In vivo hepatic localized proton magnetic resonance spectroscopy at 7T in a glycogen storage disease mouse model

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Introduction:

Glycogen storage disease type I (GSD 1) is an autosomal recessive metabolic disorder resulting in severe impairment of glucose production and large accumulation of liver fatty acids (steatosis). The phenotype of GSD I results from a defect in the glucose-6 phosphatase complex (G6pc). Despite of a strict diet, patients with GSD 1 develop, with age, multiple hepatocellular adenoma (HCA), ultimately transforming into hepatocellular carcinoma (HCC) [1]. The occurrence of this hepatic pathology appears to be related to the degree of steatosis, as in patients with GSD 1 these adenomas sometimes regress when metabolic control is tightened. Very recently, a viable mouse model of GSD I with a liver specific invalidation of G6pc has been generated [2]. GSD 1 mice exhibit hepatic pathological features (hepatomegaly, steatosis) very similar to those observed in GSD1a patients. In vivo 1H magnetic resonance spectroscopy (MRS) was used to examine the content and composition of fatty liver in the mouse model of GSD1a, in a non-invasive manner. Our objective was to evaluate the hepatic steatosis under two different diets, a standard- and a high fat diet, to further correlate the development of adenomas and the liver fatty acid composition.
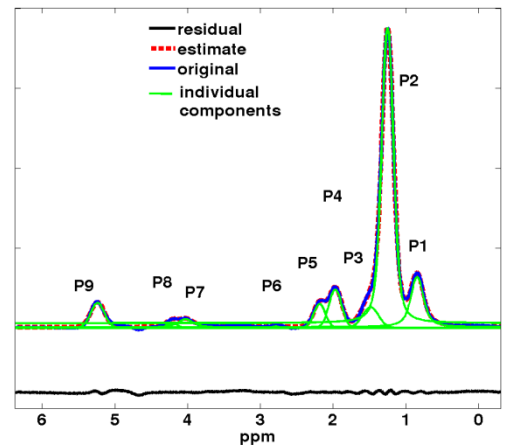
Method

Animal model: Animal experiments were performed using 9 month-old female GSD I mice (n=13), fed either on standard (3.1% lipids, 60% carbohydrate, 16.1% proteins) or on high fat (36.1% lipids, 35% carbohydrate, 19.8% proteins) diet as described in [3]. The experiments were conducted according to the procedures approved by the Institutional Animal Care and Ethical Committee of our University.

Experimental conditions: The experiments were performed on a horizontal 7T Biospec BRUKER system. The mice were anaesthetized by inhalation of isoflurane. The body temperature was maintained at 37°C by warm water circulation. A pressure sensor was used to monitor the respiratory cycle. Localized spectroscopy was performed using a short–echo time respiration-trigged PRESS sequence (TR/TE: 3000ms/16ms, 2048 data-points, bandwidth of 4kHz). The effective TR, multiple of the respiratory period of the mouse, was set to be not inferior to 3 s. The localization of the voxel (3x3x3mm^3) within the right lobe of the liver in an area free of large hepatic vessels and surrounding fat, was based on T2-weighted RARE images. Localized first- and second-order shim terms were adjusted leading to line widths for the water resonance around 60Hz. For each mouse, the MRS signals were acquired with VAPOR water suppression (128 transients, 7min scan-time). Following MR examinations, a subset of mice were sacrificed and liver tissue samples collected for histology.
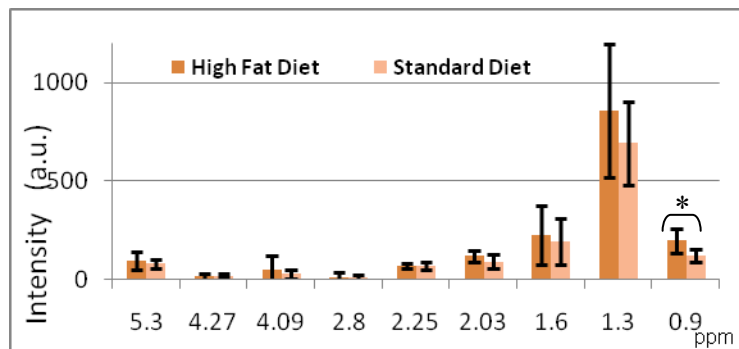
Quantification algorithm: In vivo MRS signals were processed in the time-domain. The quantification procedure, called Multiple Starting Values method (MSV) [4], is based on a nonlinear least-squares algorithm that fits the time-domain signal to a Voigt model function and uses multiple random starting values and bounds. The levels of the nine main resonating groups identified in the spectrum of Figure 1 were quantified. The fat fraction of saturated (FS) and unsaturated (FU) fatty acids were derived according to [5] (Table1).

Figure 1: ^{1}H water-suppressed spectrum acquired at 7T within the right hepatic lobe of a mouse fed on a high fat diet., Superimposed to the estimated spectrum as a sum of Voigt lineshape resonances, the individual estimated resonances and the residue.

Results

The estimated MRS profiles for both groups (Figure 2) showed significant differences for the lipid methyl resonances (P1). Both estimated levels of the methylene resonances (P2) were significantly higher than the estimates obtained for control mice fed on standard diet (results not shown). Triglyceride levels (mg/g tissue) showed strong correlations with the corresponding estimated methylene resonance levels (P2) (ρ=0.7, Spearman non parametric correlation). Liver histology confirmed that both groups developed hepatic steatosis. Based on MR imaging readings, 90% of the mice fed on high-fat diet exhibited adenomas in the liver while no adenomas were detected on mice fed with standard diet.

Table 1: **Composition of fatty acids in liver of a mouse model of GSD1a under standard and high-fat diets**

	FS (%)	FU (%)
Standard diet mice (n=7)	31±19	69±19
High fat diet mice (n=6)	18±12	82±12

Mean ± standard deviation.

Figure 2: Mean levels and standard deviation of the nine main resonances estimated for the groups of mice fed on standard and high-fat diets. * Significantly different values (P≤0.05, Wilcoxon non parametric test).

Conclusion

Accumulation of hepatic fat and fat composition within the liver were assessed using in vivo MRS. These measurements will give insight into the understanding of the onset and progression of adenomas in a mouse model of GSD1a under different diets.

References

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