Metabolic Characterization of Patients with Alcohol and Non-Alcoholic Steatohepatitis

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Introduction

Liver steatosis is an increasingly common cause of liver diseases that is largely associated with current epidemics of obesity and diabetes. Non-alcoholic fatty liver disease (NAFLD) describes conditions ranging from steatosis to steatohepatitis with inflammation of hepatocytes. Two etiologies exist for liver steatohepatitis: alcoholic steatohepatitis (ASH) and non-alcoholic steatohepatitis (NASH), both leading to similar pathological findings observed in patients. The non-alcoholic etiology is the more common form, and has recently been termed NAFLD to encompass both steatosis and NASH. Non-alcoholic steatosis is the form of fatty liver disorder that is characterised by an accumulation of fat within the liver that usually causes no liver damage. However, because NAFLD presents no symptoms, it can lead to a more serious disease: NASH, which is associated with liver-damaging inflammation. NASH can further progress to either cirrhosis (which causes progressive, irreversible liver scarring) or to liver cancer (Angulo, 2002). NASH is now the most common liver disease, and is expected to increase in prevalence in parallel with the epidemics of obesity and type 2 diabetes. Today no reliable diagnosis exists for NASH, and liver transplantation is the only treatment for advanced cirrhosis, although the procedure is rarely done in people with NASH because transplanted livers show re-development in more than two thirds of the cases.

Objective

It is not exactly known what causes NAFLD, but many researchers believe that the metabolic syndrome may play an important role in its development. However, no established treatment exists for this potentially serious disorder to stop the progression from steatosis to fibrosis and cirrhosis. In addition, pathological findings similar to NASH can be seen in patients with ASH and it is difficult to differentiate these patients on the basis of clinical and biochemical evaluations. It is becoming more and more urgent to develop new and efficient diagnostic methods for steatosis. All present methods for liver-function testing are poor predictors and invasive. Indeed, steatosis presently remains a diagnosis of exclusion that often requires the performance of invasive tests. Each of the currently applied conventional methods contain major limitations. Recently, *in vivo* magnetic resonance (MR) analysis has been attempted and research is undergoing in order to detect steatosis noninvasively (Khan *et al.*, 2005). In addition, high-resolution ^{1}H-NMR has emerged as a powerful technique to simultaneously identify and quantify multiple metabolites of medical significance without the requirement for pre-selection or separation of metabolites. Since it is becoming urgent to develop new diagnostic methods for NAFLD, we applied high-resolution NMR spectroscopy to detect metabolic profiles in body fluids in patients with NASH and ASH. Furthermore, to study other metabolites not previously detectable by conventional ^{1}H-NMR analysis, we used natural abundance ^{13}C-NMR measurements.

Methods

Thirty non-alcoholic patients (NASH) and 7 alcoholic steatotic (ASH) patients were recruited among the patients of the Hepatology Service of the Hôpital Saint-Luc du CHUM. These patients were identified from a review of patient records. Thirty control subjects matched for age and sex with the steatotic patients and with no known liver disease (among other inclusion and exclusion criteria) were recruited. Three venous blood samples were collected to obtain 1) whole blood, 2) plasma using heparin and 3) serum using heparin and anticoagulant. The plasma and serum samples were centrifuged and the supernatant retained. All samples were kept at -80°C. Urine samples were neutralised to pH 5.8, lyophilized and re-dissolved in 500 µl D_2O. We used a dual-extraction method to investigate both water-soluble metabolites and lipophilic compounds involved in fatty acid and lipid metabolism, in blood plasma and serum. To confirm chemical shifts of quantified metabolites in 1D-NMR spectra, two-dimensional {^{1}H-^{1}H} and {^{1}H-^{13}C} experiments were done. 1D-NMR spectra were recorded on a DRX-600 Bruker spectrometer. Fully-relaxed ^{1}H-NMR spectra were recorded using (trimethylsilyl)-propionic-2,2,3,3d_4-acid (TSP) as standard. Integrals in ^{13}C-NMR spectra were corrected for nuclear Overhauser enhancement and saturation effects (Aureli *et al.*, 1999).

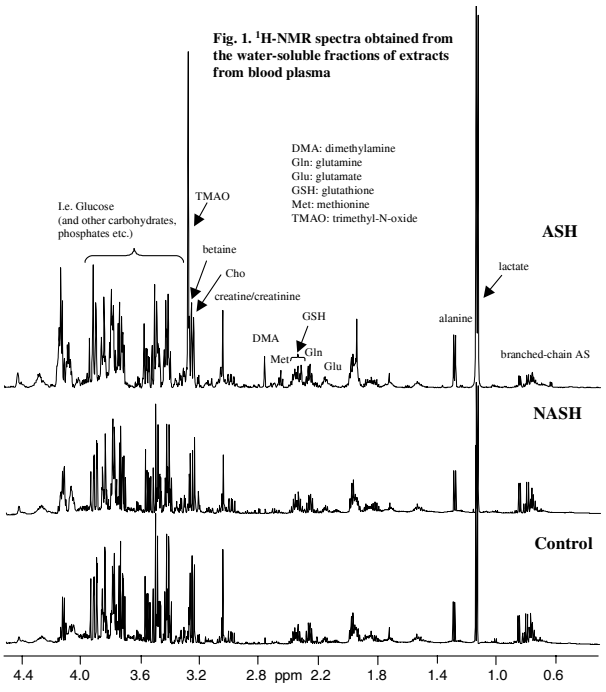

Fig. 1. ^{1}H-NMR spectra obtained from the water-soluble fractions of extracts from blood plasma

Results

^{1}H- and ^{13}C-NMR spectra of urine and blood extracts showed considerable differences in specific metabolites involved in intermediary metabolism in patients with NASH and ASH compared to healthy controls. Selective changes in patients with ASH compared to healthy controls were detected in the urine (decreases in the concentrations of hippurate (to 46 ± 13%), and urea (to 37 ± 21%), increases of TMAO (to 157 ± 28%) and tyrosine (to 322 ± 124%)) as well as accumulation of bile acids. Data analysis from blood plasma clearly showed reproducible differences in specific metabolites in ASH-patients compared to control subjects (Fig. 1). In particular these were decreases in the concentrations of branched-chain amino acids (to 24 ± 5%), increases of dimethylamine (>6-fold), trimethyl-N-oxide (>2.5-fold), and methionine (>7-fold) (which is known to play a key role in alcoholic liver diseases). These changes were not observed in blood plasma of patients with NASH. ^{1}H- NMR spectra of lipid extracts showed small but significant elevations of cholesterol (to 133 ± 16%) and phosphatidylcholine (to 180 ± 24%) in NASH-patients only. Furthermore, in NASH-patients, natural abundance ^{13}C-NMR spectra showed considerable changes in multiple metabolites involved in mitochondrial metabolism.

Conclusions

Applications of NMR-methods on human body fluids are of great potential to characterize NAFLD, a disease displaying multiple interrelated metabolic factors. This approach could provide noninvasively data to characterize steatosis and give the possibility to distinguish between NASH and ASH. The risks and discomforts presently associated with biopsies would therefore be eliminated. The application of high-resolution NMR spectroscopy would not only be able to accurately diagnose patients with NAFLD, but might also lead to new treatment protocols for individual patients.