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Pro-atherogenic medical conditions are associated with widespread regional brain metabolite abnormalities in those with alcohol use disorder

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Abstract

Aims: Widespread brain metabolite abnormalities in those with alcohol use disorder (AUD) were reported in numerous studies, but the effects of the pro-atherogenic conditions of hypertension, type 2 diabetes mellitus, hepatitis C seropositivity, and hyperlipidemia on metabolite levels were not considered. These conditions were associated with brain metabolite abnormalities in those without AUD. We predicted treatment-seeking individuals with AUD and pro-atherogenic conditions (Atherogenic+) demonstrate lower regional metabolite markers of neuronal viability [N-acetylaspartate (NAA)] and cell membrane turnover/synthesis [choline-containing compounds (Cho)], compared with those with AUD without pro-atherogenic conditions (Atherogenic−) and healthy controls (CON).

Methods: Atherogenic+ (n = 59) and Atherogenic− (n = 51) and CON (n = 49) completed a 1.5 T proton magnetic resonance spectroscopic imaging study. Groups were compared on NAA, Cho, total creatine, and myoinositol in cortical gray matter (GM), white matter (WM), and select subcortical regions.

Results: Atherogenic+ had lower frontal GM and temporal WM NAA than CON. Atherogenic+ showed lower parietal GM, frontal, parietal and occipital WM and lenticular nuclei NAA level than Atherogenic− and CON. Atherogenic− showed lower frontal GM and WM NAA than CON. Atherogenic+ had lower Cho level than CON in the frontal GM, parietal WM, and thalamus. Atherogenic+ showed lower frontal WM and cerebellar vermis Cho than Atherogenic− and CON.

Conclusions: Findings suggest proatherogenic conditions in those with AUD were associated with increased compromise of neuronal integrity and cell membrane turnover/synthesis. The greater metabolite abnormalities observed in Atherogenic+ may relate to increased oxidative stress-related compromise of neuronal and glial cell structure and/or impaired arterial vasoreactivity/lumen viability.

Keywords: alcohol use disorder; N-acetylaspartate; choline-containing compounds; atherosclerosis; medical comorbidities

Introduction

Alcohol use disorder (AUD) is associated with abnormalities in brain metabolite levels, across multiple brain regions, as measured by *in vivo* proton single volume magnetic resonance spectroscopy (MRS) or magnetic resonance spectroscopic imaging (MRSI) (Meyerhoff et al. 2013). A comprehensive meta-analysis of proton MRS and MRSI studies (Kirkland et al. 2022) indicated those with current AUD, relative to a healthy reference sample, show significantly lower N-acetylaspartate (NAA) concentrations, a marker of neuronal integrity, in several dorsolateral and mesial frontal gray matter (GM) regions, frontal and parietal white matter (WM), hippocampal, and cerebellar GM. Additionally, individuals with AUD demonstrated lower choline-containing metabolites (Cho), a marker of cell membrane turnover/synthesis, in the frontal, temporal, thalamic, and cerebellar GM, and frontal and parietal WM. Decreased creatine-containing metabolite (Cr) levels, a marker of cellular bioenergetics, were observed within temporal and occipital GM, and higher levels were noted in midbrain GM. There were no significant regional differences in myo-inositol (mI) concentration, a putative osmolyte, and marker of inflammation and astrocyte density, between individuals with AUD and controls.

In those with AUD, hypertension, hepatitis C seropositivity, type 2 diabetes mellitus and hyperlipidemia are frequent biomedical comorbidities, and individuals with AUD often concurrently experience two or more of the foregoing conditions (Mertens et al. 2003; Satre et al. 2007; Durazzo et al. 2020; Padula and Durazzo 2022). Hypertension, hepatitis C seropositivity, type 2 diabetes mellitus, and hyperlipidemia are considered pro-atherogenic, as they are each linked to significantly elevated risk for atherosclerosis (Adinolfi et al. 2014; Hurtubise et al. 2016). In persons without AUD, hypertension (Chen et al. 2016), hepatitis C seropositivity (Yarlott

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et al. 2017), type 2 diabetes mellitus (Santhakumari et al. 2014), and hyperlipidemia (Sinha et al. 2014) are associated with regional brain metabolite abnormalities. The most consistent finding, for individuals (without AUD) with the above pro-atherogenic conditions, is decreased NAA/Cr ratio in multiple brain regions.

We previously compared individuals with AUD, who had comorbid pro-atherogenic conditions (Atherogenic+), AUD negative for pro-atherogenic (Atherogenic−), and a healthy normal reference group (CON) on regional cortical thickness. Relative to CON, Atherogenic+ demonstrated the most widespread and greatest magnitude of regional thinning, while Atherogenic− had reduced thickness primarily in anterior frontal and posterior parietal lobes. Atherogenic+ also had thinner cortex than Atherogenic− in the lateral orbitofrontal and dorsal/dorsolateral frontal cortex, mesial and lateral temporal, and inferior parietal regions (Durazzo et al. 2020). Additionally, over ∼7 months of sustained abstinence, we observed Atherogenic+ showed lower recovery of cortical thickness than Atherogenic− in multiple anterior frontal, inferior parietal and lateral/mesial temporal regions (Durazzo et al. 2024). Decreased perfusion and pro-inflammatory conditions are putative mechanisms promoting regional cortical thinning, and potentially metabolite abnormalities, in pro-atherogenic conditions (Savoia and Schiffrin 2007; Alosco et al. 2013; Varbo et al. 2013; Alosco et al. 2014; Hjerrild et al. 2016).

No previous brain metabolite study in those with AUD evaluated the potential effects of the comorbid pro-atherogenic conditions of hypertension, hepatitis C seropositivity, type 2 diabetes mellitus, and hyperlipidemia on regional metabolite levels. Therefore, the relationships of pro-atherogenic conditions with regional brain NAA, Cho, Cr, and mI in individuals seeking treatment for AUD is unknown.

The main goal of this study was to assess the relationship of combined effects of the pro-atherogenic conditions of hypertension, hepatitis C seropositivity, type 2 diabetes mellitus, and hyperlipidemia on regional cortical lobar and subcortical metabolites in those seeking treatment for AUD.

We hypothesized that:

1) Compared with healthy controls (CON), individuals with AUD and one or more of the pro-atherogenic conditions of active hypertension, type 2 diabetes mellitus, hepatitis C seropositivity, and/or hyperlipidemia (Atherogenic+) demonstrate significantly lower NAA and Cho concentrations in the frontal, temporal and parietal GM and WM, lenticular nuclei and thalami.
2) Relative to CON, individuals with AUD, who are negative for pro-atherogenic conditions (Atherogenic−), show lower NAA and Cho concentrations in the frontal GM and WM, but with lower magnitude differences than observed in the same regions for Atherogenic+ versus CON.

Materials and methods

Participants

Individuals with AUD (n = 110) were recruited from the San Francisco VA Medical Center (SFVAMC) Substance Abuse Day Hospital and the San Francisco Kaiser Permanente (KP) Chemical Dependence treatment outpatient programs. All individuals with AUD were actively in treatment at the time of study, and treatment duration was typically 14–35 days [for treatment program details see (Durazzo et al. 2008)]. Forty-nine individuals with AUD completed a SFVAMC-sponsored 14–21 day residential treatment program prior to entering the San Francisco VA outpatient program. CON (n = 49) were recruited from the local community. Participants were between 28 and 71 years of age and provided written informed consent prior to engaging in study procedures. SFVAMC and University of California San Francisco approved all procedures, which were in accordance with the Declaration of Helsinki. All participants in this study were included in our previous study on atherogenic conditions in AUD and cortical thickness (Durazzo et al. 2020). All data were collected from 2001 to 2012.

Inclusion/exclusion criteria

Inclusion criteria for the AUD participants were DSM-IV diagnosis of alcohol dependence or abuse (all met criteria for dependence), average consumption of >150 standard alcohol-containing drinks (i.e.14 g of pure ethanol) per month for at least 8 years prior to enrollment for males, and average consumption of >80 drinks per month for at least 6 years prior to enrollment for females. See Table 1 for group demographic and clinical data AUD participant exclusion criteria were history of the following: intravenous drug use in the 5 years prior to study enrollment, dependence on any substance except nicotine in the 5 years immediately prior to study, opioid replacement therapy, intrinsic cerebral masses, HIV seropositivity, cerebrovascular accident, arteriovenous and cavernous malformations, myocardial infarction, cerebral aneurysm, chronic obstructive pulmonary disease (COPD), type 1 diabetes, non-alcohol related seizures, significant exposure to established neurotoxins, Wernicke–Korsakoff syndrome, current delirium, demyelinating, and neurodegenerative diseases, any penetrating head injury or head injury resulting in loss of consciousness >10 minutes. Psychiatric exclusion criteria were history of cyclothymia, bipolar disorder, schizophrenia-spectrum disorders, obsessive-compulsive disorder, panic disorder, and post-traumatic stress disorder. Depressive disorders (i.e. major depression, substance-induced mood disorder) were permitted, given their high co-occurrence in AUD (Grant et al. 2015; Durazzo and Meyerhoff 2017; Nguyen et al. 2020). AUD never-smokers were lifetime never/non-smokers (i.e. never smoked cigarettes/tobacco products or consumed <40 cigarettes over lifetime, with no tobacco use within 10 years of study). All CON were never-smokers with no history of any biomedical and psychiatric conditions known or suspected to influence brain neurobiology and neurocognition, including hypertension, type 2 diabetes mellitus, hepatitis C seropositivity, or hyperlipidemia. All participants were breathalyzed, and urine was tested for illicit substances before assessment and no participant tested positive for alcohol or any typical illicit substances. Participants were also randomly tested for illicit/non-prescribed substances and alcohol at the SFVAMC and KP treatment programs; no participant tested positive for alcohol or substances at either program prior to, or during, study participation.

Clinical assessment

All participants completed the Clinical Interview for DSM-IV Axis I Disorders, Version 2.0 (SCID-I/P) and semi-structured interviews for assessment of lifetime alcohol consumption

Table 1. Group demographics and clinical variables.

Measure	CON n = 49	Athero− n = 51	Athero+ n = 59	Group comparisons*
Age (SD) [years]	48 (9)	48 (10)	53 (9)	CON, Athero−, < Athero+
Education (SD) [years]	16 (3)	14 (2)	14 (2)	CON > Athero−, Athero+
Males [%]	94	98	92	
White [%]	78	80	70	
Days abstinent prior to study (SD)	NA	14 (13)	17 (13)	
1-year average drinks/month (SD)	19 (21)	372 (219)	401 (220)	CON < Athero−, Athero+
Lifetime average drinks/month	19 (16)	204 (99)	243 (159)	CON < Athero−, Athero+
Body mass index	26 (4)	25 (4)	28 (5)	CON, Athero− < Athero+
Systolic blood pressure	NA	121 (6)	127 (9)	Athero− < Athero+
Diastolic blood pressure	NA	80 (4)	85 (6)	Athero− < Athero+
Mean arterial pressure	NA	94 (5)	99 (7)	Athero− < Athero+
Hypertension [%]	0	0	54	
Hepatitis C seropositivity [%]	0	0	34	
Hyperlipidemia [%]	0	0	19	
Type 2 diabetes [%]	0	0	5	
Two or more concurrent atherogenic conditions [%]	0	0	22	
Antihypertensive medication use [%]:	0	0	48	
• Diuretic	0	0	60	
• Beta-blocker			25	
• Other			15	
• Two or more medications			37	
Statin medication use [%]	0	0	12	
Never-smoker [%]	100	31	27	
Former-smoker [%]	0	12	10	
Active-smoker [%]	0	57	63	
FTND	NA	5 (2)	5 (2)	
Pack years	NA	25 (18)	28 (18)	
Gamma glutamyltransferase [median; i.u.]	21	36	69	CON, Athero− < Athero+
Aspartate aminotransferase [median; i.u.]	25	29	31	
Alanine aminotransferase [median; i.u.]	23	31	33	
Prealbumin (mg/dl)	30 (5)	28 (7)	26 (8)	
Beck Depression Inventory	5 (4)	14 (10)	14 (10)	CON < Athero−, Athero+
STAI	34 (8)	48 (11)	45 (11)	CON < Athero−, Athero+
Any psychiatric comorbidity [%]	NA	40	41	
Mood disorders [%]	NA	38	40	
Substance use disorder comorbidity [%]	NA	28	29	
Antidepressant use [%]	NA	13	5	

Note. Mean (standard deviation), unless otherwise noted. Athero-: Atherogenic -; Athero+: Atherogenic +; CON: non-smoking light drinking controls. FTND: Fagerstrom Test for Nicotine Dependence; NA: not available/applicable; STAI: State -trait Anxiety Inventory – Trait. *All listed group comparisons $P < .05$. Mean (SD). Gamma glutamyltransferase, local normal range 7–64 institutional units (i.u.); aspartate aminotransferase, local normal range 5–35 i.u.; alanine aminotransferase, local normal range 7–56 i.u.; prealbumin local normal range 18–45 mg/dl.

(Lifetime Drinking History, LDH) and substance use (structured, in-house questionnaire assessing substance type, and quantity and frequency of use). Average number of alcohol-containing drinks/month over 1 year prior to enrollment and average number of drinks/month over lifetime were calculated from the LDH. Participants also completed self-report questionnaires assessing anxiety (State–Trait Anxiety Inventory, Trait form Y-2, STAI) and depressive symptomatology (Beck Depression Inventory), as well as nicotine dependence by the Fagerstrom Tolerance Test for Nicotine Dependence (FTND). See (Pennington et al. 2013) for corresponding references to the above measures.

AUD participants who had medical record-verified pro-atherogenic conditions of type 2 diabetes mellitus, hypertension, hyperlipidemia, and/or hepatitis C virus antigen seropositivity were assigned to the Atherogenic+ group (n = 59) and those without the above conditions were assigned to the Atherogenic− group (n = 51). AUD participants seropositive for hepatitis C were not prescribed medications to manage active symptomatology at the time of the study. One AUD participant was taking a sulfonylurea medication (glipizide) for type 2 diabetes. No participant with AUD was seropositive for hepatitis B. No participant with AUD had other biomedical conditions associated with atherosclerosis or other vascular diseases. Participants with AUD were considered positive for substance use disorder comorbidity if DSM-IV criteria were met for current or lifetime substance abuse or past dependence (>5 years prior to enrollment); most participants with AUD with a comorbid substance use disorder met criteria for past methamphetamine or cocaine abuse/dependence. Participants with AUD were considered positive for a psychiatric comorbidity if current or lifetime criteria for a unipolar mood or anxiety disorder were met, and most met criteria for recurrent major depressive disorder. Seated blood pressure was obtained via automated sphygmomanometer, within 2 days of magnetic resonance scan for all AUD and mean arterial

pressure ([(2*diastolic BP) + (systolic BP)]/3) was also calculated (Zahr et al. 2013).

Magnetic resonance acquisition and processing

All brain structural magnetic resonance imaging (MRI) data was acquired on a standard 1.5-T Siemens Vision platform (Siemens Medical Systems, Inc., Iselin, NJ). A volumetric magnetization-prepared rapid gradient echo was acquired with TR/TE/TI = 9.7/4/300 ms, 15° flip angle, 1×1 mm^2 in-plane resolution, and 1.5-mm-thick coronal partitions. See Gazdzinski and colleagues' study (Gazdzinski et al. 2005) for detailed structural MRI acquisition methods. MRI was followed by automated head shimming and a multislice ^{1}H magnetic resonance spectroscopic imaging (MRSI) sequence with TR/TI/TE of 1800/165/25 ms, three imaging slices, each slice 15 mm thick with a interslice gap of ~ 6 mm, a nominal in-plane resolution of 8 × 8 mm^2 (yielding a 1-ml nominal spectroscopic imaging voxel), and circular k-space sampling. Spectroscopic imaging (SI) voxels had an estimated effective spatial resolution of ~1.5 ml. The spectroscopic imaging slices were angulated parallel to the double spin-echo slices, primarily covering the major cerebral lobes, subcortical nuclei, midbrain, and cerebellar vermis. Multislice 1H MRSI data processing, fully detailed in Soher et al. 2000 and Meyerhoff et al. 2004, were employed to obtain metabolite concentrations for each SI voxel expressed in institutional units, hereafter referred to as concentrations (institutional units). In summary, metabolite concentrations for GM and WM, in each region of interest (ROI) identified on the MRI dataset, were calculated by segmenting into ROIs and tissue types [GM, WM and cerebrospinal fluid (CSF)]. ROIs were spatially co-registered to the ^{1}H MRSI dataset and reduced to MRSI resolution, taking into account the MRSI point-spread function, chemical-shift displacement, and slice profile (Schuff et al. 2001). This procedure enabled computation of the tissue composition in each voxel of the spatially registered SI acquisition volume and the metabolite concentration in each SI voxel. These concentrations were atrophy-corrected by using cerebrospinal fluid contribution and then averaged over all SI voxels from the specific ROI. Metabolite concentrations of NAA, Cho, Cr, and mI were calculated for the frontal, parietal, temporal GM and WM, occipital WM, and thalamus, lenticular nuclei, midbrain, and cerebellar vermis. Results from all major processing steps were visually inspected to ensure proper software performance and satisfactory data quality, as previously described (Meyerhoff et al. 2004). MRI/MRSI was typically acquired within 2 days of completion of the clinical assessments.

Statistical analyses

Participant characteristics

Comparisons between AUD subgroups and CON on clinical and demographic variables were conducted with univariate analysis of variance, chi-square, and Fisher's exact test, where appropriate. *P*-values <.05 were considered statistically significant for these comparisons.

Comparisons between Atherogenic+, Atherogenic−, and CON on regional brain metabolites

Comparisons between Atherogenic+, Atherogenic−, and CON were conducted with generalized linear modeling for each of the 11 regions of interest. In preliminary analyses, the findings for group comparisons were highly congruent for left and right hemisphere regions of interest, therefore, the concentration values for each of the 11 regions are the arithmetic average of the left and right hemispheres. In these comparisons, group (Atherogenic−, Atherogenic+, CON), was the between subjects factor; age and body mass index (BMI) served as continuous covariates, given their inverse relationships with the brain metabolite concentrations measured in this study (Chang et al. 2009; Gazdzinski et al. 2010). All group effects were followed-up with pairwise t-tests (two-tailed) to test our a priori predictions. In pairwise comparisons between Atherogenic− and Atherogenic+, in addition to age and BMI, alcohol consumption variables (lifetime average number of drinks/month or 1-year-average drinks/month) and smoking status (never, former, and active smoker), were also separately considered as covariates. A modified Bonferroni procedure (Sankoh et al. 1997) was used to control for multiplicity of pairwise t-tests. Pairwise t-test *P*-values for each metabolite were adjusted to account for the number of brain regions (i.e. 11) and the average Spearman inter-correlation of each specific metabolite (NAA: r = .47; Cho: r = .54; Cr: r = .55; mI: r = .52) for all participants across regions. The resulting adjusted alpha levels for pairwise t-tests were $P \leq .026$ for NAA, $P \leq .029$ for Cho, $P \leq .029$ for Cr and $P \leq .028$ for mI.

Associations between mean arterial blood pressure and regional metabolite levels in Atherogenic+ and Atherogenic− groups

Systolic and mean arterial blood pressure (BP) were log10 transformed to address their skewed distributions, which resulted in acceptably symmetrical distributions. Associations of systolic and mean arterial BP and regional metabolites were evaluated with multiple linear regression individually for the Atherogenic+ and Atherogenic−, as well as in the combined group. In the combined sample, group (Atherogenic+, Atherogenic−), age and BMI served as covariates. In the individual group assessments, age and BMI served as covariates. Associations (partial coefficients reported) were considered statistically significant at $P \leq .01$.

Results

Participant characteristics

See Table 1 for group comparisons on demographic and clinical variables. Among the 22% of Atherogenic+ who had at least two pro-atherogenic conditions, 80% had concurrent hypertension and hepatitis C seropositivity. AUD active smokers had higher systolic, diastolic and mean arterial pressures than AUD never smokers (all $P < .05$), after adjustment for age and antihypertensive use.

Regional metabolite comparisons between Atherogenic− and Atherogenic+ and CON

N-acetylaspartate

Groups (Atherogenic−, Atherogenic+, and CON) were significantly different [all group effect $P < .05$] on NAA concentration in the following regions: frontal and parietal GM, frontal, parietal, temporal and occipital WM, thalami and lenticular nuclei. Higher lifetime average drinks/month was related to lower frontal and parietal WM NAA (both $P < .03$). Pairwise comparisons indicated Atherogenic+ had significantly lower NAA than CON in all the foregoing regions and lower NAA levels than Atherogenic− in the frontal WM, parietal GM

Table 2. Significant regional metabolite (institutional units) differences between CON, Atherogenic−, and Atherogenic+.

Measure	CON n = 49	Atherogenic− n = 51	Atherogenic+ n = 59	Effect Size (Cohen's *d*)		
				Atherogenic− vs. CON	Atherogenic+ vs. CON	Atherogenic- vs. Atherogenic+
Frontal gray matter NAA	33.1 (2.7)	31.7 (2.73)	30.6 (2.8)	0.51*	0.92*	0.42
Frontal gray matter Cho	6.2 (.76)	6.1 (.8)	5.8 (.8)	0.08	0.44**	0.36
Frontal white matter NAA	31.1 (3.1)	29.3 (3.1)	27.6 (3.2)	0.60*	1.13*	0.53*
Frontal white matter Cho	6.0 (.9)	5.8 (.9)	5.4 (.9)	0.22	0.68**	0.46**
Parietal gray matter NAA	33.2 (3.2)	32.6 (3.3)	31.1 (3.4)	0.18	0.62*	0.45*
Parietal white matter NAA	29.8 (3.1)	28.9 (3.2)	26.9 (3.4)	0.30	0.88*	0.59*
Parietal white matter Cho	5.3 (.9)	5.1 (.9)	4.8 (1.0)	0.20	0.61**	0.41
Temporal white matter NAA	27.1 (5.4)	25.9 (5.6)	24.3 (5.8)	0.22	0.49*	0.28
Occipital white matter NAA	30.8 (3.7)	30.7 (3.9)	28.9 (3.8)	0.03	0.50*	0.47*
Cerebellar vermis Cho	8.8 (1.3)	8.9 (1.3)	8.2 (1.4)	0.06	047**	0.52**
Thalamus NAA	34.5 (4.2)	33.6 (4.2)	32.5 (4.3)	0.21	0.46*	0.25
Thalamus Cho	6.6 (1.0)	6.5 (1.0)	6.2 (1.0)	0.09	045**	0.36
Lenticular NAA	29.81 (3.62)	28.96 (3.49)	27.21 (3.57)	0.24	0.74*	0.50*

Note. Mean (standard deviation) *$P \leq .026$ was statistically significant for NAA for pairwise t-tests (two-tailed). **$P \leq .029$ was statistically significant for Cho for pairwise t-tests (two-tailed).

and WM, occipital WM and lenticular nuclei. Atherogenic- only showed lower NAA than CON in the frontal GM and WM (see Table 2). Effect sizes (Cohen's d) were consistently larger in Atherogenic+ than Atherogenic- in comparisons to CON. Higher BMI was related to significantly lower NAA in all regions (all $P < .04$) except the temporal WM. There were no significant regional NAA differences between AUD current, former, and never-smokers after control for multiple comparisons.

Choline-containing compounds

Groups (Atherogenic−, Atherogenic+, and CON) were significantly different [all group effect $P < .05$] on Cho concentration in the following regions: frontal GM, frontal WM and parietal WM, cerebellum, and thalami. In pairwise comparisons, Atherogenic+ had significantly lower Cho level than CON in all the foregoing regions and lower Cho than Atherogenic− in the frontal WM and cerebellum. There were no significant differences in Cho level between Atherogenic− and CON in any region (see Table 2). Lifetime average or 1-year average drinks/month were not significant predictors of Cho in any region. Higher BMI was associated with lower choline in all regions (all $P < .02$) except the occipital WM and parietal GM. There were no significant regional Cho differences between AUD current, former and never-smokers after control for multiple comparisons.

Total creatine and myoinositol

No significant differences were observed between Atherogenic+, Atherogenic−, and CON on Cr and mI in any region.

Associations between regional metabolite levels and blood pressure variables in Atherogenic+ and Atherogenic− groups

There were no significant associations between systolic and mean arterial blood pressures and NAA, Cho, Cr, and mI levels in any region in the combined group of Atherogenic+ and Atherogenic- or individually. In the Atherogenic+ group, there were trends for higher systolic BP and lower frontal GM NAA ($r = -.31$, $P = .026$), frontal GM Cr ($r = -.31$, $P = .024$), frontal WM Cr ($r = -.34$, $P = .012$), and cerebellar vermis Cr ($r = -.33$, $P = .019$).

Discussion

In this study, Atherogenic+, compared with CON, demonstrated lower NAA concentrations in all regions assessed except the temporal GM and midbrain, with the largest magnitude differences in the frontal GM and WM; Atherogenic− only had lower NAA than CON in the frontal GM and WM. The pattern of lower NAA in the frontal lobes in both groups with AUD (i.e. Atherogenic+ and Atherogenic−) was congruent with previous studies demonstrating that AUD is associated with significantly decreased NAA in the frontal GM and WM (Kirkland et al. 2022); however, the greater number of regions that Atherogenic+ showed significantly lower NAA, and the moderate-to-large magnitude NAA differences compared with both CON and Atherogenic−, indicated a more widespread, and greater compromise, of neuronal integrity in cortical GM, WM and subcortical tissue of Atherogenic+. Similarly, the significantly lower Cho in Atherogenic+, compared with CON in the frontal GM, frontal WM and parietal WM, cerebellum, and thalami suggest greater abnormal cell membrane turnover/synthesis in these regions; Atherogenic− and CON were not significantly different on Cho in any region assessed. The decreased NAA in Atherogenic+ relative to both Atherogenic− and CON aligns with previous research, in those without AUD, indicating the pro-atherogenic conditions experienced by the Atherogenic+ group are associated with compromised neuronal integrity in multiple brain regions. Notably, the NAA and Cho concentration differences in direct comparisons between Atherogenic+ and Atherogenic− were not influenced by alcohol consumption variables or cigarette smoking status. Hypertension and hepatitis C seropositivity were the most frequent conditions in Atherogenic+ and likely made the largest contribution to the significantly decreased regional NAA and Cho observed relative to CON and Atherogenic−. The pattern of results from the current study are congruent with our cross-sectional cortical thickness findings for Atherogenic+, Atherogenic− and

CON, where Atherogenic+ showed thinning in the greatest number of cortical regions, as well as the highest magnitude thinning, compared with CON (Durazzo et al. 2020).

Pro-atherogenic conditions are strongly linked to alterations of arterial lumen viability and autoregulation, which, in turn, is related to compromised cerebral perfusion (Chen et al. 2016; Hurtubise et al. 2016). Decreased perfusion is associated with brain structural abnormalities in those with hypertension and other cardiovascular diseases (Alosco et al. 2013; Alosco et al. 2014), and may be a mechanism contributing to the widespread NAA and Cho reductions observed in Atherogenic+ participants (Sahin et al. 2008; García Santos et al. 2010; Chen et al. 2016). Tissue of frontal and mesial and lateral temporal lobes appear to be highly vulnerable to decreased perfusion secondary to compromised cerebrovascular functioning [see (Payabvash et al. 2011; Durazzo et al. 2015; Durazzo et al. 2020) and references therein]. Additionally, hypertension, hepatitis C seropositivity, type 2 diabetes mellitus, and hyperlipidemia are proinflammatory; proinflammatory conditions in the cerebrovascular system may promote altered arterial integrity and function through atherosclerotic-related stenosis and/or altered hemodynamic regulation (Savoia and Schiffrin 2007; Varbo et al. 2013; Hjerrild et al. 2016) or directly compromise neuronal and glial tissue via inflammatory mechanisms (Sahin et al. 2008; Chang et al. 2013; Wu et al. 2017). Similarly, chronic, high levels of alcohol consumption and active cigarette smoking create proinflammatory conditions (likely via amplified oxidative stress) in multiple organs, including the cerebrovascular system and/or brain parenchyma (Crews et al. 2006; Durazzo et al. 2014; Gonzalez-Reimers et al. 2014). Although higher average alcohol consumption/month over lifetime was only related to lower frontal and parietal WM NAA in participants with AUD, the long-term and heavy alcohol consumption, and active cigarette smoking (63%) combined with the other proinflammatory conditions in Atherogenic+, may promote increased global brain oxidative stress burden; amplified oxidative stress in Atherogenic+, relative to Atherogenic−, may have contributed to the greater widespread compromised neuronal integrity and altered cell membrane turnover/synthesis observed in Atherogenic+.

This study has limitations that may effect the generalizability of the findings. The participants with AUD were predominantly comprised of US Armed Services Veterans. The date of onset or illness duration of the pro-atherogenic conditions for the majority of participants in the Atherogenic+ group could not be reliably obtained. Fasting glucose and serum lipids, and hepatitis C RNA viral load were not collected near time of study in this sample. Illness duration and quantitative measures of hepatitis RNA viral load, glucose and serum lipid levels may be associated with regional metabolite concentrations in the Atherogenic+ group. Accurate quantitation of the above-mentioned disease measures may provide clinically relevant information on the association between disease burden and regional metabolite levels in Atherogenic+. Examination of sex effects was precluded by the small number of female participants in this predominately Veteran sample. Blood pressure was obtained once prior to magnetic resonance scanning; at least two blood pressure measurements on two or more separate occasions is the standard for accurate quantitation (Muntner et al. 2019). Given the sample size of Atherogenic+, we could not assess for the potential individual contributions of hypertension, hepatitis C seropositivity, type 2 diabetes and hyperlipidemia on regional metabolite levels. Moreover, premorbid factors (e.g. genetic risk or resiliency factors) and comorbid factors (e.g. diet/nutrition, exercise, and subclinical hepatic, pulmonary, cardiac, or cerebrovascular dysfunction) that were not assessed in this study may have influenced the regional metabolite concentrations of the participants with AUD.

The regional metabolite findings for the frontal GM and WM in the individuals with AUD of this study were consistent with previous research; however, our novel results for the Atherogenic+ group suggested that pro-atherogenic conditions in AUD may be independently associated with widespread compromised brain neuronal integrity and cell membrane turnover/synthesis. These results, in conjunction with our cross-sectional (Durazzo et al. 2020) and longitudinal (Durazzo et al. 2024) cortical thickness findings for Atherogenic+, suggest that pro-atherogenic conditions in those with AUD are linked to abnormalities in cellular cytoarchitecture, neuronal integrity, and cell membrane turnover/synthesis across cortical nodes of multiple brain circuits. Larger-scale studies are required to assess the individual associations of hypertension, hepatitis C, diabetes, and hyperlipidemia with the regional metabolites quantitated in this study. The results from this study, combined with our previous findings for cortical thickness, provide additional evidence that common comorbid biomedical conditions, in those seeking treatment for AUD, are associated with adverse effects on brain neurobiology, and potentially, neurobiological recovery with abstinence.

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Author contributions

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Data availability

The data in this article are subject to a 12-month embargo from the publication date and will be subsequently shared upon reasonable request to the corresponding author.

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