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Case report

MEGA-PRESS and PRESS measure oxidation of glutathione in a phantom

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A B S T R A C T

Purpose: Investigate the possibility of measuring changes in glutathione (GSH) concentration using the MRS PRESS and MEGA-PRESS sequences by tracking the natural oxidation of GSH, and to examine the accuracy of the two methods.
Methods: 122 GSH edited MEGA-PRESS and PRESS acquisitions were acquired on a "braino" based phantom +3.0 mM GSH during a period of 11 days. All spectra were analyzed in LCModel. (The MEGA-PRESS data were first preprocessed in Matlab). Degradation curves were modeled. A one year follow-up on the same phantom and measurements from a similar phantom without GSH and one pure GSH phantom were also included.
Results: Both MEGA-PRESS and PRESS showed degradation of the measured GSH signal. Modeling the exponential decay of the GSH signal in MEGA-PRESS and PRESS gave for $t = 0$; 2.9 i.u. for MEGA-PRESS and 2.3 i.u. for PRESS. As t increased, the GSH concentration converged to zero for MEGA-PRESS but not for PRESS (0.7 i.u.). GSH for the one year follow up were 0.0 i.u. for MEGA-PRESS and 0.6 i.u. for PRESS. Similar phantom without GSH yielded 0.0 i.u. for both MEGA-PRESS and PRESS.
Conclusion: It is possible to measure changes in GSH concentration in a phantom using both PRESS and MEGA-PRESS techniques, however the PRESS spectrum appears to include oxidized GSH (GSSG). In addition, GSH edited MEGA-PRESS measurement gives more precise values at lower GSH concentrations.

1. Introduction

Glutathione (GSH) is the major cellular antioxidant, important for detoxification and elimination of environmental toxins and free radicals. GSH serves as a reducing agent, neutralizing the harmful substances and producing oxidized glutathione in the form of glutathione disulphide (GSSG) in the process. GSSG can then be reduced back by glutathione reductase, using NADPH as an electron donor. The GSSG molecule comprises of two GSH molecules bound together at the sulphur atoms.

An altered GSH concentration may be associated with the pathogenesis of several diseases, including schizophrenia [1–3], bipolar disorder [4–6], multiple sclerosis [7,8], Alzheimer's disease [9], autism spectrum disorder [10], amyotrophic lateral sclerosis [11] and Parkinson's disease [12,13].

GSH exists primarily in its reduced form which is present in healthy brain tissue at 1–2 millimolar (mM) concentrations [14], while a much smaller fraction (~0.01 mM) is present as GSSG. The ratio of GSH:GSSG can fluctuate between 100:1 and 10:1 in neuron, however, at a ratio 20:1, the neurons starts to degenerate [15].

While GSH can be detected by ^{1}H MRS, GSSG in vivo is virtually undetectable by MRS methods because of its low concentration. Measuring GSH by MRS is challenging since the GSH spectrum is difficult to resolve from the other compounds that may overlap in the in vivo MRS spectrum – mainly Cr, but also glutamate, glutamine, γ-aminobutyric acid (GABA), aspartate and NAA. The GSH signal-complex is in fact not visually detectable in the standard single voxel spectroscopy such as short TE PRESS and STEAM, due to the spectral overlap of metabolites with much higher concentrations. Quantification in these sequences relies on fitting the GSH signal to an a priori metabolite model [16] considering the possibility that the GSH signal lies in the spectrum.

Another approach for quantification of GSH is spectral-editing [17,18]. Spectral editing techniques such as MEscher-Garwood Point RESolved Spectroscopy [19] (MEGA-PRESS), facilitates the isolated detection of low concentration metabolites like GSH by removing overlapping signal contribution through the editing approach. In the case of GSH, an editing pulse applied around 4.57 ppm (as shown in Fig. 1) allows selective observation of the coupled cysteine - $^{7}CH_2$ resonance at 2.95 ppm, distinct from overlapping Cr, GABA and aspartate

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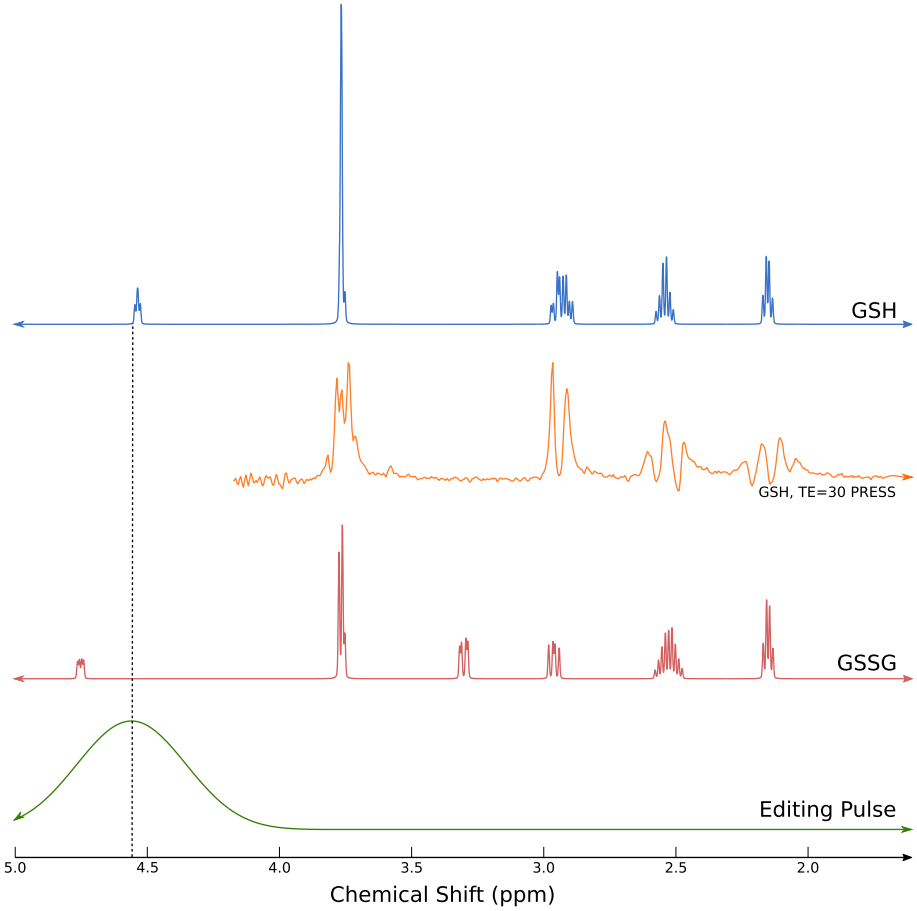

Fig. 1. Modeled ^{1}H MRS spectra for GSH (blue) and GSSG, using parameters from BRMB [27]; below, edit selectivity of the MEGA-PRESS technique. The figure is purely illustrative showing the location of the key peaks and the target of editing, not a representation of the model used for analyzing. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

resonances [20]. GSH oxidizes quickly in solution, and this must be considered when preparing phantoms containing GSH. A range of factors affect the oxidation rate, including temperature, pressure and oxygen source. GSH phantoms are, in other words, perishable, and need to be made afresh for every new GSH experiment. There are a couple of techniques that can be used to slow down the oxidization process, for example preparing the sample in an oxygen free environment (e.g. glovebox) or bubbling it with a gas such as nitrogen or argon.

In the present project, the degradation process of GSH was investigated over time and the ability to capture this process with PRESS and MEGA-PRESS measurements was assessed.

2. Methods

2.1. Phantom preparation

The metabolite solution was based on the GE "braino" MRS-HD-Sphere phantom used in Schirmer and Auer [21] with the following metabolite concentrations: 12.5 mM NAA, 10.0 mM Cr, 3.0 mM Cho, 7.5 mM myo-inositol, 12.5 mM glutamate, 5.1 mM lactate, 2.0 mM GABA and 3.0 mM GSH. The phantom also contained: Potassium phosphate monobasic at 50.0 mM and sodium hydroxide at 56.0 mM to maintain neutral pH and 0.01% sodium azide as a preservative. GSH was added to the solution roughly 40 min before the first scan. pH measurement was performed just before scanning to ensure a neutral pH (pH = 7.4). One liter of the solution was contained in a glass round-bottomed flask that was placed in the middle of the MR head coil.

2.2. MRI acquisitions

The MRS data were acquired using a 3.0T GE Discovery MR750 (Milwaukee, US) with a standard 8-channel head coil. The following MR protocol was applied:

- MEGA-PRESS with TR = 1800 ms, TE = 131 ms and 328 paired repetitions (328 ON and 328 OFF scans) of 4096 datapoints (TA = 20:24 min) at 5 kHz spectral width, phase cycling of 8 paired repetitions, with 16 unsuppressed water reference frames. 20 ms sinc weighted Gaussian editing pulse (FWHM 62 Hz) was applied at 4.56 ppm (ON) and 20 ppm (OFF) in interleaved scans.
- PRESS with TR = 3000 ms and TE = 30 ms, 256 repetitions giving a total acquisition time (TA) of 14:00 min.

In both acquisitions, a fixed voxel size of 35 × 20 × 25 mm^3 was used. The voxel was positioned in the center of the phantom using a T1w image acquired before the MRS acquisition as a scout image. Water suppression in both sequences is done with three CHESS pulses at three different flip angels (105°, 80°, 145°) giving a water suppression of 90%.

A total of 122 MEGA-PRESS and PRESS spectra were acquired over a time period of 11 days; long-term follow-up was performed after 359 days. Supplementary measurements were also performed on a similar phantom (standard "braino" phantom provided by GE) without GSH, and on a pure GSSG phantom. All phantoms were stored at room temperature (22°) next to the MRI scanner.

2.3. Data analysis and statistics

The MEGA-PRESS data were analyzed with a preprocessing tool implemented in Matlab by Felix Raschke (Division of Radiological and Imaging Sciences, University of Nottingham, UK) [22]. The unsuppressed water signal from OFF is used to obtain phase angles. Then coil combination and individual phasing to NAA are performed for both

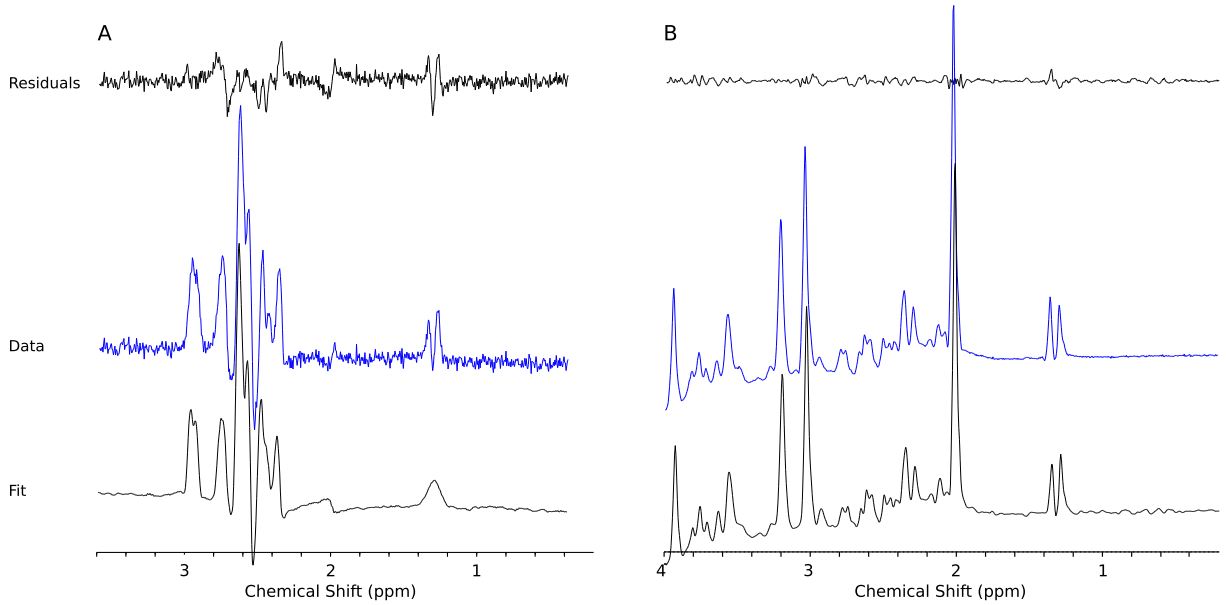

Fig. 2. Representative MRS spectrum from A) MEGA-PRESS and B) PRESS along with the LCModel fit and residual. The 1.3 ppm resonance in the MEGA-PRESS spectrum is co-edited lactate.

ON and OFF spectra. A single ON reference spectrum is made which all ON and OFF spectra are aligned to. Finally, averaging is performed. The fitting (final ON – final OFF) is performed in LCModel [16], using a measured basis set including NAA and GSH (supplied by Felix Raschke). PRESS data were analyzed in LCModel using a measured basis set consisting of seventeen metabolites (Cr, PCr, alanine, apartate, glucose, GABA, glutamine, glutamate, glucophosphocholine, phosphocholine, myo-inositol, lactate, NAA, NAAG, scyllo-inositol and taurine, GSH). Water was used as an internal reference for all GSH measurements. The oxidation process over the initial 11 - day collection was then modeled (in Matlab, R2014b) to optimize (in the least-squares sense) fitting to an exponential model:

$$GSH_{reported} = GSH_{reported,initial} \times e^{-t/\tau} + GSSG_{reported,final} \times \left(1 - e^{-t/\tau}\right)$$

3. Results

Representative MRS spectra for MEGA-PRESS and PRESS are shown in Fig. 2.

Both MEGA-PRESS and PRESS show the expected reduction of measured GSH over time, although the characteristics of the observed decay differ between the two methods: for MEGA-PRESS, the $t = 0$ intercept (reported, initial GSH) is at 2.9 i.u. GSH, with convergence to zero as time increases; the rate of oxidation is estimated with a half-life of 3.8 days. The TE = 30 PRESS acquisition reports 2.3 i.u at t = 0, converging on 0.68 i.u. as time increases; with this method, the rate of oxidation is estimated at a half-life of 6.8 days.

Long-term follow-up with both techniques yields final estimates of 0.0 i.u. with MEGA-PRESS ($n = 4$) and 0.56 ± 0.01 i.u. with PRESS (n = 4). When measuring a Braino + GABA (with no GSH) phantom, both methods repeatedly ($n = 3$) yield zero estimates for GSH.

Residuals of the measured concentration against the modeled decay may be taken as an indication of the relative precision of the two techniques; euclidean-norms of residuals for the MEGA-PRESS and PRESS models were 1.1 and 0.80 i.u. respectively. However, as can be seen in Fig. 3, variance in the MEGA-PRESS case appears to stabilize somewhat at lower concentrations. Therefore, residuals are also separated into high- and low-concentration components (splitting by time, at modeled halflife); for higher concentrations, euclidean-norms of residuals from the two techniques are 1.03 and 0.66 i.u. respectively, for lower concentrations (corresponding with nominal concentration < 1.5 mM) the values were 0.36 and 0.45 i.u.

The decaying GSH complex signal at 2.95 ppm is illustrated in Fig. 4 showing three MEGA-PRESS spectra from three different time points. Fig. 5 shows three PRESS spectra from ~ the same time points.

SNR from MEGA-PRESS and PRESS were 11–15 and 52–69 respectively. The SNR were reported from LCModel, and is defined as the ratio of the maximum in the spectrum minus baseline over the analysis window to twice the root means square residuals [16].

Linewidth (in Hz) from MEGA-PRESS and PRESS were 1.8–2.4 and 2.4–3,7 respectively.

4. Discussion

The present study shows that it is possible to measure changes in GSH concentration, in phantoms, using both PRESS and MEGA-PRESS techniques. Nonetheless, the two methods yield somewhat different results in terms of final concentration estimates, and rate of decay. It is, however noted, that the MEGA-PRESS acquisition yields initial estimates in i.u. closer to the known concentration in mM.

The MEGA-PRESS results converge on zero, suggesting that the method is measuring only GSH and is insensitive to GSSG content. In contrast, the PRESS data converge on a non-zero estimate (modeled at 0.68 i.u.), suggesting that this approach may also be miss reporting GSSG as GSH. The modeled convergence is consistent with long-term follow-up measurements, which report 0.0 i.u. GSH using MEGA-PRESS, and 0.56 i.u. using PRESS. Data from a similar phantom lacking GSH/GSSG consistently yields zero estimates, indicating that it is GSSG rather than any other overlapping spectral component which contributes to the non-zero final estimate.

Furthermore, it may be shown that the final estimate from the PRESS acquisition is in line with the expected estimate, if indeed it is a measure of the final GSSG content. Scaling the initial GSH estimate (2.3 i.u.) by the expected molar ratio of GSH to GSSG (2:1), the normalized overlap between modeled GSH and GSSG spectra (0.81 at 1 Hz linewidth over the fit range, to 0–4 ppm), and adjusting for the relative T2 times (67 ms for GSH [23] and around 40 ms for GSSG [measured locally], for a correction factor of $e^{-\left(30/40 - 30/67\right)} = 0.74$ gives an expected result of 0.69 i.u., consistent with both our modeled and measured final values (0.68 i.u. and 0.56 i.u., respectively).

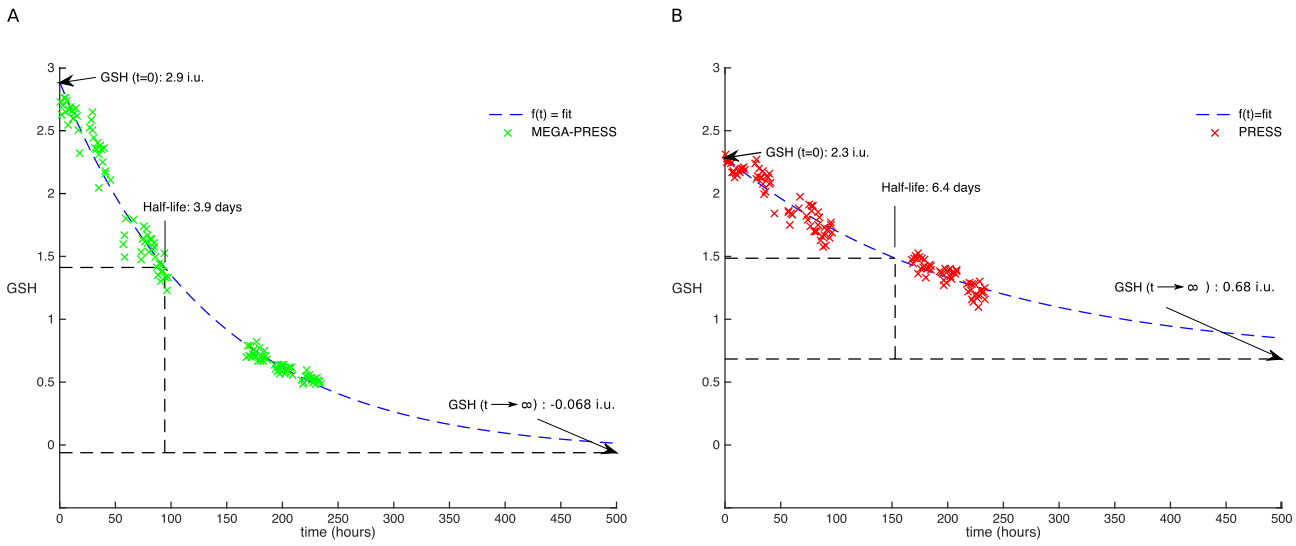

Fig. 3. GSH measurements in phantom (Braino + 3 mM GSH) by A: MEGA-PRESS (green) and B: PRESS (orange) during a time period of 11 days. The measurements are in institutional units (i.u.).
Fit for MEGA-PRESS f(t) = 2.9490 * $^{e\text{-}0.0001t}$ – 0.0680.
Fit for PRESS f(t) = 1.6081 * e $^{-0.000t}$ + 0.6789. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

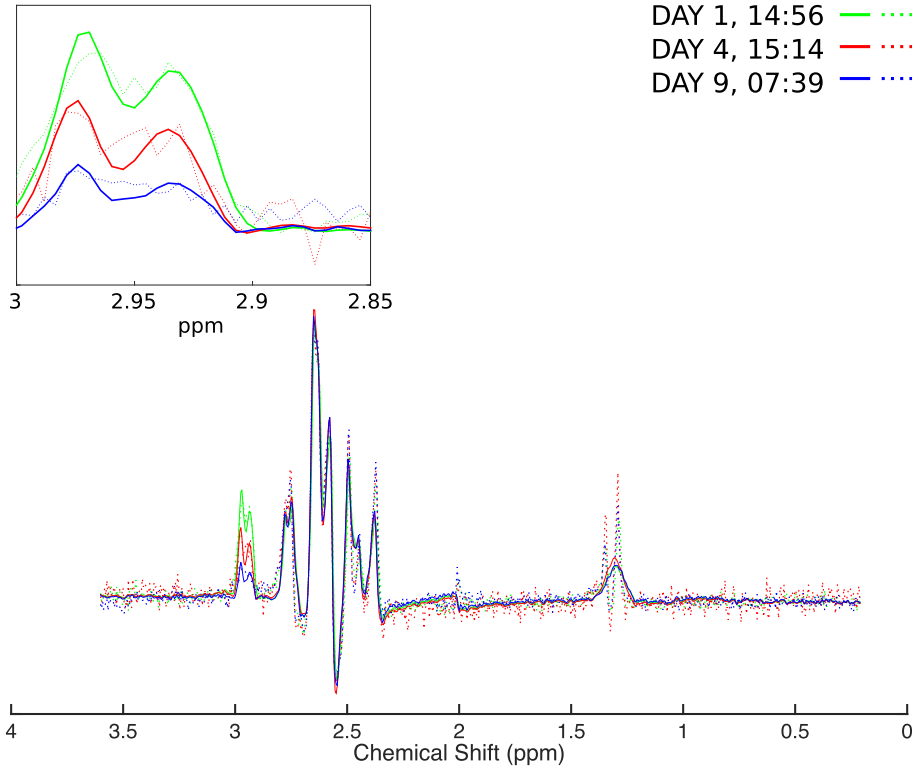

Fig. 4. MEGA-PRESS spectra from day 1 (green), day 4 (red) and day 9 (blue). The decaying GSH complex signal at 2.9 ppm is clearly illustrated. Dashed line = data, solid line = fit. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Residuals across the full-time range were slightly lower in the PRESS case; this may reflect the longer TE (hence, generally lower signal strength) of the MEGA-PRESS sequence, a concern raised by Endres et al. [24] and others. However, we also note that in the case of lower concentrations (nominal concentration < 1.5 mM) the MEGA-PRESS sequence outperforms PRESS (this is consistent with the findings of Nezhad [25]).

The inconsistency in modeled decay time between the two methods suggests that one (or both) approaches exhibits a somewhat non-linear relation between reported and actual concentration; Lagopoulos et al. [26] have previously demonstrated strong linearity of a similar PRESS approach in simple phantom solutions for higher GSH concentrations, at lower concentrations (below 2 mM) this linearity seems to break down [25]. Further investigation is needed to resolve this.

The PRESS data in the present study are acquired from a large voxel with a long acquisition time (TA), thus achieving a larger SNR than

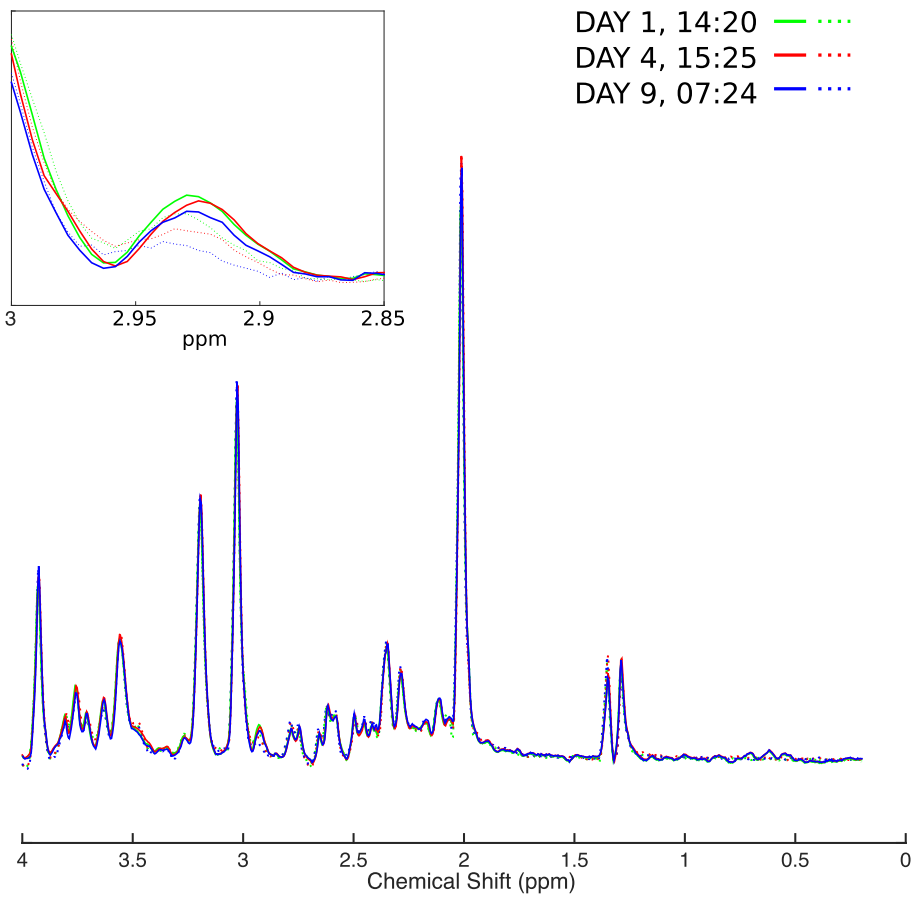

Fig. 5. PRESS spectra from day 1 (green), day 4 (red) and day 9 (blue). Dashed line = data, solid line = fit. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

with more standard voxels ($20 \times 20 \times 20\,mm^3$) and TA (~4.0 min). Lower SNR would definitely affect the preciseness of the PRESS measurements.

5. Conclusion

Both GSH edited MEGA-PRESS and TE = 30 PRESS techniques are able to quantify GSH concentrations changes from in vitro measurements. The MEGA-PRESS method is more selective, whereas the PRESS method possibly also captures signal for GSSG; this is not a concern for in-vivo applications, where the GSSG component is likely to be extremely small. The MEGA-PRESS method also offers somewhat improved precision at lower concentrations.

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