

NAFLD Metabolic Signatures by HR-MAS Molecular Profiling

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Purpose:

The liver performs a large number of metabolic functions including the use of fatty acids for energy production and interconversion of biosynthetic precursors. As a consequence, the liver plays a critical role in fat storage and retrieval. Liver pathophysiology can range from steatosis and steatohepatitis to fibrosis, cirrhosis and even liver cancer. Among these pathologies, non-alcoholic fatty liver disease (NAFLD) affects large percentages of the population and is recognized as one of the most common forms of chronic liver disease. NAFLD is associated with an increase in lipogenesis and a decrease in the ability of the liver to export lipids. Global molecular profiles, which are affected by many physiological and pathological processes, may reflect more accurately the presence of a particular disease state. The aim of this study is to measure HRMAS metabolic profiles and to find possible metabolic differences between human liver tissue with steatosis and with steatohepatitis for a better understanding of the disease.

Subjects and Methods:

We recorded 1D pre-saturation ¹H spectra in a 600 MHz spectrometer of 38 human liver tissue samples with different degrees of steatosis and inflammation. The amount of tissue analysed for each subject ranged from 5 to 25 mg. Cylindrical inserts were used limiting the rotor inner volume to 50 μ l. All measurements were performed at a temperature of 4C to minimize metabolic degradation. HRMAS spectra The chemical shift region including resonances between 0.5 and 4.7 ppm, which includes the resonances of most hepatic metabolism molecules was investigated. Statistical multivariate analysis was performed using in-house MATLAB scripts and the PLS Toolbox (Eigenvector Inc.). Orthogonal Signal Correction (OSC) and Robust Principal Component Analysis (RPCA) were applied to the set of spectral vectors. Principal components chosen explained at least 80% of the variance. Metabolite quantification was achieved by in-house peak-fitting routine over most relevant signals.

Results and Discussion

Our NMR spectra of fatty liver biopsies showed narrow signals and adequate SNR with well resolved multiplicities. Direct comparison of the spectra demonstrated that steatosis and steatohepatitis tissue are metabolically similar (Figure 1). The metabolic profile of the liver tissue determined here was highly consistent with previous studies. However, subsequent statistical multivariate analysis showed differences in signals belonging mainly to triglycerides and alanine. Overall, HRMAS allowed to discriminate between molecular profiles of steatosis and steatohepatitis and to detect corresponding metabolic differences. Metabolites closely related to the liver metabolism like lipids and amino acids display differences between tissue from fatty liver with and without inflammation. This study shows that steatosis and steatohepatitis produce metabolic alterations identifiable by HRMAS spectroscopy. The PCA analysis revealed some significant differences in metabolic composition, suggesting different degrees of dysfunction in the liver. Our results may be the basis for using HRMAS of liver biopsies as support for tissue classification.

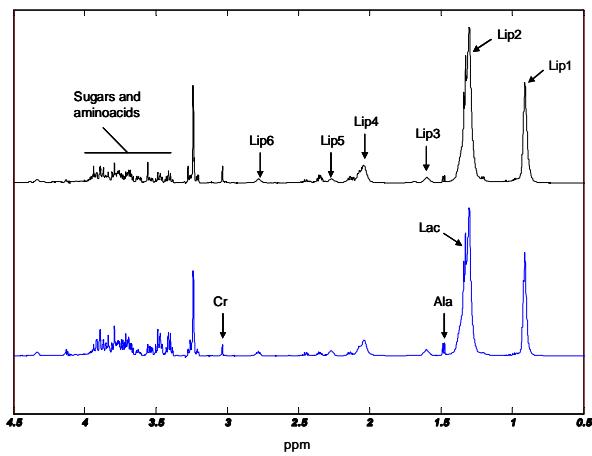


Figure 1. 1D ¹H HR-MAS CPMG spectra for steatosis (bottom) and steatohepatitis (top) liver with most relevant resonances labeled.