

# Magnetization transfer attenuation of creatine resonances in localized proton MRS of human brain *in vivo*

Gunther Helms\* and Jens Frahm

Biomedizinische NMR Forschungs GmbH am Max-Planck-Institut für biophysikalische Chemie, D-37070 Göttingen, Germany

Received 25 January 1999; revised 19 March 1999; accepted 6 April 1999

**ABSTRACT:** To assess putative magnetization transfer effects on the proton resonances of cerebral metabolites in human brain, we performed quantitative proton magnetic resonance spectroscopy (2.0 T, STEAM,  $TR/TE/TM = 6000/40/10$  ms, LCModel data evaluation) of white matter (7.68 mL, 10 healthy young subjects) in the absence and presence of fast repetitive off-resonance irradiation (2.1 kHz from the water resonance) using a train of 100 Gaussian-shaped RF pulses (12.8 ms duration, 120 Hz nominal bandwidth, 40 ms repetition period,  $1080^\circ$  nominal flip angle). A comparison of pertinent metabolite concentrations revealed a magnetization transfer attenuation factor of the methyl and methylene resonances of creatine and phosphocreatine of  $0.87 \pm 0.05$  ( $p < 0.01$ ). No attenuation was observed for the resonances of *N*-acetylaspartate and *N*-acetylaspartylglutamate, glutamate and glutamine, choline-containing compounds, and *myo*-inositol. The finding for total creatine is in excellent agreement with data reported for rat brain. The results are consistent with the hypothesis of a chemical exchange of mobile creatine or phosphocreatine molecules with a small immobilized or 'bound' pool. Copyright © 1999 John Wiley & Sons, Ltd.

**KEYWORDS:**  $^1\text{H}$  NMR; proton MRS; magnetization transfer; human brain; cerebral metabolism; brain metabolites; creatine

## INTRODUCTION

Because macromolecular components as well as protein- and membrane-bound constituents of biological tissues are motionally restricted with  $T_2$  relaxation times of less than 100  $\mu\text{s}$ , they cannot be observed by magnetic resonance imaging (MRI) or spectroscopy (MRS) sequences that cause  $T_2$  losses between radiofrequency (RF) excitation and data acquisition. It is, however, possible to detect pertinent signal contributions indirectly by selectively saturating the invisible 'immobile' pool of magnetization, e.g. by off-resonance irradiation, and measuring the attenuation imposed onto the observable NMR signal representing 'mobile' magnetization in comparison to a control experiment without irradiation.<sup>1</sup> The underlying mechanisms for this 'magnetization transfer' (MT) process are dipolar cross-relaxation between nuclear spin moments in either compartment

or chemical exchange of atoms or molecular groups. Such interactions form the basis for MT contrast of water protons, i.e. hydroxyl protons, which gives novel information on the macromolecular content of the tissue investigated.

In contrast to water MT effects, little is known about the macromolecular interactions of mobile intracellular metabolites that are detectable by localized proton MRS and the question of to what degree non-exchanging metabolite resonances are susceptible to MT attenuation. For example, the apparent underestimation of the creatine and phosphocreatine (tCr) concentration in early MRS studies of human brain relative to biochemical determinations in rats led to the hypothesis of a bound fraction of 'NMR-invisible' tCr.<sup>2</sup> Although MT attenuation of tCr proton resonances could be observed in rat brain<sup>3–5</sup> at 4.7 T, human studies at a lower field strength have been hampered by safety regulations concerning the maximum RF power deposition and technical restrictions of clinical MRI systems that limit the maximum duty cycle of the RF transmitter.

The purpose of this work was to (i) overcome the aforementioned problems by the development of a pulsed off-resonance saturation technique as an alternative to continuous-wave irradiation<sup>3,4</sup> or the use of resonant binomial pulses,<sup>5</sup> and (ii) to unequivocally identify and quantify MT attenuation effects on tCr and other metabolites in human brain *in vivo*.

\*Correspondence to: G. Helms, Karolinska MR Research Center, Karolinska Hospital, N8, S-17176 Stockholm, Sweden.  
E-mail: gunther@mrc.ks.se

**Abbreviations used:** Cho, choline-containing compounds; Cr, creatine; Glu, glutamate; Gln, glutamine; Ins, *myo*-inositol; MRS, magnetic resonance spectroscopy; MT, magnetization transfer; tNAA, *N*-acetylaspartate and *N*-acetylaspartylglutamate; NMR, nuclear magnetic resonance; PCr, phosphocreatine; RF, radiofrequency; SNR, signal-to-noise ratio; STEAM, stimulated echo acquisition mode; tCr, total creatine; VOI, volume-of-interest.

## MATERIALS AND METHODS

### Localized proton MRS

Studies of 10 healthy adults (five female/five male, age range 20–40 years) were carried out at 2.0 T using a clinical MRI system (Siemens Vision, Erlangen, Germany) and the standard circularly polarized head coil. Spectral acquisitions (STEAM localization,  $TR = 6000$  ms) focused on a 7.68 mL ( $16 \times 16 \times 30$  mm<sup>3</sup>) volume-of-interest (VOI) in the subparietal centrum semiovale excluding the cortex and lateral ventricles.<sup>6</sup> The choice of this white matter VOI was motivated by experimental care to minimize motion-related inconsistencies. For example, locations in cortical gray matter are more susceptible to variable partial volume effects with cerebrospinal fluid due to involuntary head movements. To avoid putative baseline contributions from broad macromolecular resonances while retaining a relatively short echo time for good SNR and the detectability of strongly coupled resonances, the echo time was set to  $TE = 40$  ms ( $TM = 10$  ms).

MT effects on metabolite resonances were assessed by comparing two experiments (64 scans each) performed with and without off-resonance saturation. A single scan without water suppression was used to correct for residual phase distortions.<sup>7</sup> In general, the acquired spectra showed good resolution with a mean linewidth of 0.044 ppm (3.4 Hz at 2.0 T, range 0.040–0.052 ppm). Spectra with poor linewidths or severe baseline distortions due to inadequate water suppression were excluded from the analysis. All data were evaluated by LCMoDel<sup>8</sup> yielding absolute metabolite concentrations as previously described.<sup>6</sup>

Quantitation of MT effects was based on statistical comparison (one-sided paired *t*-test) of absolute concentrations thus combining possible MT effects of all resonances of a particular metabolite. MT attenuation factors are given as the ratios of concentration values obtained with and without off-resonance irradiation. An additional check for consistency involved coherent averaging of all spectra with and without off-resonance irradiation, respectively, and subsequent LCMoDel analysis of the mean spectra with markedly improved SNR.

### Magnetization transfer by pulsed off-resonance saturation

Because continuous wave saturation is generally not available on clinical MRI systems, selective saturation of the immobile magnetization pool can be achieved in two ways: either by very short resonant pulses<sup>9</sup> which are 'transparent' for the observable magnetization, i.e. yielding an effective flip angle of zero degrees; or by off-resonance irradiation of narrowband RF pulses. Despite a certain loss of saturation efficiency, we have

opted for the latter scheme because it combines the advantages of continuous wave methods, i.e. narrowband irradiation and control of frequency-dependent effects, and pulsed saturation, i.e. limited duration of the RF irradiation.

Lacking a detailed knowledge of the physical properties of the immobile pool and the polarization transfer mechanisms, the off-resonance pulsed saturation technique is qualitatively discussed. The parameters which govern the partial saturation of the mobile pool are the saturation factor  $\sigma$  describing the result of a single pulse on the immobile pool and the pulse repetition period  $PR$  during which the transfer takes place. Assuming kinetic rates of about 1/s and a pulse duration of a few milliseconds, polarization transfer during the pulse may be neglected.

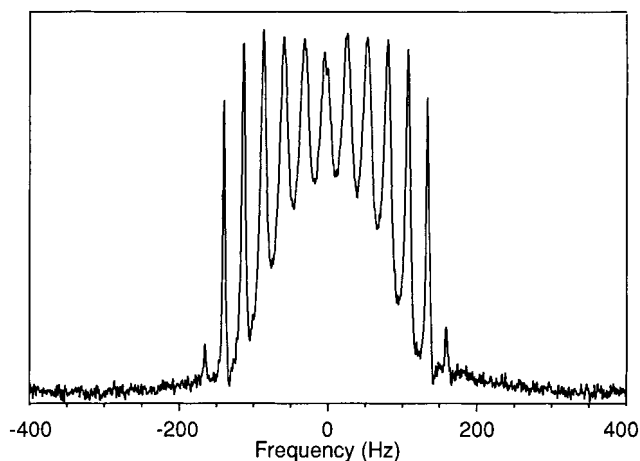
For short pulse repetition times,  $PR$ , the MT-induced decrease of the steady-state signal approaches the maximum attenuated signal reached for continuous-wave irradiation, even if the immobile pool is not completely saturated ( $1 > \sigma > 0$ ).<sup>10</sup> On the other hand, this strategy requires more pulses to reach a steady state as less time is provided for polarization transfer between successive pulses. In practical terms, a large number of rapidly repeated pulses may eventually violate the restrictions of the RF amplifier's duty cycle, which often proves to be the limiting factor in designing a suitable MT saturation protocol. Average RF power deposition, however, may be kept below safety limits by increasing the experimental repetition time  $TR$ .

### Temporal saturation protocol and frequency excitation profile

The repetitive application of saturation pulses broadens the resulting excitation profile relative to that of a single RF pulse and periodically modulates it at the reciprocal repetition period  $1/PR$ . Here we used shaped RF pulses with a Gaussian profile because their direct saturation profile decays faster than that of a rectangular pulse. Assuming an RF peak limit of 1 kHz and a rectangular pulse shape, a minimal duration of 1–2 ms has been estimated to achieve a saturation factor of  $\sigma = 0.1$ .<sup>9</sup> To ensure similar results with Gaussian pulses, the pulse duration was set to 12.8 ms yielding an irradiation strength of greater than 90% of the peak amplitude for about 2 ms.

Taking a kinetic exchange rate of creatine in rat brain<sup>11</sup> of  $k_f = 0.36^{-1}$  and  $T_{1,f} \approx 1.5$  s, a rough estimate of the approach to steady state,<sup>12</sup>  $\mu_1 \approx 1 - PR \times (1/T_{1,f} + k_f)$ , requires the application of about 100 RF pulses, i.e. a saturation pulse train of 4 s duration for a pulse repetition time of  $PR = 40$  ms. For these parameters the RF hardware monitor on our system limited the pulse amplitude to a nominal flip angle of 1080°.

Special care was taken to avoid any direct saturation of



**Figure 1.** Excitation profile of the pulse train used for off-resonance irradiation of proton metabolite spectra (100 Gaussian-shaped RF pulses, 12.8 ms duration, 120 Hz nominal bandwidth, repetition period 40 ms, nominal flip angle  $1080^\circ$ ). The profile has been obtained by taking the difference spectrum between two on-resonance scans with and without off-resonance irradiation (phantom containing tap water). The 150 Hz width of the broad excitation corresponds to the effective 6 ms temporal width of the RF pulses, whereas spikes occur periodically at the reciprocal repetition period  $1/40 \text{ ms} = 25 \text{ Hz}$

metabolite resonances in order to unequivocally discern MT effects. The frequency range affected by direct saturation was determined with use of a phantom containing tap water and application of a constant gradient during pulse off-resonance irradiation and data acquisition to broaden the water proton resonance. Figure 1 shows a corresponding difference spectrum obtained for experiments with and without irradiation. Qualitatively, the profile resembles the characteristics of both a single pulse excitation resulting in a width of about 150 Hz that corresponds to the inverse of the effective temporal width ( $\approx 6 \text{ ms}$ ) of the individual 12.8 ms Gaussian-shaped pulses and the application of serial RF pulses yielding periodic excitations at the inverse repetition period  $1/PR = 1/40 \text{ ms} = 25 \text{ Hz}$ . The frequency range between the two outer spikes covers about 330 Hz, which is narrow compared to the expected width of the MT attenuation of creatine.<sup>3,4</sup>

The lowest frequency offset at which off-resonance irradiation may be accomplished without direct saturation was determined to be approximately 0.5 kHz for the used train of 12.8 ms Gaussian-shaped RF pulses. Furthermore, any direct saturation at arbitrary frequency offsets by periodic spikes in the excitation profile was experimentally excluded by varying the RF carrier frequency in 2 Hz steps across a 50 Hz interval. For a frequency offset of 0.76 kHz no considerable change of the water signal was observed, which limits 'spiking' to a smaller frequency band in agreement with the profile shown in Fig. 1. A theoretical estimate of a possible spillover of a single  $1080^\circ$  RF pulse on the tCr resonances

**Table 1.** Magnetization transfer attenuation factors (mean  $\pm$  SD) of cerebral metabolites in white matter of young healthy subjects ( $n = 10$ ) as obtained by localized proton MRS (STEAM,  $TR/TE/TM = 6000/40/10 \text{ ms}$ , 64 accumulations) with and without pulsed off-resonance irradiation

Metabolite	Magnetization transfer attenuation Single subjects	Mean spectra
tCr	$0.87 \pm 0.05^{**}$	0.85
tNAA	$1.02 \pm 0.05$	1.01
Cho	$0.97 \pm 0.07$	0.98
Ins	$1.03 \pm 0.10$	1.04
Glu + Gln	$1.05 \pm 0.15$	0.96

\*\*  $p < 0.01$  (one-sided paired *t*-test).

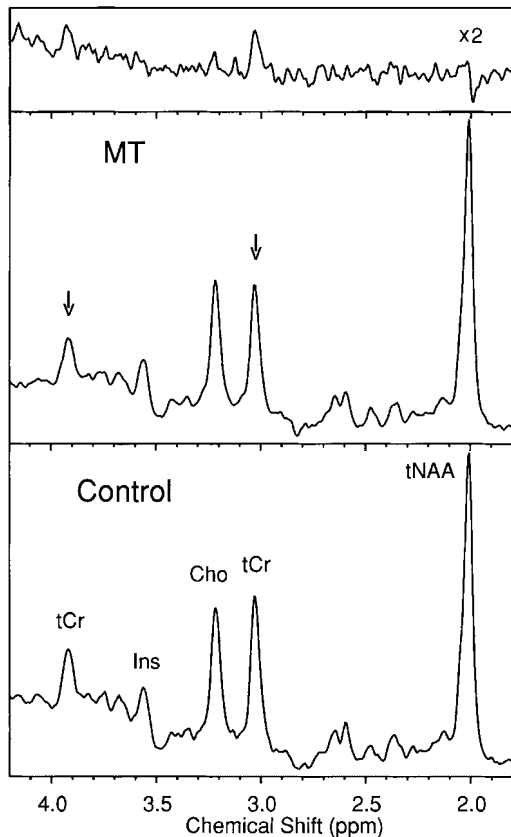
yielded about 0.1% by stepwise integration of the Bloch equations using  $T_1 = 1500 \text{ ms}$  and  $T_2 = 200 \text{ ms}$ .<sup>5</sup> This would lead to a direct saturation of less than 4% after 100 repetitions. For the practical observation of MT effects on metabolites, the offset frequency was set to 2.1 kHz and applied to the high-frequency side of the water resonance. To account for a possible saturation of the mobile magnetization pool through RF noise or leakage, the transmitter was kept unblanked during the acquisition of spectra without off-resonance irradiation.

## RESULTS

Mean MT attenuation factors are summarized in Table 1. A statistically significant attenuation factor of  $0.87 \pm 0.05$  ( $p = 0.004$ ) was found for tCr but not for tNAA, Cho, Ins and Glu + Gln. Figure 2 shows mean spectra averaged across subjects with (MT) and without (control) off-resonance irradiation as well as a corresponding difference spectrum (top trace). An LCMoDel evaluation of these high-SNR spectra confirmed the findings for individual subjects in yielding a tCr attenuation factor of 0.85 and no MT effects for other metabolites.

## DISCUSSION

Although the pulsed off-resonance saturation technique used here is less effective than continuous-wave irradiation of the same duration or high power on resonance saturation which has been employed on 4.7 T small bore animal systems,<sup>3-5</sup> an efficient attenuation of the observable mobile pool could be achieved by using a short-pulse repetition period. This allowed for a conservative choice of the frequency offset for off-resonance irradiation to minimize RF bleeding or side excitations within the frequency range of mobile proton resonances in the visible metabolite spectrum.



**Figure 2.** Mean localized proton MR spectra (10 subjects) of human white matter (2.0 T, STEAM,  $TR/TE/TM = 6000/40/10$  ms, 7.68 mL VOI, 64 accumulations) with (MT) and without magnetization transfer irradiation (control). The top trace corresponds to the difference of the summed spectra scaled by a factor of 2. Metabolite resonances include *N*-acetylaspartate and *N*-acetylaspartylglutamate (tNAA), creatine and phosphocreatine (tCr), choline-containing compounds (Cho), and *myo*-inositol (Ins)

The observed MT attenuation of the tCr pool in human white matter, i.e. of both the methyl and methylene resonances, confirms previous results obtained for rat brain at 4.7 T.<sup>3–5</sup> Notwithstanding the fact that any quantitative comparison may be hampered by the complex dependence of the MT effect on details of the saturation paradigm, the present findings are in excellent agreement with the most recent animal study reporting an attenuation factor of  $0.85 \pm 0.03$  for normal tissue.<sup>5</sup> Also, the absence of significant MT attenuation for tNAA, Cho and Ins is in line with the observations for rat brain. Inconsistent attenuations of the  $\text{CH}_2$  or CH resonances of Glu + Gln<sup>5</sup> are not seen here.

The proton resonances of tCr, tNAA, Cho and Glu/Gln stem from non-exchanging  $\text{CH}_n$  groups ( $n = 1, 2, 3$ ). The corresponding assumption that the methyl and methylene resonances of tCr experience the same MT attenuation is clearly supported by the fact that spectral evaluation by LCMODEL, i.e. by using a Cr model spectrum, yielded excellent fits of the data in the presence and absence of

off-resonance irradiation, respectively. This observation is further confirmed by the ratio of the creatine resonances in the difference spectrum. Possible explanations for the observed MT attenuation of tCr have been discussed in detail in previous publications.<sup>3,5</sup> Although the primary aim of this study was an experimental verification of putative MT effects on proton metabolite resonances in human brain, rather than an analysis of underlying mechanisms, the attenuation of tCr supports the hypothesis of chemical exchange of entire creatine or phosphocreatine molecules with a small immobilized ('bound' and 'invisible') pool.

Instead of unspecific interactions with macromolecular components that should also affect other metabolites in respective cellular microenvironments, it is more likely that the specific binding of creatine to the enzyme creatine kinase is responsible for most of the observed MT attenuation. However, using transgenic mice deficient in both cytosolic and mitochondrial creatine kinase, it has recently been shown that the tCr MT effect in mouse skeletal muscle is not solely dependent on the existence of the enzyme.<sup>13</sup> The exact nature of the tCr MT effect may not be concluded from this work. While the use of phosphorus MRS might be helpful to separate the contributions of creatine and phosphocreatine, studies of altered MT effects in disease states of the brain may lead to further insights.

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