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# Influence of muscle fibre orientation on water and metabolite relaxation times, magnetization transfer, and visibility in human skeletal muscle

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**ABSTRACT**

**Purpose:** To gain a deeper understanding of the influence of skeletal muscle fibre orientation on metabolite visibility, magnetization transfer from water, and water proton relaxation rates in  $^1\text{H}$  MR spectra.

**Methods:** Non-water-suppressed MR spectroscopy was performed in tibialis anterior muscle (TA) of ten healthy adults, with the TA oriented either parallel or at the magic angle to the 3 T field. Spectra were acquired with metabolite-cycled PRESS, and water inversion from 50 to 2510 ms prior to excitation. Water proton  $T_2$  relaxation was sampled with STEAM with echo times from 12 to 272 ms.

**Results:** Apparent concentrations of total creatine (tCr), taurine, and trimethylammonium compounds were reduced by 29% to 67% when TA was parallel to  $B_0$ . Both tCr peak areas were strongly correlated to the methylene peak splitting. Magnetization transfer rates from water to tCr  $\text{CH}_3$  were not significantly different between orientations. Water  $T_1$ 's were similar between orientations, but  $T_2$ 's were statistically significantly shorter by 1 ms in the parallel orientation ( $p=0.002$ ).

**Conclusion:** Muscle metabolite visibilities in MR spectroscopy and water  $T_2$  times depend substantially on muscle fibre orientation relative to  $B_0$ . In contrast, magnetization transfer rates appear to depend on muscle composition, rather than fiber orientation.

**Keywords:** MR spectroscopy; skeletal muscle; orientation; magnetization transfer; magic angle;

## INTRODUCTION

Skeletal muscle metabolism, metabolite compartmentation, and tissue microstructure can be probed non-invasively in vivo using MR spectroscopy. The dense muscle fibre microstructure gives rise to orientation dependent MR features, with anisotropic overall motion of the creatine (Cr) and phosphocreatine (PCr) molecules causing residual dipolar couplings first described for the total observed creatine ( $tCr=Cr+PCr$ ) resonances (1,2) while orientation dependence was later also reported for most of the other muscle metabolites. Taurine, trimethylammonium containing compounds (TMA), and carnosine resonances were all shown to be affected by muscle fibre microstructure since their spectral patterns and/or amplitudes depend on the relative orientation of muscle fibers with respect to the main magnetic field (3–7).

In addition, the exchange of water in the hydration sphere of tCr molecules with free water can be investigated via an inversion transfer experiment, where the tCr peak areas attenuate in response to frequency-selective water inversion. Previous investigations of this effect have demonstrated similar attenuation of tCr peak areas in the human brain (8), skeletal muscle (8,9), as well as in rat brain (10). Recent in vitro  $^1H$  NMR magic angle spinning observations suggest that - besides the chemical partitioning into unphosphorylated Cr and PCr - there are two pools of Cr; one motionally-free, and one motionally-restricted, where the latter may not be visible in vivo but may be involved in the transfer of magnetization with water (11). Surprisingly, our previous inversion transfer experiment in human calf muscles revealed a significant difference in magnetization transfer (MT) rates between the tibialis anterior (TA) and soleus muscles (9), which was attributed to either varying muscle composition or the differing muscle fibre orientation.

Muscle fibre orientation effects on water proton relaxation times have been investigated early on in *ex vivo* rat TA, revealing an orientation effect on  $T_2$  but not  $T_1$  (12). However, the so-called magic angle effect is only well established in vivo for cartilage tissue ((13) and refs. therein), and to date the effects of muscle fibre orientation on water relaxation in human skeletal muscle in vivo has not been characterized.

The present study sought to answer several questions regarding the NMR properties of skeletal muscle water and metabolites in humans: 1) do water-tCr MT rates depend on muscle fibre orientation or composition; 2) can the varying visibility of skeletal muscle metabolites in different fibre orientations be confirmed at 3 T and using non water suppressed MRS; and 3) do water proton relaxation times change with muscle fibre orientation?

## METHODS

### Subjects

Ten healthy adults aged 20 to 46 years, (median 26 years, 3 males and 7 females) of varying degrees of fitness and without any history of musculoskeletal disorders were recruited. The study was approved by the local institutional review board, and written, informed consent was obtained.

### MRS Data Acquisition

Subjects were scanned on a 3T Siemens VERIO MR system with the body coil for transmission and a flexible phased array coil for reception. Subjects were positioned lying on their right side on a home built support on top of the scanner table, with their lower right leg through a rotatable coil mount. The support allowed the subject's body to slide along the table when the right leg was bent or straightened, so as to keep the coil at the same position along the external magnetic field. Thus, the entire measurement could be performed twice, once with the lower leg aligned at the magic angle and once parallel with respect to the external magnetic field, without having to adjust the positioning of the coil. However, it should be noted that the flexible coil, which is partly wrapped around the calf, was rotated along with the leg such that the receive performance of the coil was smaller at the bent position than when the leg was parallel to  $B_0$  because the main axis of some of the coil elements did not remain perpendicular to  $B_0$ .  $T_2$  weighted images were acquired and used to prescribe a spectroscopy voxel (mean volume 6.9 mL) in the TA with as little contamination from extramyocellular lipid deposits as possible, as illustrated in Fig. 1. Voxel dimensions and voxel orientation with respect to the leg were kept the same within a volunteer for the two orientations, with the exception of one case in which the parallel orientation voxel was 5% smaller.

PRESS localized spectra were acquired without water suppression (WS) by presaturation, but using metabolite cycling (9,14) with TE/TR = 26/4000 ms, 2 start-up cycles followed by 32 acquisitions, 2048 samples, and a bandwidth of 4000 Hz. The metabolite cycling pulse had a duration of 22 ms, with a delay of 7 ms to the middle of the PRESS 90° pulse. Magnetization transfer between water and tCr was measured using an inversion transfer experiment in which an additional Gaussian-shaped frequency selective pulse of 12 ms duration was applied to invert the water resonance with varying delay times (TI) before the PRESS acquisition (TI = 50, 510, 660, 810, 960, 1110, 1260, 1560, 2510 ms), in addition to a non-water-inverted (NI) measurement. Individually acquired free induction decays (FID's) were stored separately for later post-processing. The measurement took 128 s for each TI time. In addition, single shot non-WS STEAM spectra with varying TE times (TE = 12, 14, 17, 20, 24, 29, 34, 40, 48, 68, 96, 136, 192, 272 ms) were also acquired. Automatic  $B_0$  shimming was performed, and the transmitter power was

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3 optimized for a  $90^\circ$  flip angle in the prescribed voxel using the manufacturer's work-in-progress  $B_1$   
4 mapping method.  
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### 7 **Data Analysis**

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10 Spectra were preprocessed as described previously (9). Briefly, individual NI shots were frequency  
11 aligned with the water peak, then eddy-current corrected with its water-only signal. Water inverted shots  
12 were frequency aligned using the 3.03 tCr methyl peak, and eddy current correction was performed using  
13 the water signal obtained from the  $TI = 2510$  water signal, then frequency and phase aligned to the NI  
14 case. Residual water and signals in the lipid region (0 to 2.7 ppm) were removed from the metabolite  
15 spectra by Hankel-Lanczos Singular Value Decomposition prior to fitting. Both metabolite and water  
16 spectra were fit in FiTAID (15), which fits peak areas at all TI times simultaneously. This is illustrated  
17 and described in Fig. 1. Base spectra had been set up heuristically, with the tCr spectrum as one doublet  
18 for  $CH_2$  and one triplet for  $CH_3$ , a singlet for TMA, and a doublet of doublets for taurine.  
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26 The longitudinal magnetizations of water and tCr were modeled as described previously (9) to extract  
27 the water and metabolite  $T_1$  relaxation times and magnetization transfer rates. In brief, the water inversion  
28 recovery curve was initially fit for each volunteer to determine the water  $T_1$  time, inversion pulse  
29 efficiency, and equilibrium magnetization. These parameters were then used as constants in the fit of the  
30 inversion transfer curves for each metabolite's peak area. The metabolite curves were modeled on an  
31 idealized pulse sequence, considering only the effects of the water inversion pulse and the  $90^\circ$  pulse in  
32 PRESS, and was described using the two-pool Bloch-McConnell equations (16). The metabolite curve  
33 fitting optimized each metabolite's equilibrium magnetization,  $T_1$  in absence of exchange, and forward  
34 transfer rate constant from water to metabolites, while the backwards transfer rate constant was set to  
35 fulfill steady state conditions. Non-WS STEAM spectra with varying TE times were fit with a single  
36 water peak, and peak areas were fit to a mono-exponential decay using least-squares minimization. The  
37 Wilcoxon signed-rank test was used to compare  $T_1$  times and magnetization transfer rates of the tCr  $CH_3$   
38 peak in between the two fibre orientations.  
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48 To determine absolute concentrations for tCr- $CH_2$  and  $-CH_3$ , TMA, and taurine, metabolite signals  
49 were scaled to the water peak area with the assumption that skeletal muscle is 78% water (17) and fully  
50 visible, independent of fibre orientation. The water peak area was corrected with the measured  $T_1$  and  $T_2$   
51 values, and scaled by a factor of 1.25 to account for the effect of direct saturation by the metabolite  
52 cycling pulses. The tCr and TMA peak areas were corrected with orientation-independent relaxation  
53 values reported previously (18). Both tCr peak areas were corrected with the same  $T_2$  values, and the  
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3 taurine peak area was corrected with the  $T_2$  value of tCr  $\text{CH}_3$ . Metabolite concentrations between the  
4 magic angle and parallel orientations were compared using the Wilcoxon signed-rank test. tCr  
5 concentrations for each resonance ( $\text{CH}_2$  and  $\text{CH}_3$ ) and  $T_2$  values of water were fit to a linear relationship  
6 with  $\text{CH}_2$  peak splitting. Signal pre-processing, model optimization, statistics, and graphing were  
7 performed in MATLAB (7.10.0 R2010a, The Mathworks, Natick, MA, USA).  
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## 10 11 12 RESULTS

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15 Creatine peak areas at 3.03 and 3.93 ppm in the tibialis anterior muscle are affected by frequency  
16 selective inversion of water, as demonstrated in Fig. 2, with maximal difference from the non-inverted  
17 spectrum approximately 1 s following water inversion. This effect is similar at both the magic angle and  
18 parallel orientations with respect to the external magnetic field. Inversion transfer modeling of the change  
19 in tCr  $\text{CH}_3$  peak area with water inversion delay time is shown in Fig. 3, and revealed MT rates of  
20 (median (25<sup>th</sup> & 75<sup>th</sup> percentiles)) 0.43 (0.37 – 0.48)  $\text{s}^{-1}$  and 0.41 (0.36 – 0.51)  $\text{s}^{-1}$  in the tibialis anterior at  
21 the magic angle or parallel to the  $B_0$  field, respectively. The median longitudinal relaxation times were  
22 determined to be 1.14 (1.00 – 1.32) s and 1.47 (1.12 – 1.69) s, respectively. No significant difference  
23 between muscle fibre orientations was detected for either the MT rate ( $p=0.85$ ) or the tCr  $\text{CH}_3$   $T_1$   
24 relaxation time ( $p=0.10$ ).  
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33 All apparent metabolite concentrations were significantly higher in the magic angle orientation as  
34 compared to the parallel orientation ( $p<0.01$ ), as illustrated in Fig. 4. The median tCr  $\text{CH}_2$  concentration  
35 exhibited a 33% decrease from 28.8 (27.7 – 30.5) mM at the magic angle to 19.2 (18.7 – 20.8) mM in the  
36 parallel orientation, while the tCr  $\text{CH}_3$  concentrations exhibited a 29% drop from 35.2 (34.0 – 37.2) mM  
37 to 25.0 (23.3 – 27.5) mM. The TMA and taurine concentrations exhibited even stronger decreases, with  
38 TMA decreasing 55% from 10.4 (8.8 – 13.4) mM to 4.7 (3.9 – 5.2) mM, and taurine decreasing 67% from  
39 15.8 (14.3 – 18.0) mM to 5.2 (4.6 – 6.1) mM.  
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45 The measured concentrations of both tCr peaks were strongly correlated to the  $\text{CH}_2$  doublet splitting  
46 of tCr ( $R^2 = 0.79$   $p<1\times 10^{-6}$  for  $\text{CH}_2$ , and 0.65,  $p<1\times 10^{-4}$  for  $\text{CH}_3$ ), as shown in Fig. 5. Furthermore, the  
47 slope of the linear relationship was similar between the  $\text{CH}_2$  and  $\text{CH}_3$  peaks.  
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51 The water proton  $T_1$  relaxation time was also extracted from the inversion transfer model, and was  
52 similar between muscle fibre orientations: 1.34 (1.32 – 1.38) s at the magic angle, and 1.34 (1.32 – 1.35) s  
53 for the parallel orientation ( $p=0.97$ ). Conversely, the water proton  $T_2$  relaxation time determined from the  
54 non-WS STEAM TE series was significantly different between orientations: 28.7 (28.3 – 29.9) ms at the  
55 magic angle and 27.6 (27.2 – 27.9) ms in the parallel orientation ( $p=0.002$ ). Its dependence on orientation,  
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3 represented by the tCr CH<sub>2</sub> peak splitting, is plotted in Fig. 6, where a strong correlation is observed over  
4 the whole cohort ( $R^2 = 0.60$   $p < 0.0001$ ).  
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## 7 8 **DISCUSSION**

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10 Selective inversion of the water resonance leads to attenuation of the total observed creatine  
11 resonances in human tibialis anterior muscle, as determined by <sup>1</sup>H MRS without water presaturation. The  
12 MT rates reported here are in agreement with our previous study in the same muscle (9), and exhibit a  
13 similar time course to earlier reports in human brain and skeletal muscle (8). Our previous work  
14 demonstrated a significant difference in water-tCr MT rates between the tibialis anterior and soleus  
15 muscles, which could be attributable to either different muscle compositions or fibre orientations. The  
16 present study found similar MT rates in the tibialis anterior muscle aligned with the external magnetic  
17 field and at the magic angle, suggesting that the varying water-tCr MT rates reported earlier (9) probably  
18 arise from different fibre type compositions between the tibialis anterior and soleus muscles, though this  
19 will need corroboration with studies including determinations of fiber type.  
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28 Histological examination of post-mortem human muscle tissue determined that the tibialis anterior is  
29 composed of roughly 78% type 1 and 22% type 2 fibres, while the soleus is composed of 96% type 1 and  
30 4% type 2 fibres (19). Given that the kinases involved in the creatine-phosphocreatine energy shuttle are  
31 bound to the mitochondria and myofibrils (20), and that there is a larger mitochondrial content in type 1  
32 fibres than type 2 fibres, type 1 fibres may have a higher ratio of bound to free creatine, which might  
33 enable a faster diffusion of magnetization from water to free creatine via the bound pool. In this case, it  
34 would be expected that muscles high in type 1 fibres would experience faster water to tCr MT rates, as  
35 was found in our previous study (9).  
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43 Indeed, high resolution magic angle spinning (HRMAS) <sup>1</sup>H NMR spectroscopy of fresh human  
44 skeletal muscle tissue resolved the tCr resonances into 3 separate peaks assigned to PCr, as well as mobile  
45 and motionally restricted creatine (11). Given that the intensities of the PCr and creatine multiplets are  
46 similar in spectra of spinning and non-spinning samples, the restricted creatine pool is likely not to be  
47 observable without magic angle spinning, i.e. in vivo. Moreover, nuclear Overhauser effect spectroscopy  
48 of these samples indicated that this motionally restricted creatine pool is confined in a small space with  
49 water molecules (11). The confirmed presence of a motionally restricted creatine pool in close association  
50 with water molecules invites the speculation that the MT-related attenuation of the tCr peak areas in the  
51 present study occurs due to transfer of the inverted water magnetization to the motionally restricted  
52 creatine pool, followed by an exchange between the restricted and free creatine pools to produce the  
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3 observed effect on the tCr peak areas. Since bound pools were not observed for any other metabolites in  
4 HRMAS and the MT is clearly much larger for creatine than other peaks, this link may be more than pure  
5 coincidence.  
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9 Regarding the observed reduced visibility of the metabolite signals off the magic angle, there are  
10 several related findings in the published literature. In terms of the creatine signals, there is an ongoing  
11 debate about the full visibility of unphosphorylated creatine. Strong correlations between exercise-  
12 induced depletion of the phosphocreatine signal in  $^{31}\text{P}$  MRS and  $^1\text{H}$  tCr peak areas in human TA  
13 suggested that the entire tCr methylene signal arises from PCr and that free creatine is MR invisible (21).  
14 For the methyl peak pattern, this study suggested a partial visibility, with changes in lineshape. This  
15 finding was corroborated in a study with electric stimulation of muscle contraction (22). The  
16 measurements on the effect of exercise were performed in the parallel orientation, which is now  
17 demonstrated to have a 29% decrease in the tCr  $\text{CH}_3$  peak area and a 33% decrease in tCr  $\text{CH}_2$  peak area  
18 compared to the magic angle setting. Given that 20-30% of total creatine is known to be  
19 unphosphorylated from chemical biopsy measurements in resting conditions for human skeletal muscle,  
20 these two observations would match up well, if one assumes that unphosphorylated creatine becomes  
21 visible at the magic angle, while at other angles the dipolar splitting could be substantially larger and/or  
22 less uniform for free creatine than for PCr, and thus free creatine signals might be broadened and  
23 distributed beyond detectability over a larger frequency range. This explanation is also supported by the  
24 determined tissue content for total creatine, which is in taking with the biopsy literature for the magic  
25 angle, but not the parallel arrangement. In contrast, the correlation between  $^1\text{H}$  and  $^{31}\text{P}$  tCr peak areas was  
26 not observed in mouse gastrocnemius muscle pre- and post-mortem (though there were some lineshape  
27 effects specific to the off-magic angle settings), and furthermore, similar tCr  $\text{CH}_2$  visibility was observed  
28 for both wild type and creatine kinase deficient mice who exhibit decreased PCr levels (23). However,  
29 these measurements were done at 7T and in another species, which still leaves room for the above  
30 interpretation for 3T and human muscle. In addition to, and in support of, the theory that creatine  
31 visibility is limited off the magic angle, it has been reported that creatine indeed shows larger dipolar  
32 couplings than PCr - at least in model systems featuring residual dipolar couplings (24,25).  
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49 The exact nature of the orientation dependence of apparent concentrations or relaxation times cannot  
50 be derived from our data as given in Figs 5 and 6. The suggested linear relation is not founded in the data,  
51 but is just the most basic correlation. Given that dipolar couplings may play a crucial role for the MR  
52 visibilities, either through residual dipolar splittings or through relaxation enhancements, it is reasonable  
53 to use the residual dipolar coupling of tCr as first approximation of an orientation measure, but the  
54 relations may equally well be non-linear.  
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3 Further evidence with regard to variable MR-visibility for metabolite peaks as function of  
4 orientation, in particular for TMA and tCr, comes from a spectroscopic imaging study of the human calf  
5 muscle. At 1.5 T the authors demonstrated the variability of tCr and TMA peak areas across different  
6 muscles, which was suggested to partially depend on fibre orientation (3). This finding was further  
7 supported by similar spectroscopic imaging of the human calf at 1.5 T with the lower leg oriented at 0°,  
8 30°, and 70° with respect to the main magnetic field, revealing nearly twice the tibialis anterior TMA/tCr  
9 ratio at 70° versus 0° (4), which is consistent with our finding of a larger orientation dependence for TMA  
10 than tCr. On the other hand, two other investigations did not find an effect of orientation on tCr peak area,  
11 but both studies experienced relevant limitations. Gao et al. did not observe changes in the tCr CH<sub>3</sub> peak  
12 areas when the human forearm muscles were placed at different angles to the B<sub>0</sub> field, however the tCr  
13 CH<sub>3</sub> peak splitting in the parallel orientation was not accounted for in the spectral fitting (26). In addition,  
14 tCr measurements in the human soleus muscle under muscle stretching caused by varying foot  
15 orientations did not reveal changing peak areas, however, the induced muscle fibre orientation changes  
16 may not have been strong enough to produce an amplitude effect (27).  
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27 Furthermore, for TMA the visibility may depend on field strength, too. TMA is a composite peak  
28 with choline, phosphocholine, glycerophosphorylcholine, betaine, carnitine, acetylcarnitine, and  
29 acylcarnitines all contributing and all with the potential for residual dipolar couplings and differential  
30 visibilities. From work at 1.5 T (28) and 3T (29), it appeared that free carnitine is only partially visible  
31 both on and off the magic angle. This was concluded from difference spectra obtained for the exercise-  
32 induced transfer of carnitine to acetylcarnitine, which showed a well resolved positive peak for the  
33 appearance of the TMA singlet (3.19 ppm) of acetylcarnitine, but only a broad negative peak for the  
34 disappearance of the free carnitine TMA contribution in TA, vastus intermedius, and rectus. Similarly,  
35 such difference spectra showed an increased signal for total TMA in soleus proportional to the appearance  
36 of acetylcarnitine, as determined from the acetyl peak at 2.1 ppm. In soleus at 7 T (30), such difference  
37 spectra feature a clear and equally sized negative contribution of free carnitine, thus suggesting full  
38 visibility of this component at 7 T – at least near the magic angle<sup>1</sup>.  
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48 The present study confirmed at 3 T the observations of the 1.5 T studies that tCr CH<sub>3</sub>, tCr CH<sub>2</sub>, TMA,  
49 and taurine peak areas are increased when the TA is at the magic angle as opposed to parallel to the main  
50 magnetic field. Moreover, the tCr CH<sub>3</sub> and CH<sub>2</sub> concentrations exhibited a strong linear correlation with  
51 the splitting of the tCr CH<sub>2</sub> resonance, reinforcing an earlier correlation of TMA/tCr ratio with tCr CH<sub>2</sub>  
52 splitting (4).  
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57 <sup>1</sup> In ref (30), spectra recorded for an off magic angle orientation in gastrocnemius muscle were mentioned, but  
58 the results not included in the report.  
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It should be noted that the orientation dependence could in principle also arise from inherent technical differences between the setups at the two orientations. For one, the receive coil, which was angulated along with the leg, has a lower sensitivity at MA than PA, but since water, which was used as an internal standard, is subject to the same field differences, no relevant net effect is expected. Secondly,  $B_0$  field homogeneity at the ROI is better with the leg stretched along the external field than when it is angulated creating substantial perpendicular air-tissue interface components. However, the median difference in water line width was less than 1 Hz, which is expected to have been compensated by the Voigt line model. In addition, a minor part of the orientation dependence of metabolite peak areas may also have arisen through inaccurate model fitting of the MR spectra, where base spectra had been adjusted heuristically for plausibility and minimal residues. In particular, the taurine peaks may not be modelled properly and some of the differences between the orientations could be attributable to the fact that there is no exact or widely accepted model for the peak pattern of taurine as a function of orientation or extent of residual dipolar coupling. Similarly, the overlapping TMA resonance may suffer indirectly somewhat from this deficit, and in addition some of the constituent molecules contributing to the overall TMA peak may actually also show a dipolar pattern off the magic angle, which was not accountable in this evaluation. Whether strictly speaking the whole apparent reduced visibility of metabolites relates to unaccountably complicated and large dipolar splitting effects for some of the constituents or whether strongly reduced  $T_2$ 's play the major role is still up for investigation.

Summarizing, the present study adds to a growing body of evidence supporting the relationship between muscle fibre orientation and measured metabolite peak areas, which is crucial for any quantitative assays of muscle metabolites in health and disease.

Finally, the water proton  $T_2$  relaxation time was found to be 1 ms longer when the muscle fibres were near the magic angle, as compared to the parallel orientation, while the  $T_1$  relaxation time was indistinguishable between orientations. The orientation dependence of  $T_2$  and independence of  $T_1$  for water protons is reminiscent of the so called magic angle effect reported for water associated with collagen fiber orientation (13,31). It has actually been reported early on for *ex vivo* rat TA muscle (12), where a longer  $T_2$  time was also found at the magic angle (12). This study, as well as ours, suggests that a hydration sphere of actin-myosin filaments exhibits a similar behavior as hydrating layers of collagen fibers and that fast exchange with the bulk of muscle water leads to the same, but largely downscaled, effects as for tendons. Whether this effect may have implications in imaging, as is the case for cartilage, is questionable because of the small extent of the  $T_2$  shortening, but not excluded.

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3 In conclusion, we have determined that our previous observation of differing water to creatine MT  
4 rates between the tibialis anterior and soleus muscles arises from differences in muscle composition and  
5 not orientation dependence. In contrast, metabolite peak areas are orientation dependent, with more  
6 accurate concentrations for tCr, TMA, and taurine determined with the muscle fibre oriented at the magic  
7 angle where dipolar coupling is negligible. Finally, a small but significant difference in water proton  $T_2$   
8 but not  $T_1$  relaxation rates was detected between muscle fibre orientations, proving that the magic angle  
9 effect also occurs for tissues that are not particularly rich in collagen.  
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For Peer Review



## Figure Captions

**Figure 1.** Tibialis anterior muscle spectra obtained with fibres oriented either parallel (upper chart) or at the magic angle to the external magnetic field (lower chart). Top black spectra for each orientation depict the measured spectra averaged over 10 volunteers, coloured lines show the fitted metabolite spectra determined by FiTAID (10) and lower black lines show the residuals. Inset: position of the spectroscopy voxel. FiTAID modeled spectra with Voigt line patterns for a baseline, total creatine (tCr) CH<sub>2</sub> and CH<sub>3</sub>, taurine, and trimethylammonium (TMA) groups. Peak frequencies, phases, and widths were optimized simultaneously for all spectra in the magnetization transfer measurement in each volunteer, while peak areas were permitted to change with water inversion delay time.

**Figure 2.** Average spectra from the water inversion recovery measurement. Top line shows the non-water-inverted (NI) spectra obtained parallel or at the magic angle to the external magnetic field, while each subsequent line shows the difference between spectra obtained with increasing water inversion delay times and the NI spectrum. Delay times are given in ms at the right of the figure.

**Figure 3.** Creatine CH<sub>3</sub> peak areas with increasing water inversion delay times or not water inverted (NI). Symbols mark peak areas fit in FiTAID, with error bars showing the Cramér–Rao lower bounds of the peak area fit, and lines demonstrating the fit of the inversion transfer exchange model, which determined the magnetization exchange rate from water to tCr CH<sub>3</sub>,  $k$  (s<sup>-1</sup>), as well as the T<sub>1</sub> relaxation time (s). Medians (25<sup>th</sup> and 75<sup>th</sup> quartiles) of the exchange rate and T<sub>1</sub> relaxation times for both fibre orientations are given, but neither were significantly different between fibre orientations. The median root mean square errors of the inversion transfer fit expressed as a percent of the non-water-inverted peak area were 8% (5 – 12%) for the magic angle, and 4% (3 – 7%) in the parallel orientation.

**Figure 4.** Comparison of apparent metabolite concentrations between the magic angle (MA) and parallel (PA) orientations. The measured concentration for each metabolite was significantly higher in the magic angle (MA) orientation versus the parallel (PA) orientation ( $p < 0.01$ ). (Tau: taurine)

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**Figure 5.** Relationship between muscle fibre orientation, represented by the splitting of the tCr CH<sub>2</sub> doublet, and apparent tCr peak concentrations. *Top:* tCr CH<sub>2</sub> concentration versus tCr CH<sub>2</sub> doublet splitting. *Bottom:* CH<sub>3</sub> concentration versus tCr CH<sub>2</sub> doublet splitting. The apparent concentration of each tCr resonance correlated with the CH<sub>2</sub> doublet splitting ( $p < 10^{-4}$ ). Diamonds represent measurements intended to be obtained at the magic angle, while circles represent measurements intended to be recorded near a parallel configuration. Standard deviations of the concentration values were always smaller than the symbol size and thus are not shown for clarity. (For abbreviations, see Fig. 4.)

**Figure 6.** Relationship between muscle fibre orientation, represented by the splitting of the tCr CH<sub>2</sub> doublet, and water T<sub>2</sub> relaxation time. (For abbreviations, see Fig. 4.)

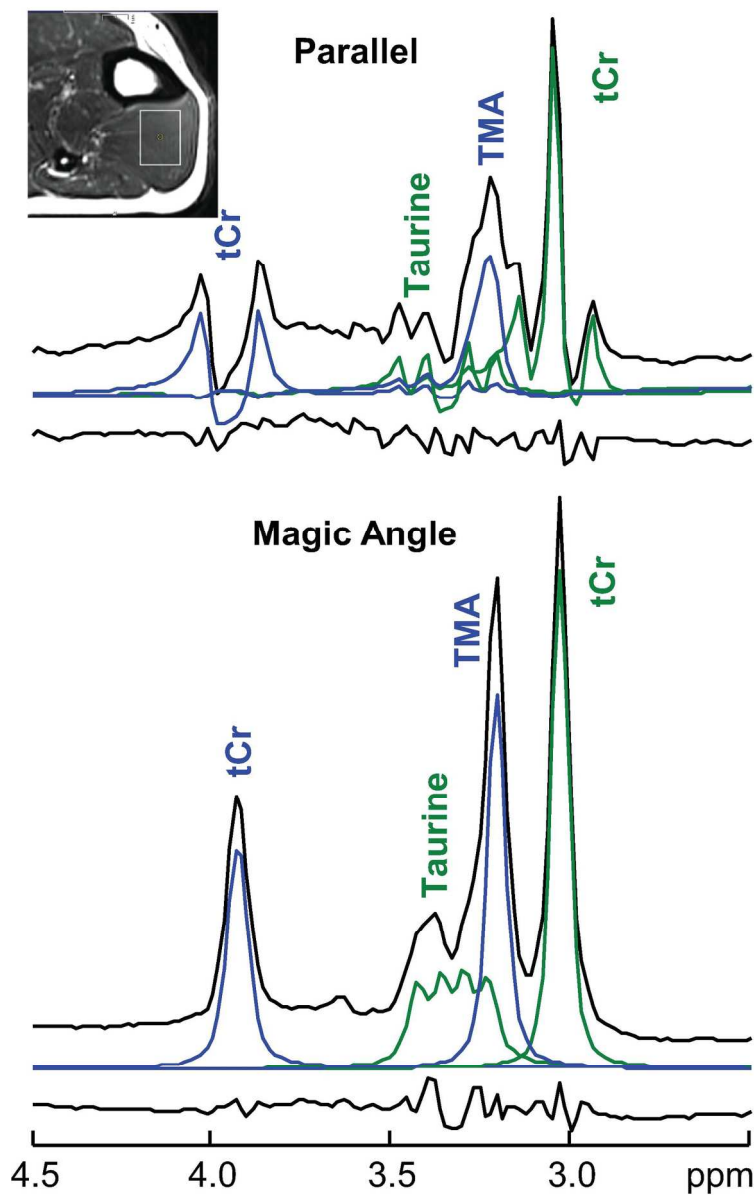


Figure 1.

Tibialis anterior muscle spectra obtained with fibres oriented either parallel (upper chart) or at the magic angle to the external magnetic field (lower chart). Top black spectra for each orientation depict the measured spectra averaged over 10 volunteers, coloured lines show the fitted metabolite spectra determined by FiTAID (10) and lower black lines show the residuals. Inset: position of the spectroscopy voxel. FiTAID modeled spectra with Voigt line patterns for a baseline, total creatine (tCr) CH<sub>2</sub> and CH<sub>3</sub>, taurine, and trimethylammonium (TMA) groups. Peak frequencies, phases, and widths were optimized simultaneously for all spectra in the magnetization transfer measurement in each volunteer, while peak areas were permitted to change with water inversion delay time.

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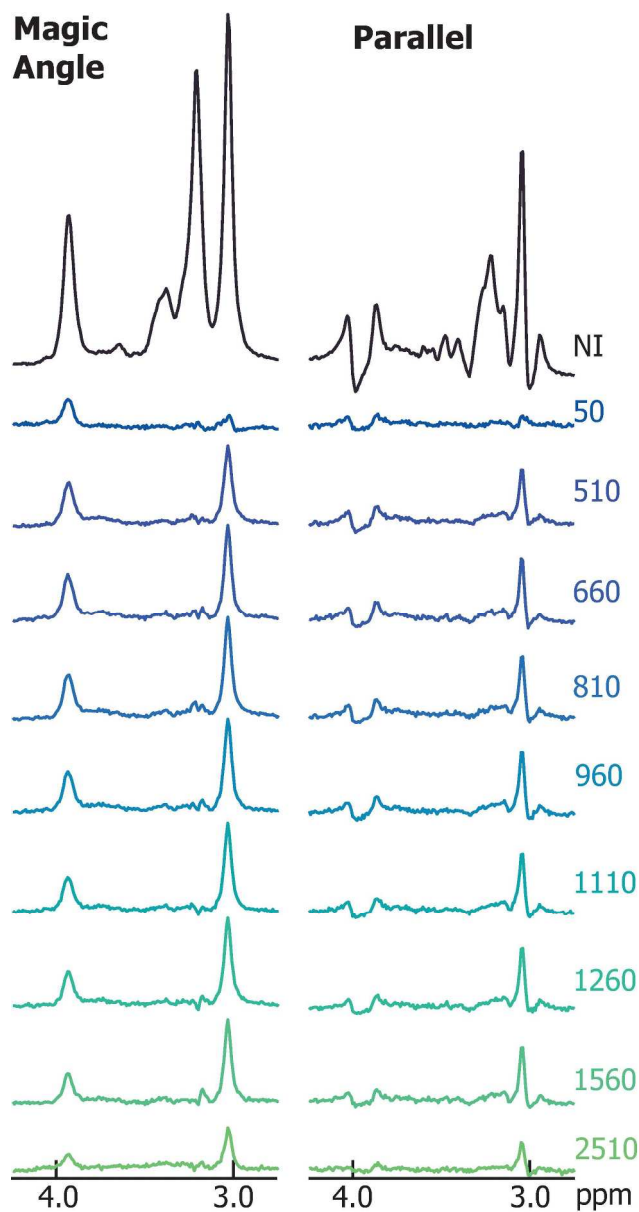


Figure 2.

Average spectra from the water inversion recovery measurement. Top line shows the non-water-inverted (NI) spectra obtained parallel or at the magic angle to the external magnetic field, while each subsequent line shows the difference between spectra obtained with increasing water inversion delay times and the NI spectrum. Delay times are given in ms at the right of the figure.

158x303mm (300 x 300 DPI)

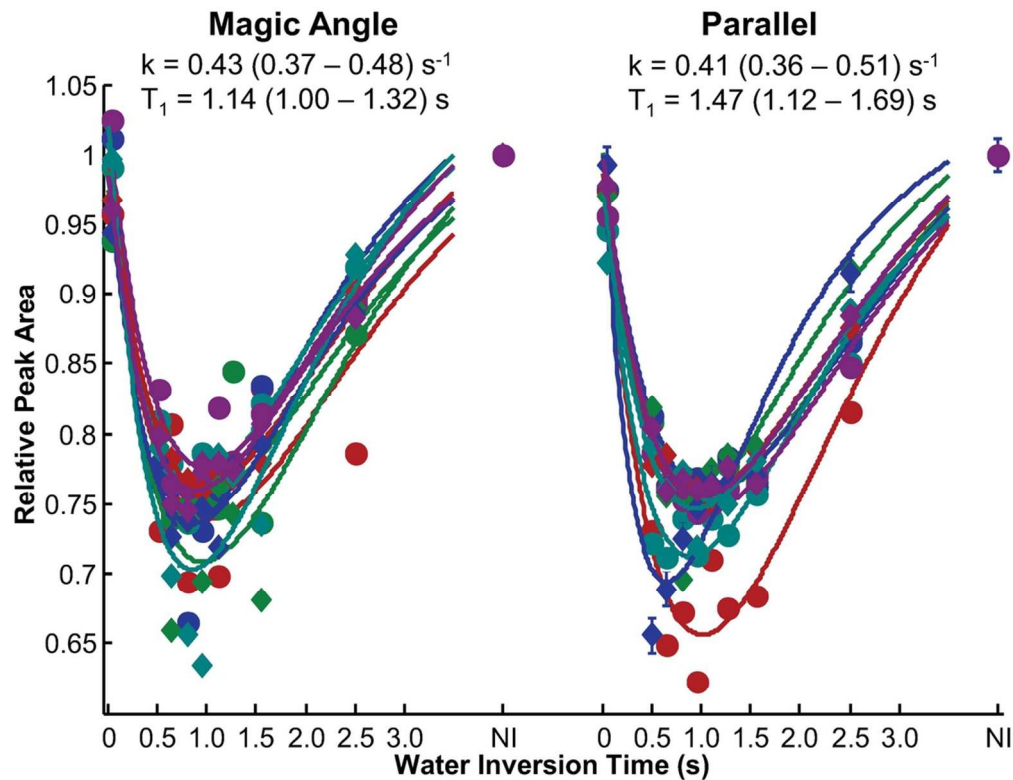


Figure 3. Creatine CH3 peak areas with increasing water inversion delay times or not water inverted (NI). Symbols mark peak areas fit in FiTAID, with error bars showing the Cramér–Rao lower bounds of the peak area fit, and lines demonstrating the fit of the inversion transfer exchange model, which determined the magnetization exchange rate from water to tCr CH3,  $k$  ( $\text{s}^{-1}$ ), as well as the  $T_1$  relaxation time (s). Medians (25th and 75th quartiles) of the exchange rate and  $T_1$  relaxation times for both fibre orientations are given, but neither were significantly different between fibre orientations. The median root mean square errors of the inversion transfer fit expressed as a percent of the non-water-inverted peak area were 8% (5 – 12%) for the magic angle, and 4% (3 – 7%) in the parallel orientation.

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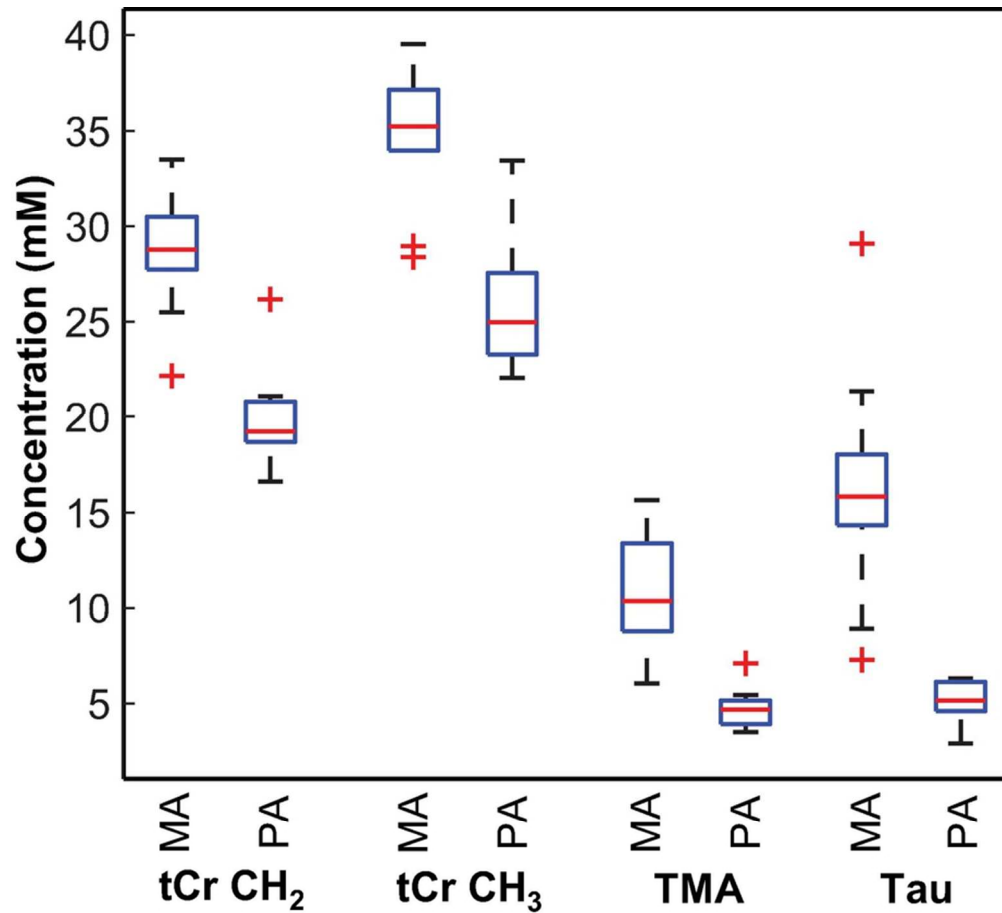


Figure 4.  
Comparison of apparent metabolite concentrations between the magic angle (MA) and parallel (PA) orientations. The measured concentration for each metabolite was significantly higher in the magic angle (MA) orientation versus the parallel (PA) orientation ( $p < 0.01$ ). (Tau: taurine)  
77x71mm (300 x 300 DPI)

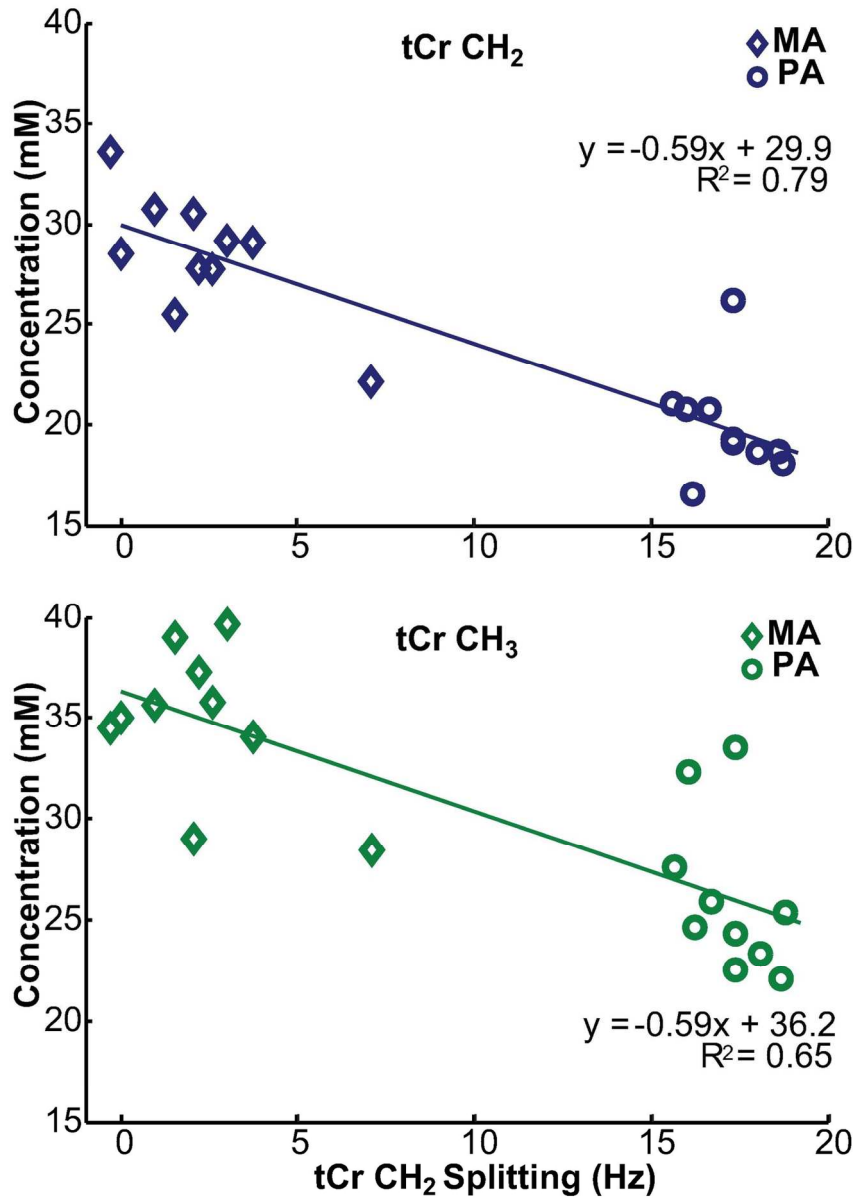


Figure 5.

Relationship between muscle fibre orientation, represented by the splitting of the tCr CH<sub>2</sub> doublet, and apparent tCr peak concentrations. Top: tCr CH<sub>2</sub> concentration versus tCr CH<sub>2</sub> doublet splitting. Bottom: CH<sub>3</sub> concentration versus tCr CH<sub>2</sub> doublet splitting. The apparent concentration of each tCr resonance correlated with the CH<sub>2</sub> doublet splitting ( $p < 10^{-4}$ ). Diamonds represent measurements intended to be obtained at the magic angle, while circles represent measurements intended to be recorded near a parallel configuration. Standard deviations of the concentration values were always smaller than the symbol size and thus are not shown for clarity. (For abbreviations, see Fig. 4.)

122x174mm (300 x 300 DPI)



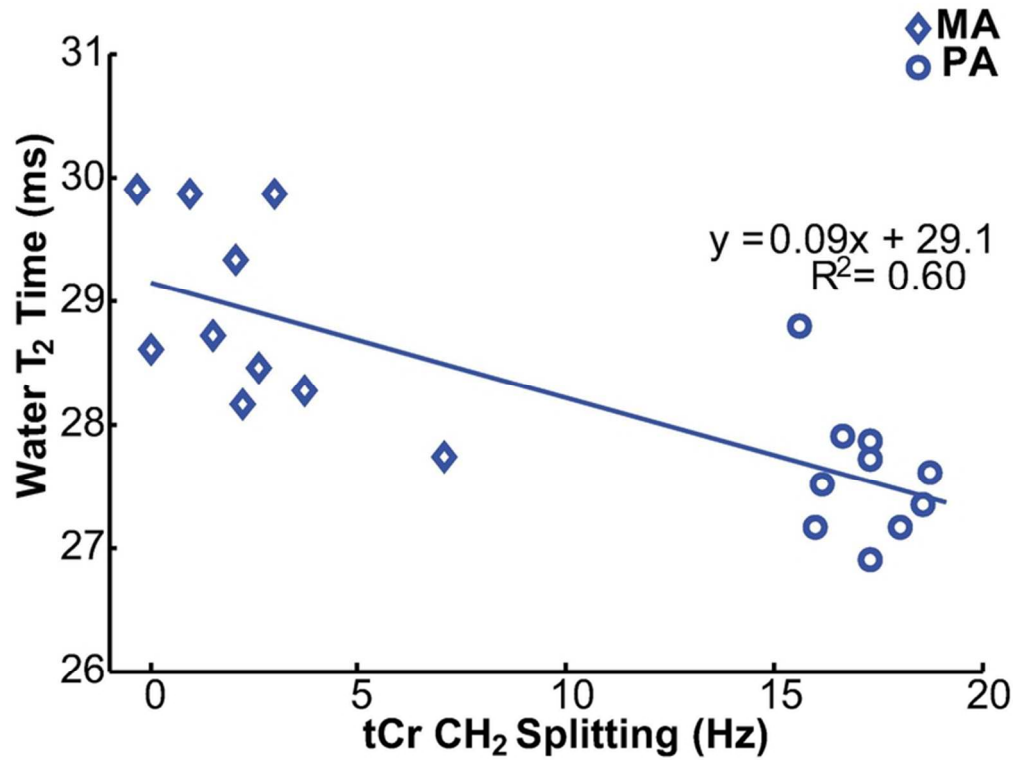


Figure 6.  
Relationship between muscle fibre orientation, represented by the splitting of the tCr CH<sub>2</sub> doublet, and water T<sub>2</sub> relaxation time. (For abbreviations, see Fig. 4.)  
65x50mm (300 x 300 DPI)