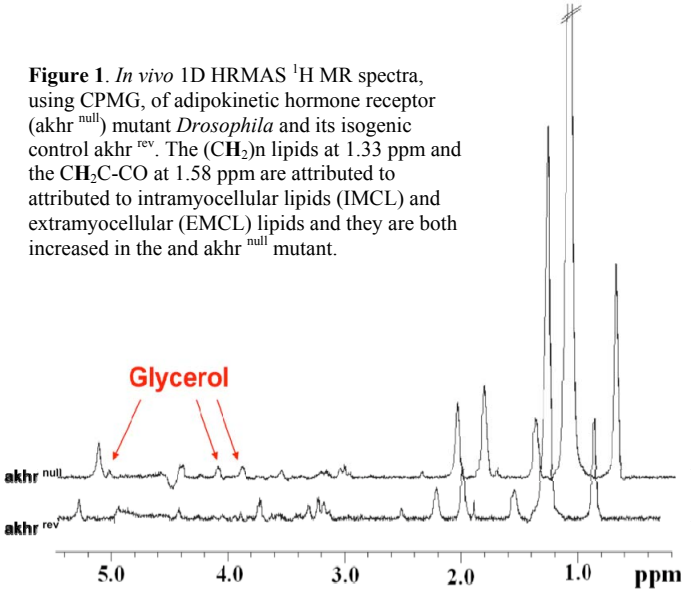

Figure 1. *In vivo* 1D HRMAS ^{1}H MR spectra, using CPMG, of adipokinetic hormone receptor (akhr ^{null}) mutant *Drosophila* and its isogenic control akhr ^{rev}. The (C**H**$_2$)n lipids at 1.33 ppm and the C**H**$_2$C-CO at 1.58 ppm are attributed to attributed to intramyocellular lipids (IMCL) and extramyocellular (EMCL) lipids and they are both increased in the and akhr ^{null} mutant.

Discussion– Chemical shift differences between lipid signals from distinct compartments in skeletal muscle are being caused by bulk magnetic susceptibility differences from IMCL and EMCL. In our study this discrimination is impossible because spinning a sample at the magic angle with respect to the static field direction averages the second-order tensors of the anisotropic chemical shift, the dipolar interaction, and the susceptibility variations. Despite this limitation, the significant increase in IMCLs and EMCLs detected in the *akhr* null mutants is in agreement with their obese phenotype and abnormal accumulation of both lipids and carbohydrates (4). Indeed, elevated IMCL levels are associated with insulin resistance, a major metabolic dysfunction of obesity (5). Another principle finding of our experiments was that ceramide (5.33 ppm) accumulated in *akhr^{null}* obese mutant flies (Table 1 and Fig. 1). Ceramide accumulation decreases insulin stimulated GLUT4 translocation to the plasma membrane and, consequently, decreases glucose transport (6), resulting in insulin resistance. Finally, from a biomedical perspective, the findings of this study suggest biomarkers for investigation of biomedical paradigms, and thus may contribute to novel therapeutic development in obesity.

References

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Table 1. Chemical shift and quantity (µmol/g) of selected metabolites in live Drosophila from 1D CPMG measurements.

Metabolite		CH3	(CH2)n	CH2CCO	CH2C=	CH2CO	CH=CH
Chemical shift (δ, ppm)		0.89 ppm	1.33 ppm	1.58 ppm	2.02 ppm	2.24 ppm	5.33 ppm
akhr	*Control-akhr^{rev}*	0.24 ± 0.02	0.89 ± 0.13	0.06 ± 0.01	0.10 ± 0.01	0.06 ± 0.01	0.06 ± 0.01
	Obese-akhr^{null}	0.35 ± 0.05	2.44 ± 0.38	0.40 ± 0.02	0.25 ± 0.04	0.21 ± 0.02	0.11 ± 0.01
	% change	45.83	174.16	566.66	150.00	250.00	83.33
	P-value	0.0002	0.00002	0.00007	0.00001	0.00005	0.00003