Lipid characterization of different organs using HR-MAS NMR spinning speed variation.

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Purpose: Lipids in biological tissues are stored in adipocytes, in lipid droplets, or confined to membranes. ^{1}H NMR spectroscopy can assess mobile lipids (ML) from different tissues [1]. However, the origin of lipid signals in ^{1}H spectra from different cell types is still subject of debate [2]. The lipid composition depends on the tissue type [3]. Recently we reported on differences in the visibility of mobile lipids in non-neoplastic brain and muscle tissue biopsies, in dependence of the spinning rate in a magic angle spinning (HR-MAS) NMR study [4]. The spinning speed variation showed a significant impact on lipid visibility from non-neoplastic brain tissue, while only a mild effect was observed in muscle tissue biopsies. The interpretation of the differences, whether it was due to lipids in membrane microdomains in brain tissue or due to size differences of intracellular lipid droplets between brain and muscle tissue was however speculative.

The purpose of our current study was to better understand the origin of the visible lipids and the cause of this lipid visibility and mobility differences, by investigating the effect of spinning speed variation in HR-MAS NMR I) in a range of different organs, comprising liver, cardiac muscle, breast, kidney cortex and medulla and visceral and subcutaneous fat biopsies, and II) in pure white (WM) and grey matter (GM) accompanied by histological visualization of lipids by fluorescence microscopy using Nile Red staining.

Methods: Sheep biopsies were collected from visceral and subcutaneous fat, liver, breast, cardiac muscle, renal cortical and medullary tissue, and from pure WM and GM from corona radiata and parietal cortex from 3 healthy sheep, collected within 1h post-mortem. Each biopsy was immediately snap-frozen in liquid nitrogen and subsequently stored at -80°C. Biopsies were placed in a 50 µl MAS rotor and D_2O-based phosphate-buffered saline (PBS) was added. A 500MHz Bruker Avance II spectrometer was used for the 1D ^{1}H HR-MAS NMR experiments. A 1D Noesy sequence with water presaturation (*noesypr1d*) was applied using spinning rates of 1.3, 1.7, 2.3, 4, 5, 6, 8 kHz at 290 K. For the brain biopsies, in order to be consistent with previous measurements, 1, 2, 4, 6, 8kHz were used as spinning speed. Topspin™ was used for spectra processing and integration of lipid resonance areas. The values were normalized to the area at the lowest spinning speed and the normalized integration of the same tissue types were averaged.

Frozen WM and GM biopsies were cut into 10 µm sections. Adjacent slides of each biopsy were used for Nile Red [5] and H&E staining, respectively.

Results: Increasing the spinning speed in HR-MAS measurements resulted for most investigated tissue types in an increase of lipid resonances. The signal increase was most pronounced for the methyl and methylene lipid peak at 0.9ppm and 1.3ppm, as visible in the renal medulla and cortex spectra (Fig.1 a, b) and the brain spectra (Fig.2a, b) while the effect was less for other lipid peaks. Comparing lipid resonances between pure GM and WM, the higher lipid visibility due to increased spinning speed was significantly stronger in WM (Fig.2a), than in GM (Fig.2b). Histology data of directly adjacent areas showed a very low amount of lipid droplets in GM (Fig.3C) and WM (Fig.3D) biopsies, under yellow fluorescence settings (Fig.3C,D). Red fluorescence settings (Fig.3E,F) demonstrate staining of membrane lipids of the myelin sheaths in WM biopsies (Fig.3F).

The lipid visibility increase was very different from one organ to another (Fig. 4) While the increase was very strong for WM, lipid signals from subcutaneous and visceral fat were almost not increased (Fig. 4 and Fig. 1c) Renal cortex showed a stronger increase than the medulla (Fig. 4 and Fig, 1a, b). The liver lipid visibility increase was intermediate between renal cortex and medulla. Heart lipid visibility was increased similarly to renal medulla, while the breast showed a very moderate increase only, close to the pure fat biopsies.

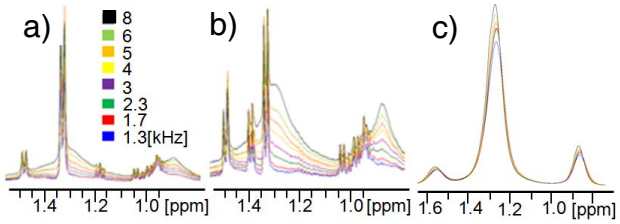

Fig.1 Spinning speed variation in a) renal medulla, b) renal cortex, c) visceral fat.

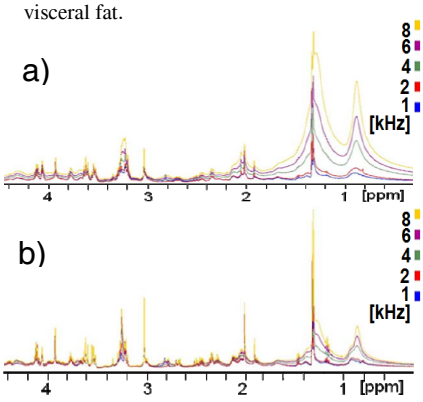

Fig.2 Spinning speed variation in a) WM, b) GM

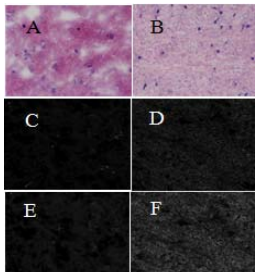

Fig3: Histology of GM (A,C, E) and WM (B,D,F) using H&E (A,B) and Nile Red (C to F) staining. Yellow fluorescence settings (C,D); Red fluorescence settings (E,F).

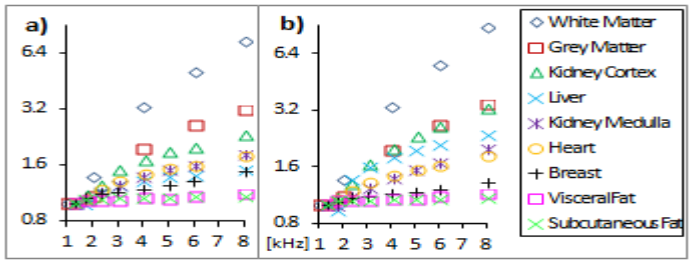

Fig.4 Increase of the lipid visibility with the spinning speed in different organs. a) methyl peak (0.9ppm), b) methylene peak (1.3ppm); log. scale on y-axis.

Discussion: Methyl and methylene groups of the lipid molecule show a higher mobility with increased spinning speed than other lipid groups, which could be explained by their location in the molecule. The lipid visibility depending on the spinning speed used is very different in the different organs analyzed.

In agreement with previous studies in rat brain [6] we detected only low amounts of lipid droplets in normal sheep brain tissue biopsies both from GM and WM by fluorescence microscopy. Therefore, the lipid resonances we detected in the brain biopsies cannot be attributed to lipid moieties located in lipid droplets. However, stronger lipid resonances and stronger increases with spinning speed were found in WM compared to grey matter. Even if the biochemical explanation remains undetermined, we assume that the observed phenomenon in absence of lipid droplets has to be linked to lipids of the myelin sheath.

The interpretation of the different lipid visibilities of the other investigated organs is challenging. As was previously shown for muscle tissue [4], the lipids of at least some lipid droplets appear to be mostly visible without spinning, as are lipids in adipocytes. Thus, if the increased visibility of lipids in various organs is due to lipids stored in lipid droplets, the differences may be explained by droplet differences, e.g. different sizes. The increased visibility may however also be due to lipids in membrane. Further measurements like diffusion scans are expected to help explaining the results.

Conclusion: The spinning speed significantly affects the NMR visibility of some lipids. Histology results support the hypothesis that lipids from myelinated membranes are NMR-visible at high spinning speed, while lipid droplets may be less affected by the spinning speed.

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References: 1.Hakumaki et al. Trends Biochem. Sci. 2000;25:357; 2 Wright et al. Eur. J. Biochem. 2003;270:2091. 3. Christie. J. Lipid Res. 1985;26:507. 4. Stahl et al. Proc.Intl.Soc.Magn.Reson.Med., 2014;22:2920. 5. Opstad et al. Magn Reson Med. 2008;60:1237. 6. Gasparovic et al. Neurosci. Lett. 2001;301:87.