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Magnetic resonance spectroscopy to measure hepatic triglyceride content: prevalence of hepatic steatosis in the general population

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NONALCOHOLIC FATTY LIVER DISEASE (NAFLD) comprises a spectrum of conditions extending from simple hepatic steatosis (fatty infiltration of the liver) to end-stage liver disease (6, 7, 15, 28, 33). It represents a significant problem among patients referred for evaluation of abnormally elevated circulating levels of liver enzymes. In this referral population, either hepatic steatosis or steatohepatitis (fatty liver accompanied by inflammation) is suspected in up to 70% of subjects (10). In addition to its role in the pathogenesis of liver disease, hepatic steatosis is associated with diabetes mellitus and many features of the so-called metabolic syndrome. Hepatic triglyceride accumulation is frequently observed in obese individuals (23, 46) and is linked to insulin resistance and hypertriglyceridemia (9, 24, 25, 39, 44). Treatments targeting obesity and insulin resistance reverse the associated accumulation of hepatic triglyceride, and weight loss reduces hepatic fat stores and improves insulin resistance (43). For patients with diabetes, rosiglitazone treatment results in a 40% reduction in plasma free fatty acid levels and hepatic triglyceride content (HTGC), which is accompanied by a marked improvement in insulin-stimulated glucose metabolism during insulin clamp studies (29, 31).

Despite its clinical importance, the prevalence of NAFLD in the general US population remains poorly defined (1) due to limitations of the available noninvasive techniques used to diagnose hepatic steatosis. In the Third National Health and Nutrition Examination Survey (37), the estimated prevalence of hepatic steatosis was 2.8%. This study used elevated serum alanine aminotransferase (ALT) activity as a surrogate for the histological diagnosis of fatty liver disease and excluded all respondents who did had a history of hepatitis B, hepatitis C, type 2 diabetes, moderate-to-high ethanol consumption, or laboratory evidence of hemochromatosis. The prevalence inferred from this study is much lower than estimates made in Japan [16–20% among adults (27, 32)] and in Northern Italy (2, 3) using hepatic ultrasound. In one of these studies (32), a large-scale (n = 2,574) population-based survey of hepatic steatosis was performed. The remainder of the studies included much smaller and selected samples, and in some studies, such as the Dionysos Study, only participants with clinical signs or symptoms of liver disease were analyzed (3).

Localized proton magnetic resonance spectroscopy (1H MRS) is an alternative, noninvasive method to measure HTGC. Because values given by 1H MRS correlate with liver biopsy results (21, 22, 41, 42), it is widely considered to be the optimal noninvasive method to assess HTGC and diagnose hepatic steatosis (10). Heretofore, measurements of HTGC using MRS have been confined to small numbers of subjects as part of research protocols (21, 22, 40, 42). The present study applies 1H MRS to quantify HTGC in a large, ethnically diverse sample of US adults, aged 30–65 yr, to describe the distribution of hepatic triglyceride accumula-
tion in the general population and to establish diagnostic criteria for defining the prevalence of hepatic steatosis in the population.

METHODS

Subjects: Dallas Heart Study

The Institutional Review Board at the University of Texas Southwestern Medical Center approved all experimental protocols, and all human subjects provided informed written consent before their participation in the study. The subjects were participants of the Dallas Heart Study (DHS), which is a large, multiethnic, probability-based population sample, aged 18 – 65 yr, of Dallas County, which is described in detail elsewhere (45). The study was designed to include equal numbers of Blacks and non-Blacks. All subjects completed a comprehensive in-home medical survey, but phlebotomy and a clinic visit were limited to subjects aged 30 – 65 yr. Blood pressure was measured in the seated position using an automatic device (series no. 52,000; Welch Allyn, Arden, NC). Body weight was measured with a portable scale (Ever Weigh Lithium Electronic Scale no. 34067; Health O Meter, Bridgeview, IL). Trained personnel determined height and waist circumference using a tape measure. Alcohol use was estimated on the basis of answers to a validated set of questions from the 1995 National Alcohol Survey (34). Forty milliliters of blood collected into serum separator or citrate-EDTA tubes were centrifuged (1,000 g for 15 min) at 4°C. Plasma was removed and stored at −80°C. Standard serum chemistries were assayed within 24 h. Magnetic resonance studies performed during the clinic visit included cine-MRI measures of cardiac mass and function, abdominal images for evaluation of subcutaneous and visceral adiposity, and spectroscopic measurements of HTGC.

Validation of Hepatic $^1$H MRS

Three subgroups of individuals with normal blood pressure, no diabetes, and a body mass index (BMI) ranging from 18 to 39 kg/m² were recruited to examine the reproducibility of the in vivo measurement of HTGC ($n = 10$), the spatial distribution of hepatic triglyceride ($n = 5$), and the impact of feeding on the HTGC ($n = 8$).

Measurements of HTGC using $^1$H MRS

Measurements of HTGC were obtained with subjects on a 1.5 Tesla Gyroscan INTERA whole body system (Philips Medical Systems). Sagittal, coronal, and axial slices through the right lobe of the liver were acquired, and a 27-cm³ spectroscopic volume of interest was positioned, avoiding major blood vessels, intrahepatic bile ducts, and the lateral margin of the liver (Fig. 1, left). The voxel size and position were optimized to prevent contamination of signal from liver by signal from abdominal adipose fat. We used a relatively large voxel to be able to collect good quality data in a short time and to minimize patient time in the magnet. After the system was tuned and shimmed, spectra were collected using a Q-body coil for radio frequency transmission and signal reception. A PRESS sequence was used for spatial localization and signal acquisition with the parameters interpulse delay $T_1 = 3$ s, spin echo time $T_2 = 25$ ms, 16 acquisitions, and 1,024 data points over a 1,000-Hz spectral width. Only signals from a selected volume element were collected. Any signals generated from tissues outside the voxel were not phased; these signals rapidly decayed and did not contaminate the measurements. Areas of resonances from protons of water and methylene groups in fatty acid chains of the hepatic triglyceride (Fig. 1, middle and right) were evaluated with a line-fit procedure and commercial software (NUTS-ACORNMR, Freemont, CA). Signal decay due to spin-spin relaxation was calculated using mean $T_2$ relaxation times for water and fat of 50 and 60 ms, respectively, and the exponential relaxation equation $I_m = I_0 \exp \left( -T_e/T_2 \right)$, where $I_0$ is the measured signal intensity obtained at the selected echo time of $T_e$, $I_0$ is the signal intensity immediately after the 90° pulse, and $T_2$ is the spin-spin relaxation time. Average $T_2$ relaxation times were used for these calculations instead of performing experiments to assess the relaxation times individually in each patient (22, 42).

A total of 622 of the DHS participants who completed the clinic visit did not have an HTGC measurement. A total of 289 of the subjects were not measured due to scheduling problems or limited time for completion of all imaging and spectroscopy procedures. Other subjects were not studied due to the presence of metallic implants ($n = 49$), claustrophobia ($n = 191$), equipment failure ($n = 19$), weight exceeding the limit of the table ($n = 58$), and patient refusal ($n = 74$). A total of 72 studies (3.1%) were excluded from the
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final analysis due to the poor quality of spectra attributable to motion artifact.

Validation. Experiments designed to address the validity of 1H MRS for measuring HTGC in a clinical setting were performed in a subset of the DHS cohort. To assess the reproducibility of 1H MRS, repeated measurements of HTGC were obtained in 10 individuals. Each subject was placed in the magnet in the prone position, the volume of interest was localized, the system was tuned and shimmed, and spectra were collected. The subject was then removed from the magnet for 10 min before the procedure was repeated.

Voxel size. In a subgroup of six people with BMI ranging from 28 to 48 kg/m², spectra were measured from five different cubic voxels to ensure that the measurements obtained were independent of the voxel size and to determine the optimal size and the length of time for data collection.

Spatial Distribution of Hepatic Triglyceride

A third subgroup of five subjects was tested to ascertain the spatial distribution of hepatic triglyceride. We selected two volumes of interest of identical size (27 cm³) in the upper right and upper left lobes of the liver. 1H MRS measurements were acquired in a single session using identical experimental parameters for both determinations.

Comparison of Hepatic Triglycerides in Fasted and Fed States

To assess the impact of food ingestion on the levels of HTGC, the subjects were overnight fasted in the General Clinical Research Center (GCRC). The following morning, a fasting blood sample was obtained, and serum glucose, cholesterol, triglyceride, nonesterified fatty acid, and leptin concentrations were measured before the volunteers underwent hepatic spectroscopy. After the first measurement of HTGC was made, the subjects consumed a high-fat meal prepared by a registered dietitian at the GCRC. The meal contained 50 g of fat and consisted of 25 g of grits, 20 g of butter, 100 g of scrambled eggs, 20 g of bacon, 15 g of cheese, and 240 g of milk. Four hours after the meal, the phlebotomy and MRS measurement experiments were repeated.

Hepatic 1H MRS in DHS Population Sample

When 1H MRS was used to measure HTGC in those individuals from the DHS population who attended the clinic visit, the MRS parameters listed above were used, and the total time of the procedure was 15 min and occurred subsequently to the subjects completing cine-MRI and abdominal imaging. Automated procedures were used to integrate the water signal over the interval of 3.0 to 5.5 ppm, whereas the fat signal was integrated over the interval from 0.5 to 3.0 ppm. All data were collected and processed by clinical MRI technologists within the Rogers Center for Magnetic Resonance Imaging. A spectroscopist constructed all procedures, supervised data collection, and maintained quality control of all results.

Calculations

Values provided by 1H MRS denote relative quantity of water and hepatic triglyceride fatty acid chains protons in the volume of interest. To convert these results to absolute concentrations expressed as percent fat by weight or volume (21, 41, 42), we used equations validated by Longo et al. (21) that incorporate the following experimentally determined factors: 1) the ratio of the number of lipid protons detected in the 0.5–3.0 ppm region of in vivo spectra to the total number of lipid protons is 0.85; 2) proton densities of fat and water are 110 and 111 mol/l, respectively; 3) liver contains 0.711 g water/g normal tissue; 4) the density of liver tissue is 1.051 g/l; and 5) the density of fat in the liver is 0.90 g/l. With these equations, a significant correlation was obtained between calculated liver fat concentration and the value measured by liver biopsy (21).

Biochemical Assays

Assays to determine serum concentrations of standard clinical chemistry analytes were performed on a Beckman CX9ALX chemical analyzer (Beckman-Coulter, Fullerton, CA). Plasma nonesterified fatty acids and leptin concentrations were assayed as described previously (14, 40).

Statistics

Results for the validation studies were compared using paired t-tests for repeated measurements in the same individual. Weighted analyses for means and distributions of the DHS database were performed using SAS software (SAS Institute, Cary, NC). Where population estimates are provided, we used a survey means procedure that accounts for the additional variation resulting from uncertainty in sample selection.

RESULTS

Validation of 1H MRS Measurements of Hepatic Triglyceride

The HTGC was measured twice in the same individuals on two independent occasions separated by ≥ 10 min. The reproducibility of the technique was high (Fig. 2) over a wide range of HTGC values (from 2.1 to 39.3% fat to total signal). The slope of the line fitting the data for the two sets of values was 1.01, and the correlation coefficient was R = 0.99 (P < 0.001). The coefficient of variation between the two measurements was 8.5%. To assess the homogeneity of liver triglyceride content, the HTGC was measured in the left lobe and in the right lobe of the liver in five individuals (Fig. 3). The coefficient of variation between these two values was 11%.

Voxel Size

To optimize the conditions for our measurements and to ensure that the measurements obtained were not dependent on voxel size, we varied the voxel size and time for acquisition of the spectra. The size of the voxel was optimized to obtain good spectral resolution in the shortest amount of time. Liver spectra were obtained from cubic voxels of differing sizes (30, 25, 20, 15, and 10 mm) in six subjects. Hepatic water and fat displayed equal scaling with the voxel size (Fig. 4). We chose a voxel...
size of 30 mm³ for these studies, because it allowed for the collection of a good quality spectrum in 1 min (mean no. of acquisitions = 16).

Comparison of Hepatic Triglycerides in Fasted and Fed States

To determine the effect of ingestion of a fatty meal on hepatic fat content, subjects were fasted for 10 h. Blood was sampled and the HTGC measured. Participants were then given a high-fat meal, and the ¹H MRS measurements were repeated. Plasma glucose, leptin, LDL cholesterol, HDL cholesterol, and nonesterified fatty acid concentrations were not statistically significantly different between the two time points. Mean plasma triglyceride levels increased from 74 ± 22 to 162 ± 46 mg/dl (P < 0.001) over the 4 h. Despite these changes in circulating triglyceride levels, no changes were seen in HTGC, as measured by ¹H MRS, after ingestion of the fatty meal, even in the subject with elevated HTGC (Fig. 5).

Population-Based Assessment of Hepatic Steatosis

Hepatic triglyceride content was measured by ¹H MRS in 2,349 of 2,971 possible subjects that completed clinical examinations as part of the DHS. Clinical characteristics of the 2,287 study participants, aged 30–65 yr, are provided in Table 1. Slightly more women than men were included, and the ethnic breakdown of the sample was as follows: 1,105 Blacks, 734 Whites, 401 Hispanics, and 47 individuals of other ethnicities (e.g., Asian Indians, American Indians, etc.). The number of participants who were overweight or obese by World Health Organization criteria was high: 76% had a BMI >25 kg/m² and 43% had a BMI >30 kg/m².

The distribution of HTGC as a ratio of methylene of hepatic triglyceride fatty acid chains and combined methylene of hepatic triglyceride fatty acid chains and water signals in the population is shown in Fig. 6, with levels ranging from 0 to 47.5%. The distribution of HTGC was non-Gaussian and

Table 1. Clinical characteristics of men and women in the Dallas Heart Study

<table>
<thead>
<tr>
<th>Clinical Characteristics</th>
<th>Men (n = 1,080)</th>
<th>Women (n = 1,207)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>44.9 ± 9.4</td>
<td>45.3 ± 9.8</td>
</tr>
<tr>
<td>Ethnicity, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>46.2</td>
<td>50.2</td>
</tr>
<tr>
<td>White</td>
<td>34.7</td>
<td>29.7</td>
</tr>
<tr>
<td>Hispanic</td>
<td>15.9</td>
<td>19.0</td>
</tr>
<tr>
<td>Other</td>
<td>3.2</td>
<td>1.1</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>29.0 ± 5.9</td>
<td>31.0 ± 7.8</td>
</tr>
<tr>
<td>Diabetes, %</td>
<td>11.3</td>
<td>10.5</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>129 ± 16</td>
<td>125 ± 18</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>79 ± 10</td>
<td>78 ± 10</td>
</tr>
<tr>
<td>Alcohol intake, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>23.8</td>
<td>36.7</td>
</tr>
<tr>
<td>Moderate</td>
<td>67.4</td>
<td>58.9</td>
</tr>
<tr>
<td>Excessive</td>
<td>8.8</td>
<td>4.4</td>
</tr>
</tbody>
</table>

Values are means ± SE, unless otherwise specified.
positively skewed with a tail extending to higher values. The median HTGC was 4.69%, and the 5th, 25th, 75th, and 95th percentile values were 0.99, 2.74, 8.56, and 22.86%, respectively. HTGC can also be expressed as a weight percent (mg/g wet wt), allowing a comparison with biochemical measurements of triglyceride from biopsy (17, 42). The median value of 4.69% (methylene:methylene and water) would be equivalent to a hepatic triglyceride concentration of 35.9 mg/g or 3.6%, and the 5th, 25th, 75th, and 95th percentile values correspond to triglyceride concentrations of 0.8, 2.1, 6.6, and 18.5%, respectively.

HTGC was positively correlated with BMI in DHS ($r = 0.3427$; Fig. 7) but did not explain most of the variation in the HTGC.

Traditionally, liver fat content >50 mg/g (5% by wet weight) is diagnostic of hepatic steatosis (17, 42). This standard can be applied to the current data set to yield an estimate of the prevalence of hepatic steatosis in this representative sample of adults aged 30–65 yr in Dallas County. Of the 2,287 members of the cohort, 34.3% have hepatic triglyceride content exceeding 5% by weight, which translates to a weighted population prevalence of 37.6%.

Alternatively, a cut-off that can be used to define hepatic steatosis can be determined by examining the distribution of HTGC in those subjects from this study who do not have a history of liver disease or risk factors for hepatic steatosis. A total of 345 subjects had low alcohol consumption (<30 g/day in men, ≤20 g/day in women), had a BMI <25 kg/m², had normal fasting serum glucose values, and were nondiabetic. These individuals also had no history of liver disease and had normal serum ALT values (≤30 IU/l in men, ≤19 IU/l in women; Ref. 33).

This low-risk group consisted of 160 men and 185 women with a racial and ethnic distribution of 140 Blacks, 143 Whites, 51 Hispanics, and 11 others. The distribution of HTGC was skewed, with the median HTGC in this subgroup being 1.9% with the 90th and 95th percentiles of HTGC being 4.3 and 5.6%, respectively. When the low-risk cohort was subdivided by ethnicity or sex, the distribution of HTGC was similar across all subgroups. If a value of 5.5% is applied to men and women of all ethnicities in the entire study cohort, 30.7% of the 2,287 subjects have hepatic steatosis. Weighting of this DHS data gives an estimated prevalence of hepatic steatosis of 33.6% in the population of Dallas County.

DISCUSSION

Our study demonstrates that $^1$H MRS, a noninvasive technique generally used in small-scale research protocols for measuring intracellular tissue triglyceride, can be used in the clinical setting to assess the HTGC. The method is reproducible and not affected by food intake. It has the advantage over the other commonly used imaging modalities to assess hepatic fat content: ultrasonography, computed tomography (CT), and magnetic resonance imaging (13, 30), since it is quantitative rather than qualitative or semiquantitative. Inasmuch as HTGC obtained by spectroscopy closely coincides with biopsy-derived triglyceride concentrations, it provides a noninvasive method for the quantitative determination of hepatic steatosis.

We also provide an “upper limit of normal” value for the HTGC using a large population of individuals ($n = 345$) with no risk factors for hepatic steatosis. A HTGC greater than 5.56% (or 55.6 mg/g liver tissue), which corresponds to the 95th percentile in the distribution of HTGC in these “normal” subjects, was defined as abnormal. These values are consistent with prior studies wherein the triglyceride levels in hepatic tissues have been measured chemically in autopsy specimens (20) or in clinical trials in which the effect of pharmacological agents on liver fat content has been monitored using spectroscopy (29, 40). By use of these criteria, over one-third (33.6%) of the adults (aged 30–65 yr) in Dallas County have hepatic steatosis.

Widely divergent estimates of the prevalence of NAFLD have been reported. In an autopsy study of 351 presumed nonalcoholic subjects, macrovesicular steatosis was observed in ≥25% of hepatocytes in 7% of nonobese and 29% of severely obese subjects (46). Of 126 healthy volunteer donors for orthotopic liver transplant, 20% had hepatic steatosis documented by percutaneous biopsy samples (26). Although hist-
tological examination specifically identifies NAFLD and provides additional information regarding the nature and severity of disease (4, 5, 38), studies using liver biopsy specimens are necessarily small and prone to selection bias. Alternative methods commonly used to assess the frequency of hepatic steatosis in the population have relied on various radiographic imaging modalities. Although some promising correlations with histomorphometric measures of HTGC have been obtained using computerized tomography \( (r = 0.90) \), the correlation between actual hepatic triglyceride levels and CT findings remains relatively low \( (r = 0.57) \) (8, 30, 36). The estimated prevalence of hepatic steatosis, using CT (8, 19, 36) or ultrasound (16, 18), vary over a wide range \((14\% - 25\%)\) due to variations in techniques and subjects selected for analysis. Ultrasound is the preferred imaging modality for the qualitative assessment of fatty infiltration, because the sensitivity of CT is highly time dependent and protocol specific (16). The largest study to date using sonography was performed in Japan, where the prevalence of hepatic steatosis was estimated to be 14\% (32). However, the results obtained using sonography do not always correlate with those obtained by biopsy (13, 16).

The other approach that has been employed to estimate the prevalence of hepatic steatosis has been to use surrogate laboratory markers, such as unexplained elevations in the serum levels of aminotransferases (11, 12, 37). The estimated prevalence of NAFLD in the US on the basis of unexplained serum ALT elevations is lower than those obtained from imaging studies. Between 2.8\% (36) and 5.4\% (11) of subjects with no identifiable cause of liver disease have an abnormal aminotransferase level attributable to hepatic steatosis. However, the use of such surrogate markers is prone to underestimation inasmuch as it is likely that all subjects with hepatic steatosis have laboratory abnormalities. Thus radiographic techniques are more sensitive at detecting the presence or absence of excess hepatic triglyceride than are surrogate serum markers. Neither method provides a quantitative measure of hepatic triglyceride.

Unlike CT scanning and ultrasound, which rely on nonspecific attenuation of X-ray and Doppler signals, \(^1\)H MRS provides a quantitative assessment of hepatic triglyceride by directly measuring the protons in the fatty acids of the triglycerides. In addition, \(^1\)H MRS is highly reproducible (Figs. 2 and 3) in contrast to CT scanning, where there is significant intrasubject variability in the measurements (19).

None of the noninvasive imaging techniques, including \(^1\)H MRS, can assess hepatic fibrosis and inflammation. Currently, liver biopsy remains the gold standard for the assessment of the stage and grade of hepatic injury. A distinct disadvantage of using liver biopsy material to assess HTGC is the small size of the sample. Any inhomogeneity in the distribution of triglyceride in the liver can introduce sampling error. A much larger volume of liver tissue is assessed using spectroscopy \((\sim 27 \text{ g} \text{ vs. } 50\% - 100 \text{ mg} \text{ in a typical biopsy sample})\), minimizing the likelihood of sampling error.

The \(^1\)H MRS technique is also more quantitative than standard histological grading of liver biopsy specimens, which can be prone to over- or underestimation of liver fat content (6). Longo et al. (21) found a satisfactory correlation between \(^1\)H MRS data and histomorphometric analysis of patient biopsy samples \( (r = 0.70) \), but a systematic error was observed when HTGC was low. This occurred because histomorphometry infers HTGC only from the presence of macroscopic lipid vesicles. Proton MRS is sensitive enough to detect small amounts of triglyceride that may not form macroscopic vesicles and thus may not be visualized histologically. When in vivo \(^1\)H MRS measurements of HTGC were compared with values obtained by chloroform-methanol extraction of liver samples excised from dogs and rabbits, the slope of the fitted line was 0.98 \( (r = 0.93) \) for values ranging from 1 to 60 mg/g (41). In similar studies in human subjects, a statistically significant correlation \( (r = 0.90) \) was found between fat concentration measured in liver biopsies and that calculated from \(^1\)H MRS experiments (42).

In this study, the prevalence of hepatic steatosis in the population was 33.6\%, which is higher than all prior estimates. The enhanced sensitivity of HTGC measurements by \(^1\)H MRS may contribute to the higher prevalence of hepatic steatosis in this study. Alternatively, the higher prevalence may reflect differences in the subject population. The present study was performed in an ethnically diverse population in which obesity is common \((\sim 43\% \text{ of the subjects in the DHS had a BMI } > 30 \text{ kg/m}^2)\) (45). Although it is debatable whether hepatic steatosis truly represents a disease (12), it is known that a yet-to-be-determined proportion of the individuals with NAFLD are at risk of developing serious liver complications, including cirrhosis and hepatocellular carcinoma. The accuracy and safety of \(^1\)H MRS make it an ideal methodology to assess and monitor changes in HTGC in response to various therapeutic interventions (29, 39, 40, 43) and to examine the natural history of the disorder.

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