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RESEARCH ARTICLE

Simultaneous detection of valine and lactate using MEGA-PRESS editing in pyogenic brain abscess

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Abstract

Valine and lactate have been recognized as important metabolic markers to diagnose brain abscess by means of MRS. However, *in vivo* unambiguous detection and quantification is hampered by macromolecular contamination. In this work, MEGA-PRESS difference editing of valine and lactate is proposed. The method is validated *in vitro* and applied for quantitative *in vivo* experiments in one healthy subject and two brain abscess patients. It is demonstrated that with this technique the overlapping lipid signal can be reduced by more than an order of magnitude and thus the robustness of valine and lactate detection *in vivo* can be enhanced. Quantification of the two abscess MEGA-PRESS spectra yielded valine/lactate concentration ratios of 0.10 and 0.27. These ratios agreed with the concentration ratios determined from concomitantly acquired short-T_E PRESS data and were in line with literature values. The quantification accuracy of lactate (as measured with Cramér-Rao lower bounds in LCModel processing) was better for MEGA-PRESS than for short-T_E PRESS in all acquired *in vivo* datasets. The Cramér-Rao lower bounds of valine were only better for MEGA-PRESS in one of the two abscess cases, while in the other case coediting of isoleucine confounded the quantification in the MEGA-PRESS analysis. MEGA-PRESS and short-T_E PRESS should be combined for unambiguous quantification of amino acids in abscess measurements. Simultaneous valine/lactate MEGA-PRESS editing might benefit the distinction of brain abscesses from tumors, and further categorization of bacteria with reasonable sensitivity and specificity.

KEYWORDS

brain abscess, difference editing, lactate, lipid contamination, MEGA-PRESS, valine

1 | INTRODUCTION

Brain abscess can be a lethal condition if appropriate treatment is delayed. Thus, early diagnosis of brain abscess is needed and is a challenge for clinicians and radiologists. Radiologically, a brain abscess in the capsule stage appears in CT and in MRI as an expansile, rim-enhancing mass surrounded by edema, which is similar in appearance to necrotic brain tumors.[1,2] Clinically, both brain abscesses and necrotic brain tumors may cause headaches, focal neurological deficits, epileptic seizures and disturbances in higher level cortical function. Also, brain abscesses induce fever in only 50% of cases.[1,2] Proton MRS has been established as a non-invasive and fast diagnostic tool to provide metabolic characteristics in discrimination between necrotic brain tumors and brain abscesses.[3–5] In addition, metabolic patterns of the amino acids (AAs) present (valine (Val), leucine (Leu), isoleucine (ILeu)) along with lactate (Lac), acetate (Ace) and succinate (Suc) have been shown to categorize aerobic and anaerobic bacterial etiology in brain abscess.[6,7] Concentrations of Val, Leu and ILeu in healthy brain are of the order of 0.1 mM and therefore below the detectability limit of clinical *in vivo* MRS. However, in abscesses the concentrations of these branched-chain AAs are typically increased, so they can be used as characteristic biomarkers for this disease. Average Val

Abbreviations AA, amino acid; Ace, acetate; Ala, alanine; Asp, aspartate; Cr, creatine; CRLB, Cramér-Rao lower bound; CSDE, chemical shift displacement error; GABA, γ-aminobutyric acid; Gln, glutamine; Glu, glutamate; GSH, glutathione; ILeu, isoleucine; Ins, myo-inositol; Lac, lactate; LASER, localization by adiabatic selective refocusing; Leu, leucine; NAA, N-acetylaspartate; NAAG, N-acetylaspartylglutamate; NSA, number of spectral averages; PCh, phosphorylcholine; Scyllo, scyllo-inositol; SNR, signal-to-noise ratio; Suc, succinate; Tau, taurine; Val, valine; WIP, work in progress.

concentrations as high as 6.20 ± 3.14 mM (ranging from 1.49 to 11.00 mM) in purulent abscesses have been reported from liquid chromatography studies.[8] Brain abscess is a rather heterogeneous pathology and AA concentrations depend on the microorganisms involved and on the stage of the disease. The spectroscopic signatures of Val, Leu and ILeu are quite similar, with dominating methyl resonances at approximately 1 ppm. Lac is a product of anaerobic glycolysis and is found in healthy brain tissue in concentrations below 1.0 mM. The Lac concentration can be greatly increased in various pathologies associated with restricted blood flow (e.g. ischemic stroke) or increased metabolism (e.g. tumors and abscesses). Lac can be detected via its dominant methyl resonance at 1.31 ppm and may therefore serve as an important MR biomarker. However, the unambiguous detection and quantification of both the above mentioned AAs and Lac is strongly hampered by major lipid resonances at 0.9 ppm and 1.3 ppm, since lipids are typically present with large concentrations in tumors as well as abscesses. In conventional PRESS experiments, the methyl resonances of AAs and Lac are often detected with an echo time of $T_E \approx 144$ ms to ensure signal inversion and thus facilitate distinction from overlapping lipid signal.[7] However, the inverted resonances can be either fully or partially cancelled out by strong lipid signals, leading to erroneous quantification results.

For a more robust detection of *J*-coupled metabolites, spectral editing techniques based on multiple quantum filtering[9] or difference editing[10] can be applied. MEGA-PRESS difference editing has been proposed for filtering out the *J*-coupled resonances of γ-aminobutyric acid (GABA) by suppression of overlapping singlet resonances and is nowadays widely used in clinical GABA studies.[11,12] This technique has also been successfully applied for the detection of glutathione (GSH),[13,14] ascorbate,[15] N-acetylaspartylglutamate (NAAG),[16] Lac[17,18] and 2-hydroxyglutarate.[19] Furthermore, double editing with MEGA-PRESS for simultaneous editing of two metabolites has been proposed and has been applied for the simultaneous quantification of GSH and ascorbate.[20,21]

In this work, simultaneous MEGA-PRESS editing of Val and Lac is proposed. The method is validated *in vitro* and applied for quantitative *in vivo* experiments in a healthy subject and two brain abscess patients.

2 | THEORY

Figure 1 shows the diagram of the MEGA-PRESS sequence used. The two frequency-selective editing pulses with an inter-pulse delay of $T_E/2$ enable *J*-coupling refocusing for weakly coupled spin systems over the whole T_E interval.

The Val spectrum arises from two methyl groups resonating at 1.03 ppm and 0.98 ppm and two methine protons at 3.6 ppm and 2.26 ppm (Figure 2). All these moieties form a single *J*-coupling network with a coupling constant of $J_{Val} \approx 7$ Hz between the methine proton at 2.26 ppm and the two almost equivalent methyl groups. This enables simultaneous editing of the two methyl groups via irradiation of a frequency-selective pulse at 2.26 ppm. The methine resonance at 3.6 ppm, which is coupled to the methine spin at 2.26 ppm ($J = 4.4$ Hz), is coedited, but compared with the strong methyl resonances at 1.0 ppm the signal can be neglected for *in vivo* Val quantification. The

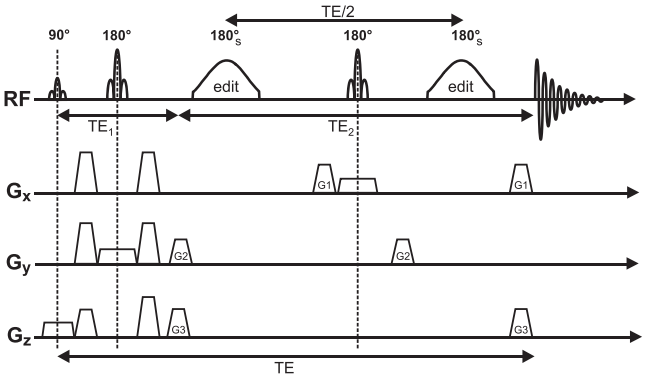

FIGURE 1 Sequence diagram of the MEGA-PRESS sequence. A total echo time $T_E = 142$ ms ($T_{E1} = 12.4$ ms, $T_{E2} = 129.6$ ms) is applied and an inter-pulse delay of $T_E/2$ between the two frequency-selective editing pulses enables complete refocusing of weak *J*-coupling evolution in the edit-on shot. The asymmetrically placed gradients (G1 and G2) dephase spins within the bandwidth of the frequency-selective pulses

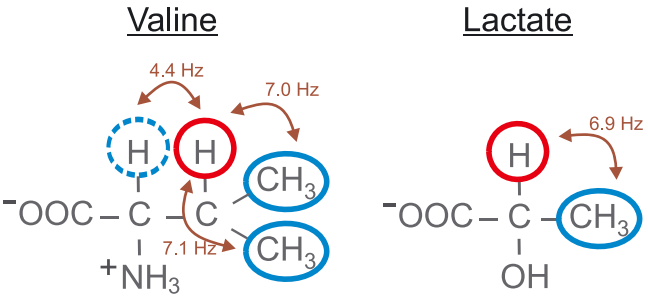

FIGURE 2 Molecular structures of Val and Lac with *J*-coupling networks (brown). Red circles designate the spin groups to which the frequency-selective pulses are applied. Blue circles designate the edited spins whose resonances are observed in the MEGA-PRESS difference spectrum. The dashed blue circle designates a methine group of the Val molecule that is coedited

Lac spectrum is characterized by a methyl resonance at 1.31 ppm and a methine resonance at 4.1 ppm, with a *J* coupling of $J_{Lac} \approx 7$ Hz between the two groups (Figure 2). Therefore the methyl resonance can be edited via irradiation of a frequency-selective pulse at 4.1 ppm.

For editing of both Val and Lac with $J_{Val} \approx J_{Lac} \approx 7$ Hz, an echo time of $T_E = 1/J \approx 142$ ms can be used for complete inversion of the target resonances at 1.0 ppm and 1.31 ppm, respectively, in the unedited spectra. This ensures high editing efficiency for both metabolites in the MEGA-PRESS experiment. The spectrally selective editing pulses are irradiated at $\nu = 2.26$ ppm for Val editing and at $\nu = 4.1$ ppm for Lac editing (Shot 1). For the acquisition of the unedited spectra (Shot 2), the frequency-selective refocusing pulses are irradiated in the downfield region at 7.14 ppm and 5.3 ppm for Val and Lac editing, respectively. These frequencies were chosen to have the same spectral separation from the water peak at 4.7 ppm as the editing frequencies in Shot 1. This ensures optimal water suppression since the water peak is equally affected by the editing pulses in the two shots and thus cancels out in the difference spectrum. For simultaneous Val and Lac editing, the frequency-selective pulses are irradiated at 2.26 ppm (Shot 1) and 4.1 ppm (Shot 2). This leads to inverted Lac/Val resonances in

Shot 1/Shot 2, respectively, and thus gives rise to upright Val and inverted Lac resonances in the difference spectra (Shot 1 − Shot 2). Shot 1 and Shot 2 are acquired in an interleaved fashion to reduce the susceptibility to frequency drifts or other experimental imperfections.

3 | METHODS

Experiments were performed on two 3 T MR systems (Trio/Skyra, Siemens Healthineers, Germany). The method was developed and validated *in vitro* on a Trio system at the University Medical Center Freiburg. Since the prevalence of brain abscesses strongly depends on the public health conditions, *in vivo* abscess spectra were exclusively acquired at the Kaohsiung Veterans General Hospital, Taiwan, where a Skyra system was available. *In vivo* experiments were approved by the institutional review boards of the University of Freiburg and the Kaohsiung Veterans General Hospital, respectively. Written informed consent was obtained from all subjects prior to MRS examinations.

The original MEGA-PRESS sequence used for GABA editing,[11] which is provided by Siemens as a work-in-progress (WIP) software package, was modified to enable complete refocusing of weak *J*-coupling evolution over variable T_E intervals by using two frequency-selective refocusing pulses with an inter-pulse delay of $T_E/2$. The Siemens editing pulses from the WIP package are based on a waveform with duration of 25.6 ms and a bandwidth of 48.2 Hz, which could be scaled to different durations/bandwidths in the protocol. Users of the WIP package should note that the adjustable editing pulse bandwidth specified in the protocol (e.g. 35 Hz for the 25.6 ms waveform) is not correct. In this work, we will henceforth refer to the real bandwidth, which was determined with Bloch simulations. The long echo time of 142 ms required for Val and Lac inversion enables the utilization of frequency-selective editing pulses with a maximum duration of 56 ms and consequently a minimum bandwidth of 22 Hz. While small editing pulse bandwidths reduce coediting of other metabolites, they also make the sequence susceptible to frequency drifts during the scan, e.g. through subject motion or temperature changes of the passive shim elements,[22] and can strongly decrease the editing efficiency. For the experiments performed on the Trio system, an editing pulse bandwidth of 41 Hz turned out to be a robust choice. However, due to larger frequency drifts with the Skyra system, an increased bandwidth of 62 Hz was used for the abscess measurements and was then also applied for the evaluation of lipid coediting. Table 1 lists the different editing pulse durations and bandwidths as well as the corresponding protocol settings.

3.1 | *In vitro* experiments

For validation purposes, *in vitro* editing experiments (Val editing, Lac editing and simultaneous Val/Lac editing) were performed in a phantom solution containing Val, Lac and a mixture of soya and olive oil.

To evaluate the degree of lipid coediting, additional MEGA-PRESS experiments were performed in a phantom solution containing only water and flaxseed oil. Flaxseed oil was used since it contains a high concentration of omega-3 lipids, which may be subject to coediting due to *J*-coupled resonances at 0.95 ppm and 2.1 ppm. An editing pulse bandwidth of 62 Hz was used for these experiments to evaluate the degree of lipid coediting in the abscess measurements. The percentage of lipid signal that survives MEGA difference editing in the region of interest was quantified by integrating the difference spectra in the spectral region between 0.6 and 1.6 ppm and dividing by the corresponding integrated signal in the sum spectrum (Shot 1 + Shot 2).

3.2 | *In vivo* experiments

MEGA-PRESS Lac editing was performed in the occipital cortex of a healthy subject with the following sequence parameters: T_R = 1.5 s, T_E = 142 ms, spectral bandwidth =1200 Hz, 1024 spectral points, editing pulse duration =30 ms (bandwidth =41 Hz), scan duration =13 min.

Simultaneous Val/Lac editing was performed in two abscess patients (without prior antibiotic treatment) using the following sequence parameters: T_R = 1.5 s, T_E = 142 ms, spectral bandwidth =2000 Hz, 2048 spectral points, editing pulse duration =20 ms (bandwidth =62 Hz), scan duration =8 min. For comparison, additional PRESS spectra with equal scan durations and T_E = 30 ms were acquired for both patients.

3.3 | Postprocessing

Both subject and patient spectra were quantified with LCModel.[23] Dedicated metabolite basis spectra were simulated for quantification of the acquired MEGA-PRESS and short-T_E PRESS data with a MATLAB-based simulation toolbox.[24] For these simulations, chemical shifts and coupling constants from literature[25] and fully shaped RF waveforms of the slice-selective and frequency-selective refocusing pulses as applied by scanners were used. For quantification of the Lac editing spectrum from the healthy subject the basis set included alanine (Ala), creatine (Cr), glutamine (Gln), glutamate (Glu), GSH, myo-inositol (Ins), Lac, N-acetylaspartate (NAA), NAAG, phosphorylcholine (PCh), scyllo-inositol (Scyllo) and taurine (Tau). The basis set for quantification of the Val/Lac editing spectra from the abscess patients additionally contained Val, ILeu, Ace and Suc. Furthermore, Leu,

TABLE 1 Editing pulse bandwidths applied in the MEGA-PRESS experiments. Since the bandwidth specified in the protocol does not reflect the real bandwidth of the pulse, Bloch simulations were performed to determine the real bandwidths

Pulse settings	Duration [ms]	Bandwidth [Hz] (Siemens protocol)	Bandwidth [Hz] (Bloch simulation)
Base waveform	25.6	35	48
Minimal bandwidth	56.0	16	22
In vitro validation/healthy subject	29.9	30	41
Lipid coediting/abscess patients	20.0	45	62

aspartate (Asp), GABA and glycerophosphorylcholine (GPC) were added for quantification of the short-T_E PRESS spectra acquired from abscess.

4 | RESULTS

Figure 3 shows phantom spectra acquired from a solution of Val, Lac and oil, using MEGA-PRESS with an echo time of T_E = 142 ms for Val editing, Lac editing and simultaneous Val/Lac editing. All shots contain contamination of lipid resonances in the region of interest. The Lac and Val resonances at 1.31 and 1.0 ppm are contaminated by lipid signal arising from methylene protons (1.3 ppm) and methyl protons (0.9 ppm) respectively. However, the difference spectra exhibit uncontaminated Val triplets and Lac doublets. Simultaneous Val/Lac editing yields a positive Val triplet and a negative Lac doublet in the difference spectrum. The Val resonance at 3.6 ppm, which is also *J*-coupled to the resonance at 2.26 ppm, is coedited and therefore appears in the difference spectra. MEGA-PRESS difference spectra acquired from a pure water/oil emulsion are displayed in Figure 4, along with the sum spectrum of the Val editing experiment as a reference. While the Val editing difference spectrum does not show any lipid resonances, the Lac and combined Val/Lac editing spectra exhibit a minor lipid signal at 1.3 ppm. Signal integration showed that 1% of the original lipid signal can be observed in the spectral region of interest (0.6–1.6 ppm) of the Val difference spectrum, 2% in the Lac difference spectrum and 3% in the Val/Lac difference spectrum.

Figure 5 shows Lac editing spectra acquired from the occipital cortex of a healthy subject. While the two shots of the editing sequence (Shot 1, editing pulse at 4.1 ppm; Shot 2, editing pulse at 5.3 ppm) show both considerable lipid contamination and baseline distortions in the spectral region between 1 and 2 ppm, the Lac resonance at 1.31 ppm was easily observed in the difference spectrum through suppression of this background signal. It should be noted that NAA with an Asp moiety resonating at 4.38 ppm and NAAG with a Glu moiety resonating at 4.13 ppm are affected by frequency-selective editing pulses irradiated at 4.1 ppm. Therefore, NAA and NAAG are coedited in Lac editing experiments and can be used as a concentration reference in the LCModel quantification of the difference editing spectra. Quantification of the difference spectrum acquired from the healthy subject yielded a Lac/NAA concentration ratio of 0.065 with Cramér-Rao lower bounds (CRLBs) of $CRLB_{Lac}$ = 7%

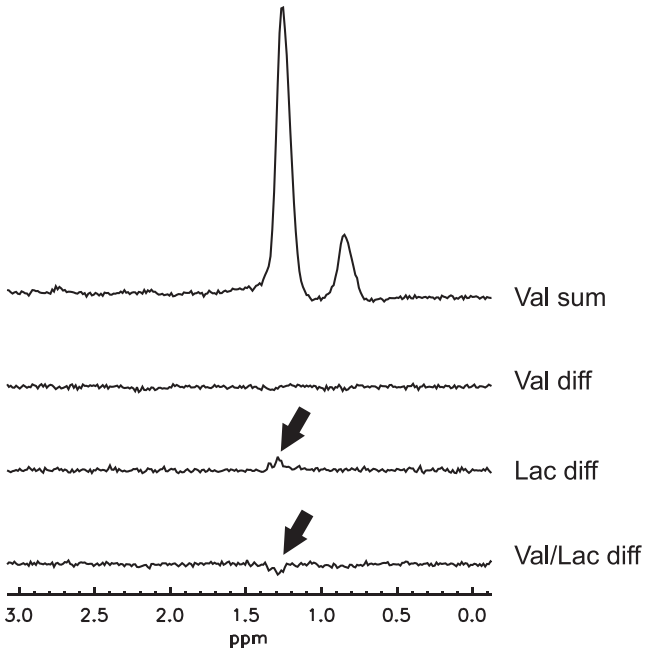

FIGURE 4 Spectra acquired from a phantom solution containing only water and oil. In addition to the editing experiments, a PRESS spectrum was acquired with T_E = 142 ms as a signal reference

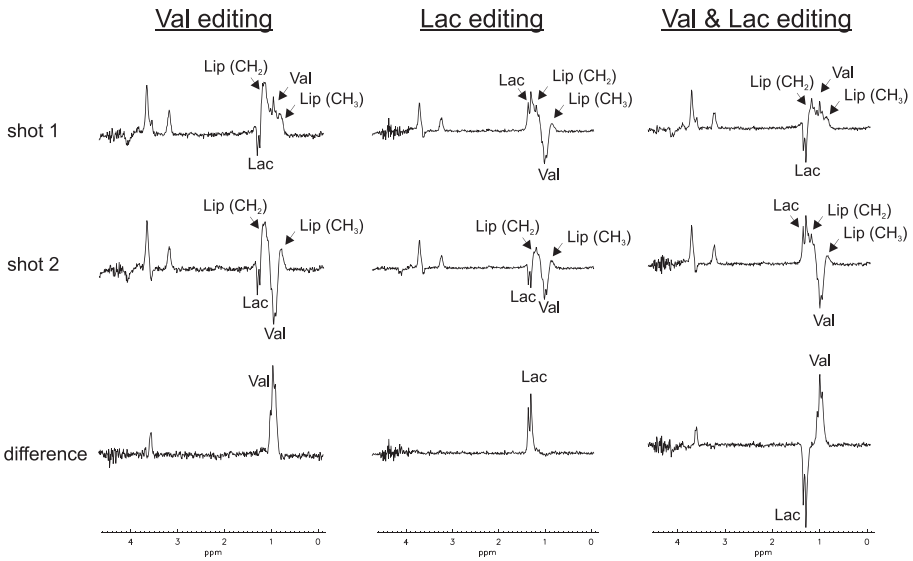

FIGURE 3 Phantom spectra acquired with an echo time of T_E = 142 ms from a solution of Val, Lac and oil, using MEGA-PRESS for Val editing, Lac editing and combined Val/Lac editing. The spectrally selective editing pulses were irradiated at 2.26 ppm (Shot 1) and at 7.14 ppm (Shot 2) for Val editing, at 4.1 ppm (Shot 1) and at 5.3 ppm (Shot 2) for Lac editing and at 2.26 ppm (Shot 1) and at 4.1 ppm (Shot 2) for simultaneous Val/Lac editing. The spectra show a prominent Lac doublet at 1.31 ppm, a prominent Val triplet at 1.0 ppm and the major lipid resonances from methyl (CH_3) and methylene (CH_2) moieties

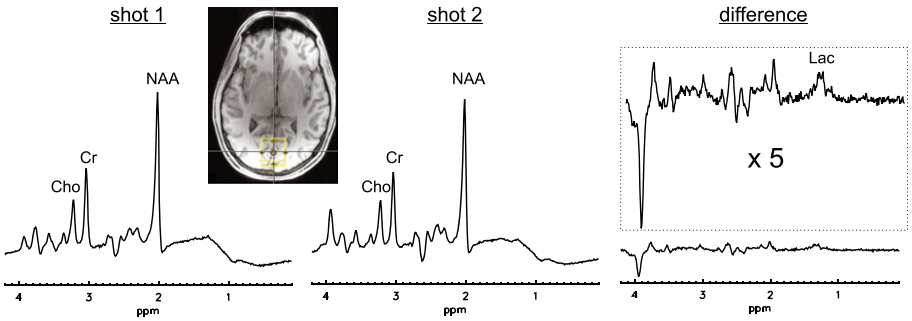

FIGURE 5 Lac editing spectra acquired with MEGA-PRESS (T_E = 142 ms) from the occipital cortex of a healthy subject. The frequency-selective pulses were irradiated at 4.1 ppm and 5.3 ppm for the two shots, respectively. The difference spectrum reveals the Lac resonance, which is hidden by the strong baseline in the two shots of the MEGA-PRESS experiment

and $CRLB_{NAA}$ = 5%, while quantification of the unedited shot (Shot 2) yielded a concentration ratio of 0.135 with $CRLB_{Lac}$ = 23% and $CRLB_{NAA}$ = 2%.

PRESS and MEGA-PRESS spectra acquired from two brain abscess patients are displayed in Figure 6. They demonstrate that simultaneous Val/Lac editing successfully filters Lac and Val resonances from a strong lipid background in these two clinical cases. While large lipid peaks (at 1.3 ppm and 0.9 ppm) are visible in the PRESS spectra as well as in the two shots of the MEGA-PRESS experiments, the difference spectra do not show major lipid signal in the spectral region between 0.6 and 1.6 ppm, enabling unequivocal detection and quantification of Val and Lac.

The difference editing spectra show that a much larger portion of the signal seen around 1.0 ppm in the PRESS spectrum can be attributed to Val for Patient 2 than for Patient 1. Figure 7 shows the LCModel fit for the difference editing spectrum acquired in Patient 2. In addition to Val and Lac, the metabolites Ala, Cr, Glu, NAA and PCh were reliably quantified (CRLBs <20%).[26] Moreover, the combined concentration NAA + NAAG could even be determined with a CRLB as low as 8%. In contrast, the MEGA-PRESS spectrum from Patient 1 only yielded reliable quantification for Lac, Val and NAA + NAAG. Some residual signal in the spectral region between 1.5 and 2.0 ppm could not be modeled with the spectral basis, probably arising from subtraction artifacts caused by frequency drifts during the acquisitions. LCModel quantification results for the abscess data are summarized in Table 2. PRESS and MEGA-PRESS spectra yield very similar Val/Lac concentration ratios for the two patients. The CRLBs of Lac as determined by LCModel are much lower for the MEGA-PRESS analysis than for the PRESS analysis. While Patient 2 also shows a reduction of $CRLB_{Val}$ for the MEGA-PRESS difference spectrum compared with

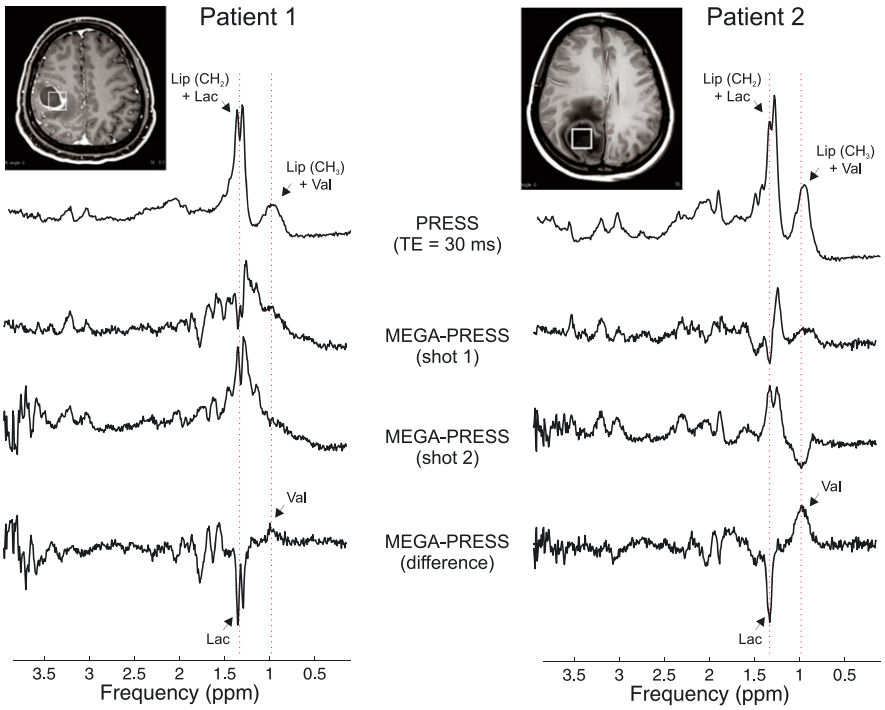

FIGURE 6 Spectra acquired from two brain abscess patients. Combined Val/Lac editing MEGA-PRESS spectra are shown along with spectra acquired with standard PRESS (T_E = 30 ms). Red dotted lines indicate the positions of Val and Lac resonances. In the PRESS spectrum the Val and Lac resonances are overlapped with lipid (Lip) signal arising from methyl (CH_3) and methylene (CH_2) moieties, respectively. In contrast, the MEGA-PRESS difference spectra show the uncontaminated Val and Lac resonances

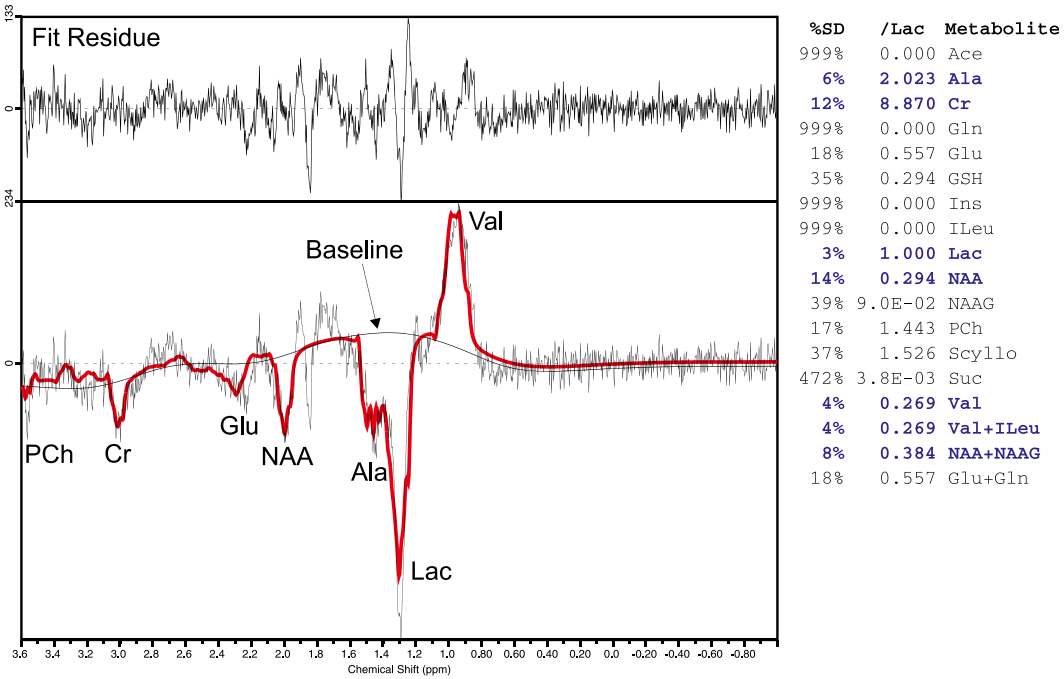

FIGURE 7 LCModel fit of the MEGA-PRESS difference spectrum acquired from Patient 2. Besides Val and Lac, the following metabolites could be quantified with CRLB <20%: Ala, Cr, Glu, NAA, PCh

TABLE 2 LCModel quantification results of the acquired PRESS and MEGA-PRESS abscess spectra. The Val/Lac concentration ratios as well as the CRLBs as determined by LCModel are presented for the two patients

		Patient 1	Patient 2
PRESS (TE = 30 ms)	[Val]/[Lac]	0.09	0.26
	$CRLB_{Val}$ [%]	15	9
	$CRLB_{Lac}$ [%]	6	7
MEGA-PRESS (TE = 142 ms)	[Val]/[Lac]	0.10	0.27
	$CRLB_{Val}$ [%]	17	4
	$CRLB_{Lac}$ [%]	3	3

conventional PRESS, $CRLB_{Val}$ is slightly larger for the MEGA-PRESS spectrum of Patient 1.

5 | DISCUSSION

MEGA-PRESS was optimized for Val and Lac editing and successfully applied *in vivo*. Val and Lac have very similar coupling constants so that without *J* refocusing their dominant methyl resonances at 1.0 ppm and 1.3 ppm both exhibit full inversion at T_E = 142 ms, which ensures a very high editing efficiency. This is in contrast to GABA detection, where only the two outer lines of the triplet resonances are inverted, resulting in an inherent signal loss of 50%. The almost identical *J*-coupling constants of Val and Lac benefit simultaneous MEGA-PRESS editing of these two metabolites. It was demonstrated in phantom experiments (Figure 3) that simultaneous editing of Val and Lac works equally well as individual editing of either Val or Lac. Since only 3% of the original (unedited) lipid signal can be observed in the Val/Lac difference spectrum in the region of interest, lipid coediting only becomes relevant when the lipid resonances are much larger than the Val and Lac resonances. However, other resonances (e.g. arising from AAs) might be coedited as well and might confound quantification of Val and Lac.

In high field systems (B_0 ≥ 3 T) the detection of weakly coupled metabolites such as Lac and Val is hampered by large chemical shift displacement errors (CSDEs).[27,28] This not only compromises the spatial localization of the acquired spectrum, but also gives rise to strong signal loss for echo times with maximum signal inversion (e.g. T_E = 142 ms for Lac and Val). Since such echo times are preferentially employed for MEGA-PRESS experiments, the difference editing spectra are strongly affected by CSDEs. The signal loss can be estimated with the analytical formulas for weakly coupled two-spin systems.[28] With the sequence parameters and refocusing pulses used in our work, this signal loss amounts to 60% and 27% for the dominant methyl resonances of Lac and Val, respectively, in the shot without *J* refocusing. The CSDEs inversely scale with the bandwidth of the spatially selective refocusing pulses and can therefore be reduced with localization by adiabatic selective refocusing (LASER).[29] MEGA editing in combination with semi-LASER localization has recently been proposed for GABA and Lac editing at 7 T.[30–32]

The noticeable residues observed in the LCModel fits of the MEGA-PRESS abscess spectra (Figure 7) might be partially due to subtraction artifacts caused by frequency drifts during the experiment. The robustness of the proposed MEGA-PRESS technique could therefore be improved through frequency and phase drift correction of singles averages, either in postprocessing[33] or with interleaved reference spectroscopy in real time during the scan.[22,34]

Leu and ILeu might be edited with MEGA-PRESS in a similar fashion as Val. In this work, Val was chosen as target metabolite since the relevant *J*-coupling constants of Val and Lac are almost identical, which is a prerequisite for simultaneous editing. Furthermore, the target resonance for Val editing (2.26 ppm) is further away from the detected resonances

than would be the case for Leu and ILeu, and the coupling strength determines the editing efficiency because refocusing of *J* evolution via spin-selective pulses only works well in the limit of weak scalar coupling.

While coediting of metabolites with resonances in the spectral region of interest is to be avoided, it is often desirable to have at least one other coedited metabolite in the difference spectrum that can be used as a concentration reference for LCModel quantification. The Asp moiety of NAA gives rise to *J*-coupled resonances with coupling partners at 2.49 ppm and 4.38 ppm, which are coedited by the frequency-selective pulses irradiated at 2.26 ppm and 4.1 ppm for Val and Lac editing, respectively, if the editing pulse bandwidth is not too small. Similarly, NAAG is coedited via resonances in the vicinity of the editing frequencies. Hence, NAA, NAAG or the total NAA + NAAG might serve as a reliable concentration reference for quantification as demonstrated for the MEGA-PRESS spectrum acquired from healthy brain (Figure 3). However, it should be noted that abscess as well as tumor spectra lack a stable reference metabolite for quantification. In abscess spectra, there are not even detectable resonances from the usual major brain metabolites NAA, Cr and choline-containing compounds. In our abscess measurements we report Val/Lac concentration ratios for validation purposes. For the distinction of brain abscess and tumor, the Val/Lac concentration ratio can serve as a metabolic marker since tumor tissue does not contain substantial Val concentrations.

The Lac editing experiment in a healthy subject as well as the abscess patient measurements demonstrate that suppression of macromolecular background signal can enhance the quantification reliability as determined by the CRLBs. Our result for [Lac]/[NAA] (=0.093) acquired by MEGA-PRESS in healthy brain roughly agrees with literature values.[25] The CRLBs of Lac as determined by LCModel (7%) were well below 20%, which is typically used as a threshold for reliable quantification. In contrast, LCModel quantification of the unedited shot (Shot 2) of the same experiment yielded $CRLB_{Lac}$ = 23%. The number of spectral averages (NSA) has to be considered for a CRLB comparison since the CRLBs inversely scale with the signal-to-noise ratio (SNR) and consequently with $\sqrt{NSA}$.[35] Taking the larger NSA (factor of 2) for the difference spectrum compared with the unedited shot into account yields a NSA-corrected $CRLB_{Lac}$ = 16% for the unedited shot, which is still substantially larger than the $CRLB_{Lac}$ = 7% for the fit of the difference spectrum. Furthermore, quantification of the difference spectrum yielded a more realistic Lac/NAA concentration ratio for healthy brain (<0.1) than quantification of the unedited shot, which seems to have overestimated the Lac concentration due to the overlapping lipid signal.

Quantification of the abscess MEGA-PRESS data yielded similar Val/Lac concentration ratios for the two patients to quantification of the PRESS data. However, despite the considerable signal loss associated with a long echo time of 142 ms, quantification of the MEGA-PRESS spectra yielded much lower CRLBs for Lac than quantification of the corresponding short-T_E PRESS spectra. This is mainly due to the fact that the macromolecular resonances are eliminated through MEGA-PRESS editing and therefore need not be taken into account in the fitting model. For Val quantification, MEGA-PRESS only yielded smaller CRLBs than short-TE PRESS in one of the clinical measurements. For Patient 1, who showed very little Val signal, short-T_E PRESS yielded slightly smaller CRLBs for Val than MEGA-PRESS. However, for quantification of the short-T_E PRESS spectra, stronger baseline distortions have to be modeled than for the MEGA-PRESS spectra, which typically have a very flat baseline. This gives rise to additional quantification confounds that are not reflected by the CRLBs. With short-T_E PRESS, the three AAs (Val, Leu and ILeu) can typically be detected in brain abscess and were therefore included in the basis set for quantification. Leu, which does not have resonances in the spectral vicinity of the editing pulses of the proposed MEGA-PRESS technique, was removed from the metabolite basis for quantification of the difference spectra. In contrast, ILeu undergoes some degree of coediting and was included in the basis set. This gives rise to a quantification confound with respect to Val since both metabolites have methyl resonances at approximately 1 ppm in the difference spectrum. As Val has two methyl groups resonating in this spectral region and ILeu only one, the total Val + ILeu cannot be detected with higher reliability, either. For the distinction of Val and ILeu, conventional PRESS has the advantage of additional resonances, which are suppressed in the MEGA-PRESS difference spectrum. A more robust distinction and quantification of Val, Leu and ILeu may be achieved with a combination of conventional PRESS and dedicated MEGA-PRESS editing experiments for the three AAs.

An additional uncertainty in the Val/Lac concentration ratio arises from T_2 decay, which affects the long-T_E MEGA-PRESS data more than the short-T_E PRESS data. In our work, we did not perform relaxation correction since the T_2 constant of Val *in vivo* is unknown and the T_2 constant of Lac has only recently been measured in tumors, with limited accuracy.[36]

The literature suggests that metabolic findings in abscess patients are rather heterogeneous. Garg et al. found large differences in the AA/Lac concentration ratios, depending on whether the abscesses were anaerobic (/[Lac] = 0.48), aerobic (/[Lac] = 0.17) or sterile (/[Lac] = 0.32).[37] Martínez-Pérez et al. reported Val/Lac concentration ratios of 0.12 and 0.22 for two abscess surgical exudates.[4] These ratios are similar to the ratios observed in our two abscess cases. While most recent studies agree that AA signal at 1.0 ppm can be used as a sensitive marker for brain abscess,[6] Pal et al reported that only 80% out of 194 brain abscess patients presented AAs in the conventional long T_E (144 ms) PRESS spectra.[7] They suggested that the absence of AAs could not rule out a pyogenic etiology. Missing AA resonances in abscess spectra may reflect the low concentration of bacteria generating these end products in the pus or confounding lipid signal in the abscess cavity.[38] In some studies, the resonances of Val and ILeu at 3.62 and 3.67 ppm have been used for AA quantification instead of the much stronger resonances at 1.0 ppm to avoid this confound.[37] MEGA-PRESS editing of Val and Lac as proposed in our work provides a solution in this regard.

In conclusion, the *in vivo* experiments performed in this work indicate that editing can considerably improve detection accuracy of Val and Lac by suppressing the overlapping macromolecular signal. Val/Lac editing might benefit the distinction of brain abscesses from tumors, and further categorization of bacteria with reasonable sensitivity and specificity.

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