Analysis of in vivo localised 1H rat muscle spectra with the Pade approximant

C. Liess[1], D. C. Williamson[2], S. M. Poucher[3], J. J. Tessier[4]

[1]GSI/Imaging, AstraZeneca, Macclesfield, England, United Kingdom, [2]Department of Chemistry, University of Manchester, Manchester, United Kingdom, [3]CVGI, AstraZeneca, Macclesfield, United Kingdom, [4]GSI/Imaging, AstraZeneca, Macclesfield, United Kingdom

Introduction

Intramyocellular lipids (IMCL) have been shown to be inversely correlated to insulin sensitivity in skeletal muscle, thus making them a possible monitoring parameter for metabolic disorders such as diabetes (1,2). ^{1}H MRS is the only technique that can selectively measure IMCL; the acquired spectra, however, exhibit peak splittings due to dipolar coupling and susceptibility based effects, which are a function of the orientation of the muscle fibres relative to B_0 (e.g. see (3)). In addition, especially when acquired in small animals, spectra suffer from low signal-to-noise ratios (SNR) as spectroscopy voxels have to be small to be muscle-specific and to avoid contributions from neighbouring extramyocellular lipids (EMCL) (1,4,5).

Thus a stable method for the estimation of peak areas is required. Previously the AMARES method (6) has been used for the analysis of rat muscle spectra (1,7), while others have used least-squares frequency domain fitting algorithms for human spectra (8); both of these methods required prior knowledge and assumptions regarding the line shape. Here we compare for the first time the standard deconvolution method based on least-squares, AMARES and the Pade approximant method (PA, (9)) in conjunction with Monte Carlo simulations for the processing of ^{1}H spectra acquired from rat tibialis anterior muscle. The PA does not require prior knowledge, avoids operator bias in form of phasing and/or baseline corrections, and it has been shown to successfully separate metabolite peaks from macromolecular baseline signal (10).

Materials & Methods

Thirty-eight localised STEAM ^{1}H spectra of the tibialis anterior were acquired from 14 rats [4 Wistar control (253 ± 13 g), 5 lean Zucker (fa/-, 311 ± 16 g) and 5 obese Zucker rats (fa/fa, 380 ± 24 g)] placed supine in a 9.4T Oxford magnet (Oxford, UK) interfaced to a Varian console (Palo Alto, CA). Rats were kept anaesthetised by the constant inhalation of 1-2% isofluorane, with their temperature kept constant at 37°C and their respiration continuously monitored.

Spectra were analysed using a standard deconvolution method, AMARES and the PA. The analysis focused on the IMCL : total creatine (tCre) ratio as a sensitive indicator for lipid metabolism (1,2). For the standard deconvolution provided by Varian, spectra were Gaussian line broadened, phased and baseline corrected before subjecting them to a least-squares based fitting routine, assuming Gaussian line shape for all resonances as suggested for muscle ^{1}H spectra by others (11,12). In the case of the Pade analysis, the residual water peak was removed using the digital suppression algorithm HLSVD. Each FID was subsequently analysed using a combination of the PA method, implemented as described by Belkic (9) and Monte Carlo simulation. This combination had been previously applied to examine short echo time spectroscopic data of the human brain (10). For the AMARES three analyses were performed in total; the first two assuming pure Gaussian and pure Lorentzian line shapes, respectively, while the third analysis (referred to as 'AMARES mixed') assumed a Gaussian line shape for the lipid region and a Lorentzian line shape for the tCre resonance (8,13). Differences were considered significant for $p<0.05$.

Results & Discussion

There was no difference in the tCre value between the different rat strains in any of the processing methods. This agrees well with biochemical data of Wistar and Zucker rats (7). The IMCL/tCre ratio for the different rat strains as determined by the various processing techniques is shown in the table. The standard deconvolution and the PA showed clear differences in the IMCL/tCre between different rat strains. Using either the AMARES(Lorentz) or AMARES(Gauss), the control and lean Zucker rats were no longer significantly different. The mixed AMARES showed a difference between the obese and lean Zucker rats, but neither between the obese and control, nor between the control and lean Zucker rats (both $p=0.12$). The data of the different rat strains were then pooled to compare the processing methods with each other. Considering individual peaks first, the AMARES(Lorentz) gave higher peak integral values then the AMARES(Gauss), both for IMCL and tCre. This is consistent with the work of Marshall and colleagues on the effects of line shape on peak integrals (14). Considering the IMCL/tCre ratio, the correlations between the processing methods were quite high, ranging from $R^2=0.73$ (AMARES mixed vs. standard deconvolution) to $R^2=0.95$ (AMARES mixed vs. AMARES(Gauss)). However, whilst the R^2 values suggest good agreement, there were systematic differences between the processing method; a Bland-Altman analysis revealed that the mixed AMARES yielded consistently lower IMCL/tCre values than any of the other methods ($p<0.002$), and that the PA showed a strong trend to producing higher IMCL/tCre values than the standard deconvolution ($p<0.055$). There was even a trend for the AMARES(Lorentz) to have higher IMCL/tCre values than the AMARES(Gauss), with $p<0.067$. The latter result is surprising since systematic bias due to line broadening resulting from B_0 inhomogeneity should be eliminated when the ratio is considered. This results points to the existence of other significant sources of line broadening which may be peak dependent.

	Standard deconvolution	Pade approximant	AMARES Lorentzian	AMARES Gaussian	AMARES mixed
Control rats (n=12)	1.11 ± 0.42	1.15 ± 0.48	1.05 ± 0.42	1.00 ± 0.40	0.81 ± 0.39
Lean Zuckers (fa/-, n=15)	0.49 ± 0.15	0.62 ± 0.23	0.78 ± 0.41	0.76 ± 0.41	0.55 ± 0.28
Obese Zuckers (fa/fa, n=11)	1.77 ± 0.70	1.91 ± 1.06	1.67 ± 0.86*	1.52 ± 0.85*	1.11 ± 0.67#
Statistics	all sign. with $p<0.002$	All sign. with $p<0.05$	* $p<0.05$ vs fa/- and control	* $p<0.05$ vs fa/- and control	# $p<0.05$ vs fa/-

IMCL/tCre ratio ± SD for the different rat strains and processing methods. Note that the rat strains can be distinguished with the standard deconvolution and the PA, but that this is progressively lost with the AMARES.

Conclusion

A number of different strategies for the processing of ^{1}H muscle spectra have been suggested in the literature, either involving some form of prior knowledge and/or operator input such as phasing and baseline corrections. Here we compare for the first time the standard deconvolution method based on least-squares, AMARES and the Pade approximant method and explore their ability to separate different strains of rats based on the IMCL/tCre ratio. Although further work is needed, the Pade approximant, which is not based on prior knowledge and requires limited operator input, was able to separate more effectively the different rats strains than the widely used AMARES.

References

(1) J Kuhlmann et al., *Diabetes* **52**: 138-144 (2003)
(2) M Krssak et al., *Diabetologia* **42**: 113-116 (1999)
(3) C Boesch & R Kreis, *NMR Biomed* **14**: 140-148 (2001)
(4) PD Hockings et al., *Dibates Obes Metab* **5**: 234-243 (2003)
(5) BM Jucker et al., *Metabolism* **52**: 218-225 (2003)
(6) S Mierisova et al., *NMR Biomed* **11**: 32-39 (1998)
(7) C Neumann-Haefelin et al., *Magn Reson Med* **50**: 242-248 (2003)
(8) C Boesch et al., *Magn Reson Med* **37**: 484-493 (1997)
(9) D Belkic et al., *J Chem Phys* **113**: 6543-6556 (2000)
(10) DC Williamson et al., British Chapter ISMRM 2004
(11) J Rico-Sanz et al., *J Appl Physiol* **87**: 2068-2072 (1999)
(12) F Gao et al., *Magn Res Imaging* **21**: 561-566 (2003)
(13) SR Stannard et al., *Am J Physiol Endocrinol Metab* **283**: E1185-E1191 (2002)
(14) I Marshall *et al Magn. Reson. Med.*, **44**, 646-649, 2000