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Simultaneous Lactate Editing and Observation of Other Metabolites Using a Stimulated-Echo-Enhanced Double-Quantum Filter

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Conventional double-quantum editing techniques recover only one metabolite at a time, and are thus inefficient for monitoring metabolic changes involving several metabolites. In this paper, a stimulated-echo-enhanced selective double-quantum coherence transfer (STE-SelDQC) sequence is described, which allows simultaneous observation of lactate and other metabolites in a single scan while leaving fat and water signals suppressed. A frequency selective double-quantum filter designed for lactate editing suppresses fat and water resonances and a stimulated-echo window of adjustable frequency and bandwidth is incorporated into the double-quantum filter for simultaneous observation of other metabolites. The performance of the sequence is demonstrated in phantoms and rat brain tissue. © 1999 Academic Press

Key Words: **double-quantum filter; stimulated-echo; lactate.**

INTRODUCTION

Accurate measurement of lactate *in vivo* using ^{1}H MR spectroscopy often requires spectral editing to isolate the lactate methyl resonance from intense lipid peaks at 1.33 ppm. Gradient-selected multiple-quantum coherence transfer techniques can generate edited spectra with good sensitivity in a single scan, so that they are less sensitive to motion than editing techniques which require subtraction of sequentially acquired spectra. Gradient-selected double-quantum (DQ) coherence methods have been used to edit MR spectra for lactate *in vivo* (*1–4*).

A general problem with editing techniques is that they recover spectra of only one metabolite at a time. They are thus inefficient in acquiring data if more than a single metabolite is of interest and the final spectrum does not contain peaks from other metabolites such as *N*-acetylaspartate (NAA) or creatine (Cr) that can serve as internal concentration standards. It would therefore be useful in many cases to be able to observe the signals from lactate and such other metabolites simultaneously. A PRESS technique has been described in which *J*-difference lactate editing allows simultaneous acquisition of the lactate methyl signal and singlets from choline, creatine, NAA, and lipids (*5*). Two other techniques based on gradient-selected DQ coherence transfer have been described for detection of edited lactate and other metabolites in a single scan (*3, 6*). In those techniques, a DQ filtered coherence transfer echo of lactate and a spin echo of the other metabolites are formed and detected either in one prolonged acquisition window (*3*) or in two sequential acquisition windows (*6*). An alternate sequence using a stimulated-echo-enhanced DQ editing acquisition to observe the edited lactate signal simultaneously with the recovery of resonances of other metabolites is described here, and the performance is demonstrated in phantoms and rat brain tissue *in vitro.*

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THEORY

A selective coherence transfer version of a metabolite-editing DQ coherence transfer sequence is shown in Fig. 1A (*7*). For lactate editing, the frequency selective excitation pulses are set to excite narrow frequency windows around 1.33 and 4.11 ppm, and the frequency selective observation pulse is centered at 4.11 ppm. The magnetization outside these regions remains oriented along the z axis at the end of the sequence. It is convenient to choose $\tau_1 = \tau - t_1$ and $\tau_2 = \tau + t_1$ in order to refocus simultaneously the coherence transfer echo and B_0 inhomogeneity experienced by the DQ coherence during the t_1 period (*8*). In practice, t_1 should be kept as short as possible to minimize effects of *J*-modulation, but it has a minimum of about 10 ms, depending on the length of the frequency selective excitation pulse and observation pulse and the duration of G_1. Then with $2\tau = 68$ ms, τ_2 is at least 44 ms, and this time is available to obtain NMR signals from the non-edited metabolites simultaneously with the edited lactate signal.

One sequence that can be used to do this is shown in Fig 1B. The insertion of three identical frequency selective 90° pulses into the τ_2 period forms a stimulated-echo window and generates a stimulated echo from the magnetization of un-edited metabolites whose resonances fall within that window (*9, 10*). The stimulated-echo window can be shifted along the chemical

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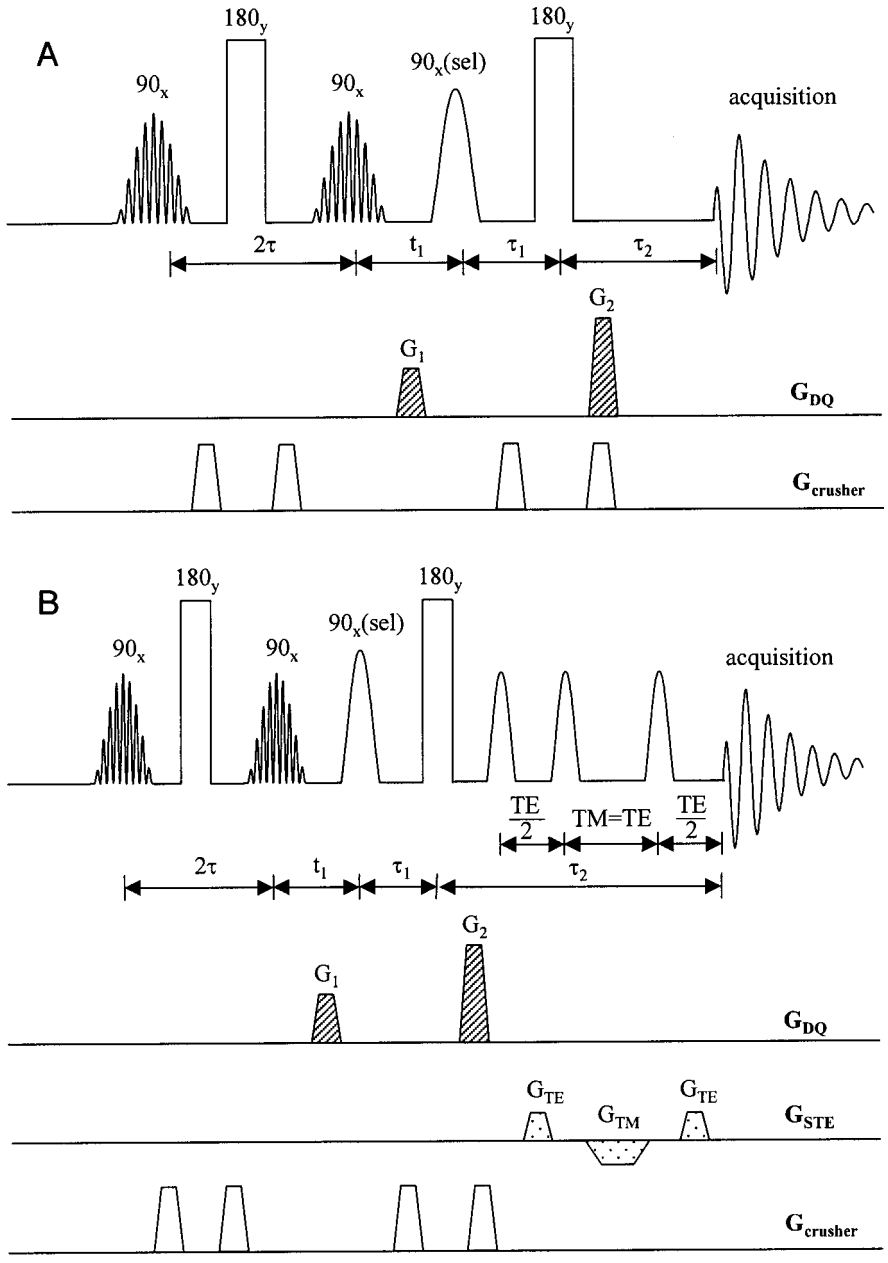

FIG. 1. The conventional selective double-quantum coherence transfer pulse sequence for lactate editing (A) and the stimulated-echo-enhanced selective double-quantum coherence transfer pulse sequence for simultaneous lactate editing and observation of un-edited metabolites (B). For the experiments described here, the gradient pulses, including the crusher pulses, the double-quantum coherence selective gradient pulses, and the stimulated-echo selective gradient pulses, were all 1 ms in duration. $G_2 = 2G_1 = 64$ mT/m, $G_{TE} = -G_{TM} = 15$ mT/m, $2\tau = 68$ ms, $t_1 = 18$ ms, $\tau_1 = \tau - t_1 = 16$ ms, $\tau_2 = \tau + t_1 = 52$ ms.

shift axis by adjusting the carrier frequency and the bandwidth of these three 90° pulses as long as the lactate methyl resonance and lipid resonance at about 1.33 ppm, the lactate methine resonance at about 4.11 ppm, and the water resonance at about 4.7 ppm are not affected. A pair of echo-time (TE) crusher gradient pulses and a mixing-time (TM) crusher gradient pulse (*9*, *10*), adjusted so that the single-quantum lactate coherence is not affected, are used to select the stimulated echo. Timing of the stimulated-echo window can be adjusted so that the stimulated echo is generated at the end of τ_2, together with the coherence transfer echo of lactate.

EXPERIMENTAL

All phantom experiments were performed on a Bruker Biospec spectrometer using a 9.4 T/21 cm Magnex magnet

equipped with a custom-made actively shielded field gradient coil. An inductively coupled Alderman–Grant RF probe with a 35 mm inner diameter was used for both transmission and reception. The performance of sequence 1B was tested on two phantoms. Each phantom consisted of a 1.2 cm diameter vial, phantom I containing lactate and GABA in saline and phantom II containing lactate and *N*-acetylaspartate (NAA) in saline. For lactate editing, a 20 ms sine-modulated single-lobe sinc pulse with double excitation bands having half-height bandwidths of 120 Hz was used as the frequency selective excitation pulse (*11*). The sine modulation and carrier frequency were adjusted so that the two excitation bands were centered at the $-CH_3$ and –CH– resonances of lactate. An 8 ms single-lobe sinc pulse with a half-height bandwidth of about 250 Hz was used as the frequency selective observation pulse. Either a 9 ms single-lobe (half-height bandwidth of about 180 Hz) or a 9 ms three-lobe (half-height bandwidth of about 700 Hz) sinc pulse was used as the stimulated echo excitation pulse. The flip angles of all pulses were carefully calibrated (*12*), and all crusher gradients and coherence transfer pathway selection gradients were adjusted experimentally to a near magic-angle setting to maximize the efficiency of water/fat suppression (*13*). Each spectrum was acquired with 16 transients into 1024 data points with TR = 2.0 s and with gradient amplitudes and delays as given in the caption to Fig 1. Spectral processing included zero-filling FIDs to 4k data points and a 5 Hz exponential line broadening.

In vitro experiments on rat brain were carried out on a Bruker Biospec/3 7 T/21 cm spectrometer equipped with actively shield gradients. A homebuilt 3 cm diameter saddle coil was used for both transmission and reception. Rat brain tissue was homogenized and placed in a 1.2 cm diameter vial for MR spectroscopy. All experimental settings for lactate editing in the *in vitro* experiments were the same as those used in the phantom experiments. For observation of creatine and choline resonances, an 11 ms single-lobe sinc pulse was used as the stimulated-echo excitation pulse with the carrier frequency set to 520 Hz upfield of the lactate methyl resonances.

RESULTS

A lactate edited spectrum from phantom I acquired by sequence 1A is shown in Fig. 2A. The water signal and GABA signals are completely suppressed due to selective coherence transfer. Figures 2B to 2D show spectra acquired using sequence 1B with the stimulated-echo windows centered on the γ-, α-, and β-GABA resonances, respectively, while in spectrum 2E, the 1.8 ppm wide stimulated-echo window covers all three GABA resoances. The amplitudes of the γ- and β-GABA peaks in 2E are smaller than those in spectra 2B and 2C because of imperfections in the excitation profile of the sinc pulse. The variation in the amplitude of lactate peaks among spectrum 2A to 2E is less than 2%.

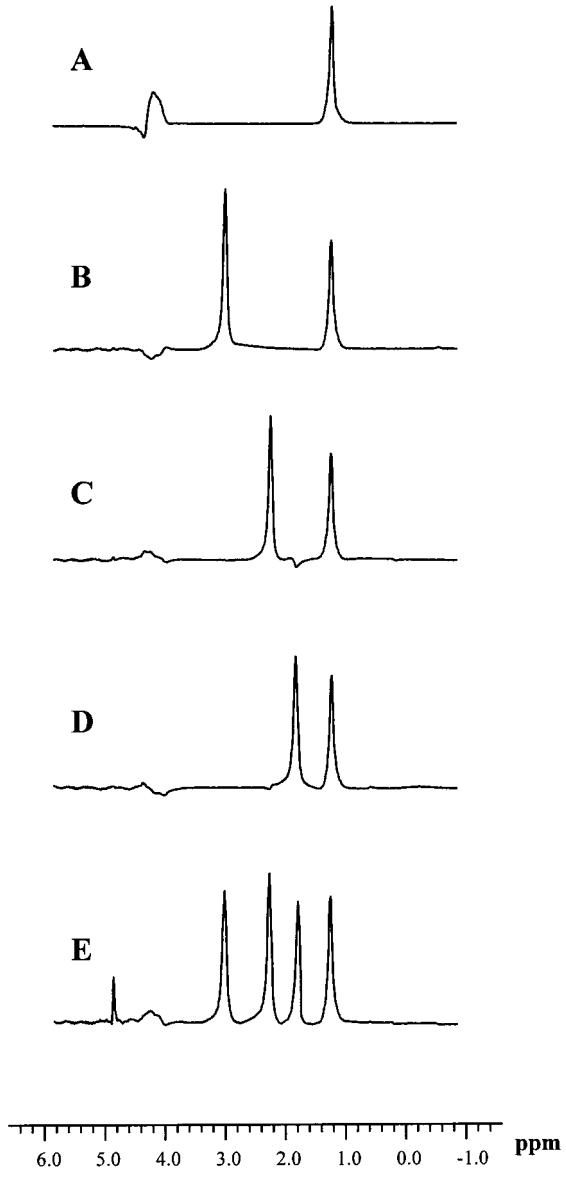

FIG. 2. Spectra obtained from Phantom I (solution of lactate and GABA in saline) using pulse sequence 1A (A) and 1B (B–E). Spectra B, C, and D were acquired with the stimulated-echo windows (9 ms single-lobe sinc pulses) centered on the γ-, α-, and β-GABA resonances, respectively. For spectrum E, the stimulated-echo window was formed by using three 9 ms three-lobe sinc pulses with the carrier frequency at 470 Hz upfield of the lactate methyl resonance (i.e., about 2.5 ppm).

Spectra 3A to 3C (see Fig. 3) show the results from phantom II. For spectrum 3A, the stimulated-echo window was centered on the 2.02 ppm NAA resonance. As expected, both the lactate methyl signal at 1.33 ppm and the NAA singlet at 2.02 ppm were recovered. For spectrum 3B, the power of the two lactate excitation pulses was reduced to a minimum, so that NAA was observed via the stimulated-echo window without lactate editing. As shown, the NAA

methyl peak was recovered, while no peak at 1.33 ppm was observed, demonstrating that the stimulated-echo window for NAA at 2.02 ppm did not excite the lactate methyl resonance. For spectrum 3C, the carrier frequency was switched to "edit" the NAA singlet at 2.02 ppm while putting the stimulated-echo window on the lactate methyl peak. The lactate methyl peak was recovered and no intensity for NAA at 2.02 ppm was found, verifying the fact that the editing efficiency was not degraded by the stimulated-echo window.

Spectra from rat brain tissue are shown in Fig 4. Spectrum 4A was acquired using sequence 1A, and only lactate signals are present in the spectrum. Spectrum 4B was acquired using sequence 1B with the frequency of the stimulated-echo excitation pulses centered between the creatine and choline resonances. As expected, both creatine and choline singlets were recovered together with the edited lactate signals.

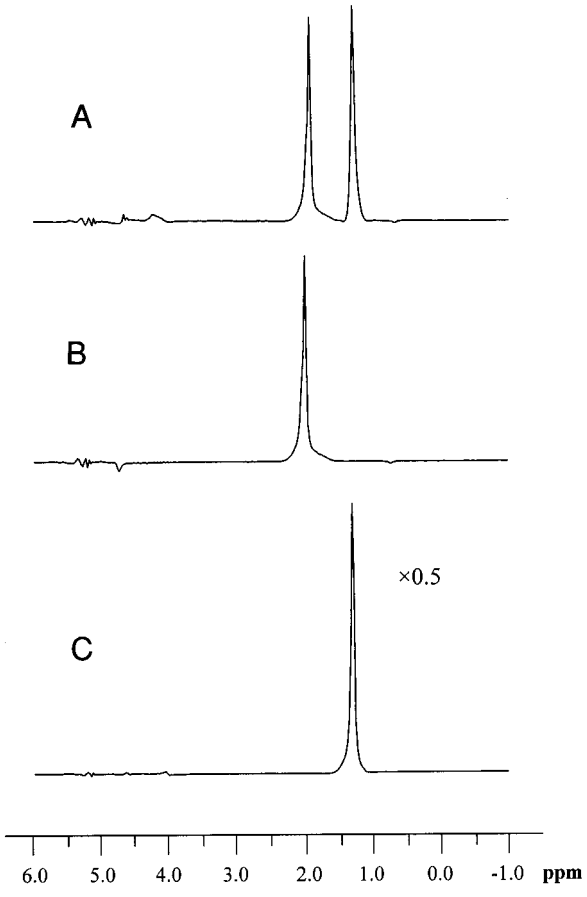

FIG. 3. Spectra obtained from Phantom II (solution of lactate and NAA in saline) using pulse sequence 1B with the stimulated-echo window formed by three 9 ms single-lobe sinc pulses with the carrier frequency centered on the NAA methyl peak at 2.02 ppm (A, B), or on the lactate methyl peaks at 1.33 ppm (C). Spectra were acquired with (A) or without (B) lactate editing or with "NAA editing" (C).

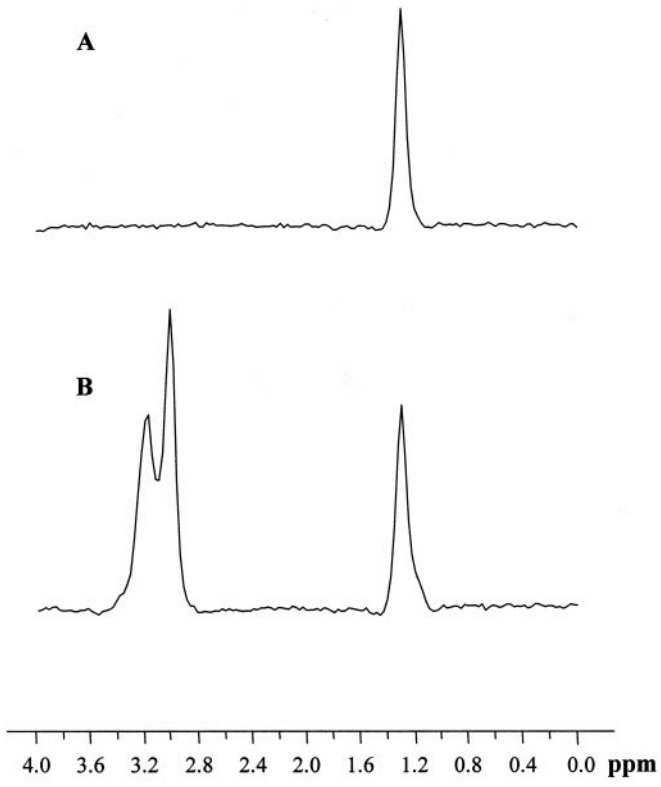

FIG. 4. Spectra obtained from rat brain *in vitro* using pulse sequences 1A (A) and 1B (B), with the stimulated-echo window formed by three 11 ms single-lobe sinc pulses with the carrier frequency centered at 3.12 ppm.

DISCUSSION

Lactate editing in combination with recovery of resonances of other metabolites has been reported previously using spin-echo-enhanced selective DQ coherence transfer (SEE-SelDQC) (*3, 6*). An alternative sequence using stimulated-echo-enhanced selective DQ coherence transfer (STE-SelDQC) has been presented here. Compared with SEE-SelDQC, STE-SelDQC has several advantages. First, in the previously described SEE-SelDQC sequences, it takes extra time to generate the spin echo, while it is possible to incorporate the entire stimulated-echo part of the sequence into the τ_2 duration of the editing sequence in STE-SelDQC. No extra time is needed. Second, in SEE-SelDQC, the minimum TE for the non-edited metabolites is long (i.e., about 160–170 ms) so that only resonances from metabolites with long T_2 can be recovered (*3, 6*). On the other hand, using STE-SelDQC, shorter TE (18–22 ms) is possible, depending on the duration of the frequency selective pulses used to excite the stimulated echo. Third, using SEE-SelDQC, the lactate coherence transfer echo and the spin echo of other metabolites cannot be refocused simultaneously. Consequently, the acquisition windows must be prolonged or two acquisition windows must be used to accommodate the two echoes. In STE-SelDQC, the timing of the stimulated-echo

window can be adjusted so that the lactate coherence transfer echo and the stimulated echo are refocused simultaneously. Finally, the STE-SelDQC sequence is more flexible than the SEE-SelDQC sequence in terms of recovering non-edited metabolites. For example, the excitation frequency and the bandwidth of the stimulated-echo excitation pulse can be adjusted to create a stimulated-echo window which captures any desired metabolite resonances as long as the lactate, lipid, and water resonances do not fall into the simulated-echo window.

However, there are also some limitations for the STE-SelDQC sequence. First, because the stimulated-echo frequency selective pulses are long (typically 8–10 ms), t_1 must be increased in order that the τ_2 period be long enough to incorporate the entire stimulated-echo part of the sequence and refocus the lactate coherence transfer echo and the stimulated echo simultaneously ($\tau_2 = \tau + t_1$). Without the stimulated-echo part, t_1 can be as short as 10 ms, while adding the stimulated-echo sequence requires that t_1 be increased to at least 18 ms. Because the edited lactate signal is modulated by a factor of $\cos^2(\pi J t_1)$, this increase in t_1 results in a lactate signal loss of about 13%. Second, because the lactate single-quantum coherence must experience the TM and TE crusher gradients during τ_2, the lactate signal intensity will be decreased by spin diffusion effects (*14*). However, because the amplitudes of the TM and TE crusher gradients were small (15 mT/m) and the duration was short (1 ms) in this study, spin-diffusion effects were minor, as can be see by comparing the lactate peaks in spectra 2A and 2B. Third, because resonances of non-edited metabolites are excited and observed during τ_2, it is essential to ensure that the magnetization of those metabolites remains intact at the beginning of τ_2 in order to obtain maximum sensitivity. Theoretically, transverse magnetization of non-edited metabolites is not excited during the first part of the sequence. However, because of RF pulse imperfections and T_1 relaxation between the first and the second 180° refocusing pulses, a 10% signal loss was found in the present study for the non-edited metabolites when the editing part of the sequence was added ahead of the stimulated-echo window. To some extent, this problem can be avoided by carefully tuning the power and bandwidth of the RF pulses used for lactate editing, particularly for the two hard refocusing pulses. Fourth, using the stimulated-echo sequence and balanced TM and TE crusher gradients, two signals are refocused at the end of the stimulated-echo window, namely, the stimulated echo and FID1 (the FID generated by the first 90° pulse of the three 90° pulses). However, the B_0 inhomogeneity experienced by FID1 is not refocused during the subsequent part of the sequence, and T_2^* is short enough for FID1 to decay completely before acquisition. This can be seen from Figs. 2B to 2D. The absence of any phase distortion in the GABA peaks demonstrates that only the stimulated echo was observed in this study.

Both the DQ coherence transfer and the stimulated-echo sequences recover 50% of the signal intensity. However, the edited lactate resonance can experience further reductions in intensity due to spin-diffusion effects and to *J*-modulation during t_1, as described above. Furthermore, the resonance of lactate and the resonances of metabolites excited by the stimulated-echo sequence experience significantly different echo times, so that differences in the extent of T_2 relaxation can also affect the relative signal intensities. Direct comparison of signal intensities to assess relative concentrations must take these features into account.

Spatial localization of the STE-SelDQC sequence can be achieved by chemical shift imaging (CSI). Two or three phase encoding gradients can be placed between the last stimulated-echo excitation pulse and the data acquisition window. If two-dimensional CSI is used, the spatial localization in the third dimension can be achieved by longitudinal Hadamard encoding (*15*), in which a Hadamard encoding RF pulse is applied at the beginning of the sequence to encode the longitudinal magnetization of both lactate and other metabolites.

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