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Original Article

Neuronal viability/astrocyte activity ratio in the dorsolateral prefrontal cortex as a biomarker of Alzheimer's dementia: a proton magnetic resonance spectroscopy study

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N-acetyl-aspartate (NAA) and myo-inositol (mI) are neurometabolites reflecting neuronal viability and astrocyte activity, respectively. These are quantified using proton magnetic resonance spectroscopy (^{1}H-MRS) and may be biomarkers for Alzheimer's disease dementia (AD). Our objectives were: 1) Compare dorsolateral prefrontal cortex (DLPFC) NAA and mI levels between AD and cognitively healthy control participants (HC) 2) assess if NAA/mI ratio can distinguish groups, and 3) explore the relationship between metabolites and cognition. The study included 64 participants over 55, 41 with AD. Bilateral DLPFC NAA and mI levels were quantified using 3 T ^{1}H-MRS and normalized to H_2O. NAA and NAA/mI ratio were lower in AD vs. HC. mI was unchanged. The NAA/mI ratio at a cut-off value of 1.69 showed 59% sensitivity and 87% specificity at distinguishing AD from HC. NAA was associated positively with cognition. In conclusion, DLPFC metabolite changes suggest altered mitochondrial function in AD. NAA/mI ratio shows good specificity in distinguishing AD from HC, suggesting its role in complementing other biomarkers. Future studies should evaluate NAA/mI ratio with other disease specific biomarkers.

Key words: cognition; diagnostic tests; metabolites; Myo-inositol; N-acetyl aspartate.

Introduction

Alzheimer's dementia (AD) is a neurodegenerative disorder primarily affecting older adults with an estimated global prevalence over 55 million (Shin 2022). Individuals present with progressive loss of memory and cognition. Amyloid-beta plaques and tau neurofibrillary tangles are established pathological hallmarks of AD (Serrano-Pozo et al. 2011). However, these markers are not sufficient to diagnose AD, as they may be present in individuals without cognitive impairment (Khosravi et al. 2019). They also do not provide information about neuronal viability or astrocyte function (Terry 2006; Cai et al. 2017).

Proton magnetic resonance spectroscopy (^{1}H-MRS) can assess tissue metabolites such as N-acetyl aspartate (NAA), a marker of neuronal viability synthesized from aspartate and acetyl-coenzyme A in neurons (Adalsteinsson et al. 2000; Moffett et al. 2007) or myo-inositol (mI), a carboxylic sugar implicated in astrocyte metabolism. (Brand et al. 1993; Parthasarathy et al. 2006; Alger 2009).

The amyloid precursor protein/pre-synuclein-1 (APP/PS1) mice model of AD has shown lower NAA/creatine (Cr) in the frontal cortex as compared to wild-type mice (Chen et al. 2009). ^{1}H-MRS of postmortem brain samples showed decreased NAA/Cr in the posterior cingulate cortex (PCC) in those with higher likelihood of AD as determined by the National Institute on Aging (NIA) Reagan neuropathological diagnostic criteria (Murray et al. 2014). Comparison of NAA in the PCC in early and late-stage AD showed lower NAA in late-stage participants, which was associated with greater amyloid and tau pathology (Chen et al. 2022). In contrast, mI was increased in the frontal cortex of APP/PS1 mouse models compared to wild-type mice and positively associated with amyloid pathology (Chen et al. 2022). ^{1}H-MRS of human brain samples showed that mI was increased in the PCC in late-stage AD compared to early stage or healthy controls (Voevodskaya et al. 2019). Thus, previous research involving participants with AD, postmortem samples, and AD disease models shows that NAA is decreased in AD while mI is increased.

Further, studies have utilized the NAA/mI ratio to encompass the distinct pathological processes that NAA and mI represent with one measurement. NAA/mI ratio in the PCC has been shown to distinguish healthy older adults who progressed to mild cognitive impairment (MCI) or AD within 7 yr from those who remained cognitively normal. A Receiver operating characteristic (ROC) analysis showed that the NAA/mI ratio had good

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sensitivity and specificity to detect progression to AD (Waragai et al. 2017). Presymptomatic carriers of APP or PS1 mutations had a lower NAA/mI ratio in the PCC compared to individuals without mutations (Godbolt et al. 2006). A postmortem study showed an association between likelihood of AD and the NAA/mI ratio in the posterior cingulate and inferior precuneate gyri. The NAA/mI ratio was a stronger predictor of the pathological likelihood of AD than NAA or mI alone (Kantarci et al. 2008). Thus, to summarize, the NAA/mI ratio shows promise as a biomarker of AD. However, metabolites (NAA and mI) in these studies were normalized to brain creatine, which has certain limitations. Firstly, creatine levels vary between controls and AD (creatine may be decreased in AD due to changes in brain oxidation) (Bürklen et al. 2006). Secondly, creatine-normalized values may have higher inter-individual variability (Li et al. 2003). Due to these problems with normalization to creatine, normalization to water is considered more accurate (Li et al. 2003).

Few studies have assessed metabolites in the dorsolateral prefrontal cortex, (DLPFC) a key area for higher cognitive function (Petrides 2000). Preliminary work by our group showed decreased DLPFC NAA in participants with AD compared to healthy controls (Kumar et al. 2020). Another study showed increased DLPFC NAA and decreased DLPFC mI in patients who responded positively to 26 wk of donepezil treatment (Henigsberg et al. 2011). Similarly, few studies have investigated the relationship between metabolites and cognitive function in AD. In one study, global cognition as measured by Mini Mental State Exam (MMSE), was associated positively with the NAA/mI ratio and negatively with mI, but had no correlation with NAA (Waragai et al. 2017). Another study, however, showed that parieto-occipital NAA levels were positively associated with MMSE scores (Huang et al. 2001). A third study showed that MMSE scores were associated positively with parieto-occipital NAA/mI but not with NAA or mI (Waldman and Rai 2003). Thus, there is a need to further study DLPFC metabolites and their relationship with cognition in AD.

Our study had three objectives: 1) Compare NAA and mI levels in the DLPFC between participants with AD and cognitively healthy control participants (HC); 2) Evaluate if the DLPFC NAA/mI ratio can distinguish AD from HC 3) Explore the relationship between DLPFC NAA, mI, and NAA/mI ratio with cognition. We hypothesize that 1) NAA is decreased and mI is increased in AD, and 2) DLPFC NAA/mI ratio has high sensitivity and specificity in distinguishing AD from HC.

Materials and methods

Participants in this study comprised of those enrolled in two separate studies at the Centre for Addiction and Mental Health (CAMH) in Toronto, Canada (clinicaltrials.gov # NCT02537496 and NCT01847586). The studies were approved by the Research Ethics Board at CAMH. All participants or their substitute decision makers provided informed consent prior to study procedures.

Participants

All individuals from one study (NCT02537496) were required to be age 55 or older, while individuals from the other study (NCT01847586) were required to be 65 or older. All individuals required corrected visual acuity that allowed them to read newspaper headlines and corrected hearing that allowed them to respond to a raised conversational voice. For the AD group, one study (NCT02537496) included those with a confirmed diagnosis of AD by National Institute on Aging and Alzheimer's Association (NIA-AA) core clinical criteria, and Montreal Cognitive Assessment (MoCA) score ≥ 10.The other study (NCT01847586) included those with a confirmed diagnosis of AD by National Institute of Neurological and Communicative Diseases and Stroke/Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA), and a MMSE score ≥ 16. Both studies required participants to be on a stable dose of cognitive enhancers (If prescribed), and free from other active mental health conditions or substance use disorders.

For the HC group, both studies required them to be free from any significant neurological or mental health disorder other than a simple phobia or adjustment disorder, or any current use of psychotropic or anticonvulsant medications besides occasional use for sleep.

Participants with AD had a standard workup including a detailed general and psychiatric history, physical examination, and ruling out of other causes for memory impairment through medical history, laboratory workup, and brain imaging as applicable.

Cognitive assessments

MMSE or MOCA were used to assess cognitive function. Both are scored out of 30 with a higher score indicating better cognitive performance (Folstein et al. 1983; Nasreddine et al. 2005). For the purposes of analyses in this study, MoCA scores were converted to equivalent MMSE scores using the conversion proposed by Roalf et al. (2013) The Executive Interview (EXIT-25) was used to assess executive functioning. It is scored from 0 to 50, with higher scores indicating increased executive dysfunction (Royall et al. 1992).

Magnetic resonance imaging and ^{1}H-magnetic resonance spectroscopy

We scanned participants in a 3T GE Discovery MR750 scanner equipped with an 8-channel head coil. Participants received a 3D IR-prepared T1 magnetic resonance imaging (MRI) scan (BRAVO, echo time [TE] = 3.00 ms, repetition time [TR] = 6.74 ms, inversion time [TI] = 650 ms, flip angle = 8°, Field of view = 230 mm, 256 × 256 matrix, slice thickness = 0.9 mm). The FIRST tool was used to segment the scan into gray matter, white matter, and cerebrospinal fluid (Beckmann et al. 2006).

Point-resolved spectroscopy (TE = 35 ms, TR = 2,000 ms, spectral width = 5,000 Hz, 4,096 datapoints, 128 water-suppressed, and 16 water-unsuppressed averages, 8 number of excitations) was used to quantify ^{1}H-MRS data. Shimming was performed to achieve a full width at half-maximum ≤12 Hz measured on the unsuppressed water signal from the voxel for fine-tuning the ^{1}H-MRS signal. Metabolite levels were corrected for CSF tissue fraction. The DLPFC voxel (size: 13.5 mL [3.0 × 3.0 × 1.5 cm^3]) was in anterior–posterior commissures alignment, markers were placed on the most anterior point of the frontal pole and the tip of the temporal pole. Using these markers, the posterior, and anterior limits of the DLPFC were determined as follows: the average distance from the tip of the temporal lobe to the posterior vertical boundary was 20% of ~50 to 10 mm; the average distance from the tip of the frontal pole and the anterior vertical boundary was 40% of 50 to 20 mm (Iwata et al. 2019). Figure 1 shows the placement of bilateral voxels. NAA and mI levels reported correspond to the averaged between right and left DLPFC voxels.

Metabolite values normalized to creatine are presented in supplementary tables (Supplementary table 1, Supplementary table 2).

Statistical analyses

Analyses were performed on Statistical Product and Service Solutions (version 27, International Business Machine). First, we assessed all included variables for normality using histograms

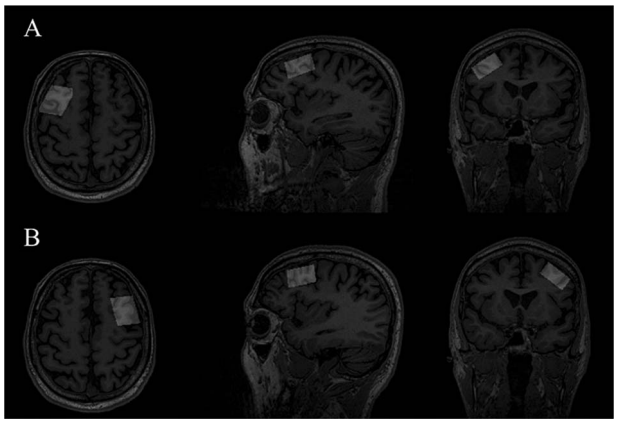

Fig. 1. Coronal, sagittal, and axial views of DLPFC voxels. A—Left DLPFC voxel. B—Right DLPFC voxel.

and calculations of skewness. Second, we used independent sample t-tests to compare demographic and clinical variables as applicable. Third, to test our main hypotheses, we used independent sample t-tests followed by analyses of covariance to compare DLPFC NAA levels, mI levels, and the NAA/mI ratio between the AD and HC groups while controlling for covariates. Fourth, we used a ROC analysis to determine the sensitivity and specificity of the NAA/mI ratio in distinguishing AD from HC using the Youden index. Fifth, we used Pearson correlations (parametric) and linear regression to assess the relationship between metabolites and global cognition (MMSE) and executive function (EXIT-25).

Results

Participants

The study included 41 participants with AD (females = 58.5%), mean (SD) age = 75.2 (7.17) years, and 23 HCs (females = 60.9%), mean (SD) age = 72.2 (7.7) years. As expected, MMSE scores were higher in HC, mean (SD) = 29.5 (0.59) as compared to the AD group, mean (SD) = 23.1 (3.2), ($t_{762} = 9.51$, $P < 0.001$), and EXIT scores were lower in the HC, mean (SD) = 4.22 (3.0) as compared to the AD group, mean (SD) = 14.27 (6.0), ($t_{62} = 4.81$, $P < 0.001$). For details of demographic and clinical characteristics, see Table 1.

Metabolite levels in AD and HC

Participants with AD had lower NAA levels in the DLPFC, mean (SD) = 11.68 (1.1) as compared to the HC, mean (SD) = 12.46 (1.38), which remained significant after controlling for age, sex, and education level ($F_{1, 63} = 4.6$ $P = 0.04$). However, there were no differences in mI levels between the two groups. Further, participants with AD had a lower NAA/mI ratio, mean (SD) = 1.69 (0.3), as compared to the HC, mean (SD) = 1.89 (0.17), which also remained significant after controlling for age, sex, and education level, F(1, 60) = 7.2, $P = 0.009$ (Table 2 and Fig. 2). We compared metabolite levels between participants taking cognitive enhancers and did not observe a significant difference in the NAA/mI ratio (Supplementary Table S3). Comparison of metabolite levels within individual trials are presented in supplementary tables (Supplementary Table S4, Table S5).

Distinguishing between AD and HC

In the ROC analyses NAA/MI ratio was able to distinguish between the AD and HC groups with an area under the curve of 0.73 (Fig. 3). Further, at a cut-off value of 1.69 (determined using Youden Index), the NAA/mI ratio showed a 59% sensitivity and 87% specificity at differentiating participants with AD from HC.

Association between metabolite levels and cognition

In the combined sample of AD and HC participants, the MMSE total score showed a positive association with NAA levels, $r = 0.47$, $n = 64$, $P < 0.001$. The association between NAA and MMSE remains significant when controlling for age, sex, years of education, and disease state (AD or HC) (B = 2.562, standard error = 0.768, 95% confidence interval = 0.725, 2.080, $P < 0.001$). Further, there was a significant interaction between the disease state and NAA levels (Supplementary Table S6). The association between NAA and MMSE also remained significant when controlling for use of cognitive enhancers (Supplementary Table S7). In contrast, in the combined sample of AD and HC participants, the EXIT score showed an inverse association with NAA levels, $r = -0.34$, $n = 64$, $P = 0.007$, which did not remain significant when controlling for age, sex, years of education, and disease state (B = −0.66, standard error = 0.676, 95% confidence interval = −2.013, 0.694, $P = 0.333$). Years of education was the only significant associate of EXIT scores in this model (B = −0.502, standard error = 0.191, 95% confidence interval = −0.885, −0.12, $P < 0.001$). There were no significant associations between mI levels and cognitive measures (Fig. 4, Supplementary Table S6). Finally, the MMSE total score showed a positive association with the NAA/mI ratio, $r = 0.312$, $n = 64$, $P = 0.012$, however this was no longer significant when controlling for age, sex, years of education, and disease state (B = 1.757, standard error = 1.292, 95% confidence interval = −0.829, 4.343, $P = 0.179$). The EXIT total score did not show a significant association with the NAA/mI ratio, $r = -0.078$, $n = 64$, $P = 0.54$ (Fig. 5). Association of metabolite levels with cognition within individual trials are presented in supplementary tables (Supplementary Table S8, Supplementary Table S9).

Discussion

This study aimed to assess DLPFC metabolites in AD and HC, and their relationship with cognition. Our results suggest that those with AD have lower NAA in the DLPFC as compared to HC after controlling for age, sex, and education level. We did not observe any differences in DLPFC mI levels between the groups. Further, those with AD had a lower NAA/mI ratio in the DLPFC as compared to HC, and the NAA/mI ratio was able to distinguish between the groups with a specificity of 87%. Finally, NAA levels were significantly associated with global cognition after controlling for relevant clinical and demographic factors with a significant interaction between metabolite levels and disease state. There were no associations between NAA and executive function, or between mI and global cognition or executive function.

The findings of decreased NAA in the DLPFC are in line with past findings of decreased NAA in AD in regions such as the frontal cortex and PCC (Chen et al. 2009; Chen et al. 2022). The differences between the DLPFC NAA and NAA/mI levels in AD and HC suggest a difference in neuronal viability in diseased vs healthy states. NAA has been postulated as a marker of neuronal health and neuronal density due to several underlying mechanisms (Schuff et al. 2006). NAA is synthesized from aspartate and acetyl-coenzyme A in neuronal mitochondria, and then transported from neurons to oligodendrocytes (Baslow 2003). Mitochondrial dysfunction is a hallmark of AD pathogenesis (Wang et al. 2020). Oligodendrocyte precursor cells also show impaired repair in Alzheimer's disease (Cai and Xiao 2016). Hence, both

Table 1. Demographic and clinical characteristics of study participants.

	Alzheimer's Disease ($n = 41$, female = 24)		Healthy ($n = 23$, female = 14)			
	Mean/n (%)	Standard Deviation	Mean/n (%)	Standard Deviation	t	*p*
Age	75.3	7.1	72.7	7.1	1.6	0.13
Age range	61–89		61–85			
Race—Caucasian	26 (61)		20 (87)			
Race—African	4 (9.8)		0			
Race—East/Southeast Asian	7 (17.1)		2 (8.7)			
Race—West Asian	1 (2.4)		1 (4.3)			
Race—Other	3 (7.3)		0			
Years of Education	13.8	3.6	15.9	2.2	−3.0	0.01
MMSE total score	23.0	3.2	29.5	0.6	−9.5	<0.001
EXIT total score	14.4	5.9	5.1	3.0	6.7	<0.001
Smoking (current) —n (%)	1 (2.4)		1 (4.3)			
Donepezil	18 (43.9)		0			
Galantamine	2 (4.8)		0			
Memantine	5 (12.2)		0			
Rivastigmine	1 (2.4)		0			
SSRIs/SNRIs	4 (9.7)		0			
Benzodiazepines	1 (2.4)		0			
Buproprion	1 (2.4)		0			
Tricyclic antidepressants	1 (2.4)		0			
Other	0		1 (4.3)			

Abbreviations: MMSE—Mini Mental State Exam, EXIT—Executive Interview, SSRIs—Selective serotonin reuptake inhibitors, SNRIs—Serotonin noradrenergic reuptake inhibitors, other (one healthy participant occasionally used trazodone for sleep).

Table 2. ^{1}H-MRS metabolite levels normalized to H_2O in AD and HC.

	Alzheimer's Disease (n = 41, female = 24)		Healthy (n = 23, female = 14)			
	Mean	Standard Deviation	Mean	Standard Deviation	t	*p*
NAA/H_2O	11.68	1.11	12.46	1.38	−2.5	0.02
mI/H_2O	7.07	1.11	6.79	1.11	−0.9	0.3
NAA/mI ratio	1.69	0.31	1.86	0.17	−2.4	0.02
MRS Full Width at Half Maximum (bilateral)	0.06	0.01	0.06	0.01	−1.2	0.24
MRS Signal to noise ratio (bilateral)	28.74	9.26	29	3.27	−0.153	0.88

Abbreviations: NAA—N-Acetyl Aspartate, mI—myo-inositol.

decreased mitochondrial generation and decreased oligodendrocyte precursor cell repair may underlie the decreased levels of NAA in AD. Secondly, human and animal studies both show that NAA synthesis is directly coupled to glucose metabolism, in the brain (Baslow 2003). Glucose hypometabolism is also seen in AD, likely as a marker of mitochondrial dysfunction. Hence, lower NAA levels in AD may be secondary to decreased mitochondrial function and decreased glucose metabolism, both of which may be associated with disease severity (Szablewski 2021).

While there were no significant differences between AD and HC in mI levels, they trended in the expected direction. The lack of significant difference in mI may be attributed to the variability in astrocytic changes depending on the stage of AD, as mI is shown to be positively associated with neuroinflammatory astrocytic activity. Course of astrocytic changes in AD remains unclear, some studies show that early stages of disease may be associated with astrocytic loss, and later stages with neuroinflammation mediated increased astrocytic activity (Verkhratsky et al. 2010). There is also evidence suggesting that neuroinflammation may precede pathological and clinical changes (Eikelenboom et al. 2010). More work is needed to understand the magnitude and direction of astrocytic changes in the DLPFC and its implication for cognition. Studies have also shown that NAA and mI are not associated with one another in AD, suggesting that the two metabolites represent distinct pathological processes (Zhu et al. 2006). Hence, it is possible that the DLPFC shows more neuronal mitochondrial dysfunction than astrocyte-mediated neuroinflammation in AD.

We found that the NAA/mI ratio shows good specificity (87%) in differentiating AD from HC. Specificity of the NAA/mI ratio increases sharply until a cut-off value of about 1.69, at which point the slope of the ROC curve flattens. An ROC analysis with just NAA does not show this same steep increase or as high an area under the curve (Supplementary Fig. S1) This cut-off value is comparable to the cut-off value of 1.67 observed in a previous study of the NAA/mI ratio in the PCC (Waragai et al. 2017). However, the ratio in our study showed lower sensitivity, as compared to the previous study (Waragai et al. 2017), suggesting regional differences. This suggests that DLPFC NAA/mI ratio may help increase specificity of AD diagnosis in conjunction with other biomarkers which are known to have a relatively high false positive rate (Khosravi et al. 2019). Specifically, our study suggests that while a good specificity might allow us to rule in a diagnosis of AD (with an NAA/mI

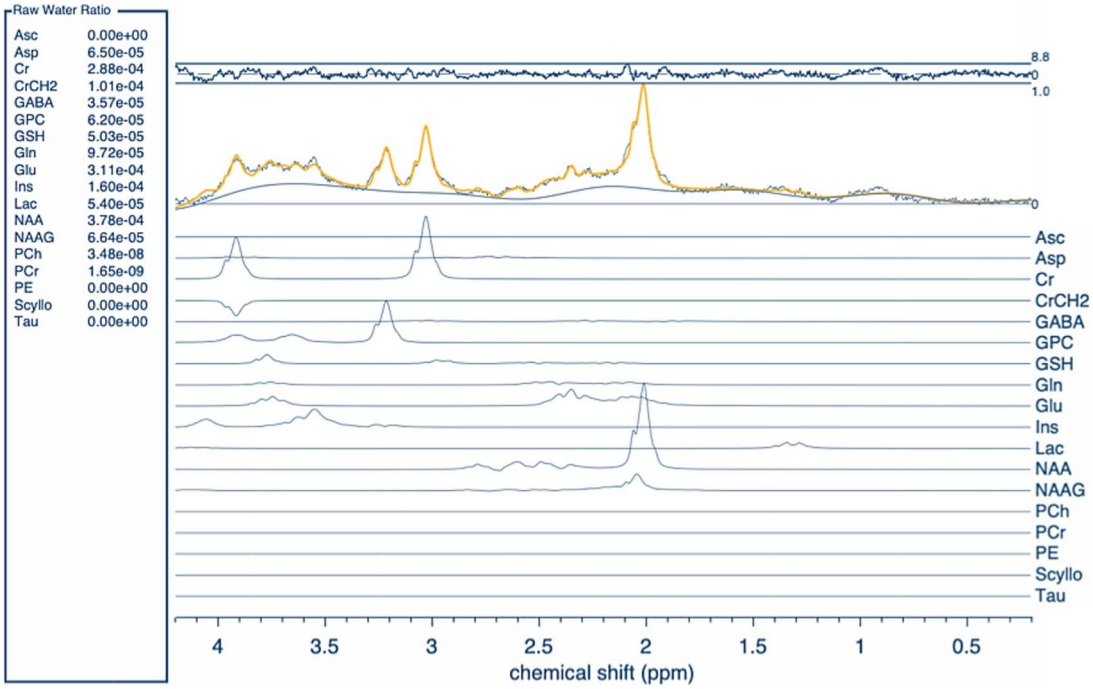

Fig. 2. Proton magnetic resonance spectroscopy (MRS) of the left DLPFC. This figure presents an MRS spectrum obtained from the left DLPFC at 3 tesla. The spectrum displays prominent metabolite peaks, including NAA at 2.0 ppm, which is a key marker of neuronal health. The highlighted line between vertical axis points 0 and 1.0 represents fit to the patient's spectral data. Individual contributions of other metabolites to the overall spectral fit are shown as separate horizontal lines. The vertical axis reflects the signal intensity, while the horizontal axis represents the chemical shift in parts per million (ppm). Data were analyzed using [Osprey version 1.4.0]. Abbreviations: Ascorbate (Asc); Aspartate (Asp); Creatine (Cr); Creatine CH2 (CrCH2); Gamma aminobutyric acid (GABA); Glycerophosphocholine (GPC); Glutathione (GSH); Glutamine (Gln); Glutamate (Glu); Myo-inositol (Ins); Lactate (Lac); N-Acetylaspartate (NAA); N-Acetylaspartylglutamate (NAAG); Phosphocholine (PCh); Phosphocreatine (PCr); Phosphoethanolamine (PE); Scyllo-inositol (Scyllo); Taurine (Tau).

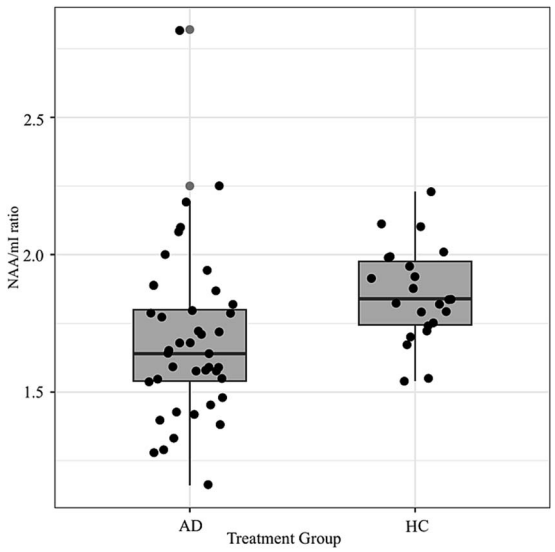

Fig. 3. NAA/mI ratio in individuals with AD vs healthy controls. NAA—N-acetyl aspartate normalized to water, mI—Myo-inositol normalized to water, AD—Alzheimer's dementia participants, HC—cognitively healthy control participants.

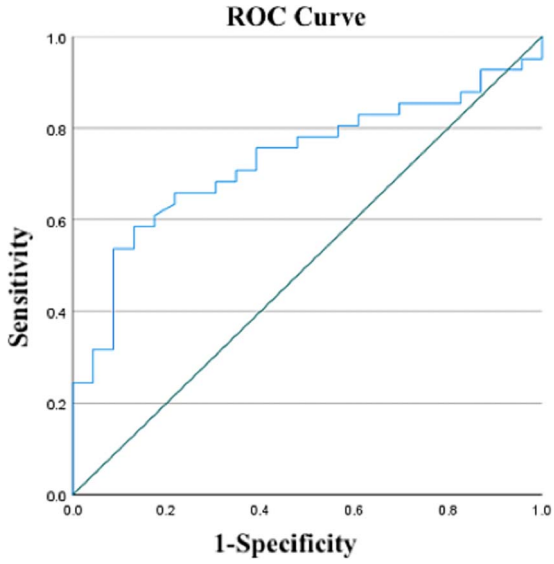

Fig. 4. ROC curve evaluating the ability of the water-corrected NAA/mI ratio in the bilateral dorsolateral prefrontal cortex to distinguish between individuals with Alzheimer's dementia and cognitively healthy controls.

ratio below 1.69) in those with a suspicion of the disease, a lower sensitivity may limit the use of the NAA/mI ratio as a screening measure for AD.

Our results showing association of NAA with global cognition are in line with previous MRS studies examining association of metabolites in the posterior cingulate cortex, occipital cortex, and parietal cortex with cognition (Huang et al. 2001). NAA has also been thought to serve as a reservoir for glutamate (a key neurotransmitter involved in neuroplasticity and learning), as it can be converted to glutamate through five energetically favorable reactions (Clark et al. 2006). The causal mechanisms underlying NAA-glutamate association remain unclear, but lower NAA levels

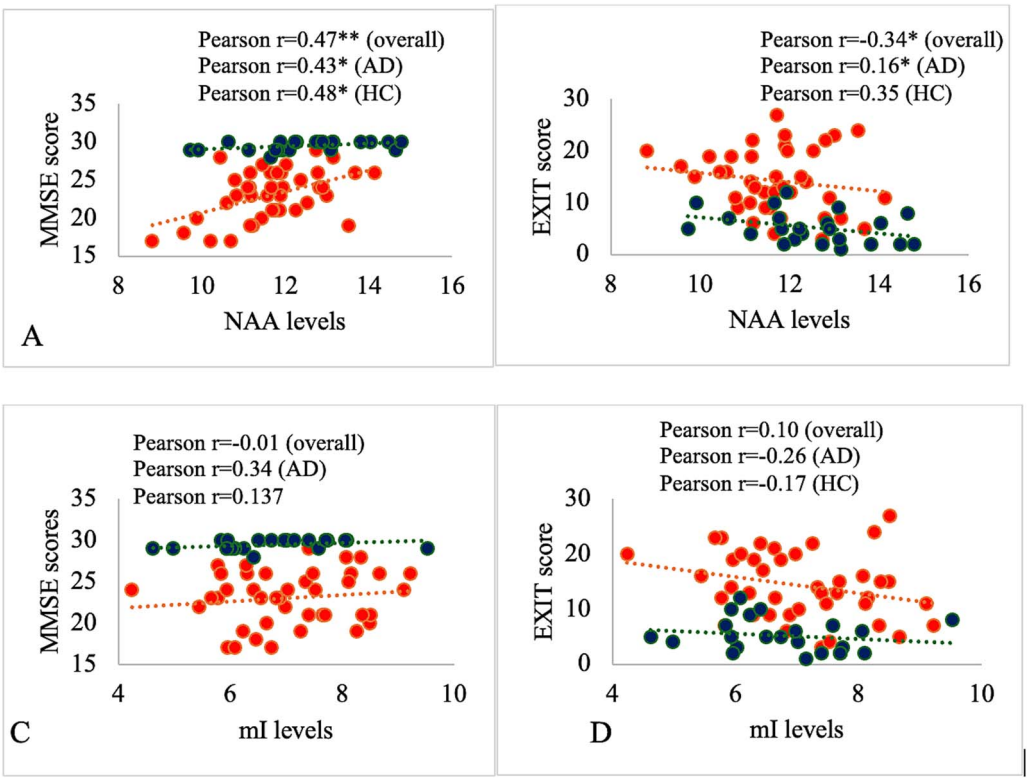

Fig. 5. Figure showing zero-order correlation between metabolite levels in DLPFC and cognitive measures. A) NAA and MMSE, B) NAA and EXIT, C) mI and MMSE, and D) mI and EXIT. * = $P < 0.01$. ** = $P < 0.001$. NAA—N-acetyl aspartate, mI—Myo-inositol, MMSE—mini mental state exam, EXIT—executive interview. All Pearson R values refer to total population. ● = Alzheimer's disease dementia, ● = cognitively healthy participants.

may indicate decreased glutamate mediated neurotransmission in AD and hence impact cognition. We observed that the association of NAA with MMSE remains significant after controlling for disease state (AD or HC) and there was a significant metabolite and disease state interaction. This implies that the relationship between NAA and global cognition is impacted by the AD. The relationship between NAA with EXIT, however, did not remain significant after controlling for disease state. Only total years of education showed an association with executive function. This could be due to lack of specificity of the EXIT abnormalities in AD, or due to a small sample size which did not allow us to detect an effect of smaller magnitude. Our post hoc power calculations suggest that to detect an effect size (Cohen's f) of 0.28 (as seen in our study) we would have required sample size of 152.

This study has several limitations, the main limitation being cross-sectional nature of our data, meaning we were not able to build longitudinal predictive models but only study the associations at one time point. Secondly, AD was diagnosed clinically, not pathologically, and existing ATN markers (amyloid beta, tau protein, and structural markers of neurodegeneration) were not assessed. Hence, we were unable to study associations between established AD pathological markers and metabolite levels. Third, while normalizing MRS metabolites to water is more accurate and current standard, creatine corrected metabolite values might be easier to use in a clinical setting. Fourth, our sample had majority Caucasian participants (71.9% of total sample) and hence the results may not be as generalizable. Lastly, this study was a secondary analysis performed on two existing clinical trials and hence was not powered to detect associations between metabolite levels and cognition.

In conclusion, findings of this study suggest that DLPFC NAA is significantly decreased in AD, without a significant change in mI, suggesting altered neuronal mitochondrial function. Further, the DLPFC NAA/mI ratio may be able to add specificity to AD diagnosis based on clinical assessment and other established markers. Finally, NAA (and not mI) is likely associated with global cognition suggesting its unique role at mild to moderate level of AD.

Future research can assess the relationship of metabolites with established neuropathological markers such as amyloid or tau to better understand the metabolic contribution to AD pathology and assess the longitudinal association between illness trajectory and metabolite levels. Future research can also assess whether the NAA/MI ratio may identify individuals with MCI that are likely to progress to AD. Future research may also consider studying motor and cognitive components of the EXIT to assess their independent relationships with metabolites. Lastly, future research can assess the relationship of DLPFC metabolites with other semantic memory tasks.

Acknowledgments

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Author contributions

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Supplementary material

Supplementary material is available at *Cerebral Cortex* online.

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