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Improved quantitative fatty acid values with correction of T2 relaxation time in terminal methyl group: In vivo proton magnetic resonance spectroscopy at ultra high field in hepatic steatosis

Kyu-Ho Song[a,b], Min-Young Lee[a,b], Chi-Hyeon Yoo[a,b], Song-I Lim[a,b], Bo-Young Choe[a,b,*]

[a] Department of Biomedical Engineering, College of Medicine, The Catholic University of Korea, Seoul 137-701, Republic of Korea
[b] Research Institute of Biomedical Engineering, College of Medicine, The Catholic University of Korea, Seoul 137-701, Republic of Korea

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ABSTRACT

Proton magnetic resonance spectroscopy (MRS) with optimized relaxation time is an effective method to quantify hepatic fatty acid values and characterize steatosis. The aim of this study is to quantify the difference in hepatic lipid content with metabolic changes during the progression of steatosis by using localized MRS sequence with T_2 relaxation time determination. Fatty liver disease was induced in C57BL/6N mice through a high-fat diet (HFD) of pellets containing 60% fat, 20% protein, and 20% carbohydrates. We used stimulated echo acquisition mode (repetition time: 3500 ms; mixing time: 10 ms; echo time: 20 ms) sequence. Using enhanced and mono exponential curve-fitting methods, the lipid relaxation time in mice was estimated at a fixed repetition time of 5000 ms and echo time ranging from 20 to 70 ms. The calculated lipid contents with incorrect and correct relaxation times were as follows: total saturated fatty acid (4.00 ± 2.90 vs 6.74 ± 2.25, $p < 0.05$ at week 0; 15.23 ± 9.94 vs 25.53 ± 10.49, $p < 0.05$ at week 4); total unsaturated fatty acid (0.40 ± 0.49 vs 0.56 ± 0.47, $p < 0.05$ at week 4; 0.33 ± 0.26 vs 0.60 ± 0.21, $p < 0.01$ at week 7); total unsaturated bond (0.48 ± 0.52 vs 1.05 ± 0.58, $p < 0.05$ at week 10). Furthermore, we determined that the correct relaxation times of triglycerides between 0 and 10 weeks were significantly altered in the resonances (~2.03 ppm: 31.07 ± 1.00 vs 27.62 ± 1.20, $p < 0.01$; ~2.25 ppm: 29.10 ± 1.52 vs 26.39 ± 1.08, $p < 0.05$; ~2.78 ppm: 37.67 ± 2.92 vs 29.37 ± 2.64, $p < 0.001$). The work presented focused on the significance of the J-coupling effect. The selection of an appropriate relaxation time considering the J-coupling effect provides an effective method for quantifying lipid contents and characterizing hepatic steatosis.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is the most common type of liver disease globally and contributes to significant health problems, such as obesity, that lead to the deposition of fat on the heart, liver, pancreas, kidney, and muscle tissue (Angulo, 2002; Bugianesi et al., 2002; Adams et al., 2005; Szczepaniak et al., 2005; Cheung et al., 2011). NAFLD is predominantly and exclusively characterized by fat accumulation in a specific organ or tissue (Cheung et al., 2011). The disease initially proceeds as steatosis (Angulo, 2002) and then develops into a more severe state, such as cirrhosis (Laubenberger et al., 1997), hepatitis (Cobbold et al., 2010), type 2 diabetes mellitus, hypertension, and dyslipidemia (Marchesini et al., 2003; Adams et al., 2005). The diagnosis of NAFLD currently relies on a biopsy to measure fat accumulation and assess the progression of fibrosis and hepatic steatosis (Manning and Afdhal, 2008). However, owing to the invasiveness of this procedure, the results are prone to inaccuracy because of observer variability and sampling errors (Manning and Afdhal, 2008).

The presence of NAFLD is strongly associated with components of metabolic syndromes such as hypertriglyceridemia and low high-

Abbreviations: NAFLD, nonalcoholic fatty liver disease; MRS, magnetic resonance spectroscopy; SNR, signal-to-noise ratio; STEAM, stimulated echo acquisition mode; HFD, high-fat diet; MRI, magnetic resonance imaging; TR, repetition time; TE, echo time; FSE, fast spin echo; TM, mixing time; OVS, outer volume suppression; VOI, volume of interest; VAPOR, variable pulse power and optimized relaxation delays; TL, total lipid; TSFA, total saturated fatty acid; TUFA, total unsaturated fatty acid; TUSB, total unsaturated bond; PUSB, polyunsaturated bond; LCModel, linear combination of model spectra; CRLB, Cramér-Rao lower bounds; ALB, albumin; GLU, glucose; TBIL, total bilirubin; AST, aspartate transaminase; ALT, alanine transaminase; ALP, alkaline phosphatase; H&E, hematoxylin-eosin; NCD, normal chow-diet; PCA, principle component analysis; PC, principle component; HR-MAS, high resolution magic angle spinning

* Corresponding author at: Department of Biomedical Engineering, College of Medicine, The Catholic University of Korea, #222 Banpo-Daero, Seocho-Gu, Seoul 137-701, Republic of Korea.
E-mail address: bychoe@catholic.ac.kr (B.-Y. Choe).

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density lipoprotein cholesterol levels (Angulo, 2002; Bugianesi et al., 2002). The pathogenesis of storage diseases or metabolic disorders depends on their specific impact on metabolism (Angulo, 2002). Hepatic steatosis in NAFLD results from lipid flux imbalance (Smith and Adams, 2011). There are complex interactions between the metabolic pathways of fatty acids, glucose, and lipoproteins (Cobbold et al., 2010; Cheung et al., 2011). Changes in the expression of metabolites, in addition to changes in saturated and unsaturated fatty acid contents following the formation of abnormal metabolites, play an important pathogenic role and can be measured using proton magnetic resonance spectroscopy (MRS) (Bugianesi, 2007; Chabanova et al., 2013; Ramamonjisoa et al., 2013; Qi et al., 2013). Using this method, liver metabolites obtained from liver tissues were found to have peaks in lipid contents ranging from methyl protons (0.90 ppm) to methane protons (5.30 ppm) (Cheung et al., 2011; Ramamonjisoa et al., 2013). For the detection of each lipid peak, multiple lipid resonance conditions can be resolved with an improved signal-to-noise ratio (SNR) by using ultra-high field strength scanners for in vivo proton MRS (Katz-Brull et al., 2003). These analytical methods are crucial for developing effective therapeutic interventions and strategies for NAFLD.

Generally, metabolites are measured using a main single-voxel MRS sequence (i.e., stimulated echo acquisition mode [STEAM]) (Bottomley, 1984; Frahm et al., 1987). The STEAM sequence has served as a standard for quantifying hepatic lipid content in clinical and preclinical settings (Hamilton et al., 2009). However, incorrect and correct relaxation times while using STEAM for quantification of liver fat tissue have resulted in contradictory results because of their sensitivities to J-coupling (Hamilton et al., 2009). In addition, the incorrect relaxation behavior of lipid resonances may lead to errors in signal quantification of lipid composition (Yahya et al., 2011; Breitkreutz et al., 2015). Previous studies on calculating the relaxation time using STEAM investigated the evidence and effects of J-coupling on lipid resonances (Yahya et al., 2011; Breitkreutz et al., 2015). However, correct relaxation times were not applied for quantification of lipid contents.

The aims of this study are as follows: (1) to validate a noninvasive MRS-based procedure for quantifying the difference in hepatic lipid contents of the liver with respect to metabolic changes; (2) to determine the possible impact of a high-fat diet (HFD) on the hepatic lipid content in mice through serum biochemistry; and (3) to demonstrate that incorrect and correct relaxation times yield contradictory quantification results of liver fat tissue.

2. Materials and methods

The animal experiments conducted were in accordance with a protocol approved by the Institutional Animal Care and Use Committee of the Daegu-Gyeongbuk Medical Innovation Foundation (No. 15072301-00) and the National Institutes of Health Guidelines for Animal Research (Guide for the Care and Use of Laboratory Animals). We used ten male C57BL/6N mice (Orient, Pyeongtaek, Korea) weighing 20–25 g that were housed in polycarbonate animal cages (260 × 420 × 180 mm; CLEA Japan, Inc., Tokyo, Japan) with ad libitum access to water and monitored their weight. The humidity and temperature of the animal care facility were controlled between 50–55% and 24–25 °C, respectively, and a 12-h light/12-h dark cycle with ventilation (10 times/h) was maintained. Hepatic steatosis was induced in the mice through an HFD of pellets containing 60% fat, 20% protein, and 20% carbohydrates (Rodent Diet with 60% Kcal% fat, D12492, Research Diets, New Brunswick, NJ) (Takahashi et al., 2012). The lipid source of the pellet-type HFD contains 32% saturated, 35.9% monounsaturated, and 32% polyunsaturated fat (with ~1% myristate, ~20% palmitate, ~12% stearate, ~34% oleate, and ~28% linoleate) (Salomäki et al., 2013). The body weight and food intake of the HFD-fed mice were recorded at 1-week intervals over a period of 10 weeks. Proton MRS was acquired at 0, 2, 4, 7, and 10 weeks after the initiation of the HFD. The mice were acclimatized to the laboratory 1 week prior to the beginning of the imaging and spectroscopy experiments to minimize the effects of transportation-induced stress.

During the imaging and spectroscopy of the mice livers, each mouse was anesthetized with isoflurane (1.0–1.5% for maintenance, ISOTROY100, Troikaa Pharmaceuticals Ltd.), which consisted of a gaseous mixture of 70% N_2 and 30% O_2, while their respiration was monitored. To maintain the mice within a normal physiological range, their respiration rates were monitored using a 1-cm-diameter animal respiration pressure pad (MR-compatible Small Animal Monitoring and Gating System, SA Instruments, Inc.). Anesthetized mice were placed in the prone position by using a palate holder equipped with an adjustable plastic nose cone. For stabilizing the body temperature, a warming system (Bruker BioSpin MRI GmbH, Ettlingen, Germany) was used.

Magnetic resonance imaging (MRI) and MRS analyses were performed using a horizontal 9.4 T MRI animal scanner (Biospec 94/20 USR, Bruker Biospin GmbH, Ettlingen, Germany) equipped with a 20-cm bore magnet with a 400 mT/m gradient (actively shielded gradient coil with integrated shims) and a four-channel receive-only array animal coil. The host software (Paravision ver. 3.0.1) was used for the reconstruction, data acquisition, and visualization of the liver images. Scout images were obtained to verify the image quality, subject position, and motion artifacts.

The voxel position of the region of interest was selected by analyzing multi-slice T_1-weighted (repetition time [TR], 1500 ms; echo time [TE], 7.2 ms) and T_2-weighted (TR, 2614 ms; TE, 33 ms) images by using fast spin echo (FSE) sequence. In both the cases, the following parameters were used during image acquisition (average, 4; repetition, 1; 1 mm slice thickness; 256 × 256 matrix; and 30 mm field of view). The MRS scan parameters were as follows: (1) standard STEAM sequence (TR, 5000 ms; mixing time [TM], 10 ms; TE, 20 ms; number of signal average, 128; acquisition data point, 2048; acquisition bandwidth, 4,401.41 Hz; repetition time, 1; scan time, ~11 min); (2) multi-TEs STEAM sequence (TR, 5000 ms; TM, 10 ms; TE, 20–70 ms; number of signal average, 16; acquisition data point, 2048; acquisition bandwidth, 4401.41 Hz; repetition time, 1). The spectra were acquired from a voxel in a region of liver fat in the mice. For animal proton MRS, a voxel size of 3 × 3 × 3 mm^3 (0.027 mL) was selected and shimmed after multi-slice scout, T_1-weighted, and T_2-weighted imaging. The shimming was performed employing a fast-automatic shimming technique using map-based projections (Gruetter, 1993). Outer volume suppression (OVS) was performed to minimize the signal beyond the volume of interest (VOI). Before the MRS scan, the water resonance signal in the VOI of the livers of the HFD-fed mice was suppressed by variable pulse power and optimized relaxation delays (VAPOR) (Tkáč et al., 1999). An unsuppressed water signal was obtained from the same voxel position and used as a reference for quantification. For the hepatic fatty acid component measurements, the total lipid (TL) quantity [$(-CH_2-)_n$/water] was measured using the water-unsuppressed spectra (STEAM: TR/TM/TE = 5000/10/20 ms; number of signal averages = 16; acquisition data points, 2048; acquisition bandwidth = 4,401.41 Hz) of the livers of the HFD-fed mice. The signals were shimmed to a line width < 22 Hz by using the automated optimization of gradient shimming, transmit-receive gain, and water suppression of the VOI.

The water suppression percentage in the spectrum was calculated as the ratio of intensity (%WS = $\{(WS_a - WS_b)/WS_a \times 100\}$) between the peaks of unsuppressed water (WS_a) and suppressed water (WS_b) (Males et al., 2000; Song et al., 2015a, 2015b). The multiple TE STEAM sequence data sets were evaluated using the curve-fitting toolbox in the matrix laboratory (MATLAB, The MathWorks, Inc., Natick, MA, USA). The following correct curve-fitting equation (enhanced curve-fitting method) was used to measure the relaxation times of the lipid resonances (Yahya et al., 2011):

$$M(TE) = M0 \times e^{(-TE/T2)}[\cos(\pi \times JE \times TE) + b] \quad (1)$$

where J_E and b are the values of the J-coupling evolution and

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