Comparison of liver steatosis quantification by MRS at 4.7 T and histology on ob/ob and db/db mice

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INTRODUCTION

Murine models of obesity such as ob/ob (leptin deficient) and db/db (leptin receptor deficient) mice are extensively used in different scientific fields including pharmacology and toxicology. Besides increased body fatness, hyperlipidemia and insulin resistance these mice develop moderate (db/db) or massive (ob/ob) steatosis (i.e. fatty liver). In some experimental settings a longitudinal follow-up of steatosis may be warranted, for instance to assess the beneficial (or deleterious) effects of chronic drug administration. Hence, noninvasive *in vivo* liver imaging methods are requested in order to perform accurate follow-up investigations without the need to sacrifice the animals for pathological examinations or lipid quantification. Several investigations in patients or animal models have demonstrated the interest of *in vivo* magnetic resonance spectroscopy (MRS) to quantify fat level in the abdominal region [1-4] and liver [5-9] in the context of obesity. However, to the best of our knowledge, there is no study in the mouse that looked for a relationship between MRS and the pathological score of steatosis.
Thus, in the present study, db/db and ob/ob mice were used to determine whether MRS could be a reliable non-invasive method to evaluate fatty liver. To this end, each animal was subjected to MRS and the "Fat/Water" ratio obtained from each spectrum was compared with the intensity of fatty liver determined by an experienced pathologist.

MATERIAL AND METHODS

Animals: This protocol included 9 male mice: 3 wild-type C57BL/6J Rj mice (8 weeks old), 3 obese C57BL/6J Rj-ob (ob/ob) mice (5, 8 and 10 weeks old) and 3 obese C57BL/KsJ Rj-bd (db/db) mice (5, 8 and 10 weeks old). Animals were put under gaseous anesthesia during MRS acquisitions. After MR experiments, animals were sacrificed and the two largest lobes (left and median) of the liver were harvested and then frozen at -80 °C.

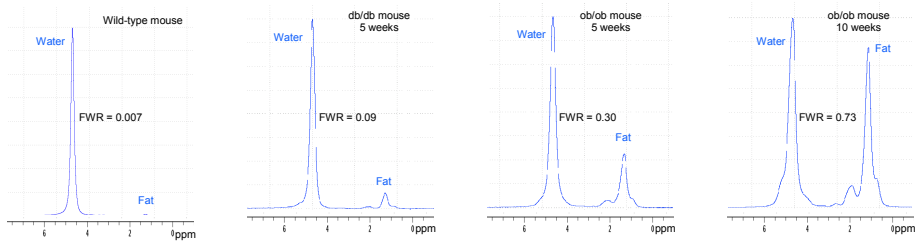
MRS: All MR experiments were performed using an imaging spectrometer equipped with a 4.7 T horizontal shielded magnet dedicated to small animal (47/40 USR Bruker Biospec), ParaVision 4.0 and Topspin 1.5 software (Bruker Biospin MRI, Wissembourg, France).
In vivo MRS acquisitions were carried out using a BGA12 gradient system and a quadrature birdcage coil (72 mm diameter). We acquired localized MR spectra (PRESS sequence – No water suppression) successively on the upper left and upper right lobes of the mouse liver, with respiratory gating. The following parameters were used: voxel of 3x3x3 mm^3, TR/TE = 2500/20 ms, 256 averages and Tacq = 10 min.
In vivo MR spectra were analyzed with Topspin. Water and fat peaks were integrated and the "Fat/Water" ratio (FWR) was calculated for each spectrum.

Histology: 7 µm-thick liver sections were fixed with a 10% formol solution and stained with Oil Red O and hematoxylin-eosin-saffron. Sections were then evaluated by one experienced pathologist and photographed at magnification 10X. ImageJ (NIH software) was then used to measure the area (fat fraction: FF) and the size of the lipid droplets on five large reconstituted fields (0.89 mm^2).

Statistical analysis: Statistical analyses of the MRS and histological data were performed using a Mann-Whitney test with a significance level of 5%. The correlation between MRS and histology regarding steatosis quantification was calculated using a Spearman test.

RESULTS

MRS: The analysis of quantitative data obtained in MRS showed a significant increase in the FWR between the wild-type mice and the obese mice ($p<0.003$). This difference was particularly large between wild-type and ob/ob mice ($p<0.001$). An increase of the FWR versus age was also observed for the ob/ob mice.



Histology: Histological data showed an increase of steatosis for obese mice compared to wild-type mice. A significant difference ($p<0.003$) between wild-type and obese mice was obtained for the FF. This parameter gave high statistical difference ($p<0.001$) between wild-type and ob/ob mice. We also observed the increase of the FF with age for the ob/ob mice.

MRS versus Histological data: Spearman test gave a high correlation ($r=0.95$, $p<10^{-6}$) between MRS and histological results considering all mice of the study (wild-type, ob/ob and db/db). A significant correlation between MRS and histological results was also obtained for obese mice ($r=0.9$, $p=0.02$).

DISCUSSION AND CONCLUSION

In the present study, we found an excellent statistical correlation between MRS and histological data regarding the assessment of intrahepatic fat in two murine models of obesity. Importantly, our preliminary investigations included db/db mice that have milder steatosis when compared to ob/ob mice, thus indicating that the MRS/histology correlation is valid over a wide range of liver lipid levels. Moreover, our study indicated that there was no major difference of liver fat accumulation between the left and median lobe.

REFERENCES

[1] Brechtel K *et al.* J. Magn. Reson. Imaging (2000). **12**: 306-317.
[2] Hu HH *et al.* Obesity (2009). Advance online publication.
[3] Walling BE *et al.* Obesity (2007). **15**: 69-77.
[4] Lunati E *et al.* Magn. Reson. Med. (2001). **46**: 879-883.
[5] Delgado TC *et al.* NMR Biomed. (2009). **22**: 310-317.
[6] Machann J *et al.* J. Magn. Reson. Imaging (2000). **12**: 306-317.
[7] Mennesson N *et al.* J. Comput. Assist. Tomogr. (2009). **33**: 672-677.
[8] Calderan L *et al.* Obesity (2006). **14**: 405-414.
[9] Garbow *et al.* J. Lipid Res. (2004). **45**: 1364-1371.