ORIGINAL RESEARCH

Regional differences in hepatic fat fractions in over-weight children and adolescents observed by 3T ^{1}H-MR spectroscopy

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Abstract

Background: Proton MR Spectroscopy (^{1}H MRS) can be used to measure hepatic fat fractions (HFF) in liver cells for assessment of non-alcoholic fatty liver disease (NAFLD). The measurement is typically performed in a single volume. For correct diagnosis it is important to know variability of HFF within liver. The purpose of this study was to investigate regional differences in HFF in a large group of overweight and obese children and adolescents using 1H MRS at 3T.

Methods: The study included 175 consecutive children and adolescents from 7 to 18 years old with a body mass index above the 97th percentile according to age and gender. ^{1}H MRS was performed at 3.0T using a point resolved spectroscopy sequence in two 11mmx11mmx11mm volumes positioned in different parts of the right liver lobe.

Results: Up to 12% difference in HFF between the two volumes was observed: the regional differences in HFF are below 1% for patients with HFF under 3%; patients with HFF between 3% and 20% have regional differences in HFF up to 5%; patients with HFF over 20% have regional differences in HFF up to 12%.

Conclusions: 1H MRS diagnosis and severity assessment of NAFLD in overweight and obese children and adolescents should take up to 12% regional differences in hepatic fat fractions into account.

Key words

Magnetic resonance, Spectroscopy, Relaxation time, Children, Non-alcoholic fatty liver disease, Obesity

1 Introduction

Obesity has become a growing problem all over the world beginning at early age in children and progressing into adulthood [1, 2]. One of the major diseases caused by obesity is non-alcoholic fatty liver disease (NAFLD). NAFLD ranges from simple steatosis through steatohepatitis (NASH) to end-stage liver disease (cirrhosis) and is defined as triglyceride accumulation in hepatocytes exceeding 5% of liver weight [3, 4]. It is hard to predict who is going to develop steatohepatitis

and liver function impairment. Liver biopsy is the gold standard of diagnosis and severity assessment of NAFLD. However, it is an invasive procedure associated with serious risks. Obese children are frequently affected by NAFLD, which can not be predicted by clinical and/or anthropometrical findings [5]. Magnetic resonance spectroscopy (MRS) is a valid alternative for the detection of NAFLD in these children [6].

MRS is a unique technique that allows the study of the metabolic tissue content in vivo noninvasively [4, 6]. Proton MRS (1H MRS) can measure the triglyceride content in liver cells directly and quantitatively analyze the fat fraction (HFF) in the liver [7, 8]. 1H MRS is more accurate in the detection of fatty liver than computed tomography (CT) and exhibit higher sensitivity in the detection of liver fat content than conventional MR imaging and ultrasound investigations [9, 10].

Since 1H MRS measures HFF in a single volume it is important to know the regional differences in HFF through the liver for assessment of NAFLD. "Within liver" variability of HFF has been studied in groups of adults ranging from 6 to 77 subjects [11-14]. The aim of the present study was to examine the variability of the HFF in a large group of overweight and obese children and adolescents.

2 Patients and methods

2.1 Patients

175 consecutive children and adolescents from 7 to 18 years of age (see Table 1) included in childhood obesity treatment [15] were referred for an MR-examination. The patients had a body mass index (BMI) above the 97th percentile according to Danish age and sex adjusted BMI charts [16].

Table 1. Age and BMI in 175 patients according to gender

Item	105 girls		70 boys	
	Age, years	BMI	Age, years	BMI
Mean	13.0	28	13.9	28
Standard deviation	2.7	5	2.3	5
Minimum	7.5	20	8.1	20
Maximum	17.9	42	17.8	42

Written informed consent was obtained from the children's parents. The study was approved by the Institutional Review Board (ID-no.: SJ-104 and SJ-98), by the data protection agency, and by ClinicalTrials.gov (ID-no.: NCT00823277 and NCT00928473).

2.2 Magnetic resonance examination

MR measurements were performed using the Achieva 3.0 T MR-imaging system (Philips Medical Systems, Best, the Netherlands) and a sense cardiac coil. Patients were examined in the supine position.

T2-weighted turbo spin echo (TSE) coronal and axial slices through the upper abdomen were acquired for positioning the spectroscopy volumes of interest. Parameters for TSE sequence were: TSE factor=93, TR=2182ms, TE=80ms, FOV=420 mm. Two spectroscopy volumes (11 mm × 11 mm × 11mm) were positioned at different levels in the right lobe of the liver. 11 mm × 11 mm × 11mm was a maximum dimension, which allowed major blood vessels and intrahepatic bile ducts to be avoided according to TSE images. Positions of the volumes were individually determined for each subject in order to avoid the vascular structures. A single voxel spectrum without water saturation was recorded using point resolved spectroscopy (PRESS) sequence with parameters: repetition time TR=4000 ms, TE=75 ms, spectral bandwidth = 2000 Hz, 1024 points, 32 averages (see Figure 1). For one volume a series echo time TE=45, 60, 75, 90 and 105 ms was applied for measuring HFF compensated for T2 relaxation, and T2 relaxation times of water and of fat. The series echo time TE was

only applied in one volume due to limited examination time. Spectra were acquired during free breathing. The complete examination time was 30 minutes and included patients entering and exiting the scanner.

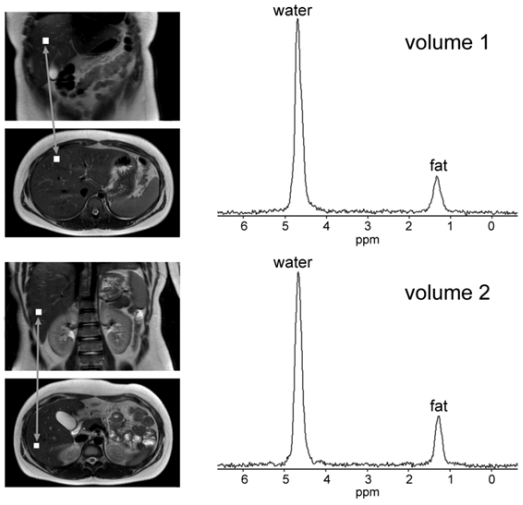


Figure 1. ^{1}H MRS spectra from the two different hepatic volumes (HFF=17% in volume 1, HFF= 23% in volume 2)

2.3 Measurement of hepatic fat fraction

The water (4.7 ppm) and fat (1.3 ppm) peaks of the acquired spectra were fitted to obtain their areas using a standard postprocessing protocol for fitting metabolite peak areas available at the Achieva 3.0T MR-imaging system.

HFF was calculated according to equation:

$$HFF = [\text{fat peak area}/(\text{fat peak area} + \text{water peak area})]\times 100$$

HFF was calculated for the two different volumes at TE=75 ms. Water and fat T2 relaxation times and HFF corrected for T2 relaxation effects (HFF at TE=0 ms) were calculated when the fat peak was sufficiently large (HFF>10% at TE=75 ms) in one of the volumes using an exponential least-square fitting algorithm to the peak areas with the series TE as described earlier [7]. TR of 4 s was considered sufficiently long to avoid influence of T1 relaxation in the post-processing calculations.

2.4 Statistical analysis

Mathematical and statistical calculations were performed using MATLAB software. Quantitative variables were processed to give group mean values, standard deviations, minimum and maximum. Pearson's correlation coefficient r was used to describe correlation. Statistical significance was described by a *P* value below 0.05. To estimate the HFF variation between the volumes, the Bland-Altman method [17] was applied, and the coefficient of variation (CV) was calculated by dividing the standard deviation of the mean difference between the two measurements by mean HFF of all measurements.

3 Results

Strong correlation was found between HFF in the two different volumes (r=0.98, P<0.0001). An up to 12% difference in HFF between the two volumes was observed: the regional differences in HFF are below 1% for patients with HFF under 3%; patients with HFF between 3% and 20% have regional differences in HFF up to 5%; patients with HFF over 20% have regional differences in HFF up to 12%. HFF variation between the two volumes is shown in Figure 2 and in Table 2. The

corresponding Bland-Altman plots are shown in Figure 3 a, b. Mean HFF in the first volume (8.2%) did not differ from the second volume (8.3%). The CV was 31%.

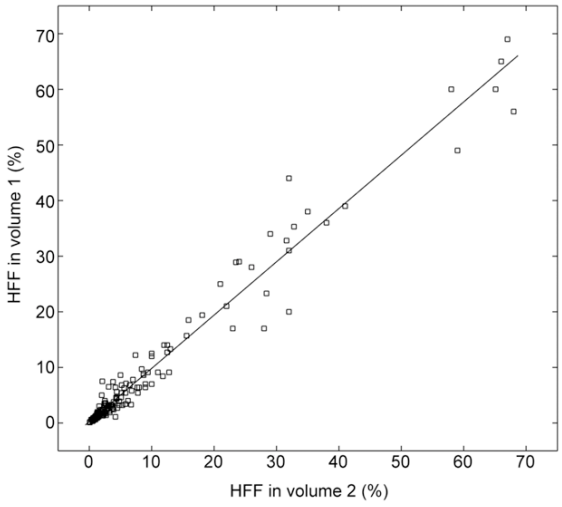
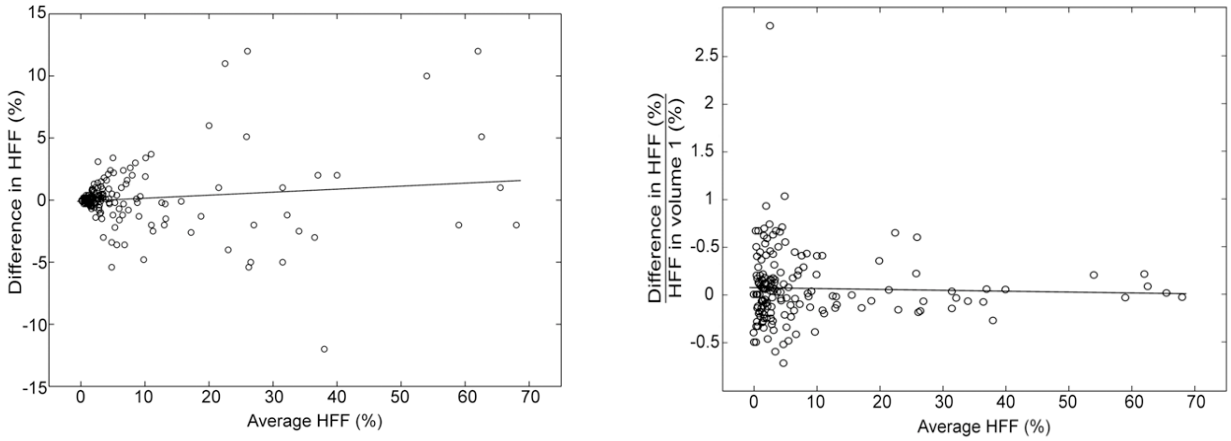


Figure 2. Correlation between HFF values measured in two different hepatic volumes at TE=75 ms (r=0.98, P<0.0001; fit: y=0.96*x+0.24, R^2=0.96).



(a) (b)

Figure 3. Bland-Altman plot of the HFF values measured in two different hepatic volumes at TE=75 ms. a) Difference of HFF values plotted against averages (r=0.13, P=0.097; fit: y=0.02*x+0.09, R^2=0.017); b) Difference of HFF values normalized by HFF in volume 1 plotted against averages (r=0.03, P=0.65; fit: y=-0.0009*x+0.07, R^2=0.001).

Table 2. Agreement between HFF measurements in the two hepatic volumes.

	HFF in volume 1 (%)	HFF in volume 2 (%)	Difference (%)	Mean (%)
Mean	8.2	8.3	0.1	8.3
Standard deviation	13.2	13.5	2.6	13.3
Minimum	0.3	0.1	-12	0.1
Maximum	69	68	12	68

HFF obtained at TE=75 ms is overestimated due to T2 relaxation effects [7]. Figure 4 shows correlation between HFF compensated for T2 relaxation (at TE=0 ms) and HFF measured at TE=75ms. The data were calculated for 30 patients

with HFF>10% (at TE=75 ms) in one of the hepatic volumes. Figure 4 shows that the correct HFF (compensated for T2 relaxation) is approximately three times smaller than HFF measured at TE=75ms.

T2 relaxation times for fat and water components were calculated for the 30 patients with HFF>10% (at TE=75 ms). T2 values for water differed with a factor of 2 ranging from 22 ms to 42 ms with an average value of 28.6 ms; and T2 values for fat differed with a factor of 2 ranging from 42 ms to 83 ms with an average value of 65.8 ms. The results are shown in Table 3.

No correlation was found between HFF, age, BMI and T2 relaxation times.

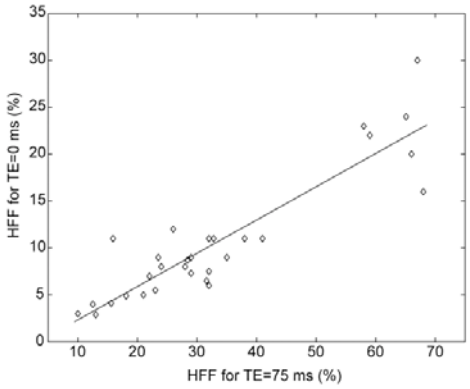


Figure 4. Correlation between HFF compensated for T2 relaxation (at TE=0 ms) and HFF measured at TE=75ms (r=0.9, P<0.0001; fit: y=0.35*x-1.19, R^2=0.81).

Table 3. T2 relaxation times of water and fat components calculated for 30 patients with HFF>10% at TE=75ms.

	T2 of water, ms	T2 of fat, ms
Mean	28.6	65.8
Standard deviation	3.8	9.4
Minimum	22	42
Maximum	42	83

4 Discussion

The technique of using 1H MRS for the evaluation of hepatic fat content is highly reproducible in the same spectroscopic volume [12, 18]. Nevertheless, liver fat is not equally distributed within liver and different "within liver" variability has been reported. Hájek et al. [14] showed that HFF obtained in three different positions varied by 20% in a group of 77 liver-transplant recipients. Van Werven et al. [12] reported regional differences in HFF up to 3.5% and CV=14% in the study of 12 subjects. Szczepaniak et al. [19] found a CV=11% for 10 subjects. The average CV calculated for spectroscopy volumes in three different positions was 12% for 20 steatosis patients and 24 control subjects [13]. Cowin et al. [11] found different CV depending on volume position in the liver for a group of 6 subjects: CV=3% in right lobe, posterior; CV=7% in right lobe, anterior; CV=6% in right lobe, central; CV=13% in left lobe.

The previously published results of adults indicate different hepatic fat content in different regions of the liver in accordance with the present study. CV=31% in the present study is higher than CV reported earlier in smaller groups of subjects. The published data may not be precisely comparable due to different experimental setups and different approaches to measure HFF by 1H MRS such as different acquisition parameters and different ways to correct/or not to correct for T1 and T2 relaxation times.

For the determination of the true HFF, spectroscopic peak areas should be corrected for T2 relaxations. Correction for T1 relaxation can be avoided by long TR. A number of studies [9, 12, 14, 19-21] used average T2 values to correct for all patients.

Since the T2 values may differ with up to a factor of 3 for different patients, it is more advisable to calculate T2 values individually for each patient [22, 23]. For 3T the following average values have been reported: T2 of water = 27 ms (range 12.4 ms – 54.3 ms) and T2 of fat = 61 ms (range 28 ms – 82.2 ms) (23); T2 of water range: 12.4 ms – 54.3 ms, and T2 of fat range 49 ms – 60 ms [14]; T2 of water = 28 ms (range 22 ms – 42 ms) and T2 of fat = 64 ms (range 36 ms – 99 ms) [7]. These values for 3T are in line with the results obtained in the present study.

It is important to correlate the HFF obtained by 1H MRS relatively to HFF determined by liver biopsy, which is the gold standard for diagnosis and severity assessment of NAFLD in clinical practice. Histological assessment of hepatic steatosis is graded according to a scale of 0-3 based on subjective visual estimation of the proportion of hepatocytes containing intracellular vacuoles of fat: 0 (normal, <5% of hepatocytes affected); 1 (mild, 5-33% of hepatocytes affected); 2 (moderate, 34-66% of hepatocytes affected); 3 (severe, >66% of hepatocytes affected) [24].

Visual histological assessment of "within liver" HFF variability showed excellent agreement for steatosis (κ=0.91 [25] and κ=0.88 [26]) between the right and left lobes of the liver according to steatosis grading scale 0-3 in morbidly obese patients. Precision of 10% was reported for determination of hepatic HFF by visual histological examination in steatosis patients[13]. Comparison of hepatic HFF variability obtained by 1H MRS with HFF variability obtained by liver biopsy is not precise due to large thresholds in the steatosis grading scale. Nevertheless, regional differences in HFF up to 12% obtained in the present study are not controversial to the biopsy results [13, 25, 26].

Assessment of HFF by histology is a 2D measurement and only the percentage of hepatocytes that show distinct fat droplets is used for quantification, HFF quantification by 1H MRS relies on determination of overall volume fraction of lipids in the liver parenchyma. Therefore, HFF obtained by the two methods can not be compared directly. The visual measurement of HFF dependent on subjective assessment by a pathologist, in average, overestimates digital measurement of HFF by 3.78 times in the same portion of the biopsy specimen [27].

Correlation between MRI and histology makes it possible to identify the steatosis grade based on 1H MRS results by linear dependency or by calibration graph [13, 14, 28]. d'Assignies et al. [13] found average HFF= 47.5±19.6 % measured by visual histology and average HFF=18.1±9 % measured by 1H MRS in 20 steatosis patients. Thus, the correction coefficient for adjustment of HFF measured by 1H MRS to histological HFF is 2.6. The ^{1}H MRS data [13] were corrected for relaxation times and therefore comparable with other studies where relaxation times correction was performed [14, 28]. Staging of steatosis based on 1H MRS was quantified by Krssák et al. [28] for 29 patients with chronic hepatitis C: 0 (normal, HFF at TE=0 <3.1%); 1 (mild, 3.1< HFF at TE=0 <5%); 2 (moderate, 5%< HFF at TE=0 <6.9%); 3 (severe, HFF at TE=0 >6.9%). Hájek et al. [14] used linear and non-linear models to describe the relationship between HFF measured by 1H MRS to histological HFF in three groups of 77 liver transplant recipients. Three different correction coefficients for linear models were obtained for the three groups: 3.3, 4.2, 6.1.

For the group of patients in the present study it was not ethically correct to perform liver biopsy. According to Fig. 4 HFF for TE=75 is about 3 times higher than HFF for TE=0. Since the reported correction coefficients for adjustment of HFF measured by 1H MRS to histological HFF are ranging from 2.6 to 6.1, the HFF calculated at 3 T directly from MR spectrum at TE=75 (see Figure 2) may be comparable with histological values.

The limitation of the present study is lack of T2 measurements for volume 2. Therefore it was not possible to investigate regional variation of T2 values and the effect of T2 correction on HFF (TE=0) regional variation. Nevertheless, since no correlation was found between T2 and HFF for one of the two volumes, it likely that T2 will not show correlation with HFF in the second volume, and it is reasonable to expect that T2 correction in volume 2 will lead to HFF for TE=75 is about 3 times higher than HFF for TE=0 as for volume 1.

In conclusion, 1H MRS diagnosis and severity assessment of NAFLD in overweight and obese children and adolescents should take up to 12% regional differences in hepatic fat fractions into account.

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