Longitudinal and cross-sectional measurements of intra-hepatic lipid levels in mouse via localized 1H MRS

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Introduction

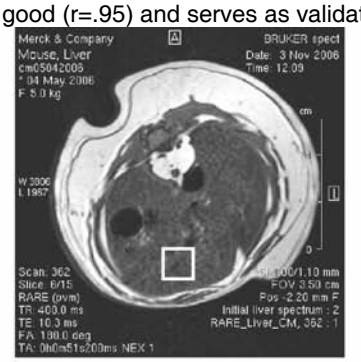
Elevated tissue lipid levels (especially in liver and muscle) are known to be correlated with symptoms of diabetes/obesity. The majority of animal models for diabetes/obesity exist in mice, yet current methods for assaying intra-hepatic lipid (IHL) levels involve harvesting large portions of the organ and sacrificing the animal, thus precluding the possibility of repeated measurements. For the evaluation of the effects of altered diets and/or novel pharmaceutical agents it is desirable to have a method by which IHL levels can be measured repeatedly. To date, however, few publications exist describing the use of MRS to measure IHL in mouse[1]. In this study, localized 1H MRS was used to estimate IHL levels in several mouse models of diabetes/obesity and also to monitor lipid levels over time in db/+ mice fed both a high fructose diet and normal chow.

Methods

Experiments were performed on a Bruker 11.7T (500MHz proton resonant frequency) wide bore NMR spectrometer with microimaging accessory (max. gradient strength = 150G/cm) using a 30mm volume coil. For this study the following mouse models were selected: db/db (a model of overeating), db/+ on high fructose diet (a model of increased IHL without increased body weight), db/+ on normal chow (a lean control), and NONcNZO on high fat diet (a combined transgenic / diet induced model of metabolic syndrome). Mice (n=3 per group) were anesthetized with 1.5-2.5% isoflurane (respiratory rate = 30-50 breaths/min) and positioned so that the liver was at coil center. Respiratory motion was monitored via pressure sensor placed on the abdomen and was used for gating image and spectral acquisition. Axial fast spin-echo images (RARE Factor = 2, TR~1.5s, TE=10ms) were acquired for localization and 1H spectra were acquired using PRESS (Voxel size = $(2.5mm)^3$, TR~4s, TE=20ms, NS=16 averages). Manual shimming was performed on the water resonance resulting in linewidths of 50-70 Hz (~1.2 ppm). Initial experiments showed lipid levels to vary little with voxel position within the liver, hence for most of the animals only one voxel location was used. For some of the animals, TE was varied from 17ms to 50ms and the spectral intensities were fit to an exponentially decaying curve to estimate T2 for the water and lipid resonances. Total scan time per animal was ~25 minutes. Following the MRS measurement, the animals were euthanized and a portion of the liver was excised and immediately frozen in liquid nitrogen for biochemical analysis of triglycerides, as described elsewhere[2].

Results

A representative image and spectrum is shown in Figure 1. Average T2 values for the water and $-(CH_2)_n-$ lipid peaks were 11ms and 25ms, respectively, and these values did not show any consistent variation with mouse strain or IHL level. Hence these average values were used to obtain the corrected values reported below. The data from the different mouse strains (Figure 2) shows lipid levels ranging from ~1% for the db/+ on normal chow to ~20% for the NONcNZO on high fat diet for five months, although the SEM within each group is relatively small. For the longitudinal monitoring of db/+ mice (Figure 3), the increase in lipid levels within the first week of the high fructose diet, and for the duration of the study, is striking. It is also interesting to note that there were no differences in body weight at any of the time points. The correlation of these MRS measurements with traditional biochemical assay of liver TG was very good (r=.95) and serves as validation of this method.



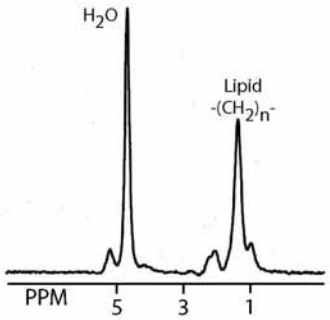


Figure 1. Axial RARE image and 1H spectrum from db/db mouse liver.

Conclusion

The use of MRS for non-invasive monitoring of IHL levels was implemented and it was shown that (1) a large window exists between endogenous IHL values for different mouse models of diabetes/obesity, (2) these lipid levels were well correlated with values obtained biochemically, and (3) this method allows sufficient throughput (~15 mice/day) for evaluation of the effects of diet and/or pharmaceutical agents over time.

References

1. Garbow, JR. et al. In vivo MRS measurement of lipid levels in mice. *J. Lipid Res.* (45) 2004.
2. Bligh, EG, and Dyer, WJ. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* (37) 1959.

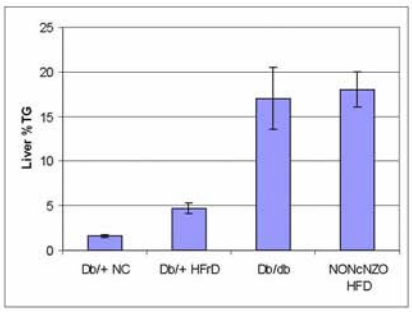


Figure 2. IHL values for the mouse strains used in this study.

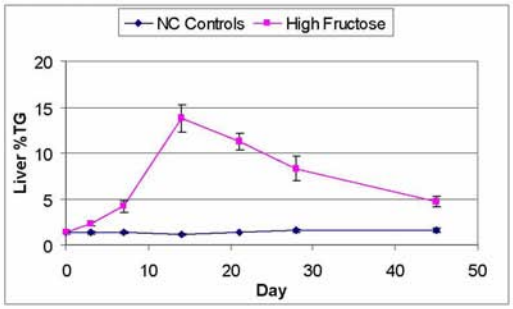


Figure 3. Longitudinal measurements of IHL from Db/+ mice on high fructose or normal chow.