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

Impact of the thesis

IMPACT

The liver plays a central role in maintaining metabolic homeostasis by regulating constant energy supply to the body through metabolism of nutrients (carbohydrates and lipids). When excess amount of fat builds up in the liver, this increases the risk for developing severe chronic liver diseases and metabolic complications including diabetes type II (T2DM) and cardiovascular diseases (CVD). Quite strikingly, 20 to 30% of the general population in Western countries have excessive storage of fat in the liver (in absence of high alcohol intake), a condition referred to as non-alcoholic fatty liver (NAFL)(1). As the obesity epidemic increases, the prevalence of NAFL increases worldwide (2). To date, our understanding of the etiology of NAFL is still limited, due to the lack of appropriate non-invasive techniques that would help us to understand the mechanisms/metabolic pathways leading to NAFL.

Magnetic Resonance Spectroscopy (MRS) provides biochemical information on cellular components and is considered a powerful tool to study metabolism *in vivo* in a non-invasive manner. Due to its safety (it does not rely on ionizing radiation) and due to the fact that dynamic information can be gained, MRS is extensively applied in experimental research settings to study hepatic lipid metabolism (3–6), however, the potential of *in vivo* MRS is not yet fully utilized. In this thesis, we have shown the potential of MRS in detecting metabolic pathways (i.e., DNL) in the liver as well as to characterize the metabolic abnormalities associated with hepatic steatosis. The interpretation of the MR derived intrahepatic lipid (IHL) % is discussed in detail in **chapter 2** and recommendations are put forward on the usage of different fat quantification formulas, to express IHL%. We advocate to express the data in a more standardized way that is directly comparable to weight/ weight %. The results described in **chapter 2** will be of great interest to a broad public including hepatologists and clinicians to better understand the exact meaning of intrahepatic lipid (IHL) %. Currently, various ways of expressing IHL are used, and it is often unclear how it is derived from the MRS signals of fat and water. This information will eventually help us (especially for clinicians) to define NAFL with known cut-off value of 5.6% and to compare data from different research sites. In addition, the observed dependency of T_2 relaxation times on the level of steatosis is important to be taken into account and therefore, it will help us to better characterize the pathophysiology of NAFLD in humans.

The innovative, non-invasive MRS protocols developed in the PhD project will be very useful overall to increase our understanding of hepatic lipid metabolism in humans, especially the

metabolic pathways (e.g., DNL) associated with NAFL. In **chapter 3,** we have shown for first time that not only total IHL content, but also hepatic lipid composition, specifically the SFA fraction can be a novel target for NAFL as the SFA fraction seems to be especially strongly related to metabolic disease in humans. We have shown that the quantification of hepatic lipid composition is feasible with our newly developed post-processing tool using the same hepatic lipid spectra as acquired usually for IHL measurement. Therefore, future interventional studies with ^{1}H-MRS can always quantify hepatic lipid composition (without any extra requirements) next to total IHL content. Indeed, the developed MRS protocols shown in **chapter3,** is already applied longitudinally in different on-going clinical studies at our department to study the efficacy of different interventions (diet, therapeutic drugs) or to test a physiological challenge (e.g., exercise, fructose) to target the metabolic pathways of DNL towards aiming treatment strategies for NAFL.

Moreover, the developed MRS protocols in this PhD project will also be interesting for pharmaceutical companies, as it provides an opportunity to test and develop new drugs in targeting SFA specifically for therapeutic purposes. Therefore, I anticipate that funding opportunities from industry will increase, who aim to implement new MR tools in clinical trials. Although our aim is to apply new MRS techniques to get more insight into hepatic lipid metabolism, the application of these MR techniques will not be limited to this particular topic and/or specific tissue. For example, the developed MRS protocol in **chapter 3** is not only used to measure hepatic lipid composition, but it could also be applied in different tissues such as adipose tissue and visceral fat or potentially also muscle. Indeed, we already demonstrated the *in vivo* feasibility of our newly developed ^{1}H-MRS tool to measure adipose tissue lipid composition and showed high correlation with biopsies, for a validation purpose. Moreover, it is known that PUFA content is implicated in breast cancer patients (7,8), so future studies may want to investigate whether our newly developed ^{1}H-MRS protocol might be applied in breast cancer patients to estimate PUFA content. Moreover, lipid composition have been implicated in regulating normal brain function (9,10) and cardiovascular diseases (11). Interestingly, our developed ^{1}H-MRS protocol can be applied in large cohort populations to determine hepatic lipid composition as it does not require any extra cost and modifications in the implementation process.

In addition, the newly developed indirect ^{13}C MRS (described in **chapter 4**) can provide valuable mechanistic insight towards understanding hepatic metabolism in more detail by

identifying the contribution of different metabolic pathways in the development of NAFL. Although this method is mainly developed to track ^{13}C labeled hepatic lipids, it can be used for other ^{13}C tracking applications. For instance, the food industries can benefit from the development of such MR tools as it provides an opportunity to study metabolism non-invasively that allows us to track specific ^{13}C incorporation of labeled target metabolites from the exogenous consumption of different ^{13}C labeled substrates. Moreover, the newly developed indirect ^{13}C MRS could be used to measure metabolic fluxes, therefore the exact contribution of different metabolic pathways can be estimated in the development of NAFL. For e.g., Future studies can use our newly developed indirect ^{13}C MR method to evaluate the dietary contribution of different sugar molecules (glucose or fructose) or specific fatty acids in the development of NAFL. While the proposed ^{1}H-MRS methodology to determine lipid composition is straightforward and easy to implement in any research site, the use of indirect ^{13}C MRS will be quite challenging. This is mainly due to the fact that requirement of an additional software and hardware (such as $^{1}H/^{13}C$ double tuned surface coil), use of expensive ^{13}C labeled substrates, sophisticated spectral editing MR sequence and extensive post-processing approach. However, efforts in developing such new MR protocols and improvements in MRS methodologies will increase the potential role of *in vivo* MRS in metabolic research. This is illustrated in this thesis by showing that the ^{1}H-MRS methodology can be employed to determine hepatic lipid composition and improved sensitivity of our indirect ^{13}C-MRS can be used for ^{13}C tracking applications in the liver. Furthermore, the developed MRS protocols at clinical field strength (3T) (rather than using high field MR scanner) in the projects of my PhD thesis will allow us to apply the new protocols directly in many research sites without any major restrictions, as 3T is commonly present in many hospitals and research institutions.

Besides developing new MR tools, the application of *in vivo* MRS in human interventional research is also shown in **chapter 5** by performing a randomized control trail to evaluate the effects of fructose restriction in liver steatosis. The observed beneficial effect of fructose restriction in lowering IHL content can be explored in more detail in future studies by measuring hepatic lipid composition, specifically targeting DNL and to check whether SFA is altered with fructose restriction. If indeed fructose is shown to induce DNL in humans (as is the case in animal models), this is highly relevant to a broad public as the high consumption of sugar sweetened beverages in our daily life may play a significant role in the development of NAFL.

We observed a negative relationship between choline and IHL content in **chapter 6,** indicating a low choline status in the overweight/ obese population, which is reported for first time in this thesis. This may suggest that correcting such low choline availability may beneficially influence metabolic health. I hope this finding will increase the interest among researchers and hepatologist to explore choline metabolism in more detail and investigate potential treatment strategies for NAFL by targeting choline status. In addition, altered choline metabolism has been implicated in several neurological disorders and tumours in different organs including brain (12), liver (13,14) and breast (15), although in cancer, choline tissue content is often elevated (12,14–17). It would be interesting to investigate whether our developed post-processing approach can be applied for MRS in breast in future studies to quantify choline content in breast cancer patients.

We expect that the role of MRS will continue to grow in metabolic research. The MR protocols described in this thesis were directly applied to humans in showing clinical relevance and we believe that the proposed MR methodology will create a paradigm for future clinical applications in the human liver, to uncover metabolic information that is currently invisible.

In addition, the results and knowledge obtained from the studies performed in this thesis had been presented in several national and international scientific conferences via oral or poster presentations. This not only helped me to improve my communication and presentation skills but also improved the awareness among scientific community to realize the potential role of MRS in metabolic research. Finally, the results presented in the current thesis are or will be published as original scientific research articles with open access in peer reviewed journals, which will be assessed by scientists worldwide. This will enhance visibility of our research group as well as sharing the advances in the MRS field in translational metabolic research among the scientific community. Thus, the knowledge obtained through this research can directly be used/shared with other researchers worldwide and the MRS protocols developed in this PhD thesis can also be applied in multiple future research studies within the department or in making national/ international collaboration with other institutes. Last but not least, the experience and knowledge that I gained through my PhD will provide potential opportunities for me to achieve major personal grants in the near future and to improve myself towards fulfilling my personal scientific goals in the path of becoming an independent successful MR researcher.

REFERENCES

1. Loomba R, Sanyal AJ. The global NAFLD epidemic. Nat Rev Gastroenterol Hepatol. 2013;10(11):686–90.

2. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. Hepatol Baltim Md. 2016 Jul;64(1):73–84.

3. Szczepaniak LS, Nurenberg P, Leonard D, Browning JD, Reingold JS, Grundy S, et al. Magnetic resonance spectroscopy to measure hepatic triglyceride content: prevalence of hepatic steatosis in the general population. Am J Physiol Endocrinol Metab. 2005 Feb;288(2):E462-468.

4. Springer F, Machann J, Claussen CD, Schick F, Schwenzer NF. Liver fat content determined by magnetic resonance imaging and spectroscopy. World J Gastroenterol. 2010 Apr 7;16(13):1560–6.

5. Longo R, Pollesello P, Ricci C, Masutti F, Kvam BJ, Bercich L, et al. Proton MR spectroscopy in quantitative in vivo determination of fat content in human liver steatosis. J Magn Reson Imaging JMRI. 1995 Jun;5(3):281–5.

6. Thomsen C, Becker U, Winkler K, Christoffersen P, Jensen M, Henriksen O. Quantification of liver fat using magnetic resonance spectroscopy. Magn Reson Imaging. 1994;12(3):487–95.

7. Bartsch H, Nair J, Owen RW. Dietary polyunsaturated fatty acids and cancers of the breast and colorectum: emerging evidence for their role as risk modifiers. Carcinogenesis. 1999 Dec;20(12):2209–18.

8. Azrad M, Turgeon C, Demark-Wahnefried W. Current evidence linking polyunsaturated Fatty acids with cancer risk and progression. Front Oncol. 2013 Sep 4;3:224.

9. Kaplan RJ, Greenwood CE. Dietary saturated fatty acids and brain function. Neurochem Res. 1998 May;23(5):615–26.

10. Bazinet RP, Layé S. Polyunsaturated fatty acids and their metabolites in brain function and disease. Nat Rev Neurosci. 2014 Dec;15(12):771–85.

11. Briggs MA, Petersen KS, Kris-Etherton PM. Saturated Fatty Acids and Cardiovascular Disease: Replacements for Saturated Fat to Reduce Cardiovascular Risk. Healthcare [Internet]. 2017 Jun 21 [cited 2021 Jan 19];5(2). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5492032/

12. Huang BY, Kwock L, Castillo M, Smith JK. Association of choline levels and tumor perfusion in brain metastases assessed with proton MR spectroscopy and dynamic susceptibility contrast-enhanced perfusion weighted MRI. Technol Cancer Res Treat. 2010 Aug;9(4):327–37.

13. Li C-W, Kuo YC, Chen C-Y, Kuo Y-T, Chiu Y-Y, She F-O, et al. Quantification of choline compounds in human hepatic tumors by proton MR spectroscopy at 3 T. Magn Reson Med. 2005 Apr;53(4):770–6.

14. Ter Voert EEGW, Heijmen L, van Asten JJA, Wright AJ, Nagtegaal ID, Punt CJA, et al. Levels of choline-containing compounds in normal liver and liver metastases of colorectal cancer as recorded by 1 H MRS. NMR Biomed. 2019 Jan;32(1):e4035.

15. Chen J-H, Mehta RS, Baek H-M, Nie K, Liu H, Lin MQ, et al. Clinical characteristics and biomarkers of breast cancer associated with choline concentration measured by 1H MRS. NMR Biomed. 2011 Apr;24(3):316–24.

16. Kobus T, Wright AJ, Weiland E, Heerschap A, Scheenen TWJ. Metabolite ratios in 1H MR spectroscopic imaging of the prostate. Magn Reson Med. 2015;73(1):1–12.

17. Glunde K, Bhujwalla ZM, Ronen SM. Choline metabolism in malignant transformation. Nat Rev Cancer. 2011 Dec;11(12):835–48.