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Comparison of post processing methods between Java Magnetic Resonance User Interface (jMRUI) and Totally Automatic Robust Quantitation in NMR (TARQUIN) software for liver fat quantification

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ABSTRACT

Background: Proton magnetic resonance spectroscopy or ^{1}H MRS is a validated and non-invasive method used for studying liver fat. However, the metabolite spectra obtained by ^{1}H MRS require a post-processing method for accurate liver fat quantification. Various spectrum analysis software has been developed and is being used in many studies. To the best of our knowledge, no comparisons between spectrum analysis software for liver fat quantification have yet been completed.

Objectives: To compare the post processing methods between java-based graphical for MR user interface packages (jMRUI) and totally automatic robust quantitation in NMR (TARQUIN) software for optimal liver fat quantification.

Materials and methods: ^{1}H MRS spectrum from the right lobe of the liver was obtained for post processing. Liver fat qualification was done by AMARES algorithms on jMRUI software, and automatic quantification algorithms was initiated by TARQUIN software. A total of 30 subjects participated in this study. Subjects were separated into a control group (n=15) and an overweight group (n=15) for liver fat quantification. Liver lipids at 0.9 ppm (-CH_3 lipids) and 1.3 ppm (-CH_2 lipids) were fitted and quantified. The results obtained from both jMRUI and TARQUIN post processing software packages for both groups were then compared.

Results: A strong and moderate correlation of signal intensity between jMRUI and TARQUIN software was found (total lipids, r=0.836, p<0.001; -CH_2 lipids, r=0.848, p<0.001; -CH_3 lipids, r=0.520, and p<0.003). Liver lipid levels were generally higher in the overweight group. There was a 2.35 times level of change in the overweight group compared to control from jMRUI, and there was a 2.16 times level of change in the overweight group compared to control from TARQUIN. There was no statistical differences between the programs (p=0.762).

Conclusion: Both jMRUI and TARQUIN are feasible post processing tools for ^{1}H MRS liver spectrum fitting for liver lipids quantification.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is a type of chronic liver disease that is caused by an accumulation of lipids in hepatocytes.[1] NAFLD is considered to be a global epidemic that leads to many other liver diseases and health complications such as liver inflammation (hepatitis), liver cirrhosis, and hepatocellular carcinoma.[1, 2] A 25% prevalence rate was found to exist in Asian counties, which is now as high as that of western countries.[3] A nationwide study in Thailand from 2015 found that liver-related diseases led Non-alcoholic fatty liver disease (NAFLD) to higher in-hospital mortality rates.[4] Liver fat

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accumulation can be induced by an excessive fat intake, metabolism impairment, and from being overweight or obese. Thus, an accurate and effective method for liver lipid determination is truly needed.

Proton magnetic resonance spectroscopy (^{1}H MRS) is a non-invasive technique that is known for its accuracy for liver lipid assessment and is also the primary validation method used for liver lipids assessment.[5-7] Being non-invasive, it is a suitable technique for follow up in longitudinal studies done on liver lipids in both clinical and research applications. Previous studies on ^{1}H MRS have found that body mass index (BMI) is associated with higher levels of liver lipids.[8, 9] However, ^{1}H MRS liver lipid signals were obtained as spectrum and require post-processing methods for ^{1}H MRS data. There are many available software packages for data analysis. Two popular ones are java-based graphical for MR user interface package (jMRUI), and totally automatic robust quantitation in NMR (TARQUIN).[10-12] jMRUI is a software package that is freely available on the jMRUI website (http://www.jmrui.eu) for non-commercial use. It provides many algorithms for spectra fitting and correction, and was included in an extensive list of literature as a very popular method of data analysis. One popular algorithm for spectrum fitting is the Advanced Method for Accurate, Robust, and Efficient Spectral Fitting or AMARES, which can import prior knowledge for better spectrum fitting.[13] However, jMRUI requires a user intervention, and a degree of knowledge during the post processing is necessary for accurate quantification. This maybe time consuming, and therefore is not suitable for liver fat assessment in clinical settings. On the other hand, TARQUIN provides an automatic quantification of metabolites based on a basis spectra model in time domain, and contains some preprocessing tools for spectrum fitting.[6] These features make it a more viable and convenient method for everday use. To the best of our knowledge, there have been no previous studies done about the comparison of liver lipid assessment between jMRUI and TAQRQUIN software packages. If this two-quantification model can be used for liver lipid assessment and can differentiate liver lipid levels between normal weight and overweight people, this data will be beneficial in both clinical and research settings. The aim of this study is to compare two software packages for liver lipid assessment in both a normal weight group (BMI 18.5-24.9 kg/m^2) and an overweight group (BMI≥25 kg/m^2).

Materials and methods

Study population

Thirty healthy subjects composed of both males and females aged between 20-35 years of age participated in this study. Inclusion criteria for study subjects is based on the following criteria: i) healthy with no known liver disease, liver injury, and chronic disease such as cancer, diabetes, or hypertension ii) no history of virus hepatitis B and C infection iii) drink alcohol <30 g/day for men and <20 g/day for women[14] iv) engage in moderate physical activity. The subjects were separated into two groups. The control group (n=15) with BMI in normal range (BMI 18.5-24.9 kg/m^2) as determined by the World Health Organization (WHO), and the overweight group (n=15) with BMIs in overweight range (BMI>25 kg/m^2).[15] 10 ml of fasting blood was drawn from subjects in both groups and basic laboratory blood tests for blood lipid level were performed. The blood tests focused on cholesterol (Cho), high-density lipoprotein (HDL), very-low density lipoprotein (VLDL), triglyceride (TG), glucose (Glu), and glycosylated hemoglobin A (HbA1c). Low-density lipoprotein (LDL) concentration was calculated from an adjustable LDL estimation equation.[16, 17] Each subject gave their written informed consent after the procedure was fully explained and understood. This study was approved by the Ethics Committee of the Faculty of Associated Medical Science, Chiang Mai University, Chiang Mai, Thailand.

Liver lipids assessment

Magnetic resonance imaging (MRI) 1.5 T Achieva (Philips Medical Systems, Best, The Netherlands) was used in this study. Subjects were told to lie down in a supine position. The SENSE cardiac coil was then placed over the liver with gated respiratory for both image and ^{1}H MRS acquisition. First, localization images were obtained, then T2 weighted images of liver were obtained in the coronal and sagittal planes (Echo time (TE) =80 ms, Repetition time (TR) =871 ms, and slice thickness =6 mm) to ensure voxel localization. The automatic shimming protocol available on MRI was performed. Later, single-voxel ^{1}H MRS acquisition for liver lipids assessment was done with Point resolved spectroscopy (PRESS) pulse sequence (TE=43 ms, TR=2000 ms, Number of signal averages =96, Data point =512, and band width=1,000 Hz) with water suppression. Voxel size of 10×10×10 mm was carefully placed in the right lobe of the liver, carefully avoiding any bile ducts and blood vessels.

Spectra analysis

^{1}H MRS spectra that were obtained from the liver were analyzed by two types of software packages: jMRUI version 6.0 beta and TARQUIN version 4.3.10. jMRUI is a highly flexible software package that provides a wind range of algorithms for ^{1}H MRS signal processing, and includes preprocessing tools and peak fitting. In this study, an AMARES algorithm was applied for spectra fitting in time domain. Water signal residual was suppressed by Hankel Lanczos singular value decomposition (HLSVD) algorithms that were provided in jMRUI software.[18] Any prior knowledge regarding estimates of liver metabolite peaks was provided for the algorithm, as described in a previous publication.[19] TARQUIN has more advantages than jMRUI since it enables automatic post processing for spectra metabolites, and wide ranges of ^{1}H MRS data can be accepted. TARQUIN is a time domain fitting algorithm by a least square projection used to determine signal amplitude. Notable features are that TARQUIN imposes soft constraints with basis in-vivo spectra data set that includes macromolecules, lipids, and metabolites to avoid possible over fitting of spectrum.[10] The signal truncation in time domain and HLSVD water removal was applied for baseline correction.[20] As it is an automatic process, it will reduce user bias and variability on spectrum analysis. The liver lipids signals of interest on liver spectra was at 0.9 parts per million (ppm) for -CH_3

lipids and 1.3 ppm for -CH_2 lipids. The lipids signals were corrected for T1 and T2 relaxation by a method described elsewhere.[21] Next, corrected liver lipids signals were calculated into a ratio of each lipid (-CH_3 lipids, -CH_2 lipids) to total lipid (-CH_3 lipids + -CH_2 lipids).

Statistical analysis

All data is expressed as mean±standard deviation (SD), unless state otherwise. All statistical analysis was performed on SPSS version 17.0 (SPSS Inc., Chicago). The Kolmogorov Smirnov test was used to determine data distribution. Comparison of blood laboratory tests between groups were performed using Mann-Whitney U test. Comparisons of lipids ratio to total lipids and liver lipids signal intensity were performed between each software package using a pair sample t-test. A Pearson correlation was used to assess the relationship between signal intensity from each of lipid group obtained by jMRUI and TARQUIN. P<0.05 were considered statistically significant.

Results

All subjects were invited to participate in this study during the period of January 2017 to March 2017. A total of 30 subjects were given blood tests and ^{1}H MRS was performed for liver fat at the Associated Medical Sciences Clinical Service Center, Chiang Mai University. The age, BMI, and laboratory blood test results of study subjects are shown in Table 1. Laboratory blood test results showed significantly higher BMI, TG, Glu, and significantly lower HDL in the overweight group compared to control group. LDL, HbA1c, and Cho was also found to be higher in the overweight group, but this tendency was not statistically significant. The representative ^{1}H MRS liver spectra fitting from both programs are shown in Figure 1. The water suppressed spectra show the liver lipids peaks at the following chemical shifts: -CH_3 lipids (0.9 ppm) and -CH_2 lipids (1.3 ppm). These two lipids signal intensities were then calculated into total liver lipids. Coefficient co-variation (CV) of liver lipids signal intensity and total liver lipids were similar for both groups (data not shown). In general, -CH_3 lipids, -CH_2 lipids, and total lipids in overweight group showed higher signal intensity than in the control group. In addition, CV values showed consistency among the signal intensities obtained from each group when analyzed across the software packages. Scatter plots of signal intensity of liver lipids between two software packages are shown in Figure 2. The Pearson correlation coefficient showed a strong positive correlation of signal intensity obtained from both programs. However, the correlation of -CH_3 lipids signal intensity between the two software packages was found to be moderate, but remained statistically significant. Total lipids of control group were set as the reference value as total lipid for the comparison of liver fat between the groups and is shown in Figure 3. The figure reveals increasing liver fat in both algorithms as having a similar pattern, and this tendency was not found to be statistically significant across the software packages. However, it should be noted that total liver lipids acquired from TARQUIN were slightly lower by around 18%. The -CH_3 lipids and -CH_2 lipids were then calculated into ratio to total lipids (-CH_3 lipids + -CH_2 lipids) as shown in Table 2. Lipids ratio from -CH_3 lipids and -CH_2 lipids to total is almost the same value in the same lipids group, with no significant correlation taking place between jMRUI and TARQUIN.

Table 1 Characterize of subjects from control group and overweight group.

	Control group (n=15)	Overweight group (n=15)	P-values
Sex (male/female)	(8/7)	(11/4)	-
age	22.0±1.0	22.8±2.1	0.762
BMI	20.8±2.2	29.1±1.4	<0.001*
Cho	195.8±37.2	212.5±37.5	0.751
Tri	71.9±20.3	110.7±58.3	0.022*
HDL	59.2±12.4	49.2±8.1	0.004*
LDL	120.2±32.9	140.7±34.4	0.496
Glu	82.2±4.6	90.3±4.7	0.002*
HbA1c	5.2±0.3	5.4±0.3	0.142

Note: *P<0.05 indicated statistically significant

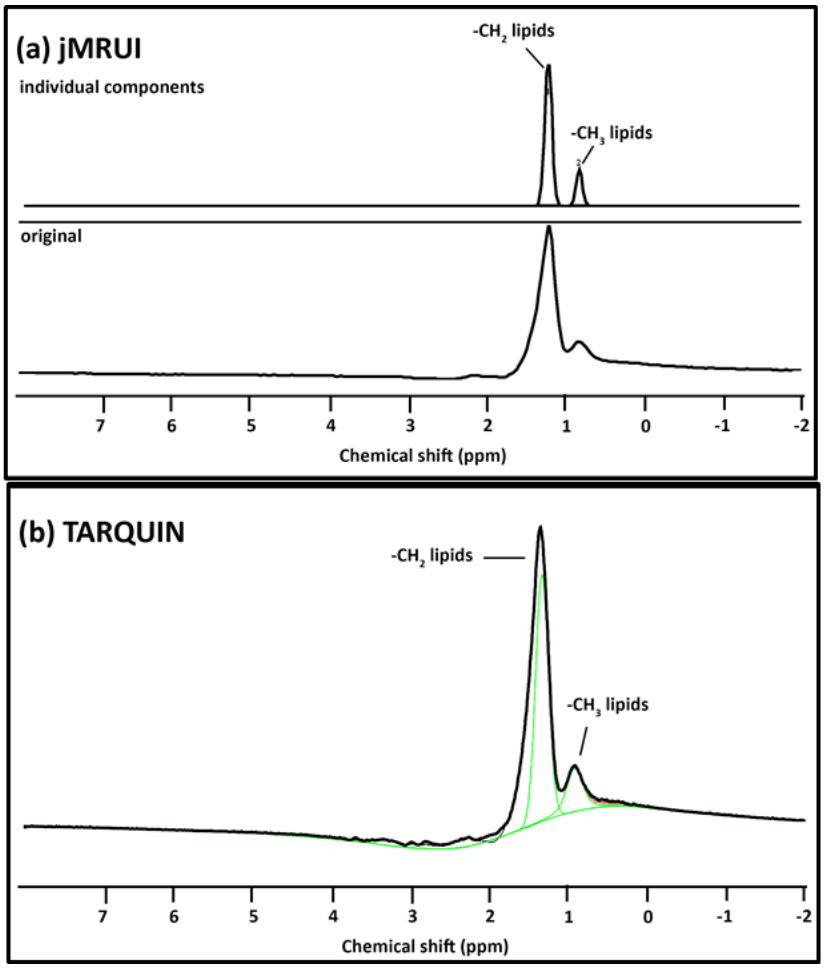

Figure 1 *Representative liver spectra with suppressed water signal. (a) jMRUI show fitted signal (top) original signal (bottom) and (b)TARQUIN software package show fitted signal (green) original signal (green). ppm; part per million.*

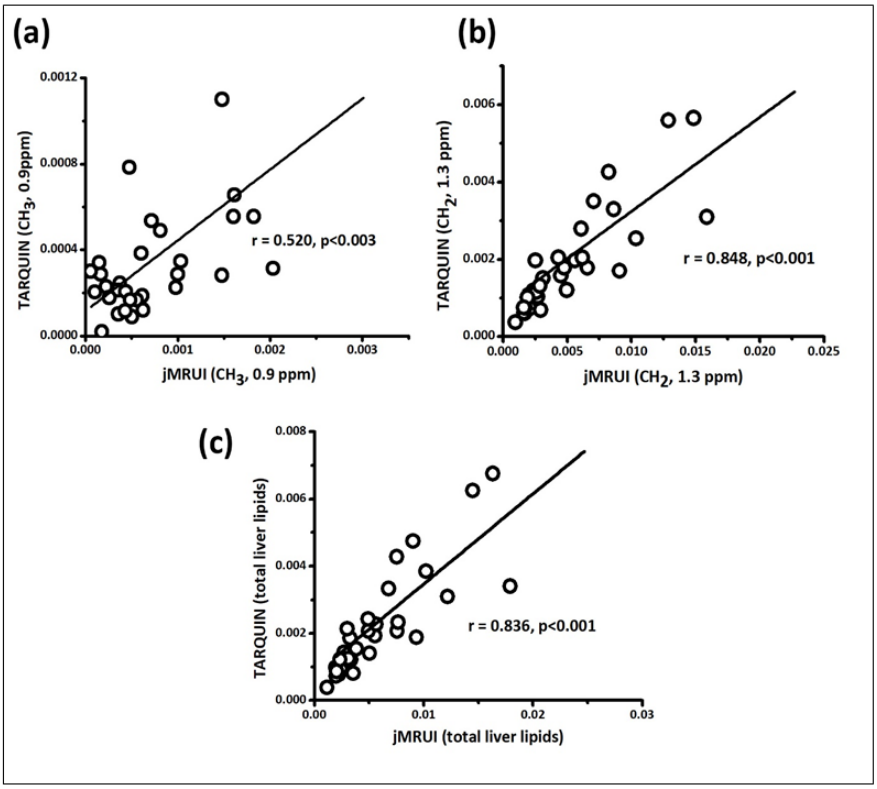

Figure 2 *Association between signal intensity of liver lipids between jMRUI and TARQUIN software package from both groups (a) signal intensity of -CH_3 lipids (b) signal intensity of -CH_2 lipids (c) signal intensity of total lipids.*

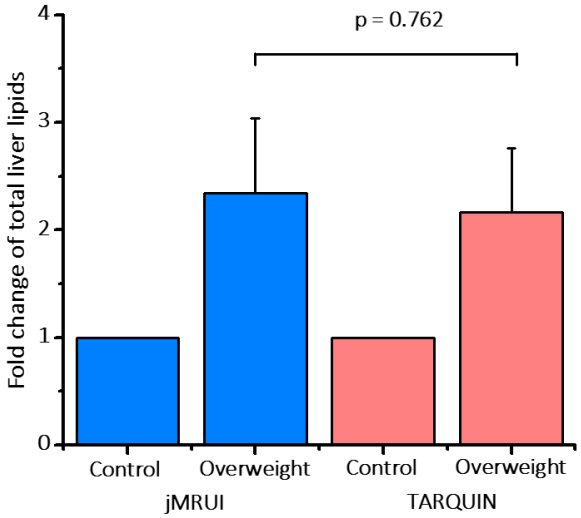

Figure 3 *Folds change of signal intensity of total liver lipids from jMRUI and TARQUIN software packages with control group as reference value.*

Table 2 Liver lipids signals ratio to total lipids and from each group.

	Control		Overweight	
	$-CH_3$ lipids/total liver lipids	$-CH_2$ lipids/total liver lipids	$-CH_3$ lipids/total liver lipids	$-CH_2$ lipids/total liver lipids
jMRUI	0.14±0.06	0.86±0.06	0.12±0.07	0.87±0.07
TARQUIN	0.16±0.05	0.84±0.05	0.13±0.03	0.87±0.03
P-values	0.182	0.182	0.977	0.977

Note: JMRUI, java-based graphical for MR user interface package; TARQUIN, totally automatic robust quantitation in NMR

Discussion

Number of NAFLD cases and its prevalence are rising significantly from 15% in 2005 to 24% globally in 2010.[1, 22] Moreover, NAFLD has found to often be accompanied with obesity, dyslipidemia, insulin resistance, and other metabolic syndromes.[23-25] Liver fat accumulation of more than 5% is considered as NAFLD.[24] This widespread problem underscores the need for an accurate and non-invasive technique for liver fat assessment for both clinical applications and research to be developed. While biopsy is the gold standard for assessing liver lipids and is widely available in hospitals, it is an invasive technique, and also is not suitable for longitudinal follow ups of liver lipids in response to therapeutic treatment. In this study, the characteristics of each subject are as expected in a study design of this type. The overweight group show statistically higher TG and lower levels of HDL, which are risk factors for cardiovascular diseases. This result was consistent with previous studies that examined a higher risk of cardiovascular disease and dyslipidemia occurring at BMI=21 kg/m^2.[26, 27]

Furthermore, blood glucose and BMI were also significantly higher in the overweight group. This results further supports the idea that increasing BMI is associated with a higher risk of insulin resistance.[28] ^{1}H MRS spectra of liver were acquired and post processing by jMRUI and TARQUIN was performed. jMRUI is a widely accepted tool used in various types of liver lipids studies, and is proven to be accurate for determining the levels of hepatic fat.[19, 29] However, the jMRUI software package require user interaction for spectrum fitting, and may result in over fitting of liver lipids. On the other hand, TARQUIN is the automated spectrum fitting algorithm that does require a bit of user knowledge, but involves less user interaction than jMRUI. In this current study, lipids signal intensity from each group is shown in the liver spectrum and total liver lipids was calculated. The signal intensity from overweight group was higher than that of the control group, which is consistent with previous studies of higher liver lipids among the overweight and obese.[30, 31] Signal intensity of each lipids group was not significantly different across the programs with the CV value also determined to be consistent; having a close relationship of signal intensity to that obtained from jMRUI and TARQUIN. The degree of change of liver lipids in the overweight group from both software package is similar, and was not significantly different. These finding suggests that both jMRUI and TARQUIN can assess liver lipids levels without noticeable differences occurring across the programs. Furthermore, both jMRUI and TARQUIN are able to give consistent results from different liver lipids levels in the overweight group. This finding, while preliminary, suggests that both jMRUI and TARQUIN are useful tools that can be

used interchangeably for liver lipids assessment. The automated nature of TARQUIN may ensure greater accessibility and ease of post processing of ^{1}H MRS spectrum without the influence or bias of user in liver lipid quantification.

Conclusion

This study reveals the correlation of liver lipids results from java-based graphical for MR user interface package (jMRUI) and totally automatic robust quantitation in NMR (TARQUIN) software packages. It can be asserted that both programs can be used for liver fat quantification study and can differentiate liver fat levels between groups of interest.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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