Research Article

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Non-alcoholic fatty liver disease: Spectral patterns observed from an *in vivo* phosphorus magnetic resonance spectroscopy study

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Background & Aims: Liver biopsy is the gold standard for diagnosing non-alcoholic fatty liver disease (NAFLD) but with practical constraints. Phosphorus magnetic resonance spectroscopy (^{31}P-MRS) allows *in vivo* assessment of hepatocellular metabolism and has shown potential for biochemical differentiation in diffuse liver disease. Our aims were to describe spectroscopic signatures in biopsy-proven NAFLD and to determine diagnostic performance of ^{31}P-MRS for non-alcoholic steatohepatitis (NASH).

Methods: ^{31}P-MRS was performed in 151 subjects, comprised of healthy controls (n = 19) and NAFLD patients with non-NASH (n = 37) and NASH (n = 95). Signal intensity ratios for phosphomonoesters (PME) including phosphoethanolamine (PE), phosphodiesters (PDE) including glycerophosphocholine (GPC), total nucleotide triphosphate (NTP) including α-NTP, and inorganic phosphate (Pi), expressed relative to total phosphate (TP) or [PME+PDE] and converted to percentage, were obtained.

Results: Compared to controls, both NAFLD groups had increased PDE/TP (p <0.001) and decreased Pi/TP (p = 0.011). Non-NASH patients showed decreased PE/[PME+PDE] (p = 0.048), increased GPC/[PME+PDE] (p <0.001), and normal NTP/TP and α-NTP/TP. Whereas, NASH patients had normal PE/[PME+PDE] and GPC/[PME+PDE], but decreased NTP/TP (p = 0.004) and α-NTP/TP (p <0.001). The latter was significantly different between non-NASH and NASH (p = 0.047) and selected as discriminating parameter, with area under the receiver-operating characteristics curve of 0.71 (95% confidence interval, 0.62–0.79). An α-NTP/TP cutoff of 16.36% gave 91% sensitivity and cutoff of 10.57% gave 91% specificity for NASH.

Conclusions: ^{31}P-MRS shows distinct biochemical changes in different NAFLD states, and has fair diagnostic accuracy for NASH.

Keywords: Non-alcoholic steatohepatitis; Simple steatosis; Liver fibrosis; Phosphorus metabolites; Adenosine triphosphate.

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Abbreviations: NAFLD, non-alcoholic fatty liver disease; ^{31}P-MRS, phosphorus magnetic resonance spectroscopy; NASH, non-alcoholic steatohepatitis; PME, phosphomonoesters; PE, phosphoethanolamine; PDE, phosphodiesters; GPC, glycerophosphocholine; NTP, nucleotide triphosphate; Pi, inorganic phosphate; TP, total phosphate; ^{1}H-MRS, proton magnetic resonance spectroscopy; NDP, nucleotide diphosphate; ATP, adenosine triphosphate; NAD/NADPH, nicotinamide adenine dinucleotide/phosphate; MRI, magnetic resonance imaging; IHTG, intrahepatic triglyceride; VOI, volume of interest; TR, repetition time; TE, echo time; AMARES, advanced method for accurate, robust, and efficient spectral fitting; PC, phosphocholine; GPE, glycerophosphorylethanolamine; AUROC, area under receiver-operating characteristics curve; PPV, positive predictive value; NPV, negative predictive value; CV, coefficient of variation; ICC, intraclass correlation coefficient; BMI, body mass index; ALT, alanine aminotransferase; ER, endoplasmic reticulum; ROS, reactive oxygen species.

Introduction

Liver biopsy remains the gold standard for the diagnosis and longitudinal assessment of non-alcoholic fatty liver disease (NAFLD), but is invasive, carries a small risk of complications, suffers from sampling error and is impractical for assessing large populations. Consequently, the development of surrogate diagnostic markers to distinguish potentially progressive non-alcoholic steatohepatitis (NASH) from benign simple steatosis has become an emerging priority [1,2]. NASH is marked by liver cell injury and inflammation, and may evolve to cirrhosis and its complications, including hepatocellular carcinoma [3].

Currently, conventional ultrasound and proton/^{1}H-magnetic resonance spectroscopy (MRS) qualifies and quantifies hepatic fat, respectively [4,5], while ultrasound elastography detects advanced fibrosis [6]. To date there is no reliable non-invasive imaging technique to identify NASH.

Phosphorus/^{31}P-MRS has been employed in the differentiation of various chronic liver diseases with thematic results [7,8]. The phosphomonoesters (PME) signal mainly represents cell

membrane precursors and is consistently elevated in states of rapid cell proliferation such as nodule regeneration in liver cirrhosis [7–9]. Whereas, phosphodiesters (PDE) mainly represent cell membrane degradation products and correlate negatively with PME.

PME also overlaps with adenosine monophosphate, which together with inorganic phosphate (Pi), reflects energy synthesis [10–12]. However energy levels are better depicted by nucleotide tri-/diphosphate (NTP/NDP) levels [13] represented by γ, α, and β peaks in the spectrum. β-NTP, comprised solely of NTP, represents most of hepatic adenosine triphosphate (ATP) [14,15] and is typically decreased in liver disease [7,8]. The γ-NTP and α-NTP resonances contain contributions from NTP and NDP, and α-NTP additionally co-resonates with nicotinamide adenine dinucleotide molecules (NAD^+/NADH, $NADP^+$/NADPH) [16,17]. Recently, NADPH was proposed as a potential marker for NASH [18].

We therefore undertook this cross-sectional study in prospectively recruited patients with biopsy-proven NAFLD to determine spectroscopic profiles and evaluate use of ^{31}P-MRS for the identification of NASH.

Patients and methods

Subjects

The study protocol received institutional review board approval with informed written consent from all subjects. Patients, recruited from a university hospital in Hong Kong and undergoing liver biopsy for persistently deranged liver function tests, were invited for ^{1}H-MRS and ^{31}P-MRS within one week before liver biopsy. Controls were healthy volunteers recruited from a population screening project [19].

Subject inclusion criteria: (a) age 18–70 years; (b) males consuming <20 g of alcohol per day; females consuming <10 g alcohol per day; (c) no active malignancy, no known acute/chronic disease except obesity or type 2 diabetes; (d) negative hepatitis B and C markers; (e) no decompensated liver disease, defined as bilirubin >50 μmol/L, albumin <35 g/L, platelet count $<100 \times 10^9$/L, international normalized ratio >1.3, no ascites or varices; (f) no contraindications to magnetic resonance imaging (MRI). Additionally, controls should have normal liver biochemistry and intrahepatic triglyceride (IHTG) content ⩽5% on ^{1}H-MRS [5].

Clinical measurements and laboratory tests

Anthropometric measurements and tests for liver biochemistry, serum lipids and glycemic parameters were performed one day prior to liver biopsy in NAFLD patients and at the first clinic visit in controls.

MRI data acquisition

MRI examinations were performed using a whole-body 3 Tesla scanner (Achieva TX; Philips Healthcare, Best, The Netherlands). Prior to MRS, a set of localizer images of the liver was acquired in the transversal and coronal planes to position volume of interest (VOI) for MRS data acquisition. Subjects were instructed to breathe normally during the examination.

^{1}H-MRS

^{1}H-MR spectra without water suppression were obtained with a stimulated echo acquisition mode sequence for spatial localization (repetition time TR/echo time TE 5000/15 ms; mixing time 18 ms; signal averages 24; bandwidth 2000 Hz and number of data points sampled 1024) using the body coil for signal excitation and reception. VOI measuring $30 \times 30 \times 30$ mm^3 was positioned in the right liver lobe, avoiding large vessels and bile ducts. Shimming was performed using an automated protocol requiring no operator input. ^{1}H-MRS took ~2 min to acquire.

^{31}P-MRS

^{31}P-MRS was performed using a 14 cm diameter transmit/receive ^{31}P surface coil securely positioned laterally over the liver with patient resting supine. VOI measuring $60 \times 60 \times 50$ mm^3 was placed in the right liver lobe (Fig. 1). The surface coil was manually matched and tuned to the operating frequency for phosphorus (51.7 MHz) prior to automatic magnetic field shimming. Spectroscopic sequence employed was a volume-selective sequence based on a modified image-selected *in vivo* spectroscopy protocol. Proton-decoupling based on a wideband alternating-phase low-power technique for zero-residual splitting four (WALTZ-4) phase cycling and nuclear Overhauser effect based on WALTZ-4 broadband irradiation technique were employed for spectral enhancement. Spectroscopic data were acquired at 2048 points with receiver bandwidth of 3000 Hz, repetition time of 5 s and 128 signal averages. Scan time was ~11 min.

For test reliability, we scanned five volunteers twice within one hour, with subjects removed from the magnet between scans.

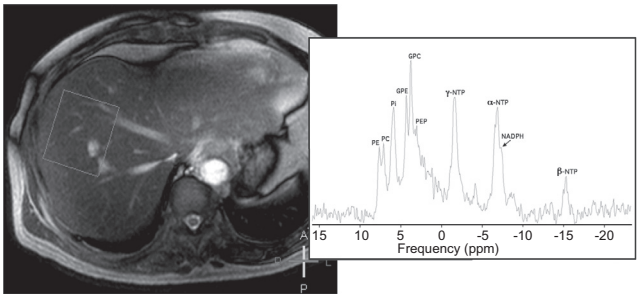

Fig. 1. ^{31}P-MRS acquisition and spectrum. (Left) Axial MRI with volume of interest in the right liver lobe. (Right) Sample ^{31}P-MR spectrum with metabolite peaks identified. PE, phosphoethanolamine; PC, phosphocholine; Pi, inorganic phosphate; GPE, glycerophosphoethanolamine; GPC, glycerophosphocholine; PEP, phosphoenolpyruvate; NTP, nucleotide triphosphate; NADPH, nicotinamide adenine dinucleotide phosphate.

MRS data processing

Spectra acquired were processed off-line using the advanced method for accurate, robust, and efficient spectral fitting (AMARES) method, a time-domain fitting routine implemented in the MRUI software (available at http://www.mrui.uab.es/mrui/) [20].

^{1}H-MRS

Water (4.65 ppm) and fat (1.3 ppm) peak amplitudes were measured using commonly adopted procedures [21]. IHTG was calculated as $[I_{fat}/(I_{fat} + I_{water})] \times 100$ where I_{fat} and I_{water} represent peak amplitudes of fat and water, respectively.

^{31}P-MRS

Prior to fitting using AMARES, spectra were apodized with a 10 Hz Gaussian filter. Broad components of the spectra were removed by truncation of a few initial points, adjusted according to the apparent linewidth of the broad component. The PME resonance was modelled as two peaks, phosphoethanolamine (PE) and phosphocholine (PC); PDE peak was modelled as two signals, glycerophosphoethanolamine (GPE) and glycerophosphocholine (GPC); while γ-NTP, α-NTP and β-NTP, Pi and NADPH were modelled with prior knowledge as described previously [22–24]. PME was calculated as the sum signal contribution from PE and PC, PDE from GPE and GPC, and NTP from γ-, α- and β-NTP. Individual signals were expressed as percentage relative to total phosphate (TP) or [PME+PDE] as appropriate. PME/PDE was also computed. Spectral data were processed and analyzed by a single operator (DKWY) blinded to clinical and histologic results.

Liver histology

For all patients, percutaneous biopsy was performed on the right liver lobe using a 16-gauge Temno needle (Cardinal Health, McGaw Park, Illinois). Histological slides were read by two pathologists (AWHC, PCLC) blinded to clinical and MRS findings. Discrepancies were resolved by slide review and consensus. For primary analysis, specimens were graded based on Matteoni classification system [25] with reclassification of Classes 1 and 2 as non-NASH and Classes 3 and 4 as NASH. Histological scores for steatosis (0–3), lobular inflammation (0–3) and hepatocyte ballooning (0–2) were additionally reported using the criteria by Kleiner [26], and fibrosis (0–4) stages were reclassified to mild (F0–2) and advanced (F3–4) fibrosis for secondary analysis.