Magnetic resonance spectroscopy to unravel metabolic alterations in hepatic steatosis in humans

SUMMARY

The prevalence of non-alcoholic fatty liver (NAFL/hepatic steatosis) is increasing rapidly worldwide and predominantly observed in obese/overweight populations. NAFL can progress into liver inflammation (non-alcoholic steatohepatitis, NASH) and end stage of all chronic liver diseases including cirrhosis and hepatocellular carcinoma (HCC). Moreover, NAFL has been shown to be strongly associated with insulin resistance and is a major risk factor for the development of diabetes type II and cardiovascular diseases (CVD). Despite the known negative health effects of NAFL, understanding primary metabolic abnormalities that drive hepatic fat accretion are still far from understood in humans and mainly rely on data obtained from animal studies. This is mainly due to lack of reliable and quantitative non-invasive methodologies to study the metabolic pathways involved in NAFL in humans.

There is increasing interest in applying *in vivo* magnetic resonance spectroscopy (MRS) in metabolic research, as it is a safe technique, and it provides an opportunity to study metabolism non-invasively. Furthermore, a major advantage of MRS is that metabolism can be studied quantitively and dynamically. However, the potential of *in vivo* MRS has not been fully utilized yet. In this thesis, I have focussed on the development of new MRS protocols to study hepatic lipid metabolism in humans and successfully applied those MR protocols to show clinical applications in metabolic research focused on improving our understanding of the origins of hepatic steatosis.

^{1}H-MRS is widely used to measure IHL content, and it has been well demonstrated in earlier studies that absolute estimation of IHL content is possible with ^{1}H-MRS. However, there are variations among different research sites as to which fat quantification formulas are used to calculate fat percentage in absolute terms. All calculations use the comparison of the lipid and water signals however, they are based on ratios of CH_2/H_2O, $CH_2/ (CH_2+H_2O)$, or on the sum of all lipid peaks/total MR signal. Also, the method of quantification (integration vs peak fitting) can vary and the type of MR sequence that is used (mainly STEAM or PRESS) can differ. Also, different T_2 correction strategies (individual vs average T_2 correction) are used. All of these differences can ultimately lead to substantial bias in the calculated IHL%. The impact that such differences have on the determination of the IHL% was investigated in **chapter 2.** This is important as differences in calculations results variations in the numerical values of fat content, while generally, the cut-off value for hepatic steatosis is set to a fixed value (5.6%). Therefore, we reviewed all the steps that are needed to determine absolute IHL%

as a weight percentage (w/w) and evaluated the bias that occurs in the calculations of IHL% when using different formulas, incorrect T_2 correction and two standard MR sequences (STEAM vs PRESS). When calculating absolute IHL% (w/w), several assumptions are made, for example in terms of typical fatty acid density and composition and liver tissue density. Interestingly, we demonstrated that simple ratio of CH_2/CH_2+H_2O yielded very similar numerical values as to the calculation of w/w% and $CH_2/(CH_2+H_2O)$ can therefore be used as a solid way to approximate w/w%. To have reliable reference values, we also determined T_2 relaxation times in a large group of volunteers and interestingly, we observed that water T_2 is reduced and lipid T_2 is increased with increasing severity of NAFL. Therefore, with fixed T_2 correction strategy, the difference in IHL% is overestimated when two groups differing strongly in liver fat content are compared.

Next to determining the accurate estimation of absolute IHL%, it is important to understand the primary metabolic pathways that drive excessive hepatic fat accumulation. De novo lipogenesis (DNL) may be an important player in this. As the end products of DNL are saturated fatty acids, determining lipid composition, specifically measuring saturated fatty acid (SFA) fraction in the liver may be an indirect measure of DNL. Therefore, we developed, validated, and successfully applied a new ^{1}H-MRS protocol based on post-processing approach that enabled us to determine the hepatic fractions of SFA, mono- and poly unsaturated fatty acid fractions (MUFA and PUFA) separately, which is described in **chapter 3** of this thesis. Our newly developed ^{1}H-MRS methodology uses appropriate prior knowledge obtained from high-resolution ^{1}H-NMR data of oil phantoms, and the overlapping allylic- and alpha-carbonyl signals were dissociated accordingly to determine lipid composition. This approach was validated in five different vegetable oil phantoms by comparing the measured to the known composition. Additionally, we validated our approach *in vivo* by measuring the lipid composition in subcutaneous adipose tissue in eight volunteers and compared it to the analysis by mass spectrometry in corresponding biopsies. Finally, we demonstrated the *in vivo* feasibility and reproducibility of our developed approach in the liver of seven healthy volunteers with varying liver fat content. Interestingly, we showed that hepatic SFA fraction as measured by ^{1}H-MRS was positively correlated with DNL (measured by deuterated water) confirming that high rates of DNL will result in higher amount of SFA in the liver. Thus, we developed a new ^{1}H-MRS methodology to determine lipid composition, specifically measuring SFA fraction in the liver can provide an indirect measure of DNL in humans.

While the newly developed ^{1}H-MRS protocol can be used as an indirect measure of the contribution of DNL to IHL over a longer period of time, it is not possible to acutely determine DNL, for example from a meal. Such measurements are usually performed by using stable isotope tracers and in principle the conversion of ^{13}C labeled glucose could be monitored by determining ^{13}C lipid signals in the liver. Although our previously developed indirect ^{13}C MRS (ge-HSQC, based on quantum-coherence) can in principle be monitor the abundance of ^{13}C labeled hepatic lipids, this method has insufficient sensitivity for such experiments. Therefore, in **chapter 4**, we optimized a J-difference editing (JDE) method, which inherently yields a two-fold higher signal as compared to the previously applied method of geHSQC. With the application of BIlinear Rotation Decoupling (BIRD) filter the subtraction artifact that hampers the application of such sequences in the liver was efficiently suppressed and the small signal of natural abundance (1.1%) of ^{13}C lipid signal could be detected, even at low fat contents. However, even though sensitivity was strongly improved, it is yet unclear whether DNL can be directly determined in humans using indirect ^{13}C MRS.

In stimulating DNL, fructose has been suggested to play an especially detrimental role in liver metabolic health. While there is growing evidence from animal studies on the prominent role of fructose in IHL accumulation, such experimental human data is very limited. Therefore, in **chapter 5,** we investigated the effects of fructose restriction on IHL content in obese/ overweight volunteers in a double-blind randomized study. Indeed, six week of fructose restriction leads to a small but significant reduction in IHL content, indicating a beneficial influence of fructose restriction on NAFL.

Next to lipids and water, also other metabolites, such as choline have resonances in the ^{1}H-MR spectrum. Choline is an interesting metabolite, as choline-deficient diets are commonly used to induce NAFL in animal models, suggesting that disturbances in choline metabolism may promote the development of NAFL. However, the choline availability in the liver of NAFL population has not yet been explored in humans. The reason is that for most ^{1}H-MRS investigations, protocols are optimized for lipid determination and generally no attention is paid to the small choline resonances, which are often affected by water suppression. Also, as choline concentrations are low, long acquisition times and dedicated post-processing and fitting is important in order to quantify the choline peaks in question. In **chapter 6**, we were able to quantify the hepatic choline content accurately in addition to lipid composition by applying our newly developed ^{1}H-MRS post-processing tool in existing ^{1}H-MRS data of obese/overweight

volunteers**.** Interestingly, we found that low choline status is associated with high IHL content, which may point at an involvement of choline metabolism in the development of NAFL in humans. Future interventional studies will have to investigate whether normalization of choline levels is beneficial to liver metabolic health.

In conclusion, we have shown that new MR protocols yield valuable information on metabolic pathways in humans. In addition, we have shown the applicability of our newly developed MRS protocols in humans to perform metabolic research towards understanding hepatic steatosis.