Is the metabolite profile of a single muscle biopsy representative for the tissue under investigation? A reproducibility study using HR-MAS

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TARGET AUDIENCE

This work is of interest for ex- and in-vivo spectroscopists, as well as for people interested in muscle research.

PURPOSE

It has been shown that high resolution magic angle spinning (HR-MAS) NMR allows to metabolically characterize biopsies from different tissues like brain, prostate, liver, or muscle [1]. However, to our knowledge, except for one study in cartilage [2], it has not been investigated yet by HR-MAS, how far the metabolite profile of a single biopsy can be considered representative for the tissue under investigation, i.e. how much do metabolites from biopsies of the same tissue vary. The aim of our HR-MAS study was therefore to investigate the metabolite variability of human muscle biopsies that are believed to represent the "same" muscle tissue.

METHODS

This study is part of an on-going clinical trial involving healthy sedentary and active older adults (60-80 years old). Skeletal muscle biopsies were obtained from the *vastus lateralis* by the Bergstrom technique, which involves needle aspiration under local anaesthesia with 2% Lidocaine. Each needle biopsy pass allows the collection of multiple specimens with possible distances of a few millimetres. From six of the subjects (5 males, 1 female, age: 66±1 years) separate tissue samples were taken from one needle puncture. From two subjects a second set of specimen was collected a few months later. Thus, in total eight sets of same needle biopsy samples were analyzed.

Biopsies with a weight of 4.1 ±2.4mg were washed and placed together with PBS in a 4 mm rotor using a 12 µl insert. The ^{1}H HR-MAS NMR experiments were performed on a Bruker Avance II spectrometer operating at 500.13 MHz at 277 K at a spinning speed of 5 kHz. A CPMG pulse sequence with water presaturation (cpmgpr1d) was used for the acquisition of 1D ^{1}H NMR spectra. For signal assignment, 2D ^{1}H-^{1}H-TOCSY was applied (dipsi2phpr). 1D CPMG spectra were phase- and baseline corrected and frequency shift corrections of individual spectral regions were performed using Icoshift [3]. AMIX software (Bruker), Matlab and Excel were used for statistical analysis, which included chemometric methods. In addition, individual peak analysis and calculation of inter- and intra-individual variabilities were performed on a collection of 28 assigned peaks over the whole spectrum including intense as well as small peaks.

RESULTS

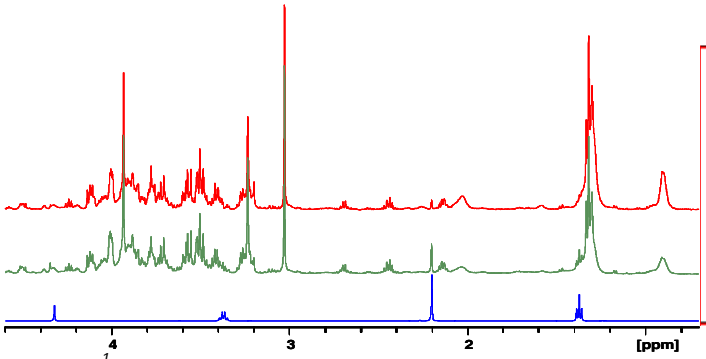

Figure 1: ^{1}H HR-MAS NMR spectra of 2 muscle samples from same needle punctures (red & green). In blue, lidocaine spectrum.

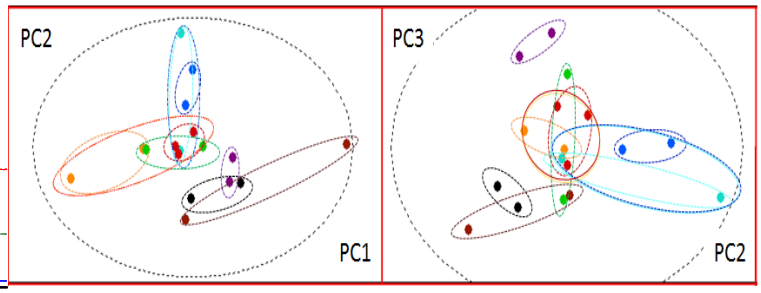

Figure 2: PCA analysis of the full spectra (excluding H_2O and lidocaine regions), for same needle biopsies.

Overall 20 metabolites were assigned based on literature and own 2D data. In Fig. 1 two spectra from same needle biopsies of one subject are shown. Visually similar patterns were obtained, but the spectra revealed also apparent differences. Some spectra were contaminated by lidocaine, as can be clearly seen by a comparison of the biopsy spectra with a reference spectrum of lidocaine (Fig. 1) and as was confirmed by the TOCSY data. The five lidocaine regions were excluded from the variance analysis.

The analysis of the 28 individual peaks yielded a smaller variation (25% ± 9%) among same needle biopsies compared to the variation among biopsies from different subjects (35% ±10%). Principal Component Analyses (PCA) of the entire spectrum (Fig.2) and on separate spectral regions demonstrated clustering for most of the same needle biopsies, both on PC1/PC2 and PC2/PC3, indicating spectral similarities between them. However, because of considerable differences among same needle biopsies in few subjects ANOVA yielded significant group differences for PC3, but not for PC1 and PC2. For the two patients with repeated same needle biopsies after a few months, an additional clustering was obtained (Fig.2, blue-cyan and red-orange).

DISCUSSION&CONCLUSION

Same needle biopsies harvested from the same muscle demonstrated similarities but also some differences in the metabolites content as obtained from HR-MAS. This variability needs to be taken into account in longitudinal HR-MAS studies on muscle tissue but also for any other study that relies on the assumption that a biopsy sample represents a region of the muscle. The variability may be due to muscle type composition, i.e. different inclusions of type I and type II fibers. Moreover, a high variability in the lipid region may occur and is probably due to varying inclusions of adipose tissue. Our results show a small variability in the lipid region suggesting that only small amount of adipose tissue was included.

Potential spectral contamination by external compounds like lidocaine should be considered in the spectral and statistical analysis. Similar contamination had been reported in breast tissue and gastric mucosa [4], but is reported in muscle for the first time here.

Acknowledgement

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