The influence of cystathionine on neurochemical quantification in brain tumor in vivo magnetic resonance spectroscopy

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Abstract

Purpose: To evaluate the ability of the PRESS sequence (T_E = 97 ms, optimized for 2-hydroxyglutarate detection) to detect cystathionine in gliomas and the effect of the omission of cystathionine on the quantification of the full neurochemical profile.

Methods: Twenty-three subjects with a glioma were retrospectively included based on the availability of both MEGA-PRESS and PRESS acquisitions at 3 T, and the presence of the cystathionine signal in the edited MR spectrum. In eight subjects, the PRESS acquisition was performed also in normal tissue. Metabolite quantification was performed using LCModel and simulated basis sets. The LCModel analysis for the PRESS data was performed with and without cystathionine.

Results: All subjects with glioma had detectable cystathionine levels > 1 mM with CRLB < 15%. The mean cystathionine concentrations were 3.49 ± 1.17 mM for MEGA-PRESS and 2.20 ± 0.80 mM for PRESS data. Cystathionine concentrations showed a significant correlation between the two MRS methods (r =0.58, p = 0.004) and it was not detectable in normal tissue. Using PRESS, nineteen metabolites were quantified with CRLB < 50% for more than half of the subjects. The metabolites that were significantly (p < 0.0028) and mostly affected by the omission of cystathionine were aspartate, betaine, citrate, GABA and serine.

Conclusions: Cystathionine was detectable by PRESS in all the selected gliomas, while it was not detectable in normal tissue. The omission from the spectral analysis of cystathionine led to severe biases in the quantification of other neurochemicals that may play key roles in cancer metabolism.

Introduction

In vivo ^{1}H magnetic resonance spectroscopy (MRS) enables the noninvasive detection and quantification of a series of neurochemicals in brain tissue. Thanks to the wider access to high magnetic field MRI systems and the significant advances in MR methodologies, in the last years, MRS has shown great promise for the noninvasive investigation of abnormal metabolic changes in brain tumors and for the identification of tumor genetic subtypes *in vivo*, providing added diagnostic and prognostic value to other MRI modalities[1–3]. In particular, since the discovery in 2009 of isocitrate dehydrogenase (*IDH*) mutations in gliomas[4,5], several molecules other than *N*-acetyl aspartate (NAA), choline containing compounds (tCho) and lactate have been shown to be detectable *in vivo* by MRS and identified to play crucial roles in the reprogramming of cancer cell metabolism. These include, 2-hydroxyglutarate (2HG), a specific marker of *IDH*-mutated gliomas[6,7], citrate, an intermediate for energy generation and biosynthesis of lipids and related molecules[8,9], and glycine, a putative marker or tumor aggressiveness irrespective of the *IDH* status[10].

Many other metabolites present in cancer cells at low concentrations and suggested to be related to malignancy can be reliably detected in tumor tissue by mass spectrometry *ex vivo* (e.g., aspartate, glutathione, phosphorylethanolamine, taurine, hypotaurine, threonine, ornithine, betaine, serine)[11]. The concentration of some of these neurochemicals may reach the threshold of detectability by MRS for high tumor cell densities or in specific brain tumor subtypes, either appearing as "unknown" signals in the spectra or being fitted by the fitting software as another compounds whose concentration is therefore overestimated. So far, due to the heterogeneity of brain tumors and their metabolism, no consensus on which metabolites should be included in the analysis of tumor spectra has been reached. Because of the large overlap between certain metabolic peaks, in conjunction with the low concentration of these molecules, the omission of a spectral component in the analysis may have a strong influence on the detection and quantification of other overlapping signals, especially when using conventional MRS. Spectral overlap can be partially removed using edited MRS methods[12], yet the analysis of non-edited spectra is useful for the quantification of the full metabolic profile and conventional MRS remains the most commonly used technique especially in clinical settings.

Recently, we reported the first detection of cystathionine in gliomas by edited MRS[13]. Cystathionine was not detectable in healthy tissue and was therefore proposed as novel noninvasive marker of tumor tissue. In addition, cystathionine was found to be present at significantly higher concentrations in *IDH*-mutated gliomas with 1p/19q codeletion compared to their non-codeleted counterparts[14].

In this study, we retrospectively analyzed the spectra acquired previously using conventional MRS optimized for 2HG detection in a cohort of patients with glioma and with detectable cystathionine levels measured from edited MRS. We evaluated the ability of optimized PRESS for cystathionine detection and reported the effect of the omission of cystathionine on the quantification of the full neurochemical profile, thus highlighting the importance of including this metabolite in the spectral fitting of *in vivo* tumor MRS data.

Methods

Human subjects

Twenty-seven subjects (18 males, median age: 41 years, range: 24 - 53 years) with a glioma were included in the study. Of these, 17 subjects were recruited at the Pitié-Salpêtrière Hospital, Paris, France, and examined at the Centre for Neuroimaging Research (Site 1), while the remaining 10 subjects were recruited at the Spedali Civili University Hospital, Brescia, Italy (Site 2).

Twenty-three subjects (1 *IDH* wild-type and 22 *IDH*-mutated, of which 14 1p/19q codeleted, 7 1p/19q intact, 1 with unknown codeletion status) were retrospectively selected based on the presence of the cystathionine signal in the *in vivo* edited MR spectrum, assessed by two expert MR spectroscopists, and the availability of both MEGA-PRESS and optimized PRESS acquisitions in tumor. For four of these subjects, the PRESS acquisition was performed also in the contralateral region outside the visible lesion. For additional four subjects, only the MRS of the healthy side of the brain was considered for this study.

Additional inclusion criteria were: age > 18 years and Karnofsky performance status > 60. Patients recruited at site 1 provided written informed consent prior to inclusion in the study, while patients recruited at site 2 were examined as part of their clinical care.

In vivo MRI/MRS acquisition

Acquisitions were performed using 3 T whole-body systems (MAGNETOM Verio – Site 1, and Skyra – Site 2, Siemens, Erlangen, Germany), using a 32- and a 20-channel receive-only head coils, respectively.

3D FLAIR images (site 1: field-of-view = 255 x 255 x 144 mm^3, resolution: 1.0 x 1.0 x 1.1 mm^3, T_R/T_E = 5000/399 ms, scan time = 5.02 minutes; site 2: field-of-view = 242 x 227 x 176 mm^3, resolution: 0.5 x 0.5 x 1.0 mm^3, T_R/T_E = 5000/394 ms, scan time = 6.27 minutes) were acquired to position the spectroscopic voxel of interest (VOI) in the glioma (hyper-intense region in the images). A 3D T_1-weighted sequence (field-of-view = 256 x 256 x 176 mm^3, resolution: 1.0 x 1.0 x 1.0 mm^3, T_R/T_E = 2300/4.18 ms, scan time = 4.28 minutes) was also acquired at site 1 and used for tissue segmentation of healthy brain tissue.

MR spectra were acquired at both sites using the same single-voxel spectral editing MEGA-PRESS (T_R = 2 s, T_E = 68 ms, 128 pairs of shots, scan time = 8.5 minutes) and PRESS sequences (T_R = 2.5 s, T_E = 97 ms, T_{E1} = 32 ms, T_{E2} = 65 ms, 128 shots, scan time = 5.45 minutes), and employing previously described procedures and parameters[15]. For both sequences, PRESS spatial localization utilized a 90º Hamming-filtered sinc pulse (duration = 2.32 ms; bandwidth = 3.83 kHz) and two 180º Mao pulses (duration = 5.80 ms; bandwidth = 1 kHz). For MEGA-PRESS, the editing pulse (180º Shinnar–Le Roux; duration = 19.2 ms; bandwidth = 62 Hz) was applied at 1.9 ppm for the edit-on condition and at 7.5 ppm for the edit-off condition, in an interleaved fashion. The final spectra were obtained by subtracting the spectra acquired at the edit-on and edit-off conditions. For each subject, the VOI size was adapted to the tumor size. The mean VOI size was 11.4 ± 7.5 mL (range: 7.2 - 33.4 mL) and 13.8 ± 2.6 mL (range: 7.5 – 15.6 mL) for Site 1 and Site 2, respectively.

Water suppression was performed using variable power with optimized relaxation delays (VAPOR) and outer volume suppression techniques[16]. Unsuppressed water scans were acquired from the same VOI for metabolite quantification and eddy current corrections using the same parameters as water suppressed spectra. B_0 shimming was performed using a fast automatic shimming technique with echo-planar signal trains utilizing mapping along projections, FAST(EST)MAP[17].

Spectral processing and quantification

Single-shots were frequency and phase aligned in Matlab (MathWorks Inc., Natick, MA) using the tCho signal at 3.22 ppm. All spectra were analyzed using LCModel v6.3-0G[18] (Stephen Provencher, Inc., Oakville, ON, Canada) with the basis sets simulated using the

density matrix formalism[19] as previously described[20]. RF duration and patterns for 90° and 180° pulses, slice-selective gradients during 180° pulses, timing and previously published chemical shifts and *J*-couplings[13,21–24] were taken into account. The basis set used for MEGA-PRESS included 2HG, cystathionine, γ-aminobutyric acid (GABA), glutamate, glutamine, glutathione, NAA, *N*-acetylaspartylglutamate (NAAG). The basis set for PRESS included 2HG, alanine, ascorbate, aspartate, betaine, citrate, cystathionine, creatine, cysteine, ethanolamine, GABA, glucose, glutamate, glutamine, glutathione, glycerophosphorylcholine, glycine, *myo*-inositol, lactate, NAA, NAAG, phosphorylcholine, phosphorylethanolamine, *scyllo*-inositol, serine, succinate, taurine and threonine. Spectra were fitted between 1.8 and 4.2 ppm for the MEGA-PRESS acquisition, and between 0.5 and 4.1 ppm for the PRESS. The DKNTMN parameter was set to 5, which results in a stiff baseline, suitable for data free from artifacts. For the PRESS data, the LCModel analysis was repeated twice, with and without cystathionine in the basis set. For tumor data, the quantification was carried out by scaling the signal using the unsuppressed water reference, assuming a tumor bulk water concentration of 43.3 M, as done previously[7]. Water and metabolite relaxation effects were compensated using water transverse and longitudinal relaxation time constants (T_2 and T_1) of 150 ms[25,26] and 800 ms[27], respectively, and previously reported metabolite T_2 and T_1 values[28,29]. For *J*-coupled metabolites with unknown relaxation time constants, glutamate T_2 and T_1 were used. For healthy tissue data, cystathionine concentrations were scaled assuming a water concentration of 41.8 M (based on tissue content in the VOI, averaged across subjects), water T_2 and T_1 of 80 ms and 800 ms, respectively, and the same cystathionine T_2 and T_1 as in tumor data. The reported concentrations are semi-quantitative.

Metabolites that were quantified from PRESS data with Cramér-Rao lower bounds (CRLB) < 50% for more than half of the subjects were included in the statistical analysis. For PRESS data, the linewidths of total creatine (tCr) at 3.03 ppm were determined from the LCModel fit as the full width at half maximum of this peak.

In vitro MRS acquisition

A cystathionine phantom ([cystathionine] = 1 mM, pH 7.2) was prepared using a phosphate buffer with 4,4,-dimethyl-4-silapentane-1-sulfonic acid added for chemical shift referencing and measured at physiological temperature. An MRS acquisition was performed using a 3 T whole-body Siemens Prisma^{fit} system (Siemens Medical Solutions, Erlangen, Germany) equipped with a 32-channel receive-only Siemens head coil. MR spectra were measured using

a single-voxel PRESS sequence (T_R = 3 s, T_E = 97 ms, 256 shots, VOI = 8 cm^3) as used in the *in vivo* acquisitions.

Statistical analysis

A two-tailed paired t-test was used to compare metabolite concentrations derived from optimized PRESS data using the full basis set to those estimated without cystathionine in the LCModel analysis. In order to account for multiple comparisons, a *p*-value of 0.05/(number of tested metabolites) was considered statistically significant. A two-tailed paired t-test was also used to compare cystathionine concentrations obtained from optimized MEGA-PRESS and PRESS data, while simple linear regression analysis was used to assess correlations between the cystathionine concentrations obtained using these two methods.

Results

All the 23 subjects selected for this study based on visual inspection of difference spectra acquired using the MEGA-PRESS sequence had detectable cystathionine levels > 1 mM in glioma tissue. An example of *in vivo* difference spectrum is shown in Figure 1A, together with the cystathionine component fitted with LCModel. The cystathionine signal at 2.72 ppm can be easily identified due to the absence of overlap with other metabolites and very characteristic spectral pattern. The mean cystathionine concentration (± SD) was 3.49 ± 1.17 mM (range: 1.73 – 5.53 mM) and the associated mean CRLB (± SD) was 13 ± 6 % (range: 7 – 33%).

The mean cystathionine concentration estimated using the optimized PRESS sequence was 2.20 ± 0.80 mM, with mean CRLB = 14 ± 6 % (range: 6 – 30%). High quality spectra were acquired at both sites. Individual spectra are shown in Supporting Information Figures S1 and S2. The mean tCr linewidth was 5.0 ± 1.2 Hz (range 3.8 – 7.2) and 5.1 ± 1.2 Hz (range 3.6 – 7.3 Hz) for Site 1 and Site 2, respectively. Despite the significant difference in the mean values (p =2 x 10^{-6}), cystathionine concentrations obtained from optimized PRESS spectra showed a significant correlation with those obtained from MEGA-PRESS data (Figure 2, r = 0.58, p = 0.004).

Figure 1B shows an example of an *in vivo* spectrum acquired in glioma using the optimized PRESS, together with the *in vitro* cystathionine spectrum, LCModel fits derived with and

without cystathionine in the basis set, separate fits of cystathionine, aspartate, betaine, citrate, GABA and serine, baselines and residuals. The simulated cystathionine signal well matched the phantom spectrum, confirming the accuracy of cystathionine chemical shifts and *J*-coupling constants used for the simulation[13].

The cystathionine signal is visible at 2.72 ppm also in the PRESS spectrum, however, due to the presence of other resonances around this frequency (aspartate, citrate), the exclusion of cystathionine from the LCModel analysis had only a slight effect on the quality of the fit and the residuals. Nevertheless, the omission of cystathionine from the basis set had a significant impact on the quantification of other metabolites. Table 1 reports the metabolite concentrations and CRLBs obtained from optimized PRESS data with and without cystathionine in the LCModel analysis. Nineteen metabolites, including cystathionine, were quantified with CRLB < 50% for more than half of the subjects. The threshold for statistical significance was then set to $p = 0.0028$ (0.05/19 metabolites). The metabolites that were most affected by the cystathionine omission were aspartate, betaine, citrate, GABA and serine, showing a percent difference in concentration between the two methods higher than 10% and $p < 0.0028$ (Figure 3). Aspartate showed a significant correlation with cystathionine ($r = 0.43$, $p = 0.04$) and the mean correlation coefficient reported by LCModel between these two metabolites was -0.53 ± 0.09 (range: -0.39 - -0.70).

In normal brain tissue, the mean cystathionine concentration was 0.27 ± 0.30 mM (range: 0-0.90 mM), with mean CRLB = 450 ± 456% (range: 53 – 999%). The mean tCr linewidth in the normal brain tissue was 5.2 ± 0.7 Hz (range: 4.0 – 5.9 Hz). An example of an *in vivo* spectrum acquired in normal brain tissue using the optimized PRESS is shown in Figure 1C. Individual spectra are shown in Supporting Information Figure S3.

Discussion

In this study, we reported on the detectability of cystathionine by a PRESS sequence optimized for 2HG detection and investigated the effect of omitting cystathionine in the spectral analysis on the quantification of the full metabolic profile. The omission of cystathionine caused significant differences in the quantification of several metabolites, notably aspartate, betaine, citrate, GABA and serine.

We previously showed that cystathionine was detectable by MEGA-PRESS at higher concentrations in 1p/19q codeleted gliomas compared to non-codeleted gliomas, in agreement with *ex vivo* mass spectrometry experiments, while it was not detectable in normal brain tissue[14]. The omission of cystathionine in the analysis of non-edited spectra may therefore lead to the wrong evaluation of other metabolic changes in tumors.

Cystathionine has a complex spectroscopic profile with several multiplets, which, at 3 T collapse into four main multiplets at around 2.2, 2.7, 3.1 and 3.8 ppm[13]. Under physiological conditions, all cystathionine resonances overlap with signals of other compounds in non-edited spectra.

For this study, patients with glioma were retrospectively selected based on the presence of cystathionine in *in vivo* edited spectra. Edited MRS removes the overlap between the cystathionine signal at 2.72 ppm and other signals resonating around the same frequency[13]. The presence of cystathionine was assessed visually from difference spectra (Figure 1A), and then confirmed and quantified by LCModel analysis.

The metabolites included in the basis set for analysis of optimized PRESS data were chosen based on previous reports on tumor tissue metabolic data measured *in vivo* by MRS and *ex vivo* by mass spectrometry[2]. Cystathionine was detectable by PRESS in glioma with CRLB $\leq$ 30%, while it was not detectable in the healthy brain tissue (CRLB > 50%). Cystathionine concentrations significantly correlated with those quantified using MEGA-PRESS. However, the mean cystathionine concentration was significantly lower from optimized PRESS than that obtained using edited MRS. This is most likely due to the ability of LCModel to correctly assign spectral patterns to different neurochemicals in different spectra. In MEGA-PRESS, there is no overlap of cystathionine with other metabolites. In optimized PRESS, there is an overlap of cystathionine with other neurochemicals, which results in the mean correlation coefficient reported by LCModel between cystathionine and aspartate of -0.53 and significant correlation between the concentrations of these two metabolites. Although, some of the discrepancy might be also coming from differences in T_E and T_R used between these methods since assumed T_2 and T_1 values were used for calculating final concentration values.

The metabolites that were most affected by the omission of cystathionine from the spectral analysis were the *J*-coupled metabolites with overlapping resonances with cystathionine signals. Aspartate was the most affected, showing a 2.69-fold higher concentration when cystathionine was not part of the basis set (Table 1), due to its multiplets, in proximity of 2.7 ppm and 3.8 ppm, both overlapping with cystathionine resonances. Similarly, citrate and

GABA showed 1.5 and 1.39-fold increases, respectively, when omitting cystathionine from the analysis, due to the overlap of their coupled resonances around 2.6-2.7 ppm with cystathionine. Betaine was 1.11 times higher when cystathionine was not part of the analysis, while serine concentration was significantly reduced, likely due to the singlets of these molecules at 3.8 ppm overlapping with cystathionine.

The precise role of these metabolites in cancer metabolisms is still not fully known. Increased aspartate and betaine were found, among other metabolites, to be significantly elevated *ex vivo* in lesions that had undergone malignant progression to grade IV compared with all other grades[30]. *Ex vivo* experiments also showed significantly lower GABA levels in *IDH*-mutated vs. *IDH*-wild-type gliomas and a positive correlation with 2HG levels in recurrent tumors[30]. GABA concentration is tightly linked with available pools of glutamate and glutamine, which were also shown to be reduced in *IDH*-mutated gliomas, from *in vivo* and *ex vivo* experiments, as well in cell cultures, possibly to compensate for the altered flux of alpha-ketoglutarate to 2HG in *IDH*-mutated gliomas[31–34].

Serine was shown, by mass spectrometry experiments, to be significantly lower in *IDH*-mutated, 1p/19q codeleted gliomas, compared to non-codeleted gliomas, and to strongly correlate with cystathionine concentrations[14]. Both serine and cystathionine are involved in glutathione biosynthesis pathways and were suggested to play crucial roles in the compensatory antioxidant mechanisms in gliomas with 1p/19q codeletion[14,35,36].

The quantification of the full metabolic profile is crucial for the characterization of brain tumors. *In vivo* MR spectroscopy may help in understanding the role of several metabolites in different tumor subtypes noninvasively. The accurate quantification of neurochemical profiles can be extremely useful for tumor diagnosis, prognosis, and monitoring of anti-cancer treatments *in vivo*. The omission from the spectral analysis of newly discovered metabolites such as cystathionine may lead to severe biases in the quantification of other key neurochemicals and may prevent from the understanding of their role in cancer metabolism.

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Figure captions

Figure 1. *In vivo* ^{1}H MEGA-PRESS and optimized PRESS spectra. (A) The *in vivo* MEGA-PRESS spectrum acquired at 3 T in one subject with an *IDH1*-mutated, 1p/19q codeleted glioma is shown together with the LCModel cystathionine contribution. The cystathionine pattern is visible at 2.72 ppm with no overlap with other metabolites. A line-broadening of 1 Hz was applied to the *in vivo* spectrum for display purposes only. (B) The *in vivo* optimized PRESS spectrum acquired for the same subject is shown together with the *in vitro* cystathionine spectrum, LCModel fits obtained with and without cystathionine in the basis set, the cystathionine, aspartate, betaine, citrate, GABA and serine contributions, the baselines and the residuals resulting from the two fits. No line-broadening was applied to *in vivo* data. The VOI is shown on a FLAIR image. A gaussian line-broadening of 0.2 s was applied to the cystathionine phantom spectrum. (C) The *in vivo* optimized PRESS spectrum acquired in the normal brain tissue of the same subject is shown together with the LCModel fit obtained with the basis set with all metabolites, the cystathionine contribution, the baseline and the residuals. The VOI is shown on a FLAIR image.

Figure 2. Correlation between cystathionine quantified with PRESS and MEGA-PRESS. Regression plot of cystathionine concentrations measured in tumor tissue by optimized PRESS and MEGA-PRESS *in vivo*. Cystathionine concentrations obtained from PRESS show a significant correlation with those derived by MEGA-PRESS ($r = 0.58$; $p = 0.004$).

Figure 3. Effect of cystathionine on metabolite quantification from optimized PRESS spectra. Box-plots of (A) metabolite concentrations and (B) CRLBs obtained with and without cystathionine in the LCModel basis set. Only metabolites showing concentration differences that are statistically significant and greater than 10% between the two fitting approaches are shown. For each box, the central mark indicates the median concentration and the bottom and top edges indicate the 25th and 75th percentiles, respectively. Circles represent values from individual subjects.
Asp: aspartate; Bet: betaine; Cit: citrate; Cth: cystathionine; GABA: γ-aminobutyric acid; Ser: serine.

Supporting Information Figure S1: Quality of MR spectra acquired at Site 1. *In vivo* ^{1}H PRESS TE = 97 ms spectra acquired in the glioma of 13 subjects at Site 1 (Centre for Neuroimaging Research Pitié-Salpêtrière Hospital, Paris, France) shown together with LCModel fits, baselines, and residuals obtained with all metabolites in the basis set.

Supporting Information Figure S2: Quality of MR spectra acquired at Site 2. *In vivo* ^{1}H PRESS TE = 97 ms spectra acquired in the glioma of 10 subjects at Site 2 (Spedali Civili University Hospital, Brescia, Italy), shown together with LCModel fits, baselines, and residuals obtained with all metabolites in the basis set.

Supporting Information Figure S3: Quality of MR spectra acquired in the healthy tissue at Site 1. *In vivo* ^{1}H PRESS TE = 97 ms spectra acquired in the healthy tissue of 8 subjects at Site 1 (Centre for Neuroimaging Research Pitié-Salpêtrière Hospital, Paris, France) shown together with LCModel fits, baselines, and residuals obtained with all metabolites in the basis set.

Table 1. Metabolite concentrations obtained with and without cystathionine in the LCModel fit. Concentrations are reported as mean value ± standard deviation. Cramér-Rao Lower Bound (CRLB) are reported in mM. If the concentration was zero for a given metabolite and subject, the corresponding CRLB (999%) was not considered in the calculation. # indicates the number of subjects (out of 23) for which each metabolite was detected with a CRLB < 50%. Statistical significance was set for $p < 0.0028$. Bold characters highlight metabolites showing concentration differences that are statistically significant and greater than 10% between the two fitting approaches.

	Fit with all metabolites			**Fit without cystathionine**			**Ratio**	**P value**
	Concentration (mM)	CRLB (mM)	#	Concentration (mM)	CRLB (mM)	#		
Asc	1.31 ± 0.56	0.28	22	1.32 ± 0.51	0.31	22	1.01	0.80
Asp	**1.33 ± 1.14**	**0.44**	**14**	**3.59 ± 1.21**	**0.40**	**23**	**2.69**	**$3 \cdot 10^{-11}$**
Bet	**0.24 ± 0.16**	**0.05**	**20**	**0.28 ± 0.19**	**0.05**	**20**	**1.11**	**$1 \cdot 10^{-4}$**
Cit	**0.74 ± 0.65**	**0.16**	**20**	**1.13 ± 0.72**	**0.17**	**23**	**1.50**	**$4 \cdot 10^{-8}$**
Cth	2.20 ± 0.80	0.27	23					
2HG	3.43 ± 2.11	0.38	23	3.65 ± 2.98	0.41	23	1.09	$1 \cdot 10^{-5}$
GABA	**0.59 ± 0.72**	**0.24**	**11**	**0.80 ± 0.78**	**0.26**	**14**	**1.39**	**$4 \cdot 10^{-7}$**
Gln	3.87 ± 1.67	0.31	23	4.20 ± 1.72	0.34	23	1.09	$7 \cdot 10^{-8}$
Glu	4.00 ± 1.55	0.32	23	4.26 ± 1.62	0.33	23	1.08	$5 \cdot 10^{-8}$

GSH	0.86 ± 0.47	0.14	21	0.83 ± 0.42	0.15	21	0.99	0.12
mIns	6.99 ± 2.22	0.30	23	6.97 ± 2.24	0.31	23	1.00	0.46
Lac	5.29 ± 2.82	0.31	23	5.35 ± 2.85	0.34	23	0.97	0.03
Ser	**2.08 ± 1.59**	**0.67**	**16**	**1.51 ± 1.88**	**0.71**	**10**	**0.70**	**$7 \cdot 10^{-4}$**
sIns	0.43 ± 0.24	0.04	21	0.44 ± 0.24	0.05	21	1.01	$2 \cdot 10^{-5}$
Suc	0.17 ± 0.09	0.07	14	0.17 ± 0.08	0.08	10	0.99	0.17
Tau	1.02 ± 0.63	0.25	19	1.08 ± 0.46	0.27	20	1.07	$3 \cdot 10^{-5}$
tCho	3.98 ± 2.19	0.05	23	3.95 ± 2.17	0.05	23	0.98	0.06
tCr	7.91 ± 2.08	0.13	23	7.79 ± 2.06	0.15	23	0.99	$7 \cdot 10^{-8}$
tNAA	5.29 ± 1.76	0.11	23	5.39 ± 1.77	0.11	23	1.03	$2 \cdot 10^{-11}$

2HG: 2-hydroxyglutarate; Asc: ascorbate; Asp: aspartate; Bet: betaine; Cit: citrate; Cth: cystathionine; GABA: γ-aminobutyric acid; Gln: glutamine; Glu: glutamate; GSH: glutathione; mIns: *myo*-inositol; Lac: lactate; Ser: serine; sIns: *scyllo*-inositol; Suc: succinate; Tau: taurine; tCho: total choline; tCr: total creatine; tNAA: *N*-acetylaspartate + *N*-acetyl-aspartyl-glutamate.