Diffusion-weighted HR-MAS of biopsies to obtain separated fat-free metabolite and lipid spectra. A feasibility study.

Gaëlle Diserens[1], Martina Vermathen[2], Christina Stahl[3], Nicholas T. Broskey[4], Chris Boesch[1], Francesca Amati[1,4], and Peter Vermathen[1]

[1]Depts Clinical Research and Radiology, University Bern, Bern, Bern, Switzerland, [2]Dept. of Chemistry & Biochemistry, University Bern, Bern, Switzerland, [3]Dept. of Clinical Veterinary Medicine, Clinical Radiology, Vetsuisse-Faculty, University Bern, Bern, Switzerland, [4]Dept. of Physiology, University of Lausanne, Lausanne, Switzerland

PURPOSE HR-MAS NMR allows to metabolically characterize biopsies [1]. Fast spinning around an axis inclined at the magic angle (54.7°) averages orientation dependent effects, reducing the linewidth. However, even when using a T2-filter with long echo times, lipid peaks remain in the spectra, overlapping metabolite regions, which could hamper metabolite estimation and may lead to misinterpretation. Metabolite quantification and metabonomical analysis would therefore benefit from lipid free spectra. Besides shorter T2, lipids differ from small metabolites in terms of much slower diffusion. The aim of our editing technique was to employ this diffusion difference to remove the lipid content in the spectra of various tissues for reliable metabolite quantification. Thus, diffusion scans were performed and pure lipid spectra obtained at strong diffusion weighting were subtracted from those obtained at low diffusion weighting, which include both, small metabolites and lipids.

METHODS ^{1}H HR-MAS NMR experiments were performed on a Bruker Avance II spectrometer operating at 500.13 MHz at 273 K at a spinning speed of 8 kHz. Diffusion measurements were performed on 59 human muscle, 2 human liver, and 36 sheep brain biopsies using a modified 2D stimulated echo experiment with bipolar gradient pulses and longitudinal eddy current delay (ledbpgp2s2d). Diffusion times Δ were 50ms and 200ms. Linear ramps of 64 different values of (sinusoidal) gradient pulses, varying from 1 to 51 G/cm were used. 16 scans were acquired at every gradient-strength with a recycle delay of 2s.

Additional measurements were performed on pork muscle combining 1D and 2D diffusion sequences with a CPMG train (TE=77ms) as a T2 filter, in order to combine both effects, i.e. diffusion and T2 differences [2], for an efficient separation of metabolites and lipids. 1D-scans were performed twice with 1024 acquisitions each, once with very low weighting (0.1 G/cm) and once with intermediate weighting (32 G/cm), yielding a mixed small metabolite / lipid spectrum and a pure lipid spectrum, respectively, both with very good SNR.

The 2D diffusion measurements were processed conventionally to create and analyze pseudo-2D DOSY spectra. In addition the individual spectra of the 64 different diffusion weightings were investigated. Spectra at stronger gradients, which exhibited purely lipid signals, were added yielding a pure lipid spectrum with good SNR. It turned out that for Δ = 50ms ideally the last 20 spectra could be added to yield a pure lipid spectrum. This summed spectrum was then subtracted from the summed spectrum at low gradient strength (20 individual spectra were selected for best SNR). For the additionally performed 1D diffusion measurements with CPMG filter the two spectra at different weightings were subtracted.

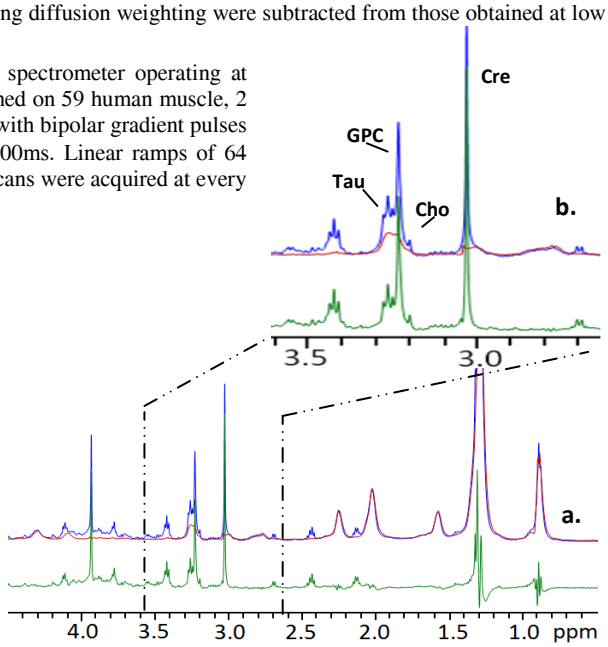

Figure 1: Example human muscle diffusion spectra. Blue: summed spectrum with low diff. weighting showing small metabolites and strong lipids; Red: summed spectrum with strong weighting showing lipids only; Green: difference spectrum.

RESULTS In Figure 1(a) is shown an example of a human muscle 2D-diffusion spectrum for fat removal. Clear improvements were obtained in spectral regions at 3.0 ppm (creatine) and 3.3 ppm (choline, taurine, and GPC) (Figure 1b, zoom), where the metabolite peaks are biased by underlying fat. The lipid spectrum subtraction removes the shoulder from the creatine peak, and suppresses the hump below the choline, taurine and GPC peaks. The strong overlap of the lactate peak at 1.3ppm or leucine, isoleucine and valine at ~1.0ppm with lipids is similarly strongly reduced. The lipid peaks at 0.9 and 2.0ppm are well suppressed, but small lipid residues remain, which are probably partly due to mobile lipids[3]. Nevertheless, the residues still impede metabolite quantification or inclusion in chemometric methods. However, the combination of the diffusion subtraction method with a CPMG T2 filter eliminates residual lipids from the spectra and permits the analysis of a completely lipid free spectrum (Fig.2). The techniques were employed as well on human liver and sheep brain biopsies, giving similar results.

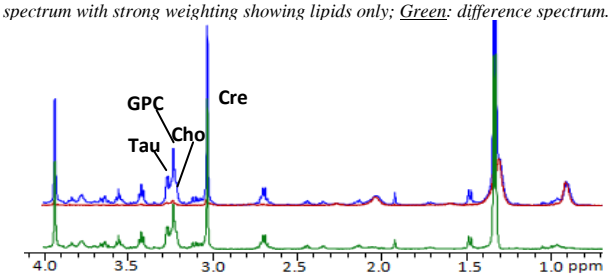

Figure 2: 1D Diffusion-CPMG spectra of a pork muscle (blue = low weighting; red = strong weighting; green = difference).

DISCUSSION The results clearly demonstrate that HR-MAS diffusion scans of muscle tissue yield lipid free small metabolite spectra by subtraction of strong from low diffusion weighted spectra, i.e. of pure lipid spectra from spectra obtained with the same sequence and parameters, but with low diffusion weighting. Residual lipids can be further reduced by a combination of the diffusion subtraction method with a T2 filter. Similar results were obtained for spectra from sheep brain biopsies (though with much less prominent lipids in brain spectra) and in spectra of human liver (with very strong lipid peaks), thus suggesting that this method is applicable in various tissue types. The removal of lipid contributions is important for quantitative spectral analysis and especially of potential value for metabonomical analyses, which generally rely on spectral binning and integration instead of fitting. The 2D diffusion experiments permitted a flexible adjustment of strong and low diffusion spectra for best balance between metabolite attenuation and SNR. However, with known diffusion thresholds 1D-diffusion scans may be preferred in the future for better SNR. The combined diffusion-CPMG sequence yielded high quality spectra with no obvious spectral impairment compared to a regular CPMG sequence and benefits from amplification of simultaneous T2 and diffusion filters in one experiment.

CONCLUSION To the best of our knowledge, the combination of different diffusion-weighted spectra to obtain fat-free spectra is presented for the first time. This technique has potential to improve chemometric analysis for biomarkers detection. Moreover, the diffusion scans permit a separate lipid analysis and a regular diffusion analysis without expanding scan time.

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