

## Evaluation of Liver Fat Content with Magnetic Resonance Spectroscopy in Overweight Subjects as an early Detector of Fatty Liver Disease and Correlation with Liver Biopsy

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to rapidly estimate the LFC in subjects and patients without or with NAFLD. The cutoff value of 5.1% enables objective rapid and reliable discrimination of normal LFC from elevated LFC.

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**Key words:** Fat Content; Spectroscopy; Overweight; Liver biopsy

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### ABSTRACT

**AIM:** To evaluate magnetic resonance spectroscopy (MRS) imaging in the estimation of liver fat content (LFC) in obese subjects as an early and noninvasive diagnostic tool for the detection of nonalcoholic fatty liver disease (NAFLD) and correlation of its accuracy with liver biopsy.

**METHODS:** A total of 45 subjects with a variable body mass index (BMI) included 30 healthy subjects and 15 patients with NAFLD underwent MRS of the liver. MRS. Subjects were divided according to the median liver fat content in a normal range ( $\leq 5.1\%$ ); low (5.2 to  $\leq 6.9$ ); moderate (7 to  $\leq 8.9$ ); and high ( $> 9$ ).

**RESULTS:** In 45 subjects and patients with BMI ranging from 19.0 to 42.9 kg/m<sup>2</sup> the mean fat contents of liver by MRS were 4.57, 6.15, and 8.7 in healthy subjects, overweight and obese subjects, and NAFLD patients respectively. There were high significant correlation between the result of MRS score and the results of liver biopsy ( $P=0.001$ ). An intercept of the regression line with the x-axis was observed at 5.1%, discriminating between normal and elevated LFC with high sensitivity (95%) and specificity (98%).

**CONCLUSION:** In-phase and out-of-phase imaging can be used

### INTRODUCTION

Fatty liver (also known as hepatic steatosis) has become a common finding over the past 2 decades. This is associated with the increased incidence of fatty liver and the increased use of radiologic examinations that reveal fatty liver, since patients with a fatty liver are usually asymptomatic at diagnosis<sup>[1]</sup>. The prevalence of fatty liver is as high as 33% in the general population<sup>[2,3]</sup>. Increased liver fat content (LFC) is related to obesity, insulin resistance, type 2 diabetes mellitus, nutritional problems, infections, metabolic and genetic disorders, and excessive alcohol consumption<sup>[2,4]</sup>. Although hepatic steatosis is benign in nature, it can progress to more severe liver disease. The LFC can be evaluated with different methods, such as histologic analysis, ultrasonography (US), computed tomography (CT), and magnetic resonance (MR) imaging<sup>[5,6,7]</sup>. Histologic analysis has been considered the most accurate method with which to stage and grade fatty liver disease; however, its invasiveness makes it less favorable, especially when patients are asymptomatic. In addition, histologic analysis is subject to sampling errors. Thus, noninvasive

radiologic methods—such as US, CT, and MR imaging—that can be used to examine the entire liver are preferred for use in the detection of fat accumulation. To date, MR imaging, particularly hydrogen 1 (1H) MR spectroscopy, has been by far the most promising and most sensitive noninvasive method with which to assess LFC<sup>[1]</sup>.

Liver imaging performed with in-phase and out-of-phase MR sequences is based on different chemical shifts of water and fat and can be used to detect fat accumulation in liver tissue<sup>[8]</sup>. Levenson *et al*<sup>[9]</sup> reported that this sequence exhibited good correlation with visually estimated histologic sampling. Mitchell *et al*<sup>[10]</sup> showed in phantom, animal, and in vivo studies that in-phase and out-of-phase imaging performed with the Dixon method can be used to estimate LFC. However, Westphalen *et al*<sup>[11]</sup> recently showed that iron accumulation alters signal intensity (SI) on in-phase and out-of-phase images and is a potential pitfall in the determination of liver fat accumulation.

The purpose of the current study was to evaluate in-phase and out-of-phase MR imaging in the estimation of LFC in overweight and obese subjects as an early and noninvasive diagnostic tool for the detection of NAFLD and correlation of its accuracy with histopathology as the reference standard.

## METHODS

This study was carried out in the gastroenterology unit of Internal Medicine, Radiology and Histopathology departments, Faculty of Medicine, Zagazig University, Egypt from January 2012 to March 2013. Our randomized crossover study included 45 subjects: 15 healthy subjects as controls (7 females and 8 males), aged 45±8.55 (Mean±standard deviation) (range, 30-58 years), with normal BMI (18.5-25), 15 subjects were overweight or obese with a BMI more than 25 (6 males and 9 females), The overall mean age was 38 years±6.31 (Mean±SD) (range, 27-47 years) and 15 subjects were patients with NAFLD, 7 men and 8 were women. The overall mean age was 38 years±8.12 (Mean±SD) (range, 27-52 years). Inclusion criteria were any healthy adult subjects with different BMI and NAFLD patients. Patients were excluded if they had a history of or current liver, renal, infectious, or malignant diseases. This study was approved by our institutional review board and performed in compliance with the Declaration of Helsinki. Written informed consent was obtained from all subjects. Everyone was interviewed to assess the status of his health and presence of any diseases which must be excluded. Body weight and height were measured, and BMI was calculated. Supine resting blood pressure was measured with mercury sphygmomanometer at 2-min intervals for 10 min, with the first reading deleted and the remaining readings averaged. All blood tests were carried out after a 12-h fast. Plasma glucose levels, total cholesterol, triglycerides, high-density lipoprotein (HDL), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were measured by enzymatic methods using either the Hitachi 917 analyzer (Roche Diagnostics, Basel, Switzerland) or the Architect ci8200 system (Abbott Diagnostics, Wiesbaden, Germany). Low-density lipoprotein (LDL) cholesterol was calculated by the Friedewald formula<sup>[12]</sup>. Highly sensitive C-reactive protein (hs-CRP) was assessed immunochemically using a Dade Behring reagent (Dade Behring, Eschborn, Germany).

### Magnetic resonance spectroscopy

Magnetic resonance studies were performed at a single visit for every subject in radiology department at Zagazig university on the same 1.5 Tesla whole-body magnetic resonance scanner (Gyrosan ACS/NT15, Philips, Best, the Netherlands) in the non-fasting state in the

morning or afternoon, with subjects at rest and in supine position<sup>[13]</sup>. 1H-MRS (hydrogen magnetic resonance spectroscopy) of the liver was performed with an 8 mL voxel positioned in the right lobe of the liver, avoiding gross vascular structures and adipose tissue depots. Sixty-four averages were collected with water suppression. Spectra were obtained with an echo time of 26 ms and a repetition time of 3,000 ms. Data points (1,024) were collected using a 1,000 Hz spectral line. Without changing any parameters, spectra without water suppression, with a repetition time of 10 s, and with four averages were obtained as an internal reference. 1H-MRS data were fitted using Java-based magnetic resonance user interface software (jMRUI version 2.2, Leuven, Belgium), as described by Naressi *et al*<sup>[14]</sup>. Hepatic triglyceride content relative to water was calculated as  $100 \times (\text{signal amplitude of triglyceride})/(\text{signal amplitude of water})$ . Subjects were divided according to the median liver fat content in a normal range ( $\leq 5.1\%$ ); low (5.2 to  $\leq 6.9\%$ ); moderate (7 to  $\leq 8.9\%$ ); and high ( $> 9\%$ ).

**Pathologic Fat Content** Pathologic fat content was measured in representative H and E slides from the resected liver specimen. In each case, one pathologist with 5 years of experience who had expertise in gastrointestinal and hepatic pathology graded the degree of steatosis using slides of tissue specimens from liver parenchyma. The degree of steatosis was graded semiquantitatively as the percentage of liver parenchyma involved by fatty infiltration, estimated to the nearest 5%. When the degree of involvement was less than 5%, it was estimated to the nearest 1%.

### Statistical analysis

Statistical analysis was performed using SPSS version 15.0 (SPSS Inc, Chicago, IL). Data are expressed as mean±SD, or geometric mean and 95% confidence interval (CI) for variables requiring logarithmic transformation. Analyses were carried out in the whole group, as well as subgroups defined according to the presence normal liver fat  $\leq 5\%$  or liver fat  $> 5\%$  (hepatic steatosis). The liver fat percentage cutoff point of 5% was based on 95 percentile values for liver fat content using HMRS in a study of control subjects without risk factors for NAFLD by Browning *et al*<sup>[15]</sup>. Statistical significance was defined as  $P \leq 0.05$ .

## RESULTS

Subject characteristics at recruitment are shown in table 1.

All groups were similar with respect to age, gender, height, albumin, bilirubin, urea, creatinine, HbA1c, an hs-CRP ( $P=0.059$ , 0.632, 0.413, 0.966, 0.962, 0.785, 0.365, 0.954, an 0.42; respectively). As expected, weight, waist circumference, BMI, ALT, AST, total cholesterol, triglyceride, LDL, and HDL differed significantly among groups ( $P=0.029$ , 0.009, 0.048, 0.001, 0.062, 0.001, 0.004, 0.002, and 0.001; respectively) (Table 1).

MRS and liver biopsy were done to all subjects in 3 groups except healthy subjects group as only 3 subjects from this group accept to do liver biopsy as they had elevated liver enzymes with exclusion of other causes of liver enzyme elevation.

Liver fat measured using MRS was higher in patients group (mean±SD=8.7±1.7) than overweight and obese group (mean±SD=6.15±1.79) and was higher in overweight and obese group (mean±SD=6.15±1.79) than healthy subjects (mean±SD=4.57±0.93). All groups differed significantly with respect to liver fat measured using MRS ( $P=0.041$ ).

In healthy subjects group number of fatty liver detected by MRS was 2 patients (13.3%), number of fatty liver detected by liver biopsy was 1 patient (6.7%), in overweight and obese group number of fatty

**Table 1** Clinical and biochemical characteristics of all study groups and comparison between them.

	Healthy subjects		Overweight and Obese subjects		NAFLD Or Patients group		r	P value
	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD		
Age (years)	30-58	45+8.55	27-52	38+8.12	27-47	38+6.31	3.504	0.059
Gender (Male/female)	8/7		6/9		7/8		χ <sup>2</sup> 0.582	0.632
Height (meter)	165-83	174+5.34	167-182	174+4.65	165-187	176+6.29	0.901	0.413
Weight (Kg)	58.5-80	67+7.25	78-129.5	101+16.4	71.5-124	67+7.3	15.3	0.029
Waist (Cm)	85-96	90+3.8	95-123	108+8.6	92-122	90+3.8	5.3	0.009
BMI (Kg/m <sup>2</sup> )	19-24.5	22+1.7	26-42.9	34+5.3	22-41	22+1.7	3.69	0.048
ALT (IU/L)	20-50	29+7.8	25-37	30+3.7	28-45	37+5.3	7.325	0.001
AST (IU/L)	16-69	26+13.2	17-33	24+4.7	25-41	32+5.67	2.325	0.062
Albumin (gm/dL)	3.9-5	4.22+0.3	3.6-5.1	4.19+0.39	3.6-4.8	4.08+0.32	0.756	0.966
Bilirubin (mg/dL)	0.7-1.1	0.8+0.12	0.7-1.1	0.8+0.12	0.7-1	0.83+0.09	0.758	0.962
Urea (mg/dL)	19-42	30.7+9.1	19-41	30.66+8.75	21-45	34.2+7.3	0.637	0.785
Creatinine (mg/dL)	0.5-1.2	0.8+0.2	0.5-1.1	0.8+0.21	0.6-1.2	0.9+0.19	1.021	0.365
Total cholesterol (mg/dL)	139-96	161+15.8	121-190	154+20.9	159-220	182.6+17.5	16.52	0.001
Triglyceride (mg/dL)	123-70	142+13.4	122-181	160+16.3	141-184	159.8+13.9	7.325	0.004
LDL (mg/dL)	68-98	81.3+8.5	79-128	98.4+14.1	76-123	98.8+13.6	9.325	0.002
HDL (mg/dL)	35-58	44.9+6.3	35-52	42.9+5.1	38-54	43.2+5.3	14.639	0.001
Hb A1c (%)	5.4-5.6	5.5+0.1	5.3-5.6	5.4+0.1	5.3-5.8	5.5+0.25	0.743	0.954
hs-CRP (mg/dL)	0.4-1.2	0.9+0.23	0.7-1.1	0.9+0.2	0.4-1.7	1.1+0.53	1.14	0.42

liver detected by MRS was 6 patients (40%), number of fatty liver detected by liver biopsy was 5 patients (33.3%), and in NAFLD group number of fatty liver detected by MRS was 15 patients (100%), number of fatty liver detected by liver biopsy was 15 patients (100%), and there was significant difference between the used methods in fatty liver measurement in the three groups, as *p*-value=0.029 (Table 2 and figure 1-4).

By comparing the value of MRS score and the results of liver biopsy, we found that; in the healthy subjects, the MRS score value was 4.57±0.93 (mean±SD) and the liver biopsy was normal in 2 subjects and grade 1 in one subjects; in the overweight and obese patients, the MRS score value was 6.15±1.79 (mean±SD) and the liver biopsy was normal in 10 patients, grade 1 in 4 patients, and grade 2 in 1 patients; and in the NAFLD patients, the MRS score value was 8.7±1.7 (mean±SD) and the liver biopsy was grade 1 in 3 patients, grade 2 in 7 patients, and grade 3 in 5 patients. There were high significant correlation between the result of MRS score and the results of liver biopsy (*P*=0.001) (Table 3).

## DISCUSSION

Hepatic steatosis is characterized by abnormal and excessive accumulation of lipids within hepatocytes. It is an important feature of diffuse liver disease and the histological hallmark of NAFLD. Liver

biopsy, the current clinical gold standard for assessment of liver fat, is invasive and has sampling errors, and is not optimal for screening, monitoring, clinical decision-making, or well suited for many types of research studies. Noninvasive methods that accurately and objectively quantify liver fat are needed. Ultrasound and computed tomography can be used to assess liver fat but have limited accuracy as well as other limitations<sup>[16]</sup>.

The current study shows that waist circumference and BMI increase significantly more in obese group and NAFLD group than healthy subjects, so fatty liver incidence is more frequent in obese subjects than lean subjects. This goes in accordance with that found in other previous studies<sup>[17-20]</sup>.

As regard laboratory investigation we found that ALT increased in overweight and obese group and NAFLD group more than in healthy subjects group. This confirm the results of other studies<sup>[21,22]</sup>. Also Clark *et al*<sup>[18]</sup> who propose that elevated ALT levels are predictive of the presence of NAFLD if two basic criteria are met: (1) exclusion of alternative chronic liver diseases; (2) presence of features of increased BMI, waist circumference and obesity. But the degree of ALT elevation does not predict the severity of disease.

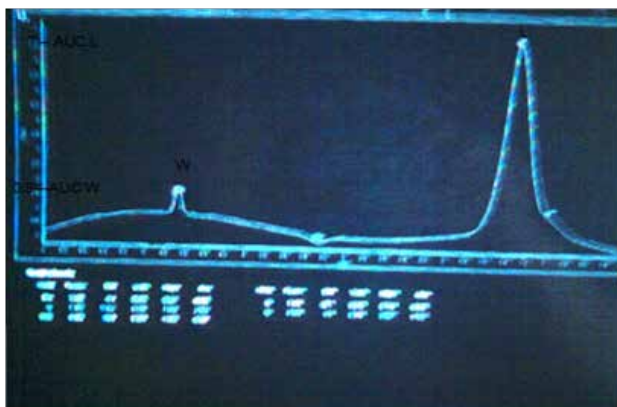
As regard lipid profile (total cholesterol, LDL and TG) which increased in overweight and obese group and NAFLD groups more than in healthy subjects group, also HDL was lesser in overweight and obese group and NAFLD group than healthy subjects group. This is

**Table 2** MRS and liver biopsy characteristics of all study groups and comparison between them.

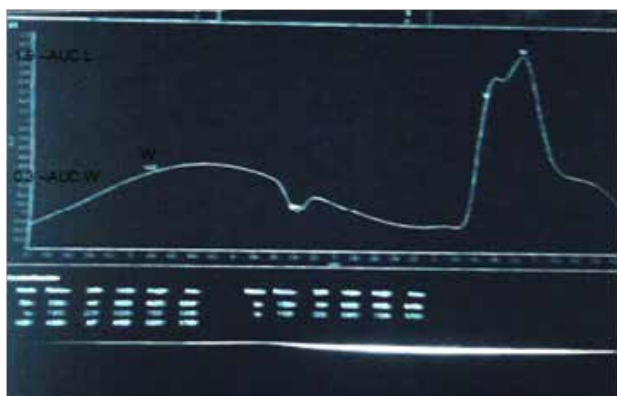
	Healthy subjects		Overweight and Obese subjects		NAFLD Or Patients group		r	P value
	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD		
MRS score	3.34-7.1	4.57±0.93	3.9-9.1	6.15+1.79	6.5-12.3	8.7+1.7	3.325	0.041
Liver biopsy result	Number of subjects	Percentage	Number of subjects	Percentage	Number of patients	Percentage	χ <sup>2</sup> 11.523	P value 0.001
Not done	12	80%	0	0%	0	0%		
Normal	2	13%	10	66.5%	0	0%		
Grade 1	1	7%	4	26.5%	3	20%		
Grade 2	0	0%	1	7%	7	46.5%		
Grade 3	0	0%	0	0%	5	33.5%		

**Table 3** Correlation of the number of fatty liver detected by MRS and liver biopsy.

	Healthy subjects		Overweight and Obese subjects		NAFLD Or Patients group		r	P value
	Number of subjects	Percentage	Number of subjects	Percentage	Number of patients	Percentage		
MRS score	2	13%	6	40%	15	100%	3.325	0.029
Liver biopsy result	1	7%	5	33.5%	15	100%		



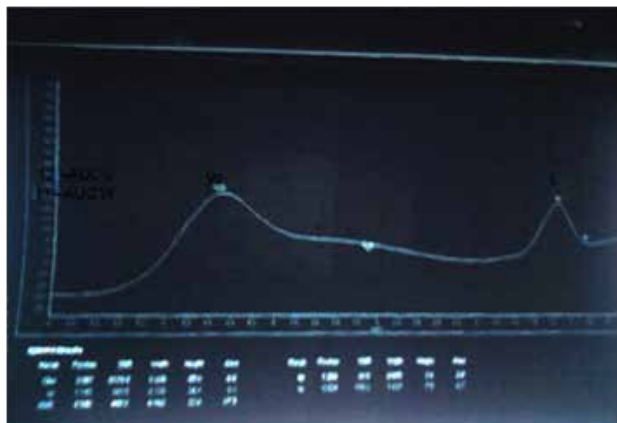
**Figure 1** MRS in patient of NAFLD group showing AUC L\AUC W=7\0.9= 7.9 (GM).



**Figure 2** MRS in subject of obese group showing AUC L\AUC W=1.9\0.3=6.4 (GM).



**Figure 3** MRS in subject of obese group showing AUC L\AUC W=0.51\0.1=5.1 (GM).



**Figure 4** MRS in subject of control group showing AUC L\AUC W=11\1=11.35 (GM).

consistent with results of previous studies<sup>[20,23]</sup>.

The current study found that the MRS results in fatty liver measurement were high in NAFLD group, overweight and obese group and less in healthy subjects group, also MRS results was higher in morbid obese subjects than moderate obese and over built subjects. This finding went with Thomas *et al*<sup>[24]</sup> who used 1H MRS to assess that the liver fat content in 11 subjects with biopsy proven hepatic steatosis and 23 normal volunteers. Hepatic fat accumulation signals were detectable in all subjects but were significantly greater in hepatic steatosis geometric mean (GM) 11.5 and interquartile range (IQR) 7.0-39.0 than in normal volunteers (GM) 2.7 and (IQR) 0.7-9.3, ( $p=0.02$ ). Hepatic fatty content levels were significantly greater in overweight compared with lean subjects with BMI 25 kg/m<sup>2</sup> ( $n=23$ ), (GM) 7.7, (IQR) 4.0-28.6 versus BMI more than 25 kg/m<sup>2</sup> ( $n=11$ ), (GM) 1.3, (IQR) 0.3-3.6, ( $p=0.004$ ). There was a significant association between Hepatic fatty content and different types of obesity.

In our study we found significant association between the result of fatty liver measurement by MRS and the value measured by liver biopsy, this goes with Noworolski *et al*<sup>[25]</sup> as he performed MRS in 8 healthy volunteers and 9 patients undergoing assessment for NAFLD. The MRS lipid: water ratios among NAFLD patients with grades 1, 2, and 3 steatosis were statistically different from each other and from healthy subjects ( $p<0.008$ ). MRS lipid: water were higher in the NAFLD patients than the healthy subjects ( $17\pm 14$  vs  $0.1\pm 0.06$ ,  $p<0.0001$ ) and increased dramatically with grade ( $p<0.008$ ). Also, Lidia *et al*<sup>[22]</sup> who found that there was significant association obtained between calculated liver fat concentration and the value measured by liver biopsy.

In the present study by comparison between different methods (MRS an liver biopsy) in their result of fatty liver detection in the three groups, we found that MRS detected 40% NAFLD patients in obese group and 13.3% in control group, however liver biopsy detected 33.3% in obese group and 6.7% in control group. So MRS was more sensitive in fat detection than liver biopsy. The same was conducted by Mireille *et al*<sup>[26]</sup> as he studied 18 White volunteers (obese), and scanned them by both ultrasonography and 1H MRS and histological scoring of liver tissue by biopsy for diagnosis of NAFLD, he detected that 1H MRS is more reliable, and more valid for quantification of hepatic fat content, so 1H MRS would be an interesting gold standard. Also, This finding also went with Fabian *et al*<sup>[27]</sup>, and Thomas *et al*<sup>[24]</sup> who found that MRS provide higher sensitivity for more accurate detection of small amounts of fat, allowing more reliable disease characterization and thus better guidance for patient management and may be a useful method for monitoring intrahepatic fatty accumulation in future interventional studies.

Perez-Daga *et al*<sup>[28]</sup> found that MRS is not widely used for these purposes, spectral analysis methods are complex and their complexity may lead to variability in results. The results also may vary because of differences in MRS systems and acquisition parameters and because fat quantification is performed in a small volume of liver tissue. The use of MRS for routine clinical applications has not yet been validated and liver biopsy still the golden method in fatty liver diagnosis and quantification.

From all above associated studies with our current study we found that, fatty liver more in obese subjects with high weight, BMI and Waist circumference than lean subjects, also the subjects who had fatty liver were with high ALT level after exclusion of all factors which may be a cause of ALT elevation, also we found them with high total cholesterol, LDL and TG, and low HDL level.

Although the difference between Perez-Daga *et al*<sup>[28]</sup> and our study in detection of the most sensitive method in fatty liver measurement,

we found that MRS was the best method in fatty liver measurement as one of the most accurate methods for noninvasive assessment of fatty liver, also are its ability to determine the absolute liver fat concentration and its high sensitivity for detecting small amounts of hepatic triglyceride and subtle changes in hepatic triglyceride content during treatment. It is also useful for revealing a necroinflammatory response in the setting of chronic liver disease.

## CONCLUSION

In-phase and out-of-phase imaging can be used to rapidly estimate the LFC in subjects and patients without or with NAFLD. The cutoff value of 5.1% enables objective rapid and reliable discrimination of normal LFC from elevated LFC. Thus, MRS provides non-invasive quantification of the intrahepatic fat fraction, and gives a reliable basis for longitudinal clinical and research studies.

## CONFLICT OF INTERESTS

There are no conflicts of interest with regard to the present study.

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