Published in final edited form as:

NMR Biomed. 2016 May ; 29(5): 600–606. doi:10.1002/nbm.3507.

Sensitivity and Specificity of Human Brain Glutathione Concentrations Measured Using Short Echo Time ^1H MRS at 7 T

Dinesh K. Deelchand¹, Małgorzata Marjańska¹, James S. Hodges², and Melissa Terpstra¹

¹Center for Magnetic Resonance Research, University of Minnesota, Minneapolis, MN, United States

²Division of Biostatistics, University of Minnesota, Minneapolis, MN, United States

Abstract

Although the MR editing techniques that have traditionally been used for measuring GSH concentration *in vivo* address the problem of spectral overlap, they suffer detriments associated with inherent long echo times (T_E). The purpose of this study was to characterize sensitivity and specificity for quantifying GSH concentration without editing at short T_E . The approach was to measure synthetically generated changes in GSH concentrations from *in vivo* STEAM spectra after *in vitro* GSH spectra were added to or subtracted from them. Spectra from five test subjects were synthetically altered to mimic changes in GSH signal. To account for different background noise between measurements, retest spectra (from the same individuals used to generate the altered data) and spectra from five other individuals were compared to the synthetically altered spectra to investigate the reliability in quantifying GSH concentration. Using STEAM spectroscopy at 7 T, GSH concentration differences on the order of 20% were detected between test and retest studies as well as between differing populations in a small sample ($n = 5$) with high accuracy ($R^2 > 0.99$) and certainty ($p < 0.01$). Both increases and decreases in GSH concentrations were reliably quantified with small impact on the quantification of ascorbate and γ -aminobutyric acid. These results show the feasibility of using short T_E ^1H MRS to measure biologically relevant changes and differences in human brain GSH concentration. While these outcomes are specific to the experimental approach used and the spectral quality achieved, this study serves as a template for analogous scrutiny of quantification reliability for other compounds, methodologies, and spectral qualities.

Keywords

GSH; antioxidant; magnetic resonance spectroscopy; LCModel; STEAM

Introduction

Glutathione (GSH) is an important antioxidant that is present in the human brain at a concentration of $\sim 1 \mu\text{mol/g}$ (1). The traditional MRS techniques for measuring GSH concentration *in vivo* use spectral editing (2-4). Although editing is invaluable for

unequivocal detection of small obstructed resonances, long echo times (T_E) render quantification of metabolite concentrations susceptible to low signal-to-noise ratio (SNR) and confounding by transverse relaxation times (T_2). While short T_E spectra are advantageous in these regards, quantification is complicated by resonance overlap. Because of higher SNR and spectral dispersion, improved quantification sensitivity and specificity are expected at higher magnetic field strength (5).

Agreement between GSH concentrations measured with edited MEscher-GARwood point resolved spectroscopy (MEGA-PRESS (6)) and non-edited stimulated echo acquisition mode (STEAM (7)) spectroscopy has been reported (8). The range of GSH concentrations in the human brain measured in that study was small. In another study (9), test-retest repeatability (i.e., standard error of measurement) of GSH concentration measured at 3 T with short T_E phase rotation STEAM was <10% (2 measurements for each of 10 subjects). Repeatability does not guarantee accuracy, especially given the plausibility of consistent but erroneous distribution of resonance intensity among overlapping resonances. Thus, quantification linearity (linear regression $R^2 = 0.99$) over large changes in GSH concentration was also demonstrated in solution (9).

However, sensitivity for picking up small differences in brain GSH concentration from spectra measured at short T_E from the living human brain is unknown. Reliability for detecting low, sub-physiologic concentrations has not been determined. The specificity with which resonances ascribed to GSH arise from this molecule instead of overlapping resonances has not been described.

In vivo spectra differ from mathematical simulations and *in vitro* spectra in many aspects that influence sensitivity and specificity. Whereas field inhomogeneity differentially impacts *in vivo* and *in vitro* spectral linewidth and line shape, simulated noise is random with even distribution throughout the spectrum. While extraneous coherences from water are present to a small extent *in vitro*, *in vivo* spectra tend to have larger extraneous contributions from incompletely suppressed water and additionally from lipids. Simulation exactness is limited by the extent to which actual radiofrequency and gradient pulses are accounted for as well as the accuracy and precision with which coupling constants and chemical shifts are known (10,11). Because of compartmentation within the human brain, these limitations are exacerbated by chemical shift displacement. The influence of model imprecision is inadvertently overlooked when the same simulated spectra are used as constituents for both building and fitting the spectra under investigation. A myriad of combinations of changes in concentrations of the other compounds in the neurochemical profile has potential to influence GSH quantification. For these reasons *in vivo* human brain spectra were studied instead of simulated spectra.

The goal of this project was to characterize the sensitivity with which synthetically imposed changes in GSH spectral intensity on spectra measured from the living human brain at short T_E can be quantified as well as the extent to which the rest of the neurochemical profile is influenced and influential.

Methods

Approach

Five short T_E *in vivo* spectra were synthetically altered by adding calibrated multiplications of an *in vitro* GSH spectrum. To account for different background noise between measurements, retest spectra from the same individuals (i.e., intra-individual test-retest) and spectra from five other individuals (i.e., inter-individual) were compared to the synthetically altered spectra to investigate the reliability in measuring the imposed changes in GSH concentration. The logic of comparing the altered test spectra with the retest spectra was to investigate if it was possible to measure a change in GSH in the same person, given that in real life at least two scans are needed in order to determine a change. Similarly, the other individuals were compared to the altered data in order to detect a difference between two groups.

Sensitivity and specificity were evaluated using LCModel to quantify GSH concentration from all fifteen baseline *in vivo* spectra as well as from the synthetically altered spectra. Spectra were fitted using a simulated basis set, thus uncoupling a key aspect of the building of the synthetic spectra (i.e., via *in vitro* spectra) from their fitting.

Human Subjects

Ten healthy volunteers were studied after giving written informed consent according to procedures approved by the Institutional Review Board at the University of Minnesota. The main test and retest spectra were measured from five subjects age 21 ± 1 years (mean $\pm$ SD, range 19 – 22 years, 2 male). The five other subjects age 21 ± 1 years (mean $\pm$ SD, range 20 – 22 years, 1 male) were scanned once. Among these subjects, no neurological or psychiatric abnormalities were detected on the day of the scan via Montreal Cognitive Assessment (all scores ≥ 26) and Beck Depression Inventory (all scores ≤ 5). There was no age, neurological test score or psychiatric test score difference between the main test and other individual groups ($p > 0.3$). All subjects consumed fewer than the RDA of fruits and vegetables and did not take vitamin supplements or medications suspected of influencing antioxidant capacity. All of them were non-smokers with no disclosed history of seizure, stroke, traumatic brain injury or hospital stay related to head injury.

In vivo NMR spectroscopy

All spectra were measured on a 7 T, 90-cm whole-body horizontal bore magnet (MagneX Scientific Inc., Oxford, UK) interfaced to a Siemens console running syngo VB17 (Siemens, Erlangen, Germany). The magnet was equipped with a body gradient coil (maximum amplitude: 70 mT/m, slew rate: 200 mT/m/ms). A 16-channel head array RF coil was used to transmit and receive (12). The transmit phase of each coil channel was controlled with an independent 1 kW RF amplifier (CPC, Brentwood, NY). An RF power monitoring system measured the forward and reflected power for each channel to ensure that the local specific absorption rate remained below 3 W/kg. Magnetization-prepared rapid gradient-echo (MPRAGE(13)) images were acquired to position 8 mL volumes-of-interest (VOI) in the posterior cingulate cortex. Proton density images were also acquired to correct for intensity field bias in T_1 images (14). To maximize the transmit B_1 in the VOI, the fast local B_1^+

shimming technique was employed (15,16). B_0 shimming of first- and second- order terms was achieved using FAST(EST)MAP (17). Spectra were measured using ultra-short T_E STEAM ($T_R = 5$ s, $T_E = 8$ ms, $T_M = 32$ ms, 128 averages) as previously described (18). Briefly, 3D outer volume suppression was interleaved with variable power and optimized relaxation delay (VAPOR) water suppression and each spectrum was individually saved (6 kHz spectral width, 2048 complex data points). A non-suppressed water spectrum was acquired for eddy current correction and absolute quantification.

***In vitro* NMR spectroscopy**

GSH (10 mM, pH = 7.2) and ascorbate (Asc) (10 mM, pH = 7.2) phantoms were prepared using 100 mM NaCl to achieve RF coil loading similar to the human head and 0.1 M phosphate buffer. 2 mM deuterated 3-(Trimethylsilyl)propionic-2,2,3,3-d₄ acid (TMSP) was added for chemical shift referencing. The phantoms were measured within 24 hours of preparation at physiological temperature (35–39°C). Spectra were measured from these phantoms using STEAM at 7 T as described above except 512 averages were acquired to increase SNR.

Synthetically altered spectra

The *in vitro* GSH spectrum was first normalized to match the amplitude and linewidth of the LCModel fitted GSH resonance in each of the five main test spectra measured during the first visit in the test-retest subjects. Then, varying multiples of this normalized spectrum were added to the corresponding *in vivo* spectrum for each person. GSH resonance intensity was changed over the range: 0, $\pm 5\%$, $\pm 10\%$, $\pm 20\%$, $\pm 30\%$, $\pm 50\%$ and $\pm 100\%$.

To investigate specificity, Asc and γ -aminobutyric acid (GABA) were also individually changed over this range. Simultaneous changes in neurochemical concentrations were investigated by changing both GSH and GABA concentrations over the grid $\pm 40\%$ in steps of 20%.

Spectral processing and quantification

All *in vivo* and phantom spectra were processed in Matlab (MathWorks Inc., Natick, MA). Eddy-current effects were corrected using the non-suppressed water spectrum. Single shot spectra were frequency corrected using a cross-correlation algorithm (maximizing the magnitude product between the mean of all spectra and individual spectra) and phase corrected using a least-squares algorithm (minimizing the phase difference between the mean of all spectra and individual spectra) before summation.

In vivo spectra were analyzed with LCModel (19) 6.3-0G (Stephen Provencher Inc., Ontario, Canada) with the following metabolites in the basis set: alanine, Asc, aspartate, creatine (Cr), GABA, glucose, glutamate, glutamine, GSH, glycerophosphorylcholine (GPC), *myo*-inositol, *scyllo*-inositol, lactate, *N*-acetylaspartate, *N*-acetylaspartylglutamate, phosphocreatine (PCr), phosphorylcholine (PCho), phosphorylethanolamine, taurine and a spectrum of fast-relaxing macromolecules (20) that was measured from a separate set of four individuals of similar age (total of 1632 averages). Basis spectra for LCModel were simulated using density matrix formalism (21) with ideal pulses and actual T_E and T_M and

previously published chemical shifts and J -coupling values (10). GSH was modeled as recently described (4): the glycine CH_2 moiety was considered as two equivalent protons resonating at 3.775 ppm and split by a 3.4 Hz J -coupling to the NH proton. 10 Hz of additional Gaussian line-broadening was applied to account for rapid exchange between the coupled NH group and water. The GABA basis spectrum was simulated as described in (10,11) with the addition of geminal coupling of -14.366 Hz between all methylene groups.

Processing parameters were optimized using an independent data set (Supplemental Data). Spectra were fitted between 0.5 to 4.1 ppm using knot spacing for the spline baseline (DKNTMN) of 5. The metabolite concentrations were determined using the unsuppressed water spectrum (corrected for T_2 relaxation of tissue water, i.e., 46 ms) as an internal reference in LCModel. These concentrations were also corrected for cerebrospinal fluid (CSF) in the VOI, which was determined by segmenting the normalized MPRAGE images using Freesurfer (22). Mean concentrations and coefficient of variation (CV) were reported without the exclusion based on the Cramér-Rao Lower Bound (CRLB) error estimate that is typically used. Metabolites that were highly correlated with each other ($r < -0.7$) were reported as sums: namely tCr (i.e., Cr + PCr) and tCho (i.e., GPC + PCho).

SNR was calculated as the peak height of the metabolite of interest (measured in the frequency domain) divided by the root-mean-square (RMS) noise (measured between -2 to -4 ppm). The RMS noise of the *in vivo* spectra over all imposed changes ($\pm 100\%$) in GSH resonance intensity was measured for one representative individual.

To investigate interrelated phenomena involving GABA without preparing and measuring an additional phantom, GSH, Asc and GABA spectra were simulated (see basis spectra above). These simulated spectra were only used when investigating GABA alongside Asc or GSH.

Statistical analysis

Baseline versus main test spectra with synthetic GSH changes were compared using two tailed Student's t -tests. Paired tests were used when comparing the same baseline spectra (i.e., baseline main test spectra versus main test spectra with synthetic GSH changes) or the same people (i.e., retest spectra versus main test spectra with synthetic GSH changes), and unpaired tests were used when comparing spectra from other individuals to the main test spectra (i.e., spectra from other individuals versus main test spectra with synthetic GSH changes). The least squares method was used to fit a line to known GSH signal changes versus concentrations measured from the main test spectra with synthetic GSH changes. The coefficient of determination, R^2 , of the linear fit was calculated. Sample sizes needed for reliably finding differences of 10% and 5% were computed using standard power-computation methods for paired and two-sample t -tests.

Results

The imposed change in GSH concentration from baseline to $\pm 100\%$ of its initial value was precisely measured among the five main test spectra. The line fitted through measured % change versus imposed % change had slope 0.99 and intercept -0.07%. A high correlation was found between the imposed and measured changes in GSH concentration ($R^2 > 0.99$).

For example, the measured GSH level was $99 \pm 1\%$ (mean $\pm$ SD) when the imposed increase was 100% and $-99.5 \pm 0.4\%$ (mean $\pm$ SD) when the imposed decrease was -100%. Figure 1 illustrates the agreement between imposed and measured changes from the five main test spectra. The linearity of GSH quantification was preserved even at -100% imposed GSH where the measured GSH concentration was $0.01 \mu\text{mol/g}$ and the CRLB maximized at 999%. The average CRLB were $0.12 \mu\text{mol/g}$ for all changing levels. The imposed versus measured means fall close to the unity line with SD bars that include it. Table 1 lists GSH concentrations and measurement errors for all of the study groups. The mean test-retest CV was 5% for glutathione.

Changes at or larger in magnitude than $\pm 20\%$ in GSH concentration between the main test and retest data were detected at level $p = 0.01$. Changes at or larger in magnitude than $\pm 20\%$ were detected ($p < 0.01$) between main test spectra and spectra measured from five additional individuals. When comparing the original test spectra against the same with changes made (i.e., under conditions of identical random and non-random noise), all differences were measured with high certainty ($p < 10^{-5}$). Regarding sample sizes needed to improve sensitivity compared to the sample of five used here, a sample size of 10 per group would give 80% power to detect a 10% change in GSH, while 33 per group would be needed to detect a 5% change.

In addition to the observed changes in GSH quantification as synthetic GSH changes were made, Asc and GABA quantification were slightly impacted. Figure 2A shows how measured Asc and GABA concentrations changed as synthetic GSH changes were made. Analogous changes in all of the other neurochemicals were within the range -2 to 1% over the entire imposed GSH change span of $\pm 100\%$.

Figure 2 demonstrates a reciprocal relationship between impacts of change in one neurochemical upon measured change in another. That is, imposing a change in GSH resonance intensity caused a change in measured Asc concentration that was equal in magnitude and opposite in sign to the measured change in GSH concentration that arose from imposing a change in Asc resonance intensity. A comparable reciprocal relationship was present between GSH and GABA. Imposed changes in GABA on all five *in vivo* spectra over the range $\pm 100\%$ caused increases and decreases in GSH concentration that were within 3%. The imposed versus measured change relationship for each of GABA and Asc was comparably linear to that of GSH. As a collection, these findings demonstrate that change in the resonance intensity from a given neurochemical has the expected major impact on the measured concentration of that same neurochemical and also a minor impact on the measured concentration of other neurochemicals. Asc concentration would have to differ from physiologic by more than 50% to bias GSH quantification by more than 2% (Figure 2B). At that point the abnormal Asc concentration would be detected outright. Simultaneous changes in GSH and GABA caused only minor errors in quantification of GSH concentration, with the largest 3% mismatch occurring for imposed changes in GSH of 20% and GABA of -40%. This 3% mismatch was equal to the sum of the mismatches caused by GSH and GABA separately.

Highly dispersed ^1H spectra were consistently measured *in vivo* in this study without extraneous coherences (Figure 3). The quality control parameters reported by LCModel were $\text{SNR} = 47 \pm 3$ (mean $\pm$ SD, range 42 - 50) and full width at half maximum = 0.023 ± 0.003 ppm (range 0.02 - 0.03 ppm or 6 - 9 Hz). SNR and linewidth were not different (p 0.2) among any of the subgroups (i.e., main test, retest, and other individuals). The chemical shift displacement of the VOI was 4% per ppm.

The spectra from a representative subject that resulted from synthetic GSH changes are shown in Figure 4. Changes in resonance intensities were visible at the expected chemical shifts without affecting other regions. Although plotted for all levels of GSH addition and subtraction, the residual, spline, Asc and GABA fits overlap such that only one line is apparent for each component. The RMS noise of the *in vivo* spectra for all imposed changes in GSH was within $\pm 0.5\%$ of that of the baseline spectrum (i.e., for 0% change in GSH).

Excellent agreement was observed between simulated spectra and those prepared at physiologic pH and scanned at physiologic temperature for both GSH (Figure 5) and Asc (not shown), affirming the accuracy of the chemical shifts and J -coupling constant values that were used to generate the basis spectra. The SNR of the GSH resonance in the phantom spectrum (after matching it to the *in vivo* linewidth) was 116 (as measured using Matlab).

Discussion

GSH concentration differences on the order of 20% were detected in a small sample ($n = 5$) with high accuracy ($R^2 > 0.99$) and certainty ($p < 0.01$) using short T_E ^1H MRS at 7 T. Both increases and decreases in GSH were detected reliably. This sensitivity was achieved for both incipient difference and test-retest change *in vivo*. The constancy of brain GSH concentration and its small variance among healthy young adults were remarkable (Table 1). The p values for detecting change (main data compared to retest) and difference (main data compared to other individuals) were similar because retest error was larger than between-person variation. Changes and differences on the order of 10% and 5% should be detectable in sample sizes of 10 and 33 people per group, respectively. These estimates from the retest data are associated with measuring change, but serve as a conservative estimate for measuring difference where the projected sample size estimate was smaller but potentially impacted by the small variation in GSH concentrations among the individuals studied. Biological relevancy of changes $\sim 20\%$ has been demonstrated as follows. Human occipital cortex GSH concentration was found to be 35% lower in healthy elder subjects than in young subjects (23), although that finding may have been confounded by differing T_2 given the long T_E used for edited ^1H spectroscopy. Additionally, human occipital cortex GSH concentration can be boosted by as much as 55% by intravenous delivery of *N*-acetylcysteine, a membrane permeable cysteine precursor that serves as the rate-limiting substrate for GSH biosynthesis (24). GSH concentrations lower by 30–40% have been measured in several brain regions of individuals with autism using HPLC-MS of brain tissue samples (25). Brain GSH concentration was recently found to be $\sim 20\%$ lower in elderly individuals with low dairy consumption compared to those following healthy dietary recommendations (26).

The accuracy with which differing GSH concentration can be detected as shown in this study agrees with that reported previously. Linearity of measured versus imposed changes in GSH in this study ($R^2 > 0.99$) was the same as that measured previously in solution ($R^2 = 0.99$) at a large and coarse scale (0 to 10 mM, (9)).

The impact of imposed changes in Asc on GSH was a mirror reflection of that of imposed changes in GSH on Asc. An analogous relationship was observed between GABA and GSH. Therefore adding of GSH was sufficient to identify the other neurochemicals that were most expected to confound findings and to estimate the magnitude of potential bias.

Specificity for detecting an abnormal GSH concentration that is not an artifact from other neurochemicals is within 3% of physiologic as long as the other neurochemical concentrations are in a reasonable range. This observation is based on the measured impact of non-physiologic Asc and GABA concentrations on GSH quantitation and the reciprocal relationships. Analysis comparable to that undertaken in this study is advisable when any neurochemical concentration is beyond 50% of physiologic. When simultaneously changing GSH and GABA by $\pm 40\%$, the measured GSH concentrations all remained very close to those measured under imposed GSH change separately. In fact, the largest error observed in GSH concentration was the sum of the errors caused by changing each of GSH and GABA individually. As such, simultaneous changes did not combine to produce errors of unpredictable magnitude.

Figure 5 illustrates outstanding agreement between measured and simulated GSH spectra. Noise multiplication from adding (or subtracting) *in vitro* GSH spectra was negligible based on: absence of noise changes in spectra (Figure 4), constant fit residuals (Figure 4) and the similarity of the SNR over all imposed changes.

Quantification of neurochemical concentrations from MR spectra is limited by the accuracy and precision with which the simulated or phantom measured model spectra match those arising from the associated compounds *in vivo*. Since simulated and phantom measured GSH spectra were used to artificially add and subtract GSH signal from *in vivo* spectra, the measured GSH concentration was influenced by the match between the simulated or phantom and *in vivo* spectral patterns of GSH. Although simulated and *in vitro* spectra were calibrated to the *in vivo* environment by using carefully determined chemical shifts and coupling constants (4) and scanning at body temperature and pH, an exact match cannot be guaranteed. While Kaiser et al. showed good comparability of *in vitro* and *in vivo* spectra at 4 T and absence of undesired superimposed signal components which were not covered by the fitting procedure, comparability under those circumstances cannot be directly applied to this study at 7 T. The fine details of the spectral pattern are influenced by line widths and relaxation times that were not measured *in vivo*. While Figure 5 shows an exemplary match between simulated and measured spectra, it also illustrates that agreement is rarely perfect on a fine scale. Analogously, the match between the model and *in vivo* spectra cannot be guaranteed. The extent to which the mismatch of fine details between model and *in vivo* spectra impacts quantification is unknown, especially when quantification is complicated by overlapping signals of many substances.

Spectral quality is expected to influence sensitivity and specificity of GSH quantification. The spectra used in this study had high spectral dispersion, narrow line width, high SNR and good water and lipid suppression. Imposed changes in GSH had a negligible influence on the spline (Figure 4). Variance in the fitted spline is increasingly recognized as an aspect of quality control, especially with regard to differences between experimental groups. Of course, sensitivity and specificity should be evaluated under matched circumstances, such as localization techniques and achieved spectral quality. This approach for evaluating the sensitivity and specificity of GSH quantification serves as a template for evaluating sensitivity and specificity of the other neurochemicals. It is especially warranted for resonances that are weakly represented and highly overlapped. While GSH concentration measured at baseline was in good agreement with other studies (2,8), inaccuracy in this estimate would shift and skew the relationship between imposed and measured changes. Verification of measured concentration is always an important quality control step.

To our knowledge this is the first time that spectra have been altered by subtracting resonances. Soundness of this approach is evidenced by quantification of a non-negative GSH concentration $0.01 \mu\text{mol/g}$ when 100% of the GSH resonance was subtracted (i.e., -100% resonance intensity was added). When the entire GSH resonance was removed, a negligible concentration was measured. The subtracting aspect contributes novel insight into sensitivity and quality control for detecting sub-physiologic concentrations. In particular, linearity of quantification was preserved when CRLB maximized at 999% (at -100% imposed resonance addition). These observations support that removing data on the basis of high percent CRLB might bias results (27).

Conclusions

Short T_E ^1H MRS can be used to measure biologically relevant changes and differences in human brain GSH concentration. Sensitivity on the order of 20%, which was demonstrated for a sample size of just five people, is relevant to investigation of oxidative stress. Good specificity of measured changes and differences to synthetically imposed differences in brain GSH concentration was demonstrated, even in the presence of reasonable non-physiologic concentrations of the other constituents of the neurochemical profile. Accurate detection of sub-physiologic GSH concentration was demonstrated. Since the accuracy and precision achieved in this study are valid only for the high spectral quality that was achieved at 7 T, they should be analogously verified for different circumstances. *In vivo* spectra were used as the starting point upon which changes were imposed. This is in contrast to prior studies in which *in vitro* or simulated spectra were used as the starting point. As such, *in vivo* noise conditions were accounted for in this study.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by funding from the NIH R01AG039396, P41 EB015894, S10 RR026783, R21 EB009133 and the W.M. Keck Foundation. The authors would like to thank Andrea Grant, Ph.D. for developing

and implementing the process for segmenting images and calculating the CSF content of the VOI and Edward J. Auerbach, Ph.D. for implementing STEAM and FAST(EST)MAP sequences on Siemens scanners.

References

1. Dringen R. Metabolism and functions of glutathione in brain. *Prog Neurobiol.* 2000; 62(6):649–671. [PubMed: 10880854]
2. Choi C, Zhao C, Dimitrov I, Douglas D, Coupland NJ, Kalra S, Hawes H, Davis J. Measurement of glutathione in human brain at 3T using an improved double quantum filter in vivo. *J Magn Reson.* 2009; 198(2):160–166. [PubMed: 19261496]
3. Terpstra M, Henry PG, Gruetter R. Measurement of reduced glutathione (GSH) in human brain using LCModel analysis of difference-edited spectra. *Magn Reson Med.* 2003; 50(1):19–23. [PubMed: 12815674]
4. Kaiser LG, Marjanska M, Matson GB, Iltis I, Bush SD, Soher BJ, Mueller S, Young K. ¹H MRS detection of glycine residue of reduced glutathione in vivo. *J Magn Reson.* 2010; 202(2):259–266. [PubMed: 20005139]
5. Deelchand DK, Iltis I, Henry P-G. Improved quantification precision of human brain short echo-time ¹H magnetic resonance spectroscopy at high magnetic field: A simulation study. *Magn Reson Med.* 2014; 72(1):20–25. [PubMed: 23900976]
6. Mescher M, Merkle H, Kirsch J, Garwood M, Gruetter R. Simultaneous in vivo spectral editing and water suppression. *NMR Biomed.* 1998; 11(6):266–272. [PubMed: 9802468]
7. Frahm J, Merboldt K-D, Hanicke W. Localized proton spectroscopy using stimulated echoes. *J Magn Reson.* 1987; 72(3):502–508.
8. Terpstra M, Vaughan TJ, Ugurbil K, Lim K, Schulz SC, Gruetter R. Validation of glutathione quantitation from STEAM spectra against edited ¹H NMR spectroscopy at 4T: application to schizophrenia. *Magn Reson Mater Phys.* 2005; 18(5):276–282.
9. Wijtenburg SA, Gaston FE, Spieker EA, Korenic SA, Kochunov P, Hong LE, Rowland LM. Reproducibility of phase rotation STEAM at 3T: Focus on glutathione. *Magn Reson Med.* 2014; 72(3):603–609. [PubMed: 24151202]
10. Govindaraju V, Young K, Maudsley AA. Proton NMR chemical shifts and coupling constants for brain metabolites. *NMR Biomed.* 2000; 13(3):129–153. [PubMed: 10861994]
11. Govind V, Young K, Maudsley AA. Corrigendum: Proton NMR chemical shifts and coupling constants for brain metabolites. *NMR Biomed.* 2000; 13:129–153. Govindaraju V, Young K, Maudsley AA. *NMR Biomed* 2015; 28(7):923–924. [PubMed: 10861994]
12. Adriany G, Van de Moortele P-F, Ritter J, Moeller S, Auerbach EJ, Akgün C, Snyder CJ, Vaughan T, Ugurbil K. A geometrically adjustable 16-channel transmit/receive transmission line array for improved RF efficiency and parallel imaging performance at 7 Tesla. *Magn Reson Med.* 2008; 59(3):590–597. [PubMed: 18219635]
13. Brant-Zawadzki M, Gillan GD, Nitz WR. MP RAGE: a three-dimensional, T1-weighted, gradient-echo sequence--initial experience in the brain. *Radiology.* 1992; 182(3):769–775. [PubMed: 1535892]
14. Van de Moortele P-F, Auerbach EJ, Olman C, Yacoub E, Ugurbil K, Moeller S. T1 weighted brain images at 7 Tesla unbiased for Proton Density, T2* contrast and RF coil receive B1 sensitivity with simultaneous vessel visualization. *NeuroImage.* 2009; 46(2):432–446. [PubMed: 19233292]
15. Metzger GJ, Snyder C, Akgun C, Vaughan T, Ugurbil K, Van de Moortele PF. Local B1+ shimming for prostate imaging with transceiver arrays at 7T based on subject-dependent transmit phase measurements. *Magn Reson Med.* 2008; 59(2):396–409. [PubMed: 18228604]
16. Van de Moortele PF, Akgun C, Adriany G, Moeller S, Ritter J, Collins CM, Smith MB, Vaughan JT, Ugurbil K. B₁ destructive interferences and spatial phase patterns at 7 T with a head transceiver array coil. *Magn Reson Med.* 2005; 54(6):1503–1518. [PubMed: 16270333]
17. Gruetter R, Tkac I. Field mapping without reference scan using asymmetric echo-planar techniques. *Magn Reson Med.* 2000; 43(2):319–323. [PubMed: 10680699]
18. Tkac I, Andersen P, Adriany G, Merkle H, Ugurbil K, Gruetter R. In vivo ¹H NMR spectroscopy of the human brain at 7 T. *Magn Reson Med.* 2001; 46(3):451–456. [PubMed: 11550235]

19. Provencher SW. Estimation of metabolite concentrations from localized in vivo proton NMR spectra. *Magn Reson Med*. 1993; 30(6):672–679. [PubMed: 8139448]
20. Tkac I, Gruetter R. Methodology of ¹H NMR spectroscopy of the human brain at very high magnetic fields. *Appl Magn Reson*. 2005; 29(1):139–157. [PubMed: 20179773]
21. Henry PG, Marjanska M, Walls JD, Valette J, Gruetter R, Ugurbil K. Proton-observed carbon-edited NMR spectroscopy in strongly coupled second-order spin systems. *Magn Reson Med*. 2006; 55(2):250–257. [PubMed: 16402370]
22. Fischl B, Salat DH, Busa E, Albert M, Dieterich M, Haselgrove C, van der Kouwe A, Killiany R, Kennedy D, Klaveness S, Montillo A, Makris N, Rosen B, Dale AM. Whole Brain Segmentation: Automated Labeling of Neuroanatomical Structures in the Human Brain. *Neuron*. 2002; 33(3): 341–355. [PubMed: 11832223]
23. Emir UE, Raatz S, McPherson S, Hodges JS, Torkelson C, Tawfik P, White T, Terpstra M. Noninvasive quantification of ascorbate and glutathione concentration in the elderly human brain. *NMR Biomed*. 2011; 24(7):888–894. [PubMed: 21834011]
24. Holmay MJ, Terpstra M, Coles LD, Mishra U, Ahlskog M, Oz G, Cloyd JC, Tuite PJ. N-acetylcysteine Boosts Brain and Blood Glutathione in Gaucher and Parkinson Diseases. *Clin Neuropharmacol*. 2013; 36(4)
25. Chauhan A, Audhya T, Chauhan V. Brain Region-Specific Glutathione Redox Imbalance in Autism. *Neurochem Res*. 2012; 37:1681–1689. [PubMed: 22528835]
26. Choi I-Y, Lee P, Denney DR, Spaeth K, Nast O, Ptomey L, Roth AK, Lierman JA, Sullivan DK. Dairy intake is associated with brain glutathione concentration in older adults. *Am J Clin Nutr*. 2015; 101(2):287–293. [PubMed: 25646325]
27. Kreis R. The trouble with quality filtering based on relative Cramér-Rao lower bounds. *Magn Reson Med*. 2015; 1002/mrm.25568

Abbreviations

Asc	ascorbate
CRLB	Cramér-Rao Lower Bound
GABA	γ-aminobutyric acid
GSH	Glutathione
SNR	signal-to-noise ratio
VOI	volume-of-interest

Figure 1.

Mean and SD ($n = 5$) of GSH concentrations measured from the 5 main test spectra (0% imposed change) and the spectra after the GSH resonance pattern measured from a phantom was added to and subtracted from (up to $\pm 100\%$) the main test spectra. The diagonal unity line marks theoretical agreement between imposed change in units of percent and measured concentration in units of $\mu\text{mol/g}$. All of the GSH concentrations differences between the main baseline test spectra and those to which *in vitro* GSH spectra were added or subtracted were significant ($p < 10^{-5}$).

Figure 2.

Influence of imposed changes in the magnitude of one resonance on concentrations measured from other resonances. A) Imposed change in the magnitude of the GSH spectrum versus changes in measured concentrations of Asc and GABA averaged over 5 individuals. B) Imposed change in the magnitude of the Asc spectrum versus changes in measured concentration of GSH for one individual.

Figure 3.

Overlaid 15 unaltered spectra (5 test and 5 retest from the same individuals and 5 from other individuals). From top to bottom: *in vivo* measured spectra, LCMoel fits, spline baselines and residuals. The small variations observed in these data demonstrate consistent spectral quality among subjects. For display purposes, all spectra were normalized based on the amplitude of the NAA singlet peak.

Figure 4.

In vivo spectrum at baseline and with imposed GSH changes and fitting outcomes from one representative subject. *In vivo* spectrum (STEAM, $T_R = 5$ s, $T_E = 8$ ms, 64 averages, no apodization) and the spectra that resulted from adding and subtracting several levels ($\pm 100\%$, $\pm 50\%$, $\pm 30\%$, $\pm 10\%$, 0%) of phantom measured GSH. LCModel fit outcomes for the residual, spline baseline, GSH, Asc and GABA are shown. Although fit outcomes are plotted for all levels of added and subtracted GSH resonance intensity, the differences for the residual, spline, Asc, and GABA are so small that only one line is visible. Inset: representative image and VOI location.

Figure 5.

Spectrum measured (STEAM, $T_R = 5$ s, $T_E = 8$ ms, $T_M = 32$ ms, 512 averages, 8 mL VOI size) from a phantom containing GSH prepared at physiologic pH and scanned at physiologic temperature and spectrum simulated using the density-matrix formalism. The simulated spectrum was line-broadened to match the measured spectrum (apodized with a 3 Hz exponential decay function) for display purposes only.

Table 1

Human brain GSH concentrations ([GSH]) measured in each group of five subjects and the average CRLB estimate of measurement error that is provided by LCModel listed in units of concentration (i.e., %CRLB times measured concentration).

Group	[GSH] ($\mu\text{mol/g}$) (mean $\pm$ SD, $n=5$)	CRLB ($\mu\text{mol/g}$) (mean, $n=5$)
Main test	1.35 ± 0.09	0.12
Retest	1.33 ± 0.09	0.12
Other individuals	1.35 ± 0.08	0.13