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# Effects of Age and Sex on Brain Glutamate and Other Metabolites

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### Keywords

proton magnetic resonance spectroscopy; TE-averaged PRESS; glutamate

In a recent paper (1), we reported the effects of sex and age on brain glutamate (Glu), as well as other brain metabolite concentrations, measured with a new technique called TE-averaged PRESS (2) on a 3 Tesla Siemens scanner in four brain regions of 50 healthy subjects. While revising the original IDL processing script for a scanner upgrade, we found a programming error in the original code to calculate the metabolite concentrations. Specifically, the revised code used the unsuppressed water signal corrected for T2-decay and percentage of CSF in each voxels as a reference for calculating metabolite concentrations (3). In contrast, the original processing lacked the use of a proper reference signal in calculating metabolite concentrations; consequently, the metabolite values presented were dependent on factors such as coil loading, B1 sensitivity, and receiver gain. We therefore re-analyzed the original data, and included data from 12 additional subjects to further increase the sample size. We report here the re-analyzed metabolite concentrations of Glu and other metabolites that differ from our original manuscript, based on measurements performed on the original 50 as well as the 12 new subjects (total 62 healthy subjects: 39 males and 23 females).

## Statistical considerations

Statistical analyses were performed using SAS version 9.1 (SAS Institute, Cary, NC, USA). All statistical tests were two-sided and p-values less than 0.05 were considered significant. The t-test was used to assess gender differences in metabolite concentrations. Linear regression was used to examine the association between age and the metabolite concentrations. Analysis of covariance was performed to assess the interaction effect between age and gender on the metabolite concentrations. The Simes procedure was used to adjust for multiple comparisons (4).



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## Effect of sex on brain metabolites (Table 1)

The Sailasuta et al paper described significant sex differences in the total creatine (Cr) concentration in the parietal gray matter (M: 6.22±0.13, F: 5.95±0.16, p=0.05) and basal ganglia (M: 4.62±0.11, F: 4.63±0.11, p=0.04). Trends for sex differences were also reported for glutamate (Glu) in the frontal white matter, parietal gray matter and the basal ganglia. None of these sex differences for Cr or Glu remained significant in the re-analyzed data of the 50 subjects or the larger sample of 62 subjects. The re-analyzed data with 62 subjects showed that men had higher choline (Cho) concentrations in the parietal gray matter (+6.7%, p=0.04) and higher NA concentrations in the basal ganglia (+6.5%, p=0.01) compared to women, but these differences were not significant after correction for multiple comparisons. Since higher Cho was reported in subjects with more recent alcohol use (5) and higher NA were reported in those with higher intelligence (6), we additionally evaluated for possible differences in these variables. We found no differences in the National Adult Reading Test-estimated verbal intelligence quotient (VIQ: 111.4±1.7 vs. 108±3.3, p=0.41) or average alcohol drinks per week (5.8±1.5 vs. 4.9±1.7, p=0.73) between these men and women. Parietal gray matter Cho also did not correlate with the averaged number of alcoholic drinks per week (r=0.04, p=0.78), and basal ganglia NA did not correlate with VIQ (r=−0.14, p=0.45).

## Effect of age on brain glutamate and other metabolites (Table 2)

While age-dependent decreases in parietal gray matter Glu and basal ganglia Glu and Cr remained significant, the previously reported age-dependent decreases in frontal white matter NA and Glu, and age-dependent increase in frontal white matter Cho, were no longer significant on our re-analyses.

## Sex-differences on age-related changes in brain metabolites (Table 3)

The Sailasuta et al paper also stated that men but not women showed significant age-related decreases in parietal gray matter Glu (r=−0.67, p<0.001 in men), although the originally reported sex-by-age interaction was not significant (p=0.11 in the original Table 4). In the re-analyzed data set with 50 subjects, this sex-by-age interaction was similar (p=0.12) but became less significant with the larger sample size (p=0.27). The Sailasuta et al paper also found a greater age-related decrease in basal ganglia Cr in men than in women (p=0.02); this sex-by-age interaction remained significant in the revised analysis with 50 subjects (p=0.04) but became non-significant with the larger sample size (p=0.12).

However, we observed a trend for age-related decline in frontal white matter Glu in men (r=−0.38, p=0.02, based on the larger sample size) but not in women (r=0.07, p=0.77), although the interaction was not significant (p=0.15). Lastly, in the re-analyzed data, the sex-by-age interaction for the basal ganglia Glu became more significant (p=0.0004, based on the larger sample size), with men showing age-related decreases in Glu (r=−0.78, p<0.0001) and women showing no change in Glu with age (r=0.11, p=0.66).

## Discussion

The re-analyzed data no longer show sex-differences in Cr or trends for sex differences in Glu in any brain regions. However, we observed higher parietal gray matter Cho and higher basal ganglia NA in men than women. The higher brain Cho in men compared to women is consistent with two prior studies. One study evaluated healthy subjects on a 1.5 Tesla scanner (7), and another study evaluated subjects on a 4 Tesla scanner (8) using conventional single-voxel PRESS. We additionally evaluated for possible confounds, differences in alcohol use or intelligence, for these sex-differences on brain Cho and NA and

found none, and our subjects were all screened and evaluated carefully to ensure they were healthy with no major medical problems or drug dependence prior to the study.

The age-dependent decreases in Glu levels remain significant in the parietal gray matter and basal ganglia regions, but Glu now shows only a trend to decrease with age in the frontal white matter. Since both the parietal and basal ganglia regions are often affected in degenerative brain diseases that are associated with aging, changes in brain glutamate may play a key role in these disorders. For example, Alzheimer's disease affects the parietal brain region while Parkinson's disease affects the basal ganglia. Prior studies of Alzheimer patients indeed documented lower glutamate levels (9,10) or glutamate transmission (11). Few studies, however, have evaluated changes in glutamate levels in the basal ganglia of Parkinson patients; one recent study of patients with Parksinon's disease using the TE-averaged PRESS technique found no abnormality in glutamate; however, the sample size was small (n=10) (12). Our findings are also consistent with the age-related decline of Glu in the motor cortex in normal aging (13).

Lastly, our re-analyzed data no longer show a significant sex-difference for the age-related decline in basal ganglia Cr, although a larger sample size of women in future studies may demonstrate this difference. However, our re-analyzed data also showed that only the men, but not women, had an age-related decline in frontal white matter and basal ganglia Glu levels. The sex-difference for the age-related decline in the basal ganglia Glu became even more significant due to the lesser variability in the re-analyzed data.

The major error in the original processing software involved lack of a proper reference signal (e.g. unsuppressed water amplitude) to calculate the metabolite concentrations. The consequence of this mistake was probably an increased variance of the (apparent) metabolite levels, and possibly the introduction of "outliers" that may have caused false-positive findings. The fact that the original (n=50) and larger cohorts (n=62) yielded similar results with the revised processing suggests that most of the differences between the original and revised findings can be attributed to the differences in processing.

In summary, our re-analyzed data no longer show sex-differences in brain glutamate levels in the four brain regions measured, but we continue to observe significant age-related declines in Glu, especially in the parietal gray matter and basal ganglia, and to a lesser degree in the frontal white matter. Further analyses confirm that the basal ganglia and frontal white matter Glu declines were predominantly due to a decline in men, but not women. These findings indicate that Glu concentrations decline markedly with age, and may be especially useful as a marker for brain diseases that are affected by aging.

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## Table 1

Sex-differences on brain metabolite concentrations (mean±S.E., % difference, p-values)

| Brain Region | Sailasuta et al* (n=50) | Re-analyzed results (n=50) | Re-analyzed results (n=62) | Revised Conclusion |
|---|---|---|---|---|
| ***Parietal gray matter*** | | | | |
| Glu | M=7.35±0.24; F =6.64±0.24 +10.7%, p=0.06 | M=7.15±0.23; F=6.75±0.22 +5.9%, p=0.25 | M=7.30±0.21; F =6.93±0.21 +5.3%, p=0.25 | Sex-difference no longer significant |
| Cr | M=6.22±0.13; F =5.95±0.16 +4.5%, p=0.05 | M=6.02±0.08; F=6.08±0.11 −1.0%, p=0.66 | M=6.08±0.08; F =6.12±0.09 −0.7%, p=0.72 | Sex-difference no longer significant |
| Cho | M=1.50±0.05; F =1.44±0.04 +4.2%, p=0.37 | M=1.59±0.04; F=1.50±0.03 +6.0%, p=0.10 | M=1.60±0.03; F =1.50±0.03 +6.7%, p=0.04 | Larger sample shows higher Cho in men than women |
| NA | M: 8.26±0.13; F: 7.98±0.25 +3.5%, p=0.17 | M=8.14±0.10; F=8.20±0.18 −0.7%, p=0.79 | M: 8.18±0.10; F: 8.17±0.15 +0.1%, p=0.97 | No longer show a trend |
| ***Basal ganglia*** | | | | |
| Cr | M=4.62±0.11; F =4.63±0.11 −0.2%, p=0.04 | M=4.57±0.11; F=4.52±0.09 +1.1%, p=0.71 | M=4.60±0.10; F =4.63±0.10 −0.6%, p=0.85 | No longer significant |
| NA | M=7.90±0.11; F =7.29±0.15 +8.4%, p=0.12 | M=7.73±0.12; F=7.08±0.14 +9.2%, p=0.001 | M=7.59±0.12; F =7.13±0.13 +6.5%, p=0.01 | Became significant (Higher NA in men than women) |
| ***Frontal gray matter*** | | | | |
| NA | M: 7.85±0.12; F: 7.49±0.16 +4.8%, p=0.40 | M=7.72±0.14; F=7.54±0.12 +2.4%, p=0.38 | M: 7.74±0.12; F: 7.43±0.13 +4.2%, p=0.10 | Larger sample shows a trend for higher NAA in men than women |
| Cr | M: 5.83±0.15; F: 5.85±0.21 −0.3%, p=0.85 | M=5.65±0.13; F=5.92±0.17 −4.6%, p=0.22 | M: 5.58±0.11; F: 5.91±0.15 −5.6%, p=0.08 | Larger sample shows trend for higher Cr in women than men |
| ***Frontal white matter*** | | | | |
| NA | M: 8.05±0.14; F: 7.92±0.19 +1.6%, p=0.17 | M=7.86±0.13; F=7.86±0.14 0%, p=1.0 | M: 7.82±0.13 F: 7.83±0.12 −0.1%, p=0.95 | No longer show a trend |
| Glu | M: 5.21±0.17; F: 5.71±0.23 −8.8%, p=0.11 | M=5.36±0.16; F=5.40±0.21 −0.7%, p=0.88 | M: 5.29±0.14 F: 5.38±0.19 −1.7%, p=0.71 | No longer show a trend |

## Table 2

Age-related changes on brain glutamate and other metabolites

| | Sailasuta et al (n=50) | Re-analyzed results (n=50) | Re-analyzed results (n=62) | Revised Conclusion |
|---|---|---|---|---|
| ***Parietal gray matter*** | | | | |
| Glu | r=−0.56, p<0.001 | r=−0.55, p<0.001 | r=−0.55, p<0.001 | Unchanged |
| ***Basal ganglia*** | | | | |
| Glu | r=−0.50, p<0.001 | r=−0.51, p<0.001 | r=−0.47, p<0.001 | Unchanged |
| Cr | r=−0.37, p<0.01 | r=−0.36, p=0.02 | r=−0.42, p=0.001 | Unchanged |
| ***Frontal White matter*** | | | | |
| Glu | r=−0.267, p=0.01 | r=−0.28, p=0.06 | r=−0.25, p=0.06 | No longer significant (trend only) |
| NAA | r=−0.35, p<.01 | r=−0.24, p=0.11 | r=−0.24, p=0.07 | No longer significant (trend only) |
| Cr | F=0.07, p=0.79* | r=0.33, p=0.03 | r=0.24, p=0.07 | Significant for age-dependent increase (only a trend with larger sample) |
| Cho | r=0.30, p=0.01 | r=0.18, p=0.22 | r=0.20, p=0.13 | No longer significant |

*F-value and p-values from ANCOVA (Table 4, original paper).

## Table 3

Sex-differences in age-related changes in brain metabolites

| Brain Region | Sailasuta et al paper (n=50) | Re-analyzed results (n=50) | Re-analyzed results (N=62) | Revised Conclusion |
|---|---|---|---|---|
| ***Parietal gray matter*** | | | | |
| Glu | M: r = −0.667, p=0.0002<br>F: r = −0.167, p=0.568<br>Interaction: p=0.11 | M: r=−0.65, p=0.0003<br>F: r=−0.17, p=0.53<br>Interaction: p=0.12 | M: r= −0.61, p=0.0001<br>F: r= −0.35, p=0.14<br>Interaction: p=0.27 | Men no longer show a trend to have steeper age-related decline than women with a larger sample size |
| ***Frontal white matter*** | | | | |
| NAA | M: r & p-values not reported<br>F: r & p-values not reported<br>Interaction: p=0.15 | M: r=−0.17, p=0.36<br>F: r=−0.47, p=0.07<br>Interaction: p=0.38 | M: r=−0.22, p=0.17<br>F: r=−0.30, p=0.21<br>Interaction: p=0.94 | No longer showing a trend |
| Glu | M: r=−0.286, p=0.125<br>F: r=−0.231, p=0.388<br>Interaction: p=0.29 | M: r=−0.40, p=0.03<br>F: r=−0.01, p=0.97<br>Interaction: p=0.32 | M: r=−0.38, p=0.02<br>F: r=0.07, p=0.77<br>Interaction: p=0.15 | Only men showed age-related decline in Glu but not women with a larger sample size |
| ***Basal ganglia*** | | | | |
| Cr | M: r & p-values not reported<br>F: r & p=not reported<br>Interaction: p=0.02 | M: r=−0.54, p=0.006<br>F: r=0.08, p=0.76<br>Interaction: p=0.04 | M: r= −0.54, p=0.001<br>F: r= −0.20, p=0.38<br>Interaction: p=0.12 | The steeper age-related decline in men compared to women is no longer significant with a larger sample size |
| Glu | M: r=−0.748, p<0.0001<br>F: r=0.036, p=0.894<br>Interaction: p=0.03 | M: r=−0.86, p<0.0001<br>F: r=0.04, p=0.89<br>Interaction: p=0.002 | M: r=−0.78, p<0.0001<br>F: r=0.11, p=0.66<br>Interaction: p=0.0004 | Only men showed age-related decline but not women (sex-difference more significant) |