Monitoring hepatic lipid in response to CB1R inverse agonist treatment with MRS

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Introduction Based on CDC data, almost 30% adult Americans and 14% children are overweight. A long term health effect of obesity is an increased risk for developing heart disease, stroke, diabetes, and cancer. Animal models, especially mice, are widely used in preclinical research for studying obesity to investigate complications and treatment paradigms of obesity. Besides weight gain due to ubiquitous accumulation of adipose tissue, fat content in liver tissue also tends to increase in humans with the development of obesity. Liver is an important organ actively involved in fatty acid metabolism. Abnormal fat deposition in liver and in skeletal muscle tissue has been frequently associated with the progression of complications associated with obesity. Many anti-obesity drugs are being developed to treat abnormal body weight and body composition through various mechanisms. Because of the sensitivity of MR spectroscopy (MRS) to various biochemical contents in tissue, such as hepatic lipid, we were able to use a MR spectroscopy technique to detect liver lipid content in mouse liver non-invasively, and monitor its change during treatment with an anti obesity compound. The aims of the study were 1) to evaluate the distribution of hepatic lipid in a group of DIO mice; 2) to monitor the change in hepatic lipid content induced by a CB1R inverse agonist treatment in mice.

Methods *in vivo MRS*: We used a spatially-localized MR spectroscopy (MRS) method to obtain a hydrogen spectrum from mouse liver non-invasively. The resulting hydrogen spectrum was free of contamination by surrounding subcutaneous and visceral adipose tissues outside of the mouse liver. From the MR spectrum, water and fat signals were quantified numerically from their corresponding peaks. This MRS technique for quantifying liver lipid was implemented and validated on a Bruker 9.4T/31cm NMR system(Bruker Biospin, Germany), which was equipped with a 12-cm high performance gradient coil insert (maximum gradient strength =40G/cm; rise time = 80usec). The MRS method used was based on point resolved spectroscopy (PRESS). The relevant PRESS acquisition parameters are as follows: TR/TE=7sec/7ms, BW=10081Hz, voxal size = 1μliter, NA=16. Manual shimming was performed immediately prior to each acquisition with 3 linear magnetic field gradients if necessary.

Animal: Two groups (control and treated) of diet induced obese (DIO) mice were singly-housed and fed with a high fat diet(60 kcal% fat). The mice in control and treated groups (n=6 per group) were administered vehicle and AM251 at 3mg/kg per day for 10 days. Hepatic lipid content was measured with MRS immediately prior to and post treatment. MRS measurements were performed on mice under a gas anesthesia (1.0-1.5% isofluorane / air).

Data Analysis: Typical localized MR spectra obtained from mouse liver tissue reveal water and lipid components as two distinct NMR spectral peaks. Lipid and water contents were quantified by integrating spectral area under their corresponding peaks. The hepatic lipid percentage was defined as: 100 x lipid / (lipid +water). Normalized liver fat change was calculated as follows: $(\text{liver\_lipid\%}_{pre} - \text{liver\_lipid\%}_{post})/ \text{liver\_lipid\%}_{pre}$.

Results Average liver lipid percentage of a group of DIO mice at baseline (N=18) was found to be 29.3% with a standard deviation of 7.5% (max=38.1% and min=19.3%). Hepatic lipid contents of two groups of mice measured at the beginning and the end of the study are summarized in Figure 1A and 1B (error bar: SEM). A broad distribution of individual liver fat at baseline was clearly noticed and their individual trend due to the treatment was revealed. Group averages of liver fat content before and after vehicle treatment group was 28.5+/-1.5% and 29.4+/-1.1% respectively, and for the AM251 treated group was 30.1+/-1.4% and 23.1+/-2.2% respectively. The drug treated group showed a significant reduction in liver fat ($p<0.013$) while the control group did not ($p>0.31$). Furthermore, if each mouse was followed individually and used as its own control, percent change in liver lipid content from that of pre-treatment showed a statistically significant treatment effect (-35.2 +/- 10.8 % vs 5.3+/- 8.0 % shown in Fig.1C) with an improved p-value ($p<0.007$). Based on the result, it can be estimated that for achieving a statistical significance value of 0.05, there may be some opportunity to potentially reduce the sample size or increase the detection limit.

Conclusions Hepatic lipid content was measured before and after a CB1R inverse agonist (AM251) treatment in DIO mice non-invasively. Our MRS result shows there is a relatively wide distribution in hepatic lipid content, which limits the statistical power of a cross-sectional study to resolve a change. In a typical treatment study, using this MRS method to follow liver lipid content serially and individually we were able to improve the statistical power compared with that of the cross-sectional approach. The study also showed the feasibility and sensitivity of the method in revealing the change in hepatic lipid content in a routine *in vivo* study involving mice. Lastly, the method is translatable to humans and of value in the study of metabolic disease and discovery of anti-obesity medications.

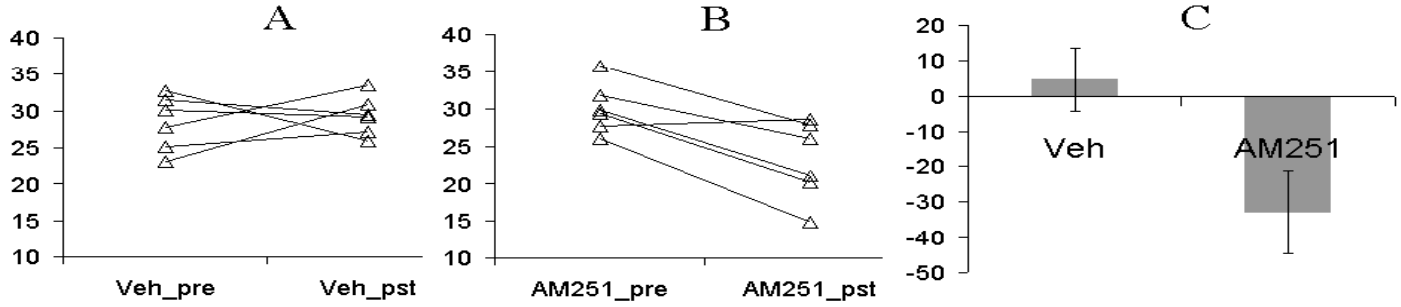

Figure 1. A: Liver fat percentages of mice in the control group before (_pre) and after (_pst) vehicle treatment. B: Liver fat percentages of mice in the AM251 group before and after treatment. C: Averaged percentage changes in liver fat (normalized to baseline) for two groups of mice before and after the treatment (p=0.007).