The effect of spinning rate variation on lipid resonances in HR-MAS spectra of brain and muscle biopsies

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Purpose

HR-MAS NMR spectroscopy is an established technique to investigate small molecule metabolites of tissue biopsies. Lipid resonances from mobile lipids (ML) can be observed by ^{1}H NMR spectroscopy in multiple tissues[1], e.g in muscle, liver, neoplastic and non-neoplastic brain tissue, and also in cells.[2,3,4] In order to use lipid resonances as a marker for malignant or metabolic disease, a reference standard from healthy tissue has to be established taking the influence of variable factors like the spinning rate into account. It has been shown that the spinning rate affects the visualization of ML in cultured glioma cells[2] and glioma tissue biopsies.[3] To our knowledge, the spinning rate dependent variability of mobile lipids in non-neoplastic brain and muscle tissue biopsies has not been examined. Therefore, the purpose of our study was to investigate the effect of spinning rate variation on the HR-MAS pattern of lipid resonances in non-neoplastic brain and muscle biopsies.

Methods

Sheep brain biopsies (7.5-10.1mg) were collected from the brainstem and thalamus from 5 sheep free from brain malignancy (3 histopathologically normal and 2 with listeria brainstem encephalitis, no differences between the two groups detected) under general anesthesia using a minimally invasive stereotactic brain biopsy technique. In addition 1h post-mortem biopsies of three healthy sheep were also investigated. Human skeletal muscle (M. vastus lateralis) biopsies (0.23-11.8mg) were collected from 4 healthy volunteers using Bergstrom technique. Both brain and muscle biopsies were immediately snap-frozen in liquid nitrogen and subsequently stored at -80°C. Biopsies were placed in a 15 µl MAS rotor and D_2O-based phosphate-buffered saline (PBS) was added. ^{1}H HR-MAS NMR experiments with water presaturation applying the 1D noesy sequence (*noesypr1d*) of biopsies were performed on a Bruker Avance II spectrometer (500.13 MHz) using spinning rates of 1000, 2000, 4000, 6000 and 8000 Hz at 285 K. To test for reversibility of changes spectra were measured at 4 kHz at the beginning and end of the experiment in two brain biopsies. Spectra were postprocessed and integration of lipid resonance areas was performed using Topspin software (Bruker). Integrals were assessed relative to the constant creatine area to compare variability between spinning rates.

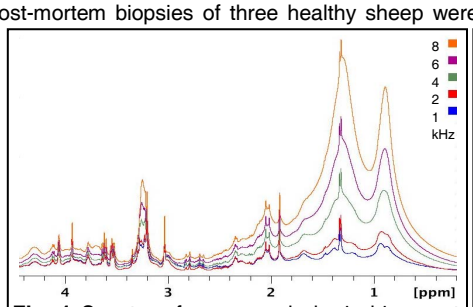

Fig. 1 Spectra of an example brain biopsy at different spinning rates. Obvious area increase of lipid resonances between 0.5 and 2.2ppm with increasing spinning rate (1-8 kHz color coded)

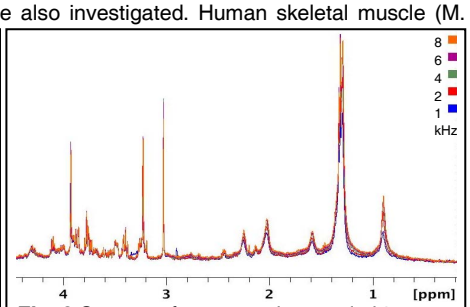

Fig. 2 Spectra of an example muscle biopsy at different spinning rates. Only mild area increase of lipid resonances with increasing spinning rate (1-8 kHz color coded)

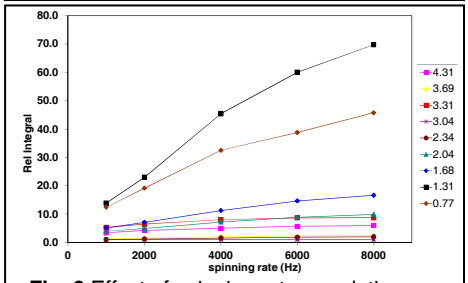

Fig. 3 Effect of spinning rate on relative integrals (mean of all biopsies) of various lipid resonances of brain biopsies.

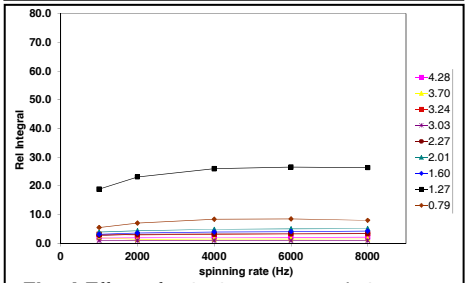

Fig. 4 Effect of spinning rate on relative integrals (mean of all biopsies) of various lipid resonances of muscle biopsies.

Results

Between 1 and 8 kHz brain biopsies showed a substantial and almost linear increase in lipid resonances (Figures 1 and 3), the area of the mobile lipids at 0.77 ppm was increased 3.7-fold, at 1.31 ppm 5-fold, at 1.68 ppm 3.3-fold and at 2.04 ppm 2.5-fold, respectively. This increase was reversible as no changes between brain biopsy spectra recorded at 4 kHz at the beginning and end of the experiment occured. In contrast to the brain tissue, only a mild increase in lipid resonances of muscle biopsies (Figures 2 and 4) could be observed in the lipids resonating at 0.79 ppm and 1.44 ppm with an 1.4-fold increase of both between 1 and 4 kHz and no or minimal additional increase between 4 and 8 kHz.

Discussion

The substantial increase in the relative integral of the most characteristic ML resonances with high spinning rates has been described in cultured glioma cells. This was ascribed to an enhanced visibility of intracellular lipid droplets that are prevalent in neoplastic tissue.[2] In our study, a similar strong rotation-dependent increase of visible lipids was also observed in non-neoplastic brain tissue biopsies. In skeletal muscle biopsies only a mild lipid increase was found, suggesting that the intramyocellular lipid droplets are already mostly visible without spinning. The discrepancy of spinning rate influence on visualization of ML between non-neoplastic brain and muscle tissue remains speculative. Possible contributors could be membrane microdomain characteristics of neural tissue myelin sheaths or possibly size differences of intracellular lipid droplets. Further investigation of factors responsible for the different behavior of lipid resonances in muscle and brain biopsies is needed.

Conclusion

In HR-MAS NMR, the spinning speed has a significant impact on lipid visibility in non-neoplastic brain tissue biopsies as indicated by rotation-speed dependent reversible changes in the lipid resonance areas. However, this effect seems to be only mild in muscle tissue biopsies. Using lipid contents as a marker for disease, the variable behavior of lipid resonances from different tissues and at different spinning rates has to be considered.

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References 1. Hakumäki et al., Trends Biochem Sci. 25(2000);357-362. 2. Martin-Sitjar et al., Magn Reson Mater Phy. 25 (2012); 487-496. 3. Griffin et al., Cancer Research. 63 (2003); 3195-3201. 4. Ramani et al. Magn Reson Imaging. 21(2003) ;1039-1043