Received: 9 August 2020 | Accepted: 14 November 2020

DOI: 10.1002/nbm.4453

RESEARCH ARTICLE

NMR IN BIOMEDICINE WILEY

# An investigation of glutamate quantification with PRESS and MEGA-PRESS

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Glutamate is an important neurotransmitter. Although many studies have measured glutamate concentration in vivo using magnetic resonance spectroscopy (MRS), researchers have not reached a consensus on the accuracy of glutamate quantification at the field strength of 3 T. Besides, there is not an optimal MRS protocol for glutamate measurement. In this work, both simulation and phantom scans indicate that glutamate can be estimated with reasonable accuracy (<10% error on average) using the standard Point-RESolved Spectroscopy (PRESS) technique with TE 30 ms; glutamine, however, is likely underestimated, which is also suggested by results from human scans using the same protocol. The phantom results show an underestimation of glutamate and glutamine for PRESS with long TE and MEGA-PRESS off-resonance spectra. Despite the underestimation, there is a high correlation between the measured values and the true values ($r > 0.8$). Our results suggest that the quantification of glutamate and glutamine is reliable but can be off by a scaling factor, depending on the imaging technique. The outputs from all three PRESS sequences (TE = 30, 68 and 80 ms) are also highly correlated with each other ($r > 0.7$) and moderately correlated ($r > 0.5$) with the results from the MEGA-PRESS difference spectra with moderate to good shimming (linewidth < 16 Hz).

KEYWORDS

glutamate, MEGA-PRESS, MRS, phantom, PRESS, simulation

## 1 | INTRODUCTION

Glutamate (Glu) is the most abundant excitatory neurotransmitter in vertebrates. It plays an important role in many neural functions, including in reward/addiction circuits of the brain. Although MR spectroscopy (MRS) has the potential to quantify Glu concentrations in vivo, whether Glu can be quantified at 3 T remains controversial due to overlapping peaks with glutamine (Gln), gamma-aminobutyric acid (GABA) and N-acetylaspartate (NAA). Because of that, studies tend to report the combined concentration of Glu and Gln, denoted as Glx. Given that many published Glu studies were conducted at 3 T and that most research scanners today operate at 3 T and are equipped with the standard Point-RESolved Spectroscopy (PRESS) sequence, there is a need to investigate the accuracy and reliability of Glu quantification using PRESS at 3 T.

Over the last 15 years, many studies have attempted to measure Glu accurately and reliably in vivo based on the PRESS sequence at 3 T. For instance, an echo time (TE)-averaged PRESS method was proposed to quantitatively measure Glu and Gln at 3 T.[1] While this method is reliable for Glu/Gln quantification, it sacrifices other metabolites due to J-evolution and the basis set must take into account different $T_2$-weightings at

**Abbreviations used:** GABA, gamma-aminobutyric acid; Gln, glutamine; Glu, glutamate; Glx, combination of glutamate and glutamine; MQF, multiple-quantum-filter; M_diff, MEGA-PRESS difference spectrum; M_off, MEGA-PRESS off-resonance spectrum with TE 68 ms; NAA, N-acetylaspartate; PRESS, Point-RESolved Spectroscopy; P_TE30, PRESS with TE 30 ms; P_TE80, PRESS with TE 80 ms; SPECIAL, spin echo, full intensity-acquired localized spectroscopy; STEAM, stimulated echo acquisition mode; tCr, total creatine.

  

different TEs. In other studies, the PRESS sequence was considered optimized to separate Glu and Gln at specific TEs such as 40 or 80 ms.[2,3] In Mullins at al,[2] although the coefficients of variation for Glu were 7% and 5% for TE 30-ms and 40-ms PRESS, the ratios of Glu/Gln in the anterior cingulate cortex were 6.5 and 5.6, respectively, which are out of the typical physiological range. The Glu concentrations estimated from TE-30 and TE-40 PRESS were nearly 40% higher than those from TE-averaged PRESS although the difference in Gln concentration was much smaller (−14% for TE 30 ms and 3.5% for TE 40 ms). Similarly, in Schubert et al's study using TE 80-ms PRESS,[3] the coefficients of variation for Glu concentration were of the order of 10% and the ratio of Glu/Gln was 4.6-4.8 in the anterior cingulate, which is still higher than expected. Based primarily on simulation, Hancu suggested that short-TE PRESS results in the most reproducible Glu measurements were comparable with TE-averaged PRESS and stimulated echo acquisition mode (STEAM) sequences, while slightly overestimating the Glu concentration.[4] Henry et al showed that at 4 T, LCModel can reliably quantify Glu but not Gln or GABA for the PRESS sequence based on phantom data.[5] Their simulation suggested an overestimation of Glu but an underestimation of Gln.

PRESS has the advantage of simplicity and high signal-to-noise ratio (SNR). Other sequences have also been explored as alternative approaches to quantifying Glu and Gln. For instance, Yang et al proposed using a STEAM sequence with optimized timing parameters to achieve good separation of Glu and Gln at different field strengths, which was verified by simulated, phantom and in vivo results at 3, 4 and 4.7 T.[6] The ratio of Glu/Gln was measured to be 1.8 around the medial occipitoparietal junction using an optimized STEAM sequence at 3 T. However, STEAM loses 50% SNR compared with PRESS. In addition, the optimal TE to quantify Glu with STEAM at 3 T is 72 ms, suffering further SNR loss from $T_2^*$ decay compared with short-TE PRESS. Lately, two new techniques have been developed based on PRESS to minimize the overlap of Glu, Gln or NAA. One is adding a J-suppression pulse to an TE-optimized PRESS pulse sequence to minimize the NAA signal at 2.49 ppm,[7] the other is J-modulated spectroscopy.[8] Although the results seem promising, these techniques are not readily available on clinical scanners. There are many other MRS techniques attempting to quantify Glu/Gln in vivo, as summarized by Ramadan et al in their review.[9] Most studies reported a Glu concentration of ∼10 mM in the gray matter despite a large variability in Gln concentration.[10] Moreover, the Glu/Gln ratio derived from a PRESS-based sequence (PRESS, TE-averaged PRESS, optimal-TE PRESS, SPECIAL) tends to be larger than that derived by other methods.

Recently, the Mescher–Garwood point-resolved spectroscopy (MEGA-PRESS) sequence has become widely used to quantify GABA.[11] MEGA-PRESS edits GABA near 1.89 ppm and as a consequence coedits both Glu and Gln.[12] Hence, Glu and Gln can be estimated from GABA-edited MEGA-PRESS spectra when quantifying GABA. Sanaei Nezhad et al argued that Glu and Gln can be accurately quantified using MEGA-PRESS with appropriate phantom calibration and quality control of the spectrum (eg, linewidth < 8 Hz).[13] More recently, Maddock et al[14] suggested that MEGA-PRESS off-resonance spectra can replace PRESS for Glu quantification. Comparing the results between PRESS at TE = 80 ms and the MEGA-PRESS off-resonance spectrum at TE = 68 ms, a high correlation value was obtained for Glu (0.87) and Glx (0.84), much higher than the correlation between PRESS and the MEGA-PRESS difference spectrum (<0.3).

Despite the ambiguity in the quantification accuracy of Glu at 3 T, the reproducibility of Glu measurement has been extensively studied for those sequences,[2–4,15,16] and high reproducibility of Glu measurements has been confirmed in many experiments, including simulation, phantom and in vivo validation. On the other hand, there is still no standard protocol for measuring Glu. Since researchers use different approaches to measure Glu (ie, short-TE PRESS vs. long-TE PRESS), it is important to know to what level the measurements from different methods are correlated. Moreover, there is little discussion on how the quantification accuracy is affected by the shimming quality. In this study, the accuracy of Glu quantification is investigated by simulation, phantom and in vivo experiments using LCModel.[17] Four different methods—PRESS with TE 30 ms (P_TE30), PRESS with TE 80 ms (P_TE80), MEGA-PRESS off-resonance spectrum with TE 68 ms (M_off) and MEGA-PRESS difference spectrum (M_diff)—are compared and the correlations between the measurements of these methods are computed. The effect of shimming quality is also discussed.

## 2 | METHODS

### 2.1 | Simulation

Spectra of 12 different metabolites were synthesized using the simulation module of Vespa (https://scion.duhs.duke.edu/vespa/project). The 12 metabolites were NAA, N-acetylaspartylglutamate (NAAG), aspartate, choline, creatine, GABA, Glu, Gln, myo-inositol, phosphocreatine, scyllo-inositol and taurine. These metabolites are included in the LCModel basis set. We simulated the PRESS sequence with two different TE values (30 and 80 ms) as well as the MEGA-PRESS sequence. All pulses were modeled by ideal GAMMA pulses. The other parameters for PRESS simulation were: TE1 = TE2 = 15 ms for TE = 30 ms; TE1 = TE2 = 40 ms for TE = 80 ms; data points = 1024 and bandwidth = 1200 Hz. MEGA-PRESS was simulated with TE = 68 ms, number of data points = 1024, bandwidth = 1200 Hz and editing frequencies at 1.9 ppm for the "on" condition and at 7.5 ppm for the "off" condition. The concentration of different metabolites was derived from an in vivo scan of one subject in a study we conducted previously,[18] and total creatine (tCr: creatine + phosphocreatine) was used as the internal reference. Metabolite concentrations were tuned by the area scale factor in the Prioset module of Vespa. Table 1 lists all the metabolites and the corresponding concentrations used in the simulation. The concentrations of the metabolites were fixed, except for Glu and Gln, which were varied in six different combinations, as listed in

**TABLE 1** Metabolites included in the simulation and corresponding relative concentration with respect to total creatine (tCr: creatine + phosphocreatine) and $T_2$ relaxation values. The concentration of NAA, choline, GABA and myo-inositol with respect to tCr is the same as in the phantom.

| Metabolites | Relative concentration (met/tCr) | $T_2$ (s) |
|---|---|---|
| N-acetylaspartate (NAA) | 1.1 | 0.250 |
| N-acetylaspartylglutamate (NAAG) | 0.15 | 0.250 |
| Aspartate | 0.377 | 0.125 |
| Choline | 0.3 | 0.200 |
| Creatine | 0.49 | 0.150 |
| GABA | 0.2 | 0.088 |
| Glutamate | variable | 0.160 |
| Glutamine | variable | 0.160 |
| Myo-inositol | 0.75 | 0.167 |
| Phosphocreatine | 0.51 | 0.150 |
| Scyllo-inositol | 0.05 | 0.140 |
| Taurine | 0.25 | 0.140 |

**TABLE 2** Various combinations of Glu/Gln/GABA concentrations in phantom experiments along with fixed concentration of NAA (12.5 mM), creatine (10 mM), choline (3 mM), GABA (2 mM) and myo-inositol (7.5 mM). The relative concentration to tCr is the same as in the simulation.

| Phantom | Glu (mM) | Gln (mM) | Glu/Gln |
|---|---|---|---|
| 1 | 8 | 2 | 4 |
| 2 | 9 | 3 | 3 |
| 3 | 10 | 4 | 2.5 |
| 4 | 12 | 6 | 2 |
| 5 | 7 | 5 | 1.4 |
| 6 | 11 | 2.5 | 4.4 |

Table 2. The $T_2$ relaxation values were chosen based on several references.[8,19,20] Four macromolecule peaks (2.346, 2.142, 1.357 and 0.900 ppm) were selected in simulation to account for the baseline signals. The software uses Ta to represent $T_2$ relaxation and Tb to represent $T_2^*$ that characterizes the line-broadening effect. Five different Tb values were used to achieve different linewidths. The noise level was fixed and the final mean SNR was 57 (SNR fluctuates with the linewidth).

## 2.2 | Phantom scans

Six MRS phantoms mimicking the in vivo brain were made using an aqueous solution with several metabolites including choline, GABA, myo-inositol, creatine, NAA, Glu and Gln. Choline chloride was ordered from Alfa Aesar (Haverhill, MA, USA). All the other chemicals, namely, GABA, myo-inositol, monopotassium phosphate, potassium dihydrogen phosphate, creatine monohydrate, N-acetyl-L-aspartic acid, L-Glutamine and L-Glutamic acid, were ordered from Sigma Corporation (St. Louis, MO, USA).

In making the phantom, first, a 1000 ml buffer of pH = 7.2 was prepared using 5.239 g of monopotassium phosphate and 10.712 g of dipotassium phosphate. Six batches of phantom solutions were prepared in plastic bottles by adding other metabolites to 125 ml buffer that filled the bottle. The phantoms had various combinations of Glu/Gln/GABA concentrations (Table 2) but fixed concentrations for NAA (12.5 mM), creatine (10 mM), choline (3 mM) and myo-inositol (7.5 mM). The concentrations of the metabolites in the phantom were identical to those in the simulation, except that the simulation included more metabolites. The phantom was stored in the scanner room after being prepared then was scanned 6 hours later. The temperature in the scanner room was 20°C.

The MRS scans were performed on a Siemens Prisma scanner using a 64-channel head/neck coil. For each phantom, three single-voxel MRS pulse sequences were used to acquire the MRS spectra on a voxel of 2 cm × 2 cm × 2 cm centered in the phantom in the order of P_TE30, P_TE80, and the Siemens work in progress, MEGA-PRESS. The scan parameters for PRESS were TR = 2 seconds, bandwidth = 2000 Hz, 2048 data points and number of measurements = 128. The scan parameters for MEGA-PRESS were TR = 1.5 seconds, bandwidth = 2000 Hz, 1024 data points and number of measurements = 128. The editing frequency was 1.7 ppm for "On" and 7.7 ppm for "Off" spectra; the editing pulse bandwidth was set to 50 Hz. We changed the editing frequency to accommodate the temperature difference between the phantom and in vivo brain tissue. The magnetic field around the voxel was very homogeneous and therefore high-quality shimming could easily be achieved. The shimming quality was first set to achieve a full width at half maximum (FWHM) linewidth of 8 Hz of the water peak, which was realized using fastmap.[21] After all three MRS scans were completed under this shimming condition, the shimming quality was manipulated by manually changing the

shimming parameters to simulate in vivo scans in the brain with poorer shimming quality. Two shim levels (linewidth = 16 and 24 Hz) were targeted and MRS spectra were subsequently acquired. In the end, nine sets (3 sequences × 3 shim values) of MRS spectra were obtained of each phantom. All simulation and phantom data are available upon request.

## 2.3 | In vivo scans

In vivo data were collected on three human subjects using the same protocols as the phantom scans (ie, P_TE30, P_TE80 and MEGA-PRESS). The three subjects were all males (aged > 18 years) without any neurological disorders. The voxel of interest was selected in the region of the dorsal anterior cingulate cortex (voxel size 15 × 20 × 25 $mm^3$). The FWHM of the water peak during shimming was between 12 and 16 Hz. The editing frequency was 1.9 ppm for "On" and 7.5 ppm for "Off" spectra.

## 2.4 | Data analysis

All the MRS data were analyzed in LCModel (version 6.3 1 L for the phantom and simulated data; version 6.3 1 N for the in vivo data). For P_TE30 spectra, the basis set provided by LCModel was used for fitting. For P_TE80, MEGA-PRESS off-spectrum (M_off) and MEGA-PRESS diff spectrum (M_diff), the basis sets were generated by Dr. Jim Murdoch from density matrix simulations of the sequence using published values for chemical shifts and J-couplings.[22] The fitting range was set to 0.2-4 ppm. Eddy current correction and water scaling were employed for phantom data and in vivo data, but not for synthesized data. The concentrations of Glu, Gln and Glx were all expressed as the ratio to tCr.

The Glu, Gln and Glx values from the output of LCModel were compared with the real value to quantify the errors for both simulation and phantom results. Pearson correlation coefficients were computed between the measured and real values as well as between the measured values of the different methods for the phantom experiment. For in vivo data, since there was no ground truth, only the mean values of Glu/tCr and Gln/tCr were computed.

# 3 | RESULTS

Figure 1 compares the simulated 3 T PRESS spectra of GABA, Glu and Gln for TE = 30 ms. The three spectra overlap between 2.0 and 2.6 ppm. Glu and Gln signal also overlap near 3.75 ppm. Figure 2 compares a simulated spectrum and a phantom spectrum, along with one in vivo P_TE30

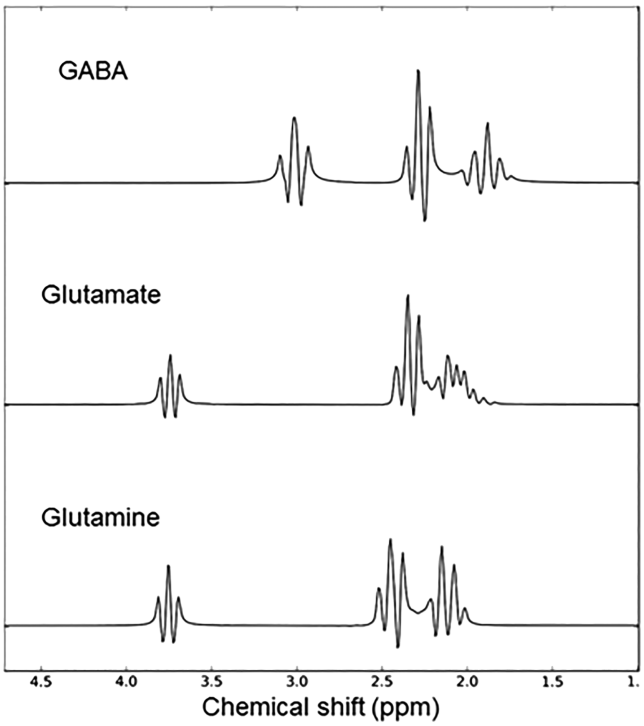


**FIGURE 1** Simulated PRESS spectra of GABA, glutamate and glutamine at 3 T (123.26 MHz) and TE 30 ms. Spectra are scaled individually and not according to concentration

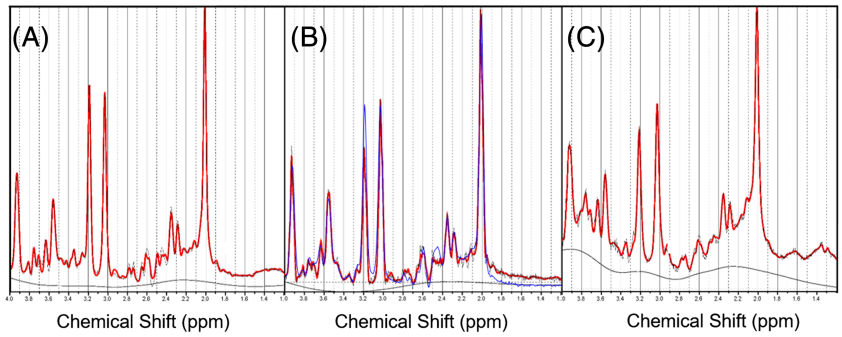


**FIGURE 2** Comparison between A, a synthesized spectrum, B, a phantom spectrum and C, an in vivo spectrum using PRESS at TE = 30 ms. The ratio of glutamate and glutamine concentration to creatine is set to 1.1 and 0.25 in Figures 2A,B. In Figure 2B, the phantom spectrum is overlaid by a synthesized spectrum (blue), in which only the metabolites present in the phantom are included in the synthesized spectrum. The ratio of glutamate and glutamine concentration to creatine is quantified to be 1.14 and 0.2 from the LCModel in Figure 2C. The FWHM of the NAA peak is 0.029 ppm in Figure 2A and 0.032 ppm in Figure 2C. The SNR is 41 in Figure 2A, 39 in Figure 2B and 40 in Figure 2C

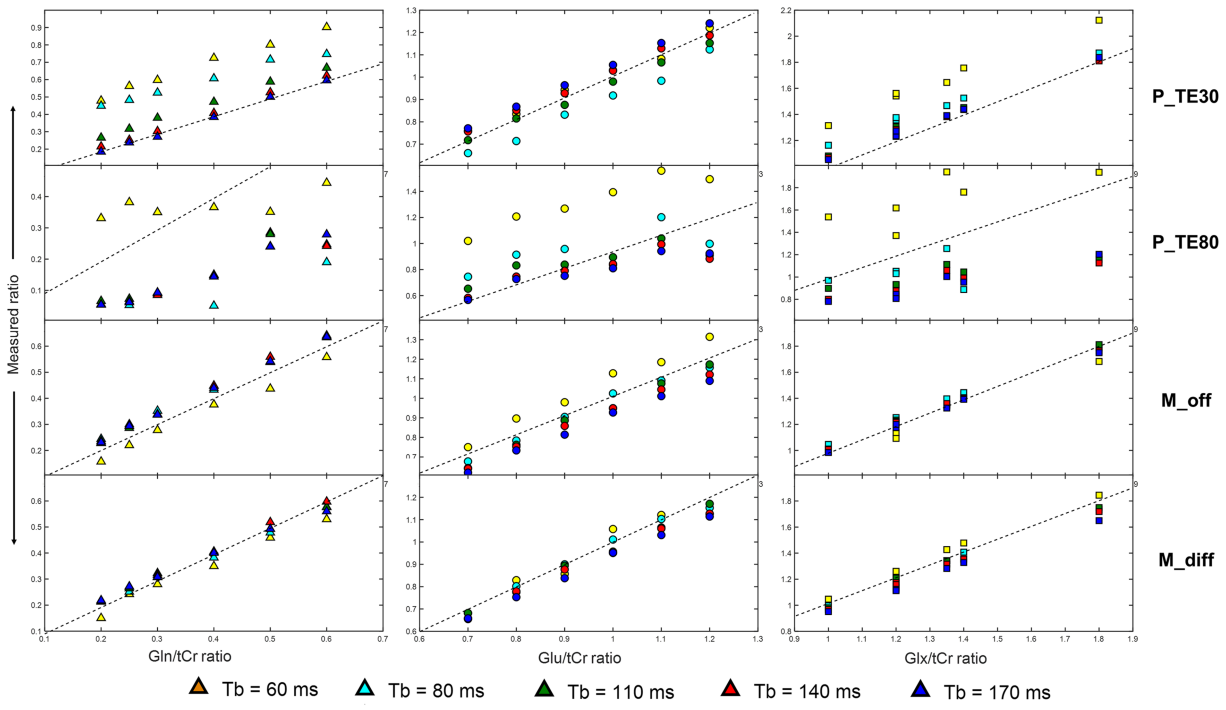


**FIGURE 3** Simulation results: scatterplots of measured ratios of Gln/tCr, Glu/tCr and Glx/tCr vs. corresponding real ratios from the synthesized data (dotted line) for P_TE30, P_TE80, M_off and M_diff

spectrum under comparable shim conditions. The three spectra resemble each other in terms of major metabolite peaks, shim values and SNR, except that the in vivo spectrum has some residual water signal and additional macromolecules and lipids in the baseline.

Figure 3 plots the measured ratio of Gln/Glu/Glx to tCr vs. real ratio for P_TE30, P_TE80, M_off and M_diff from synthesized data. The corresponding linewidth of the biggest NAA peak calculated from LCModel for different Tb values used in the simulations (60, 110, 140 and 170 ms) are 0.029, 0.033, 0.043, 0.057 and 0.068 ppm, respectively. The results show that data points for P_TE30, M_off and M_diff align well along the dotted line, indicating good estimation of Gln and Glu, especially with good shimming quality (NAA linewidth ≤ 0.043 ppm). Among them, P_TE30 is more sensitive to shimming quality. By contrast, the quantification of Gln or Glu with P_TE80 is not well aligned.

Figure 4 plots the measured ratio of Glu/Gln/Glx to tCr vs. real ratio for P_TE30, P_TE80, M_Off and M_diff from phantom data. Unlike the results of the synthesized data, there is a prominent trend of underestimation of Glu/Gln/Glx for PRESS with TE 80 ms and MEGA-PRESS. PRESS with TE 30 ms can quantify Glu and Glx well, even with relatively poor shimming, but it underestimates Gln, especially when shimming is poor.

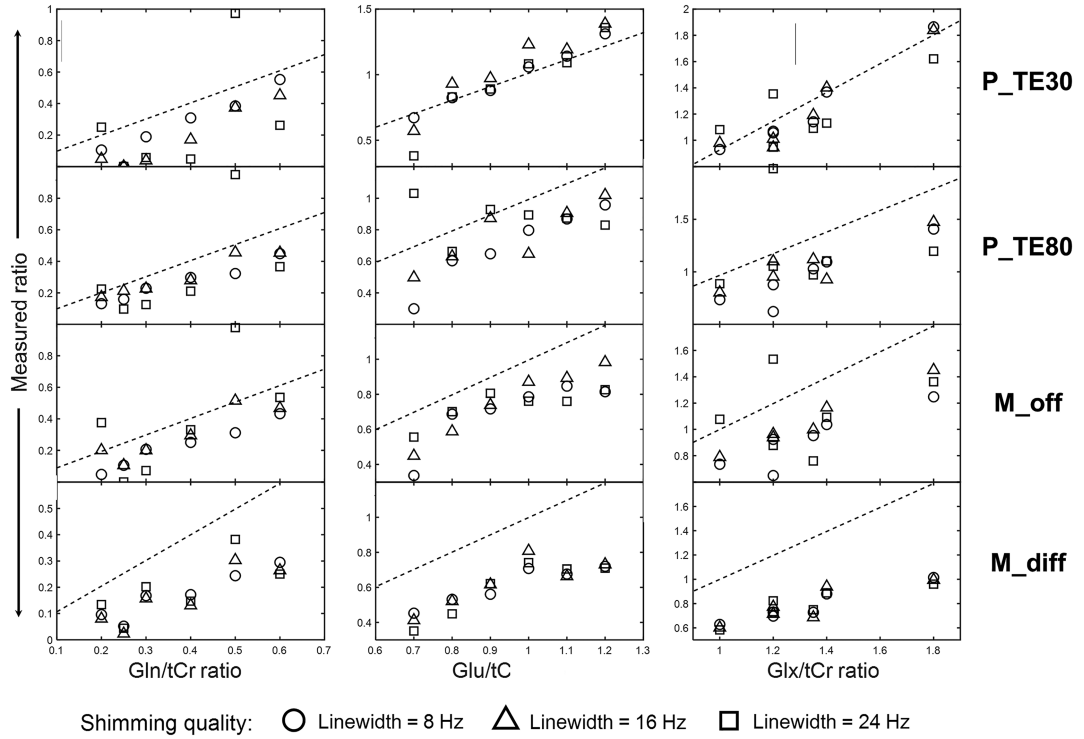


**FIGURE 4** Phantom results: scatterplots of measured ratios of Gln/tCr, Glu/tCr and Glx/tCr vs. corresponding real ratios from the phantom experiments for P_TE30, P_TE80, M_off and M_diff

Although there is a significant underestimation of Gln in general and underestimation of Glu for PRESS with TE 80 ms and MEGA-PRESS methods, there is an overall relatively high correlation between the LCModel-quantified values and the true values ($r > 0.8$). Table 3 lists the Pearson correlation coefficients of quantified Glu, Gln and Glx with corresponding true values under different shimming conditions for the phantom data. With good to moderate shimming quality, the correlation coefficients are higher than 0.8 for Glu, Gln and Glx. This value is even higher than 0.95 for PRESS with TE 30 ms. The correlation decreases as the shimming quality degrades. However, a high and linear correlation still exists for Glu and Glx derived from the difference spectrum of MEGA-PRESS ($r = 0.90$ and 0.88, respectively).

The quantified Glu/Gln/Glx values from these four methods are also highly correlated. Figure 5 shows the correlation matrices between the four different methods on the phantom data with different shimming quality. The correlation is higher than 0.8 for linewidth = 8 Hz, and higher than 0.5 for linewidth = 16 Hz. Interestingly, although underestimated in general, Gln has high correlations between methods for all three shimming conditions (>0.8), and is higher compared with Glu and Glx.

**TABLE 3** Pearson correlation coefficients of quantified glutamate, glutamine and Glx with corresponding true values at different shimming conditions of the phantom data. # indicates that the correlation is not significant ($P > .05$).

| | P_TE30 | P_TE80 | M_off | M_diff |
|---|---|---|---|---|
| Shim = 8 Hz | | | | |
| Glu | 0.99 | 0.96 | 0.84 | 0.94 |
| Gln | 0.96 | 0.98 | 0.98 | 0.94 |
| Glx | 0.97 | 0.92 | 0.89 | 0.96 |
| Shim = 16 Hz | | | | |
| Glu | 0.95 | 0.86 | 0.98 | 0.83 |
| Gln | 0.96 | 0.96 | 0.91 | 0.87 |
| Glx | 0.95 | 0.88 | 0.98 | 0.86 |
| Shim = 24 Hz | | | | |
| Glu | 0.95 | –0.18# | 0.81 | 0.90 |
| Gln | 0.48# | 0.59# | 0.66# | 0.71# |
| Glx | 0.74# | 0.01# | 0.24# | 0.88 |

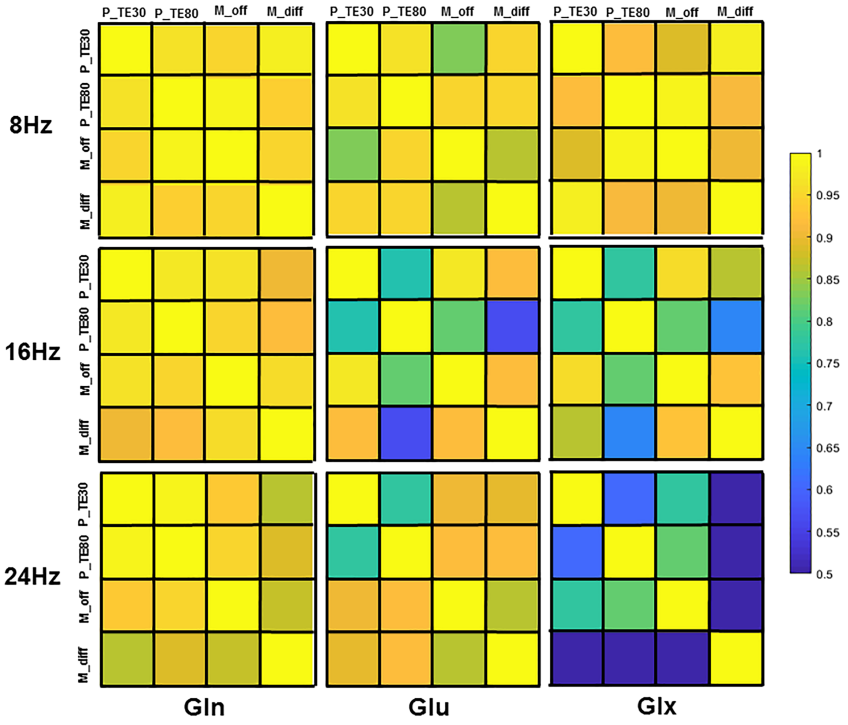


**FIGURE 5** Correlation matrices between four different methods for Gln, Glu and Glx on the phantom data with different shimming quality (row 1: 8 Hz; row 2: 16 Hz, row 3: 24 Hz)

To investigate whether the quantification error is related to the ratio of Glu to Gln, Figure 6 plots the quantification error as a function of the true ratio of Glu to Gln for the phantom data. Except for PRESS at TE 30 ms, Glu was underestimated for the other three methods and the level of underestimation was not affected by the ratio of Glu/Gln. For PRESS at TE 30 ms, the relative error is within 10% at good shimming quality and within 20% for moderate shimming quality. There is no particular relationship between the relative error and the Glu/Gln ratio.

The results of Glu and Gln quantifications on three human subjects are shown in Figure 7. P_TE30 gave the highest Glu/tCr ratio for all three subjects compared with the three other methods, which is consistent with the phantom study. For P_TE30, the average value of Glu/tCr was 1.15 and the average value of Gln/tCr was 0.20. The Glu/tCr ratio was very close to those reported previously.[6–8] The ratio of Glu/Gln was larger than expected, as discussed by Sanaei Nezhad et al.[13] The Glu values from P_TE80 were higher than those from M_off but lower than those from M_diff. This is consistent with the findings by Maddock et al.[14]

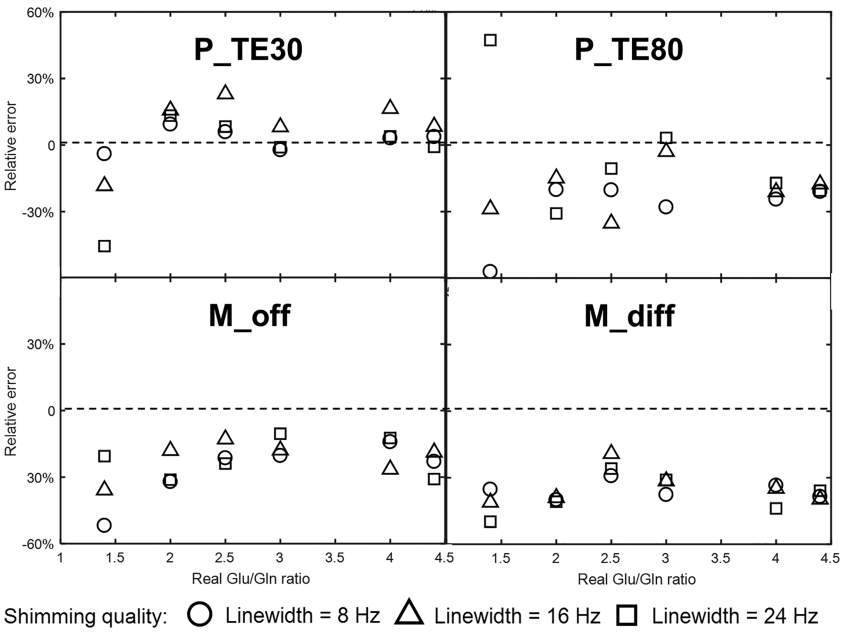


**FIGURE 6** Relative error of estimated glutamate as a function of real Glu/Gln ratio for phantom data of P_TE30, P_TE80, M_off and M_diff. The dashed line indicates 0% error

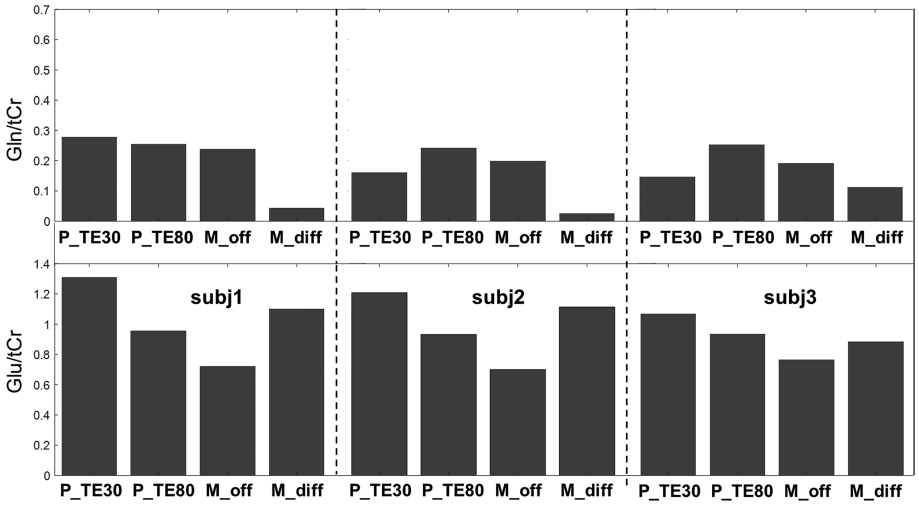


**FIGURE 7** The ratios of Gln/tCr and Glu/tCr of three human subjects quantified by P_TE30, P_TE80, M_off and M_diff

## 4 | DISCUSSION

We have used simulation and phantom experiments to investigate the accuracy in quantifying Glu and Gln for different acquisition schemes. The simulations show that both Glu and Gln can be quantified with good accuracy with adequate shimming (NAA linewidth < 0.05 ppm); however, quantification is poor using PRESS at TE 80 ms. The results of P_TE30 and M_diff are in line with the simulation results at 4 T.[5] However, the results from phantom experiments are not as good as the simulations in terms of Glu and Gln quantification accuracies. As phantom experiments are closer to the settings of in vivo studies, the following discussion mainly focuses on the phantom results, in order to be conservative and practical.

Both the simulation and phantom results indicate good accuracy in quantifying Glu with LCModel for the PRESS sequence with TE 30 ms at 3 T. The Glu concentration is slightly overestimated (<10% on average). This finding agrees well with most previous reports.[4,5,8] Other methods, including PRESS with a long TE and MEGA-PRESS difference spectrum, tend to underestimate Glu based on phantom data. This is contradictory to the simulation results. Although it was suggested that TE 80 ms is optimal for PRESS in measuring Glu, there were no in vitro results to confirm this in the original publication.[3] To our knowledge, the quantification accuracy for PRESS at long TE has never been investigated in vitro. However, the underestimation of Glu and Gln by M_diff agrees well with another study using phantoms.[13] An interesting observation from the phantom study is that the accuracy of Glu quantification is not sensitive to the true Glu/Gln ratio or shimming quality. Intuitively, the error of Glu quantification comes from the contamination of Gln signal. Therefore, it would be assumed that the error was smaller if Gln makes less contribution to the overlap. This is not the case in our data, probably because of the intrinsic similarity of the Glu and Gln basis spectra in LCModel fitting, as well as the lower concentration of Gln compared with Glu. In addition, the level of similarity between the two basis spectra does not vary much with linewidth.

The linearity of quantification is the most important characteristic for an in vivo study. It is more important to accurately reflect increases or decreases in a linear manner. A very interesting observation is that with good shimming quality, there is a good correlation between the measured Glu and Gln values with the true concentration. Sanaei Nezhad et al[13] reported similar findings for M_off, which is believed to be insensitive to Glu or Gln alone, but rather sensitive to Glx. This is encouraging because it means that the measurement is off by a scaling factor. Therefore, the overestimation or underestimation of Glu or Gln values can be corrected by a factor that might be determined by a phantom scan. Moreover, the correction factor is not critical if comparing the Glu between groups. High correlation of the Glu or Gln values between four different methods is also observed. Maddock et al first reported a high correlation of measured Glu and Glx values between P_TE80 and M_off, which is essentially a PRESS sequence with an TE of 68 ms.[22] These high correlations support the possibility of simultaneously measuring Glu and GABA using MEGA-PRESS off-resonance spectra and difference spectra, as suggested by Maddock et al.

The phantom experiments show a significant underestimation of Gln concentration for all methods, which is contradictory to the simulation results, except for P_TE80. The underestimation of Gln is supported by the in vivo scans using P_TE30, from which the average ratio of Glu/Gln was measured as 5.75, much higher than its typical physiological range of 1.5-4.5.[13] In fact, many in vivo studies using PRESS, short TE or long TE, reported higher than normal Glu/Gln ratios that were mainly caused by an underestimation of Gln. For instance, Schubert et al[3] reported similar Glu values between PRESS and multiple-quantum-filter (MQF) methods, but the Gln value from PRESS is nearly half of that from MQF. In another study using PRESS, the ratios of Glu/Gln in the anterior cingulate cortex were 6.5 and 5.6 for TEs of 30 and 40 ms, respectively. Hence, by checking the ratio of Glu/Gln, as was done by Sanaei Nezhad et al,[13] it is easy to tell if the quantification fails for at least one metabolite of Glu or Gln.

Nevertheless, since the phantom data show an underestimation of both Glu and Gln, the ratio itself cannot be used as a single index to determine if the quantification of Glu or Gln is accurate in vivo.

Because of the difficulty in separating Glu and Gln, some researchers recommend reporting the combined Glx value as opposed to Glu or Gln. From our results, especially those of the phantom experiments, there is no apparent advantage in doing that. Because the Glu concentration is higher than Gln in vivo, most of the Glx fits are related to Glu, rather than to Gln. In addition, it involves fitting Glu and Gln independently in LCModel and errors already exist before combining them. Without knowing the actual ratio of Glu/Gln, it is difficult to create a basis for Glx.

It is worth discussing the discrepancy between phantom results and the results of synthesized data. First, the simulation results suggest that Gln is not underestimated for P_TE30 while the phantom results clearly show an underestimation, which is confirmed by the in vivo data. Simulations usually provide better quantification accuracy than real scans. The errors of Glu or Glx are all lower than 10% for five simulations of PRESS, with TE ranging from 15 to 144 ms.[4] Another Monte-Carlo simulation obtained Gln/Cr = 0.32 against the ground truth of 0.35 for PRESS with TE 35 ms.[8] The reason could be that the basis set is derived from simulation, and therefore provides better fitting for simulated data. Because Glu and Gln have relatively smaller contributions to the spectra than other metabolites, Glu and Gln are more sensitive to this effect. Second, the simulation results suggest that Glu and Gln can be accurately measured using MEGA-PRESS difference spectra and LCModel for narrow linewidth (<8 Hz at 3 T). However, the phantom data show an underestimation of both Glu and Gln, which agrees with the work of Sanaei Nezhad et al.[13] In addition to the previous reason, another reason could be low-editing efficiency of Glu/Gln in practice. Our human scan results show an obvious underestimation of Gln for M_diff compared with the other three methods, while the Glu concentrations are only slightly lower than those from P_TE30. This is possibly related to the difference in editing efficiency in humans and also phantoms.

There are some limitations to this study. First, the $T_1/T_2$ values of metabolites in the phantom are not measured. This might cause some small errors when computing the ratios of Glu and Gln to tCr compared with the ground truth, especially at longer TE. Second, neither simulation nor phantom can mimic the in vivo condition perfectly. The phantom does not include macromolecules or many other metabolites, while the simulation does not include any scan-related issues such as eddy current, water suppression or baseline drift. Actually, there remain some differences between the phantom results and those from the synthesized spectra that include only the components present in the phantom (Figure S1). Third, the acquisition parameters (eg, the number of data points, bandwidth) are not identical between simulation, phantom scans and human scans, resulting in different spectral resolution and SNRs. Again, these factors will not produce a striking difference on the quantification as both the spectral resolution and SNR are sufficiently high for good fitting with LCModel (Table S1). In addition, two different versions of LCModel were used in the analysis. However, the results are identical when fitting one phantom spectrum with LCModel version 6.3-1 L or 6.3-1 N (Figure S2).

In summary, at the field strength of 3 T, both simulation and phantom scans indicate that Glu can be estimated with reasonable accuracy by PRESS with TE 30 ms, although Gln is probably underestimated. The phantom results show an underestimation of Glu and Gln for PRESS with long TE and MEGA-PRESS off-resonance spectra. Despite the underestimation, there is a high correlation between the measured and true values. Therefore, the quantification of Glu and Gln can be considered "accurate" up to a scaling factor. The outputs from all three PRESS sequences (TE = 30, 68 and 80 ms) are also highly correlated with each other ($r > 0.7$) and moderately correlated ($r > 0.5$) with the results from the MEGA-PRESS difference spectra with moderate to good shimming (linewidth < 16 Hz). However, the current conclusions cannot be uncritically generalized to in vivo measurements as neither simulation nor phantom experiments could precisely mimic the in vivo conditions.

## ACKNOWLEDGEMENTS

We are very grateful for Dr. Brian Soher's technical support on Vespa. We thank Dr. Jim Murdoch for providing the LCModel basis sets for this study. We also thank Siemens Healthineers support team for providing the MEGA-PRESS sequence. We truly appreciate the constructive comments and suggestions from all reviewers.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

**How to cite this article:** Cheng H, Wang A, Newman S, Dydak U. An investigation of glutamate quantification with PRESS and MEGA-PRESS. *NMR in Biomedicine.* 2021;34:e4453. https://doi.org/10.1002/nbm.4453