Applications of magnetic resonance spectroscopy for noninvasive assessment of hepatic steatosis
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Chapter 9: Summary and implications

SUMMARY AND IMPLICATIONS
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SUMMARY

In this thesis several aspects of the application of $^1$H-MRS (primarily at 3T) for noninvasive assessment of hepatic steatosis were studied.

In chapter 1 a general introduction and outline of this thesis is provided.

In chapter 2 the available literature on the diagnostic accuracy of US, CT, MRI and $^1$H-MRS for the evaluation of hepatic steatosis is summarized. Forty-six articles were included. Mean sensitivity estimates are 73.3–90.5% for US, 46.1–72.0% for CT, 82.0–97.4% for MRI and 72.7–88.5% for $^1$H-MRS. Mean specificity ranges are 69.6–85.2% (US), 88.1–94.6% (CT), 76.1–95.3% (MRI) and 92.0–95.7% ($^1$H-MRS). Our results show that MRI and $^1$H-MRS perform better than US and CT over the total range of cut-off values that were analyzed.

When evaluating a new technique for noninvasive assessment of hepatic steatosis for clinical use, reproducibility of the studied technique is relevant. In chapter 3 the reproducibility of $^1$H-MRS for measuring hepatic fat content is studied and $^1$H-MRS showed to be very reproducible. We report a coefficient of variation of between weeks reproducibility of 9.5% and an intra class correlation coefficient of 0.998. The coefficient of variation in fatty livers is 4.1% with an intra class correlation coefficient 0.997. Within day coefficient of variation is 4.5% with an intra class correlation coefficient 0.999. The coefficient of variation for within liver variability is 14.5%.

In chapter 4 we evaluate fully paired measurements of four different hepatic steatosis imaging techniques with histopathological confirmation in patients undergoing liver resection. We demonstrate that both MRI and $^1$H-MRS show stronger correlation (r=0.85, p<0.001 for MRI and r=0.86, p<0.001 for $^1$H-MRS) with the reference standard than US (r=0.66, p<0.001) and CT (r=-0.55, p<0.001) and yield superior diagnostic accuracy (91% for MRI and 89% for $^1$H-MRS) than US and CT (71% for US and 72% for CT). Both MRI and $^1$H-MRS could assess hepatic steatosis with good sensitivity (90% for MRI and 91% for $^1$H-MRS) and specificity (91% for MRI and 87% for $^1$H-MRS). CT and US are less suitable for the quantitative assessment of hepatic steatosis; sensitivity, specificity and likelihood ratios show insufficient diagnostic performance. MRI and $^1$H-MRS correlate with histopathological steatosis grades: none vs. mild (p=0.001, p=0.001), mild vs. moderate (p<0.001, p<0.001) and moderate vs. severe steatosis (p=0.04, p=0.01). Therefore we conclude that MRI and $^1$H-MRS are more accurate for diagnosis and assessment of liver steatosis than US and CT in patients undergoing liver resection.
In the study reported in chapter 5, the assessment of hepatic steatosis in morbidly obese patients before and after laparoscopic Roux-en-Y gastric bypass surgery using open magnet $^1$H-MRS is investigated with histopathological comparison from intra-operative laparoscopic liver biopsies. It is shown that, with an open clinical 1T MR scanner, hepatic steatosis can be accurately measured with $^1$H-MRS in morbidly obese patients. The accuracy of $^1$H-MRS is 89%: sensitivity is 85% and specificity is 94%. $^1$H-MRS significantly correlates with histopathological assessment of hepatic steatosis ($r=0.85$, $p<0.001$). After laparoscopic Roux-en-Y gastric bypass surgery patients show a significant decrease in hepatic steatosis. Three months after surgery, median steatosis decreased from 5.8% to 3.1% ($p<0.001$). The prevalence of hepatic steatosis measured by $^1$H-MRS decreased from 53% to 32%. Patients show significant improvements in relevant clinical parameters associated with hepatic steatosis. Also $^1$H-MRS at 1T is able to discriminate none from mild steatosis ($p=0.011$), mild from moderate steatosis ($p<0.001$) and moderate from severe steatosis ($p<0.001$).

In chapter 6 $^1$H-MRS in a hepatic steatosis model in rats is investigated using a clinical 3.0T MR scanner. A significant correlation is found between $^1$H-MRS and macrovesicular steatosis at histopathology ($r=0.93$, $p<0.001$) and between $^1$H-MRS and total hepatic fatty acids ($r=0.94$, $p<0.001$) at biochemical analysis (gas chromatography). We demonstrate that $^1$H-MRS is capable of discriminating between clinically relevant degrees of steatosis (i.e., mild, moderate, and severe). It is also shown that the development of steatosis in rat livers occurred at the expense of hepatic water content. Consequently, the most frequently employed $^1$H-MRS methods to calculate hepatic fat content are re-evaluated.

In chapter 7 we show that 3.0T $^1$H-MRS in rats is able to noninvasively detect total unsaturated (rTUFA) and polyunsaturated (rPUFA) fatty acids irrespectively from the total hepatic fat content. These measurements strongly correlate with biochemical data from gas chromatography. During diet periods, hepatic steatosis at histopathology significantly increases ($p<0.001$). Total unsaturated fatty acids (TUFA) estimated with $^1$H-MRS strongly correlated with the biochemical unsaturated fatty acids ($r=0.90$, $p<0.001$). Polyunsaturated fatty acids (PUFA) estimated with $^1$H-MRS strongly correlated with biochemical polyunsaturated fatty acids ($r=0.91$, $p<0.001$). The proportion of total unsaturated fatty acids relative to the amount of total fatty acids (rTUFA) measured with $^1$H-MRS strongly correlated with the biochemical amount of unsaturated relative to total hepatic fatty acids ($r=0.81$, $p<0.001$). The proportion of polyunsaturated fatty acids relative to the amount of total fatty acids (rPUFA) measured with $^1$H-MRS correlated with the biochemical amount of polyunsaturated fatty acids relative to total fatty acids ($r=0.59$, $p=0.005$),
and with the biochemical amount of omega-6 polyunsaturated fatty acids relative to total fatty acids ($r=0.73$, $p<0.001$). PUFA at $^1$H-MRS correlated with the histopathologically assessed degree of lobular inflammation in the liver ($r=0.57$, $p=0.001$).

In chapter 8, we investigate hepatic unsaturated fatty acids with 3.0T $^1$H-MRS in diabetic and non-diabetic patients with NAFLD and its correlation with clinical and metabolic parameters. In these patients hepatic unsaturated fatty acids correlate with AST/ALT ratio ($r=-0.46$, $p=0.02$), glucose levels ($r=0.46$, $p=0.018$), insulin resistance (HOMA-IR) ($r=0.59$, $p=0.004$) and hepatic fat content ($r=0.81$, $p<0.001$). In diabetic patients ($n=12$) hepatic unsaturated fatty acids correlate with alkaline phosphatase levels ($r=0.72$, $p=0.01$), insulin resistance (HOMA-IR) ($r=0.73$, $p=0.01$) and total hepatic fat content ($r=0.83$, $p=0.002$). Compared to non-diabetic patients with NAFLD, hepatic unsaturated fatty acids levels are increased in patients with DM2 and NAFLD ($p=0.03$).

**IMPLICATIONS AND FUTURE RESEARCH**

Our results show that MRI and $^1$H-MRS perform better than US and CT for evaluating hepatic steatosis over the total range of cut-off values that are analyzed. The findings also show that MRI and $^1$H-MRS perform better than US and CT for detecting separate disease grades, especially for mild disease ($<30\%$ steatosis). This is of value in clinical practice when an accurate estimation of the amount of hepatic steatosis is needed, for example in patients undergoing liver resection or in potential liver donors to determine the condition of the future liver graft. In living donor liver transplantation procedures, moderate and severe macrovesicular steatosis are considered exclusion criteria. Also, given the relationship between the growing obesity epidemic and the prevalence of NAFLD/NASH in both adults and children, there is an increasing need for noninvasive monitoring of those patients who are at risk. We noted that $^1$H-MRS is increasingly used as the reference standard instead of liver biopsy and that the sequences used show great variability. We therefore recommend that the role of $^1$H-MRS as the reference standard for quantifying hepatic steatosis needs to be further substantiated. Also, we recommend standardisation in many aspects of the research on MRI and $^1$H-MRS for hepatic steatosis, including the MR sequences and histopathological classification used.

Assessment of reproducibility in time is essential to detect abnormal variation in longitudinal studies. 3.0 Tesla $^1$H-MRS for the measurement of hepatic fat content is highly reproducible in a spectrum varying from low to high hepatic fat content and is comparable with reproducibility of $^1$H-MRS at 1.5 Tesla. Our data can be used for monitoring hepatic steatosis in individual patients and for calculating sample sizes in intervention trials.
However, it would be interesting to investigate in future research how fast hepatic fat content responds to dietary changes. Due to heterogeneity of hepatic fat content voxel positions are relevant when performing $^1$H-MRS. Our results indicate a difference in hepatic fat content in different parts of the liver (coefficient of variations of 14.5\%). In performing repeated $^1$H-MRS measurements of hepatic fat content one should be aware of this and should perform measurements in the same voxel positions in the liver. During the projects included in this thesis our experience with $^1$H-MRS increased. Therefore, we would like to point out some suggestions for future research in relation with $^1$H-MRS in general. We did correct our $^1$H-MRS data for T2 relaxation, but only in a fixed number of patients and animals or by using T2-values reported in literature. This ignores the variability in T2 relaxation values between individual patients or animals. Since T2 differences can be present between patients and animals, more accurate $^1$H-MRS measurements can be performed when $^1$H-MRS data is corrected for T2 relaxation in every single patients or animal. Increasing evidence suggests that the use of a STEAM sequence is more accurate and reliable than a PRESS sequence, mainly because of the decreased T2-weighting in STEAM acquisitions. We used a PRESS sequence which can be confounded by J-coupling effects. For future research, a STEAM sequence is recommended. Furthermore, in our studies $^1$H-MRS was performed during free breathing. This comprises a potential limitation because the volume interrogated by $^1$H-MRS is blurred in the longitudinal direction by respiratory excursions of the liver. Although more time consuming, future research could implement respiratory gating to minimize motion artifacts and further increase quality of $^1$H-MR spectra. To assess heterogeneity of hepatic fat one should ideally perform multiple voxel measurements or spectroscopic 2D imaging of the whole liver. However, this would be time consuming and poses higher demands on the shimming of the volume of interest making it unpractical in clinical practice at this moment. For future research spectroscopic 2D imaging of the whole liver could be of value. Although spectroscopic 2D imaging undoubtedly has advantages, one should be aware that, in perspective of heterogeneity, the amount of liver tissue analyzed for hepatic fat content in single voxel $^1$H-MRS is approximately 400 times larger than the amount of liver tissue examined by biopsy. Furthermore, because the assumption that water content in the liver is constant and can be used as an internal reference standard to calculate hepatic fat content does not hold true, our recommendation is to abolish this $^1$H-MRS calculation method. For future research we recommend the method that incorporates all the fat peaks in $^1$H-MR spectra instead of only the total fatty acid peak at 1.3 ppm. This method takes into account possible variations in unsaturated fatty acids in patients with steatosis.
Chapter 9: Summary and implications

Given the increasing prevalence of obesity with subsequently an increasing prevalence of NAFLD, the use of open MR scanners is an attractive solution to measure hepatic fat content with $^1$H-MRS in the (morbidly) obese population. The assessment of hepatic fat content with $^1$H-MRS in (morbidly) obese patients is feasible in an open MR scanner at 1.0 Tesla and should be considered in morbidly obese patients that do not fit in standard cylindrical MR scanners. The use of standard cylindrical MR scanners is limited by an aperture diameter of 60 cm and in some 3.0T scanners even 55 cm. The aperture is decreased by the thickness of the table and coils, leading to considerably reduced maximum patient circumference in standard cylindrical MR scanners. However, recently large bore (70 cm) MR scanners became available for clinical use, allowing more obese patient to fit in cylindrical MR scanners and offering higher magnetic field strengths. Polyunsaturated fatty acids detected by $^1$H-MRS are associated with lobular inflammation of the liver. We showed that the assessment of total unsaturated fatty acids and polyunsaturated fatty acids at $^1$H-MRS is feasible on a clinical 3.0T MR scanner and that results are in agreement with biochemical data obtained from gas chromatography. The comparison between the assessment of hepatic lipid composition with $^1$H-MRS and biochemical control provides more insight in noninvasive assessment of the different $^1$H-MRS signal resonances in the liver. We showed that omega-6 polyunsaturated fatty acids increase more than omega-3 polyunsaturated fatty acids, resulting in a significantly increased omega-6/omega-3 ratio. Omega-6 polyunsaturated fatty acids are pro-inflammatory and could play a role in the development of NAFLD towards more severe nonalcoholic steatohepatitis (NASH). Hepatic polyunsaturated fatty acids could be a biomarker in this process because NASH induced in animal models show increased polyunsaturated fatty acids and this parameter is also positively correlated to the amount of lobular inflammation on histopathology.

Our experimental animal study provides evidence for the use of 3.0T $^1$H-MRS as a noninvasive diagnostic tool to assess hepatic lipid composition. In future research more knowledge about hepatic lipid composition could provide more insight in the partly unexplained pathophysiologic pathways in NAFLD/NASH.

Using $^1$H-MRS correlations between hepatic unsaturated fatty acids and clinical and metabolic parameters associated with NAFLD/NASH could be demonstrated, e.g. HOMA-IR, serum AST/ALT-ratio and total hepatic fat content. In patients with type 2 diabetes, hepatic unsaturated fatty acids are significantly higher than in patients without type 2 diabetes, whereas for total hepatic fat content no differences were detected. It is known that the presence of NASH is more frequent in patients with type 2 diabetes.
Hypothetically, this increase in unsaturated fatty acids in patients with type 2 diabetes may be attributed to a decreased omega-3/omega-6 ratio with a substantial hepatic accumulation of omega-6 polyunsaturated fatty acids. In the steatotic liver hepatic fat content is sufficiently high enough for additional resonances like unsaturated fatty acids at 5.4 ppm to become detectable. Our results show that unsaturated fatty acids were also detectable in human livers at 3.0 Tesla. In future research hepatic unsaturated fatty acids could be a predictive parameter to discriminate simple steatosis from NASH. Both our experimental animal study as well as our human study provide evidence for the use of 3.0T $^1$H-MRS as a noninvasive diagnostic tool to assess unsaturated fatty acids in vivo in human livers in future research.

**THIS THESIS DEMONSTRATES THAT:**

1) In contrast to US and CT, MRI and $^1$H-MRS strongly correlate with histopathological steatosis assessment and are able to show differences across steatosis grades. MRI and $^1$H-MRS show best diagnostic accuracy in detecting hepatic steatosis. MRI and $^1$H-MRS can be considered as the techniques of choice for accurate evaluation of hepatic steatosis.

2) Reproducibility of $^1$H-MRS to measure hepatic fat content is high. Within the liver parenchyma, variability is considerable, meaning that liver fat is not equally distributed and during consecutive measurements the same voxel position should be used.

3) $^1$H-MRS sequences and calculations need to be standardized before $^1$H-MRS can be used as the standard imaging technique for quantification of hepatic steatosis.

4) 3.0T $^1$H-MRS is able to noninvasively measure (poly)unsaturated fatty acids in the liver and these measurements strongly correlate with biochemical assessment. This provides evidence for the use of noninvasive $^1$H-MRS to assess hepatic unsaturated fatty acids in vivo.

5) $^1$H-MRS for assessment of hepatic steatosis and changes in steatosis after gastric bypass surgery is feasible in morbidly obese patients using an open 1.0T MR scanner. $^1$H-MRS measurements are accurate and correlate with histopathology results.