# Orientation Dependence is the Rule, Not the Exception in $^1$H-MR Spectra of Skeletal Muscle: the Case of Carnosine

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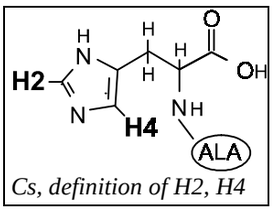
## INTRODUCTION

Up to a few years ago, in vivo MR spectroscopy had been considered to be equivalent to NMR of liquids Now, it has become evident that several components of $^1$H-MR spectra of muscle are orientation dependent[1]. The creatine[2] (Cr) and lactate[3] resonances have been shown to be subject to dipolar coupling. Taurine[1,4] and possibly other resonances near 3.2 ppm are also orientation-sensitive. The lipid resonances are split into two patterns based on their orientation-dependent susceptibilities[5,6]. Even water shows anisotropic MR effects[7]. This contribution deals with the orientation-dependence of carnosine (Cs), a dipeptid consisting of histidine (His) and alanine (Ala). The two downfield peaks (H4 and H2, c.f. Fig.) of His at 7 and 8 ppm can easily be detected in spite of the fairly low concentration of Cs and provide a means to determine intracellular pH by $^1$H-MRS[8].

## METHODS

Localized $^1$H-MR spectra were recorded on a 1.5T MR scanner (Signa, GE) using PRESS: TE 20ms, TR 3.0s, 1953 Hz spectral width, outer volume suppression, 16-step phase cycle, water presaturation and RF coils adapted for best SNR. Data treatment included eddy current correction and scaling with unsuppressed water. Because it was unclear what lineshape should be used for model fitting, Cs peak areas needed for $T_2$ evaluations were determined by peak integration after baseline correction. Three muscles were investigated: tibialis anterior (TA), soleus, vastus lateralis (VL). Six subjects were investigated explicitly for this study: 3 for $T_2$ measurements at the magic angle (MA = 54°), and 3 for determination of signal shapes of H2 and H4 as a function of muscular orientation (in TA). 90 spectra (in 21 subjects) recorded for other studies were used to corroborate the findings.



*Cs, definition of H2, H4*

## RESULTS

Fig. 2 contains averaged spectra from TA and VL. Those for TA had been obtained with the lower limb parallel to $B_0$ (upper trace) and at the MA (lower trace). All spectra of VL were recorded with the leg parallel to $B_0$, but spectra were sorted according to the dipolar splitting observed for the Cr $CH_2$ resonance (Cr2) which reflects the local orientation of muscle fibers in the selected ROI (dependent on subject and exact choice of ROI). Near MA spectra were averaged for the lower trace, those with Cr2 as a doublet yielded the upper trace. In both muscles the Cs peaks are broader for orientations off the MA. This is more pronounced for TA, where the Cr2 splitting is maximal (fibers parallel $B_0$). For H4, the amplitude of the remaining peak is reduced relative to H2. Whether the total peak area of H4 is reduced is presently not clear because of unclear lineshapes and limited SNR. These features are consistently seen in individual subjects. Data from soleus was more difficult to analyze because of lower SNR. $T_2$ obtained near the MA in TA could be determined very reproducibly for H2 (153±11ms), while for H4 $T_2$ came out lower and with much more spread (85±53s).

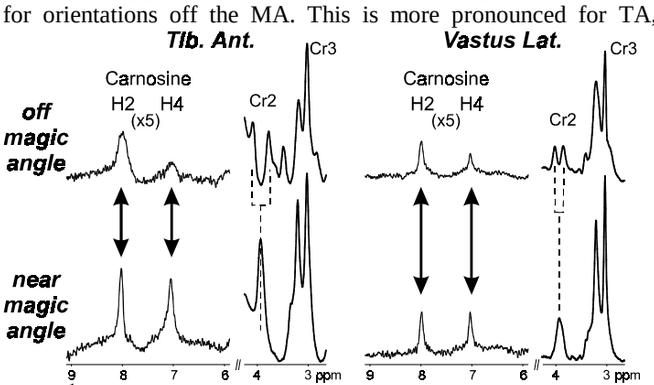


*Fig 2. $^1$H-MR spectra of TA and VL as function of orientation. Off the MA both Cs resonances broaden and the H4 peak decreases.*

## DISCUSSION

These data show that Cs is subject to orientational effects also. Several explanations are possible. *1 Dipolar coupling:* Similar to Cr and taurine, Cs could feature incomplete motional averaging leading to residual dipolar coupling depending on the net orientation. As the order parameter is expected to be quite different for Cr and Cs, it is impossible to predict the size of dipolar coupling expected for Cs based on the Cr2 splitting, even though it is known that the interproton distances between H2, H4 and the β His protons are considerably larger than for the geminal protons in Cr. However, one can expect the H2 peak to be less susceptible to dipolar splitting than H4, because H2 is further from nearest protons than H4 (neglecting the N-H proton, which is in fast exchange). This would agree with the observations. *2. Chemical shift anisotropy (CSA):* Because His forms an asymmetric aromatic system, it is conceivable, that the orientation dependence of the chemical shifts of H2 and H4 could lead to peak broadening, if Cs is partly ordered and has a distribution of preferred orientations. CSA, however, has not been observed so far *in vivo*. *3. Relaxation anisotropy:* $T_2$ could be orientation-dependent as seen for tendons[9]. Dropping from 150 to <50ms might suffice and would inherently include reduced areas and amplitudes in a spin echo experiment which was indeed observed for H4.

## CONCLUSIONS

All major peaks in the $^1$H-MR spectrum of skeletal muscle, except for intramyocellular lipids and the acetyl peak of acetylcarnitine[10], show anisotropic features. The reason for the orientation dependence of the His resonances in Cs are presently not clear. Further experiments including $T_2$ measurements as a function of orientation are needed, but are obviously difficult to perform because of reduced SNR off the MA, and are difficult to interpret because residual dipolar couplings would also lead to apparent $T_2$-reductions. If Cs is used to measure pH, these orientational effects have to be kept in mind. H2 is a better indicator of pH than H4, not only because of longer $T_2$ and greater pH dependence[8] but also, because it changes less with orientation. Furthermore, broadening of the Cs peaks after exercise may not represent inhomogeneous pH distributions, but rather imperfect repositioning of a subject.

## REFERENCES

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