

Hepatic fatty acid quantification using MRS and GC in a mouse model of GSD1A under two different diets

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Introduction: Glycogen storage disease type I (GSD1) is an autosomal recessive metabolic disorder resulting in severe impairment of glucose production and large accumulation of liver fatty acids. Very recently, a viable mouse model of GSD1 with a liver specific inactivation of G6pc has been generated [1,2]. GSD1 mice exhibit hepatic pathological features very similar to those observed in GSD1a patients. The analysis of fatty acid composition in tissues is commonly performed by gas chromatography [3]. ¹H magnetic resonance spectroscopy (MRS) allows non invasive assessment of in vivo triglyceride composition [4]. Recently, the triglyceride composition of subcutaneous human adipose tissue has been estimated using long echo-time (TE) MRS and validated with gas chromatography (GC) [5]. In this paper, short-TE in vivo MRS and gas chromatography analysis were used to evaluate the content and composition of fatty liver in the mouse model of GSD1a under two different diets, a chow and a high fat high sucrose (HFHS) diet.

Methods

Animal model: Animal experiments were performed using 9 month-old female GSD1 mice, fed either on chow (3.1% lipids, 60% carbohydrate, 16.1% proteins) (N=10) or on HFHS (36.1% lipids, 35% carbohydrate, 19.8% proteins) diet (N=10) [6]. The experiments were conducted according to the procedures approved by the Institutional Animal Care and Ethical Committee of our University.

¹H MRS experiments: 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-TE respiration-triggered PRESS sequence (TR/TE: 3000ms/16ms, 2048 data-points, bandwidth of 4kHz, 128 accumulations, 7min scan-time). Water suppression was achieved with VAPOR. The effective TR, multiple of the respiratory period of the mouse, was set to be greater to 3 s. The localization of the voxel (3x3x3mm³) 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. Following MR examinations, a subset of mice were sacrificed (N=9) and liver tissue samples were collected for histology and Gas Chromatography (GC).

MRS data analysis: *In vivo* MRS signals were processed in the time-domain. The quantification procedure, called Multiple Starting Values method [7], 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. Nine components were selected to fit the resonances corresponding to lipid contribution : methyl group (0.9ppm), methylene group(1.3ppm), β-methylene to carboxylic group (1.6ppm), allylic group (2ppm), α-methylene to carboxylic group (2.25ppm), diallylic group (2.8ppm), glycerol backbone (4.07ppm and 4.23ppm) and olefinic group (5.3ppm). The amplitude estimates of the components were normalized to the estimated methylene amplitude. The fat fraction of saturated (FS) and unsaturated (FU) fatty acids and also the mean chain length (MCL) were derived from the quantification estimates as described in [8].

Gas Chromatography Analysis: Fatty acids assay was performed as described in [9]. Following homogenization of tissue samples in methanol/5 mM EGTA (2:1, v/v), lipids corresponding to an equivalent of 1 mg of liver were extracted in the presence of glyceryl triheptadecanoate (0.5 g) as an internal standard. The lipid extract was transmethylated with 1 ml of BF₃ in methanol (1:20, v/v) for 150 min at 100°C, evaporated to dryness, and the fatty acid methyl esters (FAMES) were extracted with hexane/water (3:1). The organic phase was evaporated to dryness and dissolved in 50 μl ethyl acetate. One microliter of FAME was analyzed by gas-liquid chromatography on a 5890 Hewlett-Packard system using a Fawax fused-silica capillary column (30 m, 0.32 mm i.d., 0.25 mm film thickness; Restek, Belfast, UK). Oven temperature was programmed from 110 to 220°C at a rate of 2°C/min, and the carrier gas was hydrogen (0.5 bar). The injector and the detector were at 225 and 245°C, respectively. Identification of the FAMES was based upon retention times obtained for methyl ester standards. In order to obtain comparable results between the GC and MRS methodologies, the proportions of the chemically equivalent groups of proton were calculated from the GC fatty acid data, then fatty acid indexes were derived.

Results: The lipidic profiles derived by both by MRS and GC are in good agreement as shown on Figure 1, except for the diallylic and olefinic groups. The group resonating at 5.2ppm was successfully quantified although the estimated values may be affected by the water suppression deficiency. The 2.8ppm resonance clearly measured by MRS. The derived fatty acid indexes are displayed in Table 1. Significant differences were found for the fraction of unsaturation between the two studied groups (Wilcoxon test, *p<0.05). Liver histology confirmed that both groups developed hepatic steatosis.

Indexes	HFHS diet		Chow diet	
	GC	MRS	GC	MRS
FS(%)	26±1	22±2	30±2	31±3
FU(%)	75±1	78±2*	68±3	69±3*
MCL	18±1	20±1	18±1	19±2

Table 1 : Mean values and standard error of mean of the estimated fatty acid indexes derived from MRS and gas chromatography measurements for the two studied mouse groups.

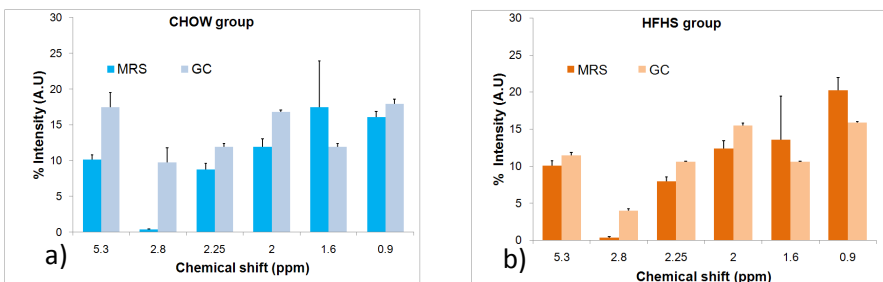


Figure 1: Mean levels and SEM of the lipid profiles normalized to the methylene group level estimated from MRS and GC measurements for the a) CHOW and b) HFHS groups. Rajouter * sur les 2.8ppm

Conclusion: Strong agreements between the quantitative lipid profiles measured by MRS and Gas Chromatography were demonstrated with GSD1 mouse model evaluating the impact of two diets on the disease evolution. Our results confirm that ¹H MRS is a suitable non invasive tool for hepatic lipid quantification and lipid fraction estimation.

References

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