

## SYSTEMATIC REVIEW



# Brain metabolite alterations related to alcohol use: a meta-analysis of proton magnetic resonance spectroscopy studies

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Alcohol misuse and alcohol use disorder (AUD) have neurobiological consequences. This meta-analysis of proton magnetic resonance spectroscopy (MRS) studies aimed to assess the differences in brain metabolite levels in alcohol misuse and AUD relative to controls (PROSPERO registration: CRD42020209890). Hedge's *g* with random-effects modeling was used. Sub-group and meta-regression techniques explored potential sources of demographic and MRS parameter heterogeneity. A comprehensive literature review identified 43 studies, resulting in 69 models across gray and white matter (GM, WM). Lower N-acetylaspartate levels were found in frontal, anterior cingulate cortex (ACC), hippocampal, and cerebellar GM, and frontal and parietal WM, suggesting decreased neuronal and axonal viability. Lower choline-containing metabolite levels (all metabolites contributing to choline peak) were found in frontal, temporal, thalamic, and cerebellar GM, and frontal and parietal WM, suggesting membrane alterations related to alcohol misuse. Lower creatine-containing metabolite levels (Cr; all metabolites contributing to Cr peak) were found in temporal and occipital cortical GM, while higher levels were noted in midbrain/brainstem GM; this finding may have implications for using Cr as an internal reference. The lack of significant group differences in glutamate-related levels is possibly related to biological and methodological complexities. The few studies reporting on GABA found lower levels restricted to the ACC. Confounding variables were age, abstinence duration, treatment status, and MRS parameters (echo time, quantification type, data quality). This first meta-analysis of proton MRS studies consolidates the numerous individual studies to identify neurometabolite alterations within alcohol misuse and AUD. Future studies can leverage this new formalized information to investigate treatments that might effectively target the observed disturbances.

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## INTRODUCTION

There is a clear connection between alcohol misuse, such as binge drinking ( $\geq 5$  drinks for males,  $\geq 4$  drinks for females on an occasion) and heavy drinking (binge drinking  $\geq 5$  days/week), and an increased risk of developing alcohol use disorder (AUD) [1, 2]. In fact,  $\sim 40\%$  of alcohol consumers endorse heavy episodic drinking [3], and one-third of adults in the US meet lifetime criteria for AUD [4, 5]. The personal and societal costs of alcohol misuse and AUD are high [3], yet efforts to prevent or treat these behaviors have only been modestly effective [5–8], necessitating improvement in current treatments and development of more efficacious treatments.

One step in the treatment development process is to investigate the neurobiological consequences of alcohol misuse and AUD. Chronic alcohol use is associated with complex, maladaptive neurobiological changes that contribute to the escalation and continuation of use [5, 9, 10]. The neural effects of alcohol use in humans can be measured through proton magnetic resonance spectroscopy (MRS) [11], a non-invasive in vivo imaging technique that quantifies the chemical composition of tissues ('metabolites') in localized regions. MRS can routinely detect relatively concentrated neurometabolites, including glutamate (Glu), glutamine

(Gln), gamma-aminobutyric acid (GABA), N-acetylaspartate (NAA), choline-containing metabolites (Cho), creatine-containing metabolites (Cr), and *myo*-inositol (ml). Of note, the MRS signal is not able to be separated into distinct choline metabolites (phosphorylcholine, glycerophosphorylcholine, free choline, or acetylcholine) or creatine metabolites (creatine and phosphocreatine), thus we use the term "choline-containing metabolites" or "creatine-containing metabolites" to refer to any metabolite that will contribute to the choline or creatine peaks, respectively. See Supplementary Table 1 for an overview of these MRS metabolites and their relation to alcohol use ([11–16] for comprehensive reviews). Derived neurometabolite information can be leveraged when studying alcohol misuse and AUD to assist in characterizing neurometabolic alterations and informing development of treatment options that can specifically target detected abnormalities. Future work can then be dedicated to understanding if or how such changes in neurometabolite levels may correspond with treatment outcomes.

To our knowledge, this is the first published meta-analysis summarizing the extant literature on brain metabolite alterations associated with human alcohol use, relative to controls, measured with in vivo proton MRS. Broad inclusion criteria captured studies

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of alcohol use and AUD to comprehensively cover relevant populations (i.e., binge drinkers, treatment-seeking and treatment-naïve AUD individuals), brain regions, and neurometabolites. Sub-group analyses and meta-regressions explored potential sources of heterogeneity, including population demographics (abstinence duration, age, sex, and treatment-seeking status) and MRS parameters (echo time (TE), absolute versus relative quantification, and data quality metrics).

## METHODS

### Search strategy and study eligibility

This meta-analysis followed the Preferred Reporting Items for Systematic Reviews and Meta-Analysis guidelines [17]. The protocol was pre-registered with PROSPERO (protocol number: CRD42020209890).

Relevant extant literature assessing the differences in brain metabolite levels between alcohol misuse/AUD groups relative to controls was identified by systematically searching PubMed, PsychINFO, Scopus, Google Scholar, CINHALL, and TRIP using the terms: “magnetic resonance spectroscopy” or “MRS” and “alcoholism”, “alcohol abuse”, “alcohol dependence”, “AUDs”, “polysubstance use” (See Supplementary Materials). Inclusion criteria were as follows: (1) use of in vivo proton MRS; (2) alcohol-using group defined by DSM, ICD, or other study-specific criteria; (3) healthy control group defined by study-specific criteria (note: some control groups did consume alcohol, but at a lower quantity/frequency with no history of AUD. See Supplementary Table 3a for more details); (4) direct comparison between the alcohol-using group and control group; (5) appropriate data to complete meta-analysis; and (6) published in English. Exclusion criteria were: (1) carbon or phosphorus MRS, preclinical NMR imaging; (2) other human neuroimaging technique (e.g., fMRI); (3) metabolite measures from body fluids (e.g., CSF, blood); and (4) comorbid psychiatric disorder or other illness (e.g., HIV, bipolar disorder, liver disease, Fetal Alcohol Syndrome) as the main population of interest. Studies were not excluded based on year published, age of participants, or metabolite quantification method (i.e., absolute metabolite concentration and metabolite resonance intensity relative to water or Cr). Systematic searches were conducted by one author (BDB) on May 17, 2021. All identified studies were then reviewed for potential inclusion by two reviewers (BDB and AEK) using Covidence Systematic Software (Veritas Health Innovation, Melbourne, Australia. Available at [www.covidence.org](http://www.covidence.org); Supplementary Materials). Inter-rater reliability was high for title/abstracts (96% agreement, Cohen’s kappa  $k=0.836$ ) and full-text screening (92% agreement,  $k=0.625$ ).

### Data extraction

Both study reviewers independently extracted data from all eligible studies using EXCEL. Spreadsheets were compared (97% agreement), and conflicting data entries were resolved via consultation between reviewers (BDB and AEK, with LMS as tiebreaker). See supplementary materials for complete list of variables extracted. To determine methodological quality of the studies, both reviewers independently completed the Appraisal Tool for Cross-sectional Studies (AXIS) [18] modified for proton MRS studies [19] within Covidence (Supplementary Table 2).

### Meta-analysis statistical analyses

Comprehensive Meta-Analysis Version 3.0 [20] was used for all analyses. Significance was set at  $p < 0.05$ .

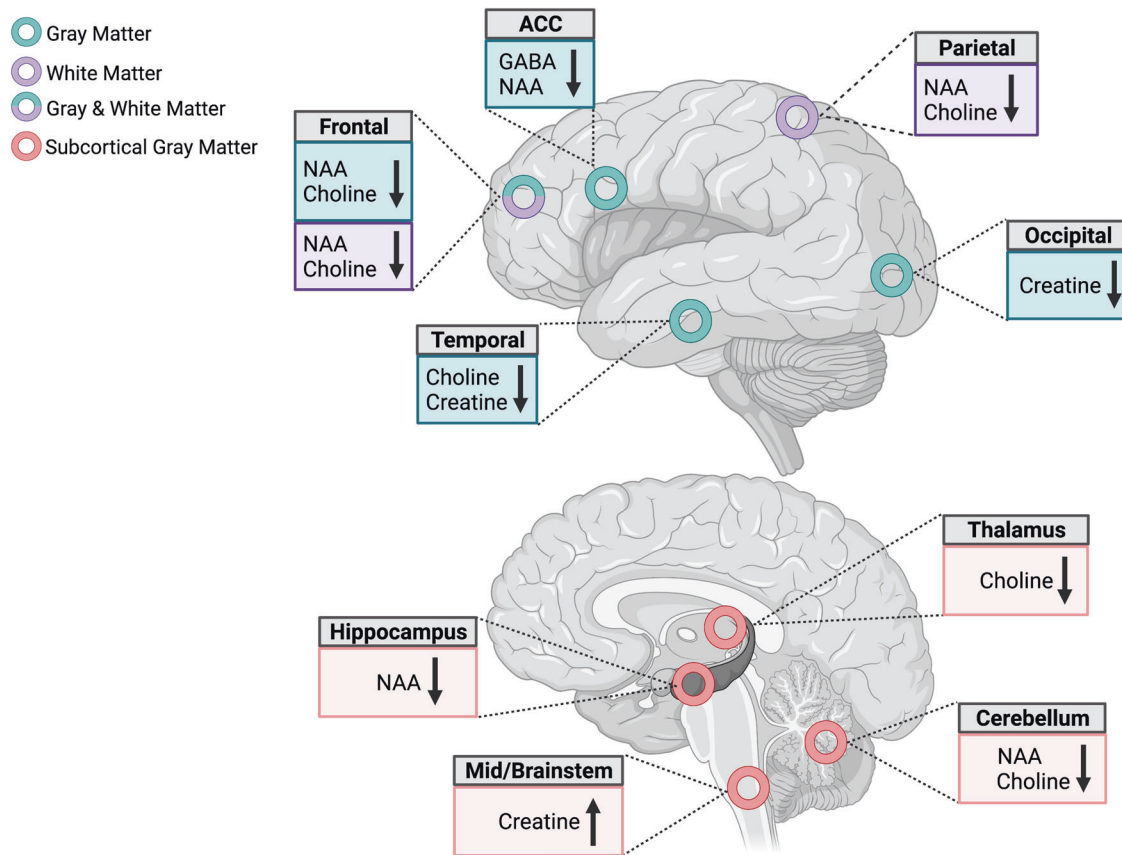
**Main meta-analytic models.** The main model required at least two distinct studies within a brain region per metabolite. For longitudinal studies, only baseline data were included to maintain independence of data [19]. If a study had more than 1 measurement within the

same brain region, the data were treated as independent [19]. A remove-one analysis was performed as a sensitivity analysis to assess the impact of each study individually on the overall estimate; additionally, this was used to check for the influence of studies with multiple groups within the same brain region. Hedges’  $g$  (unbiased standardized mean difference estimate) was used as the measure of effect size [21] with random-effects modeling [22]. Heterogeneity within studies was assessed through the Cochran’s  $Q$ ,  $I^2$ , tau, and tau<sup>2</sup> statistics. Publication bias was assessed in models with >2 studies by: (1) funnel plots, (2) Duval and Tweedie trim and fill method [23]; and (3) Egger’s regression [24]. See Supplementary Materials for more details.

**Sub-group analyses.** The sub-group analyses required at least two studies within each group and ten studies between groups [22, 25]. Random-effects modeling with pooled estimates was used [22, 26]. Two sub-group analyses examined the potential effects of (1) treatment-seeking status (treatment-seeking v. naïve/non-treatment seeking) and (2) quantification type (absolute v. relative concentration) on effect sizes. Treatment-seeking status may be a proxy for severity of alcohol use [27–30], potentially affecting neurometabolic alterations [31–33]. Treatment-seeking status was applied to a study if it was explicitly described in the text, which ranged from stating the participants were seeking treatment to stating that participants were recruited from alcohol-specific programs, clinics, or hospitals. If it was not explicitly stated, studies were labeled as treatment-naïve (explicitly stated) or non-treatment seeking samples (if not explicitly stated as treatment-seeking or treatment-naïve).

MRS-derived metabolites can be quantified using absolute or relative (e.g., normalized to Cr) concentrations [34, 35]. Relative concentrations often use the Cr peak as an internal concentration reference in calculation of metabolite ratios, assuming Cr levels are stable and not affected by pathology. However, this assumption can be invalid, as Cr levels vary with brain activity, age, and different pathologic states [13, 36, 37]. (Note: Relative concentrations can also be calculated using water as the reference, but not many studies included used this method and thus is not included in the sub-group analysis).

**Meta-regression.** Meta-regressions required a minimum of ten studies for each model (Cochrane standard), and random-effects modeling was used. We identified five variables assumed to introduce major heterogeneity into our main models: duration of abstinence (days; alcohol group only), age (years), sex as a biological variable (SABV), MRS sequence-specific echo time (TE in milliseconds (ms) and log-transformed TE), and coefficient of variation (COV). Abstinence duration has been associated with variations in neurometabolite levels (for review see refs. [13, 16]), reflecting at least partial reversibility of metabolite level alterations with abstinence. Most studies included middle aged to older participants, yet a select few reported on young adult alcohol use; thus, given that metabolite levels can be age-dependent, age also introduces heterogeneity. Similarly, most studies included mostly male participants, so SABV (defined as percent of males included in the study) was assessed as clinical research has historically over-sampled males [38], which can have treatment ramifications [39, 40], and there have also been SABV differences in neurometabolite levels across clinical populations [41] and healthy control populations [42]. The TE was included as a variable with potential impact on ability to properly quantify metabolite signals, as its standard has changed over the years with improvements in technical developments [36, 37, 43]. COV is an MRS quality metric that has been shown to moderate metabolite quantification within clinical populations. In a recent meta-analysis, MRS studies with lower COV (considered to be of higher quality) had reduced heterogeneity and increased sensitivity within meta-analytic



**Fig. 1 Overall significant results across all brain regions and metabolites.** ACC Anterior cingulate cortex. NAA N-acetylaspartate. Choline Choline-containing metabolites. Creatine Creatine-containing metabolites. Figure created in BioRender.com.

modeling [44]. COV was derived from the SD divided by the corresponding group mean, calculated [1] with alcohol and control groups combined for overall study data quality and [2] separately for alcohol and control groups.

## RESULTS

### Study selection

Forty-three studies were included in the meta-analysis (Supplementary Fig. 1). The average sample size (alcohol and control groups combined) for each study was 53 (SD = 43). Eleven studies included only male participants (25.6%), with most studies having more male participants than female participants. The average age of participants included in each study was 42.20 (SD = 8.33; min average age = 21.6, max average age = 64.9). Race/ethnicity were rarely reported, thus was not able to be reported. Approximately half ( $n = 22$ , 51.2%) of the studies explicitly reported inclusion of participants who reported tobacco use, while other substance use was not consistently reported. The average standard alcoholic drinks per month for participants in the control group (reported in 37 studies) was 15 (SD = 12) and for the alcohol group (reported in all studies) was 318 (SD = 188). Abstinence duration ranged from active drinking to 1.7 years (median of sample); abstinence duration was not reported for control groups (Supplementary Table 3a).

For MRS, scanner types included Siemens ( $n = 27$ ; 62.8%), GE ( $n = 8$ ; 18.6%), Philips ( $n = 4$ ; 9.3%), Bruker ( $n = 1$ ; 2.3%), Varian Unity ( $n = 1$ ; 2.3%), and the magnetic field strengths were 1.5 T ( $n = 21$ ; 48.8%), 2 or 2.1 T ( $n = 3$ ; 7.0%), 3 T ( $n = 16$ ; 37.2%), and 4 T ( $n = 3$ ; 7.0%). Twelve studies (27.9%) used magnetic resonance spectroscopic imaging (MRSI) to cover multiple brain VOIs, and 31 studies (72.1%) used single-voxel spectroscopy. Acquisition sequences were PRESS ( $n = 19$ ), MEGA PRESS ( $n = 3$ ), STEAM

( $n = 4$ ), short echo ( $n = 3$ ), J-editing sequence ( $n = 1$ ), 17-slice turbo spin-echo ( $n = 1$ ), 2D JD-PRESS ( $n = 3$ ), and CT-PRESS ( $n = 1$ ). Software used for analysis consisted of LC Model ( $n = 15$ ), MATLAB ( $n = 4$ ), MRUI ( $n = 2$ ), SITOOLS ( $n = 3$ ), LUISE ( $n = 1$ ), manufacturer software ( $n = 2$ ), automated spectral fitting program ( $n = 1$ ), or in-house code ( $n = 18$ ). For metabolite quantification, 25 studies (58.1%) used absolute concentration methods, 12 studies (27.9%) used ratio-to-creatine methods, 2 studies (4.7%) used both absolute concentration and ratio-to-creatine, 3 studies (7.0%) used ratio-to-Water, and 1 study (2.3%) used ratio-to-NAA. Cramér-Rao Lower Bounds (CRLB; data quality metric) cut-offs were reported in 14 studies (32.6%) and Full Width at Half Maximum or shimming values were reported in 2 studies (4.7%) (Supplementary Table 3b).

### Overall results from main model

Data were organized by metabolite and volume-of-interest (VOI) (Supplementary Fig. 2), and the overall findings are presented in Fig. 1. Specifically, in alcohol-users compared to controls, lower NAA and Cho metabolite levels were found in many regions of the brain. Lower Cr levels were found in temporal and occipital GM regions, with higher levels only in midbrain/brainstem GM. Lower GABA levels were restricted to the anterior cingulate cortex (ACC). There were no significant differences for ml or glutamate-related (Glu, Gln, or their combination [Glx]) neurotransmitter levels. Only significant findings are discussed within the metabolite-specific sections below, with full results detailed in Table 1.

### N-acetylaspartate (NAA)

**Cortical GM.** Altered NAA levels were found within the ACC [32, 45–53] and frontal GM [33, 45, 47, 50, 54–61].

**Table 1.** Main meta-analytic model results for all brain regions and metabolites.

Metabolite	Meta-analysis			Heterogeneity							Publication bias	
	N ALC/CON	K	Hedge's g	Lower limited	Upper limit	p	Q	I <sup>2</sup>	T <sup>2</sup>	T	t-stat	p
ACC												
NAA	477/342	14	-0.376	-0.671	-0.081	<b>0.021</b>	44.209	70.594	0.202	0.449	2.178	0.050
Cho	455/315	13	-0.066	-0.303	0.172	0.588	24.296	40.160	0.085	0.292	0.727	0.482
Cr	444/302	11	-0.018	-0.303	0.268	0.903	29.430	66.021	0.142	0.377	2.312	<b>0.046</b>
ml	374/223	10	0.195	-0.173	0.563	0.300	31.121	71.081	0.226	0.476	0.722	0.491
Glu	352/355	15	0.051	-0.217	0.319	0.711	41.459	66.231	0.176	0.107	2.521	<b>0.026</b>
Gln	56/57	4	0.385	-0.665	1.436	0.472	23.07	86.996	0.985	0.992	2.266	0.151
Glx	257/107	3	0.001	-0.426	0.428	0.996	4.644	56.937	0.081	0.284	0.974	0.508
GABA	140/110	6	-0.351	-0.638	-0.064	<b>0.016</b>	6.379	21.618	0.028	0.167	0.323	0.763
FRONTAL GM												
NAA	374/356	15	-0.479	-0.757	-0.201	<b>0.001</b>	44.809	68.756	0.193	0.440	1.796	0.096
Cho	348/346	15	-0.255	-0.463	-0.047	<b>0.016</b>	25.286	44.633	0.072	0.269	0.366	0.720
Cr	292/268	11	-0.068	-0.262	0.125	0.488	13.059	23.426	0.025	0.157	0.515	0.619
ml	234/192	9	0.020	-0.165	0.205	0.832	7.967	0.000	0.000	0.000	1.624	0.148
Glu	52/28	2	-0.104	-0.564	0.357	0.659	0.516	0.000	0.000	0.000	-	-
GABA	52/28	2	0.004	-0.456	0.463	0.987	0.013	0.000	0.000	0.000	-	-
PARIETAL GM												
NAA	252/226	8	-0.111	-0.336	0.114	0.336	10.367	32.477	0.033	0.183	0.522	0.621
Cho	166/145	5	0.273	-0.041	0.587	0.089	7.362	45.669	0.058	0.240	0.066	0.951
Cr	166/145	5	0.133	-0.216	0.483	0.455	9.182	56.438	0.088	0.297	0.820	0.472
ml	166/145	5	0.078	-0.146	0.302	0.495	3.944	0.000	0.000	0.000	2.427	0.094
Glu	86/59	3	-0.182	-0.558	0.193	0.342	2.499	19.966	0.022	0.149	0.164	0.896
GABA	86/59	3	0.078	-0.255	0.411	0.647	0.412	0.000	0.000	0.000	0.029	0.981
TEMPORAL GM												
NAA	60/61	2	-0.349	-0.704	0.006	0.054	0.852	0.000	0.000	0.000	-	-
Cho	60/61	2	-0.468	-0.825	-0.111	0.010	0.136	0.000	0.000	0.000	-	-
Cr	60/61	2	-0.415	-0.771	-0.059	0.022	0.144	0.000	0.000	0.000	-	-
ml	60/61	2	0.082	-0.27	0.434	0.648	0.612	0.000	0.000	0.000	-	-
OCCIPITAL GM												
NAA	96/102	5	0.115	-1.471	1.701	0.887	81.247	95.077	2.966	1.722	0.886	0.441
Cho	92/92	4	0.518	-0.742	1.778	0.420	44.151	93.205	1.520	1.233	0.053	0.963
Cr	48/44	2	-0.462	-0.872	-0.053	<b>0.027</b>	0.844	0.000	0.000	0.000	-	-
ml	71/71	3	0.611	-1.178	2.400	0.503	41.73	95.207	2.327	1.526	0.004	0.998
Glx	47/41	2	-1.089	-2.744	0.566	0.197	9.245	89.183	1.275	1.129	-	-
GABA	17/17	2	-1.062	-4.050	1.926	0.486	12.982	92.297	4.296	2.073	-	-

Table 1. continued

Metabolite	Meta-analysis		Heterogeneity							Publication bias			
	N	ALC/CON	K	Hedge's g	Lower limited	Upper limit	P	Q	I <sup>2</sup>	T <sup>2</sup>	T	t-stat	P
INSULA													
NAA	66/70		3	-0.323	-0.68	0.035	0.077	2.235	10.531	0.011	0.103	1.511	0.372
Cho	66/70		3	-0.276	-0.612	0.059	0.107	0.697	0.000	0.000	0.000	1.590	0.357
Cr	66/70		3	0.040	-0.294	0.374	0.815	0.455	0.000	0.000	0.000	1.566	0.362
ml	66/70		3	0.287	-0.049	0.622	0.094	0.412	0.000	0.000	0.000	13.521	<b>0.047</b>
FRONTAL WM													
NAA	461/367		17	-0.533	-0.747	-0.319	<b>0.000</b>	33.815	52.683	0.100	0.316	0.975	0.345
Cho	412/322		16	-0.295	-0.516	-0.073	<b>0.009</b>	31.005	51.621	0.099	0.315	0.505	0.622
Cr	403/312		14	0.040	-0.133	0.212	0.652	16.884	23.005	0.025	0.157	0.295	0.386
ml	339/274		13	0.091	-0.085	0.267	0.312	13.927	13.838	0.014	0.12	0.106	0.918
Glu	41/24		2	0.006	-0.490	0.501	0.982	0.000	0.000	0.000	0.000	-	-
PARIETAL WM													
NAA	259/223		7	-0.342	-0.582	-0.102	<b>0.005</b>	10.236	41.384	0.043	0.207	1.068	0.335
Cho	210/178		6	-0.278	-0.518	-0.037	<b>0.024</b>	6.947	28.031	0.025	0.159	0.543	0.616
Cr	174/149		5	0.000	-0.287	0.287	0.999	6.567	39.089	0.042	0.204	0.466	0.673
ml	175/149		5	0.059	-0.304	0.421	0.751	10.375	61.447	0.104	0.322	0.047	0.966
TEMPORAL WM													
NAA	92/82		3	-0.277	-0.573	0.02	0.068	0.376	0.000	0.000	0.000	2.532	0.240
Cho	92/82		3	-0.250	-0.546	0.047	0.099	0.577	0.000	0.000	0.000	0.230	0.856
Cr	92/82		3	-0.017	-0.313	0.278	0.909	0.890	0.000	0.000	0.000	2.263	0.265
ml	92/82		3	-0.002	-0.297	0.294	0.990	0.721	0.000	0.000	0.000	0.358	0.781
OCCIPITAL WM													
NAA	92/82		3	-0.191	-0.487	0.104	0.205	0.059	0.000	0.000	0.000	0.471	0.720
Cho	92/82		3	-0.118	-0.414	0.179	0.436	1.874	0.000	0.000	0.000	0.249	0.845
Cr	92/82		3	-0.085	-0.381	0.21	0.571	0.284	0.000	0.000	0.000	0.771	0.582
ml	128/111		4	0.09	-0.162	0.342	0.484	1.023	0.000	0.000	0.000	4.183	0.053
CEREBELLUM													
NAA	324/284		12	-0.357	-0.645	-0.069	<b>0.015</b>	32.929	66.594	0.165	0.406	2.961	<b>0.014</b>
Cho	285/248		12	-0.536	-0.826	-0.246	<b>0.000</b>	29.12	62.226	0.156	0.397	1.501	0.164
Cr	248/216		9	-0.022	-0.204	0.160	0.813	5.829	0.000	0.000	0.000	0.447	0.668
ml	153/137		6	0.086	-0.163	0.335	0.498	5.676	11.916	0.012	0.108	1.274	0.272
THALAMUS													
NAA	139/145		7	-0.253	-0.525	0.019	0.068	7.358	18.462	0.025	0.157	1.221	0.277
Cho	175/174		8	-0.292	-0.529	-0.054	<b>0.016</b>	8.303	15.696	0.018	0.135	1.091	0.317
Cr	126/128		4	-0.123	-0.407	0.162	0.397	3.906	23.200	0.020	0.141	8.193	<b>0.015</b>
ml	115/123		5	0.221	-0.228	0.670	0.335	9.648	58.539	0.136	0.368	2.122	0.124
Glu	34/29		2	0.238	-0.768	1.244	0.643	4.095	75.579	0.398	0.631	-	-



Table 1. continued

Metabolite	Meta-analysis		Heterogeneity					Publication bias				
	N	ALC/CON	K	Hedge's g	Lower limited	Upper limit	p	I <sup>2</sup>	T	t-stat	p	
MIDBRAIN/BRAINSTEM												
NAA	112/122		4	-0.171	-0.425	0.083	0.188	2.195	0.000	0.000	0.478	0.680
Cho	112/122		4	0.015	-0.302	0.333	0.925	4.251	0.031	0.176	1.333	0.314
Cr	112/122		4	0.828	0.245	1.412	<b>0.005</b>	8.834	<b>0.032</b>	0.469	2.241	0.154
ml	106/113		3	0.305	-0.074	0.684	0.115	3.922	0.055	0.234	4.264	0.147
STRIATUM												
NAA	172/174		6	-0.216	-0.502	0.07	0.139	9.176	0.058	0.241	1.568	0.192
Cho	148/152		5	-0.214	-0.438	0.01	0.061	0.751	0.000	0.000	2.358	0.100
Cr	149/152		5	0.010	-0.317	0.336	0.953	8.335	0.072	0.268	0.596	0.593
ml	120/122		4	-0.139	-0.546	0.268	0.503	7.758	0.105	0.325	4.178	0.053
HIPPOCAMPUS												
NAA	42/50		2	-1.794	-3.283	-0.304	<b>0.018</b>	5.099	<b>0.024</b>	0.964	-	-

Bold = significant ( $p < 0.05$ ), *italicized* = trending ( $p > 0.05$  but  $< 0.10$ ).

Both models indicated lower levels of NAA associated with the alcohol-using group (ACC:  $k = 14$ ;  $g = -0.38$ ; 95% CI  $-0.67$  to  $-0.08$ ;  $p = 0.02$ ;  $I^2 = 70.6\%$ . Frontal GM:  $k = 15$ ;  $g = -0.48$ ; 95% CI  $-0.76$  to  $-0.20$ ;  $p = 0.001$ ;  $I^2 = 68.8\%$ ) (Fig. 2).

*White matter.* Alcohol use also affected NAA levels within the frontal and parietal WM, with both models indicating significantly lower NAA levels within the alcohol-using group (Frontal WM:  $k = 17$ ;  $g = -0.53$ ; 95% CI  $-0.75$  to  $-0.32$ ;  $p = 0.000$ ;  $I^2 = 52.7\%$ . Parietal WM:  $k = 7$ ;  $g = -0.34$ ; 95% CI  $-0.58$  to  $-0.10$ ;  $p = 0.005$ ;  $I^2 = 41.4\%$ ) (Fig. 2).

*Subcortical GM.* Altered levels of NAA were noted within the cerebellum (combined VOIs from cerebellar cortex [56, 57, 59, 62, 63], vermis [47, 54, 55, 57, 61, 64], and dentate nucleus [57]) and the hippocampus [65, 66]. Both models indicated lower NAA levels within the alcohol-using group (Cerebellum:  $k = 12$ ;  $g = -0.36$ ; 95% CI  $-0.65$  to  $-0.07$ ;  $p = 0.015$ ;  $I^2 = 66.6\%$ . Hippocampus:  $k = 2$ ;  $g = -1.79$ ; 95% CI  $-3.28$  to  $-0.30$ ;  $p = 0.018$ ;  $I^2 = 80.4\%$ ) (Fig. 2). The model for the thalamus approached significance ( $k = 7$ ;  $g = -0.25$ ; 95% CI  $-0.53$  to  $0.02$ ;  $p = 0.068$ ) (Table 1). Removing the only study in treatment-naïve individuals [33] during the remove-one sensitivity analysis yielded a significant model for thalamic NAA.

### Choline-containing metabolites (Cho)

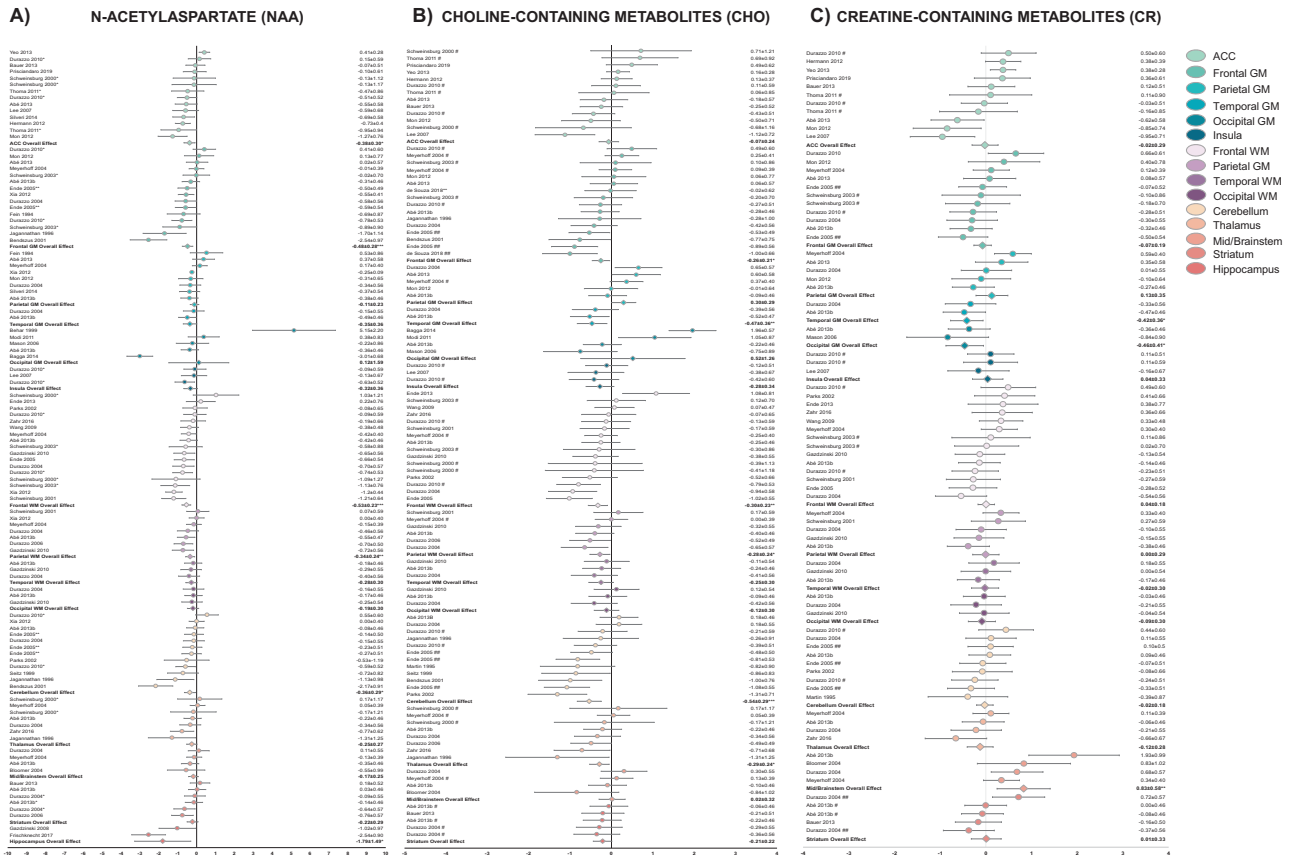
*Cortical GM.* Cho levels were lower in alcohol-using groups compared to controls in the frontal [33, 45, 47, 50, 54–60, 67] and temporal GM [45, 55] (Frontal GM:  $k = 15$ ;  $g = -0.26$ ; 95% CI  $-0.46$  to  $-0.05$ ;  $p = 0.016$ ;  $I^2 = 44.6\%$ . Temporal GM:  $k = 2$ ;  $g = -0.49$ ; 95% CI  $-0.83$  to  $-0.11$ ;  $p = 0.010$ ;  $I^2 = 0.0\%$ ). The effect of alcohol use on lower parietal GM Cho levels was marginal ( $k = 5$ ;  $g = -0.27$ ; 95% CI  $-0.04$  to  $0.59$ ;  $p = 0.089$ ;  $I^2 = 45.7\%$ ) (Fig. 2).

*White matter.* Main models for frontal [33, 47, 51, 54, 55, 57, 60, 62, 68–72] and parietal WM [33, 54, 55, 70, 71, 73] were significant, indicating lower levels of Cho associated with the alcohol-using group (Frontal WM:  $k = 16$ ;  $g = -0.30$ ; 95% CI  $-0.52$  to  $-0.07$ ;  $p = 0.009$ ;  $I^2 = 51.6\%$ . Parietal WM:  $k = 6$ ;  $g = -0.28$ ; 95% CI  $-0.52$  to  $-0.04$ ;  $p = 0.024$ ;  $I^2 = 28.0\%$ ) (Fig. 2). For parietal WM, the remove-one sensitivity analysis resulted in an insignificant model when four effects were individually removed from the model [54, 55, 70, 73]. Therefore, as the significance of this model is strongly influenced by individual studies, parietal WM Cho loss should be interpreted with caution.

*Subcortical GM.* The cerebellum (combined VOIs from cerebellar cortex [56, 57, 59, 62, 63], vermis [47, 54, 55, 57, 64], and dentate nucleus [57]) and thalamus models also indicated lower Cho in alcohol-using groups (Cerebellum:  $k = 12$ ;  $g = -0.54$ ; 95% CI  $-0.83$  to  $-0.25$ ;  $p = 0.000$ ;  $I^2 = 62.2\%$ . Thalamus:  $k = 8$ ;  $g = -0.29$ ; 95% CI  $-0.53$  to  $-0.05$ ;  $p = 0.016$ ;  $I^2 = 15.7\%$ ) (Fig. 2). For the cerebellum, the trim and fill method indicated two additional effects would correct funnel asymmetry from publication bias (adjusted effect size  $g = -0.41$ ). The model for the striatum approached significance ( $k = 5$ ;  $g = -0.21$ ; 95% CI  $-0.44$  to  $0.01$ ;  $p = 0.061$ ;  $I^2 = 0.0\%$ ), becoming significant when one effect [54] was removed in a follow-up sensitivity analysis.

### Creatine-containing metabolites (Cr)

*Cortical GM.* Both occipital [54, 74] and temporal GM [45, 55] models resulted in significantly lower levels of Cr within alcohol-using groups than controls (Occipital GM:  $k = 2$ ;  $g = -0.46$ ; 95% CI  $-0.87$  to  $-0.05$ ;  $p = 0.027$ ;  $I^2 = 0.0\%$ . Temporal GM:  $k = 2$ ;  $g = -0.42$ ; 95% CI  $-0.77$  to  $-0.06$ ;  $p = 0.022$ ;  $I^2 = 0.0\%$ ) (Fig. 2).



**Fig. 2 Forest plots for three of the major neurometabolites measured with proton MRS. A** N-Acetylaspartate (NAA) findings across all volumes-of-interest (VOIs). **B** Choline-containing metabolites (Cho) across all VOIs. **C** Creatine-containing metabolites (Cr) across all VOIs. Circle = individual study effect. Diamond = overall effect size. # same study with >1 alcohol groups. ## same study with >1 VOI in brain region. \**p* < 0.05, \*\**p* < 0.01, \*\*\**p* < 0.001.

**White matter.** There were no significant alterations in Cr levels associated with alcohol use in white matter VOIs (Fig. 2).

**Subcortical GM.** Within the midbrain/brainstem VOIs [33, 54, 55, 75], the main model indicated significantly higher Cr levels in alcohol-using groups relative to controls (*k* = 4; *g* = 0.83; 95% CI 0.25 to 1.41; *p* = 0.005; *I*<sup>2</sup> = 66.0%) (Fig. 2).

**Myo-Inositol (ml)**

There were no significant models with any VOI (Supplementary Fig. 3).

**Glutamate, Glutamine, Glx (Glu, Gln, Glx)**

The meta-analytic models for Glu, Gln, and Glx were all insignificant (Supplementary Fig. 4). Possible reasons for this are considered in the Discussion section.

**GABA**

The only significant model for GABA was from the ACC [31, 32, 45, 50, 76, 77]; GABA levels were lower in the alcohol-using group than controls (*k* = 6; *g* = -0.35; 95% CI -0.64 to -0.06; *p* = 0.016; *I*<sup>2</sup> = 21.6%) (Fig. 3). The remove-one sensitivity analysis resulted in an insignificant model when three studies were individually removed [31, 32, 77]. Each of these studies investigated younger, treatment-naïve samples, suggesting that age and symptom severity affect GABA levels.

**Sub-group analyses**

**Treatment v. treatment-naïve studies.** There was one significant effect for treatment-seeking status, where studies including only

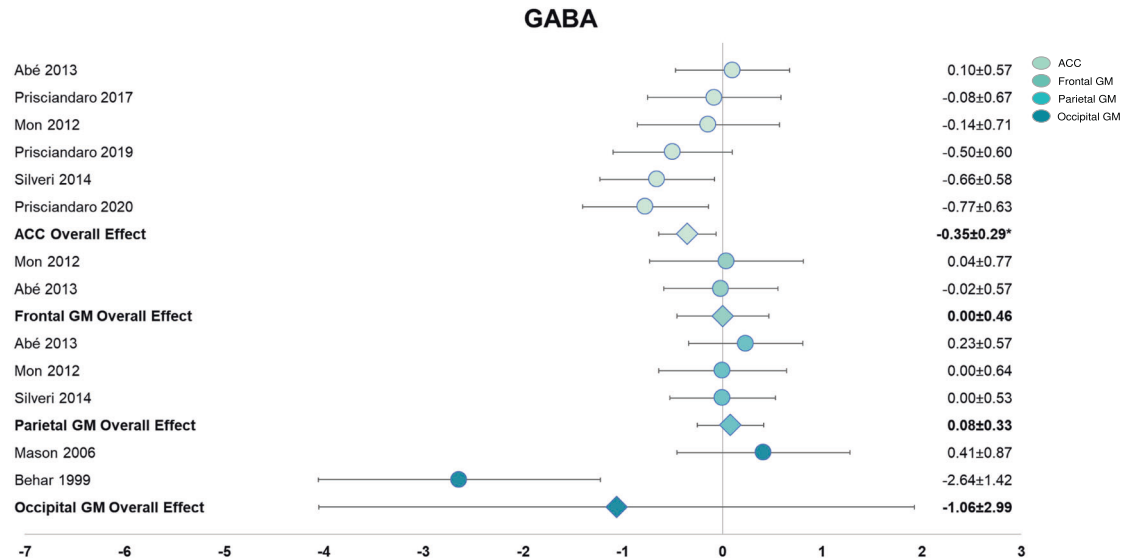
treatment-seekers had significantly lower levels of NAA within frontal WM of alcohol groups compared to controls (treatment seeking *k* = 14, *g* = -0.63, *p* < 0.001, non-treatment seeking *k* = 3, *g* = -0.02, *p* = 0.953; *Q* = 4.73, *p* = 0.03; Table 2).

**Absolute concentration v. relative concentration (Ratio-to-Cr).** Sub-group analysis found a significant difference for metabolite quantification type within frontal GM NAA. Only ratio-to-Cr studies showed significantly lower NAA levels in alcohol groups compared to controls, whereas the absolute concentration studies were only marginally different from controls (absolute concentration *k* = 11, *g* = -0.28, *p* = 0.06, ratio-to-Cr *k* = 4, *g* = -1.16, *p* < 0.001; *Q* = 7.92, *p* = 0.005; Table 2).

Corresponding exploratory sub-group analyses (when *k* < 10) were conducted for parietal GM NAA; occipital GM NAA, Cho, and ml; and cerebellar NAA and Cho. Analyses for occipital GM Cho and cerebellar NAA indicated that only ratio-to-Cr studies were significantly different from controls, while the corresponding absolute concentration studies were not. This is particularly consequential for occipital GM, where the main model resulted in significantly lower levels of Cr for alcohol-using groups, as compared to controls. The possibility that Cr levels in the brain are affected by alcohol use demands caution when interpreting reports of ratio-to-Cr group differences.

**Meta-regression**

**Abstinence duration, age, and SABV (% Male).** Abstinence duration (reported only for alcohol groups; Table 3) showed a significant positive association (longer abstinence duration, greater positive study effect size) with frontal WM NAA levels (*k* = 17;  $\beta$  = 0.01, *p* =



**Fig. 3 Forest plot of GABA results across all VOIs.** Circle = individual study; diamond = overall effect size. # same study with >1 alcohol groups; ## same study with >1 VOI in brain region. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

0.045;  $R^2 = 0.21$ ). Conversely, abstinence duration showed a negative association (longer abstinence, greater negative study effect size) for ACC Glu levels ( $k = 15$ ;  $\beta = -0.004$ ,  $p = 0.036$ ;  $R^2 = 0.26$ ). Both models became insignificant when an outlying long-term abstinent group was removed (mean time = 260 days from ref. [51]; mean time = 365 days from ref. [52], respectively; Supplementary Figs. 5 and 6).

Age (average across alcohol and control groups) was positively correlated with effect sizes for cerebellar NAA ( $k = 12$ ;  $\beta = 0.13$ ,  $p = 0.01$ ;  $R^2 = 0.38$ ) and Cho levels ( $k = 11$ ;  $\beta = 0.15$ ,  $p = 0.000$ ;  $R^2 = 0.95$ ) (Table 3; Supplementary Fig. 7).

SABV (defined as percent of males included in the study) was negatively associated with effect sizes for Cho levels in the ACC ( $k = 13$ ;  $\beta = -0.02$ ,  $p = 0.01$ ;  $R^2 = 0.68$ ) and positively associated with effect sizes for Cho levels in the cerebellum ( $k = 12$ ,  $\beta = 0.03$ ,  $p < 0.001$ ;  $R^2 = 1.00$ ) (Table 3; Supplementary Fig. 7).

**MRS specific TE and data quality variables (COV).** The log-transformed TE was found to affect study effect sizes within GM (Table 4, Supplementary Fig. 8). Positive associations (largest positive study effect size at longer TE) were found between log-transformed TE and study effect sizes for ACC Glu ( $k = 12$ ,  $\beta = 1.40$ ,  $p = 0.002$ ,  $R^2 = 0.66$ ) and Cr ( $k = 10$ ,  $\beta = 1.14$ ,  $p = 0.039$ ,  $R^2 = 0.42$ ). Negative associations (largest negative study effect size at longer TE) were found between log-transformed TE and study effect sizes for frontal GM NAA ( $k = 14$ ,  $\beta = -1.07$ ,  $p = 0.004$ ,  $R^2 = 0.29$ ) and Cho ( $k = 15$ ,  $\beta = -0.71$ ,  $p = 0.004$ ,  $R^2 = 0.77$ ); and cerebellar Cho ( $k = 12$ ,  $\beta = -0.90$ ,  $p = 0.005$ ,  $R^2 = 0.68$ ). Meta-regressions with the raw TE (ms) support these results, except for the meta-regression within ACC which became insignificant (Supplementary Table 4).

MRS data quality (COV; lower number reflects higher quality data) had no significant effects when the alcohol and control groups were combined (Table 3). When looking at COV within alcohol and control groups separately, the alcohol group had a negative association between COV and study effect sizes for Cr within the ACC ( $k = 11$ ,  $\beta = -5.91$ ,  $p = 0.005$ ,  $R^2 = 0.65$ ). This suggests lower-quality data (higher COV) within the alcohol group was related to more negative effect sizes. For the control group, there was a positive association (higher-quality data related to more positive effect sizes) between COV and study effect sizes for NAA in the frontal GM ( $k = 15$ ,  $\beta = 9.01$ ,  $p = 0.019$ ,  $R^2 = 0.18$ ). See

Supplementary Materials for tables and figures (Supplementary Tables 4, 5; Supplementary Fig. 9).

## DISCUSSION

The objective of this meta-analysis was to assess the difference in MRS-determined brain metabolite levels in alcohol misuse and AUD groups relative to controls. Combining studies allowed for identification of significant neurometabolite alterations above and beyond the individual study level, providing better consolidated insights for future neuroscience-informed alcohol treatment development. The main findings reflect alcohol-related alterations in systems representing neuronal/axonal/mitochondrial function (NAA), membrane synthesis and structure (Cho), bioenergetics (Cr), and inhibitory neurotransmission (GABA). Neither brain regions nor metabolites were affected ubiquitously, supporting the idea that we are not capturing generalized toxic effects of alcohol misuse. NAA and Cho had the most alcohol-related aberrations throughout the brain. In alcohol users compared to controls, lower levels of NAA were observed in GM (ACC, frontal, temporal, hippocampal and cerebellar VOIs) and WM (frontal and parietal VOIs). Lower Cho levels were also observed in GM (frontal, temporal, and thalamic VOIs) and WM (frontal and parietal VOIs). Lower Cr levels were detected in temporal and occipital GM cortices, whereas Cr levels in midbrain/brainstem GM were higher. Alterations in GABA were restricted to the ACC, with (mostly younger, treatment naïve) alcohol users having lower levels of GABA than controls. There were no significant group differences for myo-inositol or Glu-related metabolite levels; the latter may be due to uncontrolled factors affecting Glu levels across studies. This meta-analysis is the first to provide formalized evidence that alcohol and AUD-related neurobiological alterations measured by MRS are present in many brain regions and tissues, affecting specific metabolites typically measured by MRS.

Importantly, some main models in this meta-analysis revealed significant neurometabolite alterations related to alcohol use above and beyond the individual study findings. This was particularly apparent for Cho (thalamus, frontal and parietal GM and WM), NAA (ACC), and Cr (occipital, temporal, and midbrain/brainstem GM), highlighting the importance of meta-analytic applications. Recognizing the influence of alcohol misuse on these metabolites may open new research lines into direct (e.g., pharmacotherapy) or indirect (e.g., efficacy markers) treatment targets.



**Table 2.** Sub-group results.

Metabolite	Non-Tx seeking				Tx-seeking				Absolute concentration				Relative concentration (Ratio-to-Cr)			
	K	Hedge's g	P		K	Hedge's g	P	Q	K	Hedge's g	P	Q	K	Hedge's g	P	Q
ACC																
Glu	6	-0.028	0.901	0.097	9	0.097	0.584	0.190	0.663	12	0.000	0.998	3	0.211	0.485	0.538
NAA	5	-0.189	0.403	-0.464	9	0.005	0.957	0.328	-	-	-	-	-	-	-	-
Cho	3	0.310	0.298	-0.136	10	0.294	1.886	0.170	-	-	-	-	-	-	-	-
Cr	3	0.316	0.239	-0.145	8	0.389	2.118	0.146	-	-	-	-	-	-	-	-
ml	3	0.454	0.149	0.063	7	0.777	1.031	0.301	-	-	-	-	-	-	-	-
FRONTAL GM																
NAA	2	-0.543	0.198	-0.476	13	0.003	0.022	0.883	0.057	11	-0.278	0.057	4	-1.164	0.000	7.92
Cho	2	-0.001	0.561	-0.295	13	0.009	0.905	0.341	0.117	11	-0.183	0.117	4	-0.527	0.020	1.83
FRONTAL WM																
NAA	3	-0.015	0.953	-0.633	14	0.000	4.731	0.030	-	-	-	-	-	-	-	-
Cho	3	0.097	0.722	-0.374	13	0.002	2.472	0.116	-	-	-	-	-	-	-	-
Cr	2	0.317	0.121	-0.016	12	0.860	2.209	0.137	-	-	-	-	-	-	-	-
ml	2	0.021	0.927	0.106	11	0.309	0.108	0.742	-	-	-	-	-	-	-	-

All other VOIs and metabolites did not reach the inclusion criteria of  $\geq 10$  effects overall with  $\geq 2$  effects within each subgroup. Bold = significant ( $p < 0.05$ ), *Italicized* = trending ( $p > 0.05$  but  $< 0.10$ ).

Tx Treatment.

While the lack of Glu-related metabolite findings may surprise at first glance, considering the well-studied relationship between alcohol use and glutamatergic neurotransmission (e.g., [78–80]), many factors may explain the non-significant Glu-related models. Glutamate and Gln levels are intrinsically dynamic, related to highly regulated Glu homeostasis across different phases of consumption, withdrawal, recovery, and relapse [78–80]. In addition, there are known methodological factors influencing the ability to quantify Glu and low-intensity Gln multiplets reliably with MRS (e.g., TE, magnet strength) [81]. Together, they may account for the null findings. The detection of substantial heterogeneity across the Glu-related models was not surprising, and our exploration of potential sources of heterogeneity provided some insights for Glu within the ACC (the only region with enough data to complete analyses). In contrast with existing longitudinal studies measuring Glu in treatment-seekers after alcohol cessation [48, 50, 52], we did not find a strong association between abstinence duration and levels of Glu across studies. While the meta-regression with all studies indicated a negative association between study effect size in the ACC and abstinence duration (longer abstinence, lower Glu levels in the alcohol groups v. controls), this was driven by one study that included individuals abstinent for at least 365 days [52] and became insignificant during a sensitivity analysis. This finding is consistent with varying Glu levels across different phases of alcohol use and remission. Most studies assessing Glu levels were between 0 and 14 days of abstinence, so this relationship may be better understood with the inclusion of future cross-sectional and longitudinal studies across varying levels of abstinence. Significant meta-regressions were found for TE, suggesting an influence of echo time Glu metabolite levels. This finding speaks to the importance of using sequence parameters optimized for metabolites of interest, which are often updated through expert consensus recommendations (see refs. [36, 37, 82]). Taken together, the demographic and methodological heterogeneity may contribute to the difficulty to understand the relationship between alcohol misuse/AUD and Glu and its intermediates with the available cross-sectional MRS studies since the low number of effects limited further investigation (meta-regression or subgroup analysis) into such heterogeneity. Future conduct of longitudinal studies together with standardization of MRS parameters and quantification methods, or the adaptation of novel imaging techniques (i.e., GluCEST[83, 84]), may provide more meaningful insights on glutamatergic alterations in AUD.

Eight studies reported on GABA levels across four cortical brain regions, revealing significant differences between alcohol users and controls in the ACC only. As indicated by the remove-one analysis, younger adults with relatively short abstinence duration appear to drive the lower GABA levels, an observation that warrants follow-up. The measurement of GABA via MRS is relatively difficult given its low concentration and spectral overlap with other resonances [82]. All but two studies reporting GABA levels used an appropriate edited sequence (MEGA-PRESS or 2D J-PRESS) increasing the confidence in the findings [82, 85]. As with glutamatergic metabolites, the small number of studies (compared to NAA, Cho, Cr, and ml) and methodological complications may limit the current findings; thus, future standardized clinical MRS studies should further clarify alcohol effects on both Glu and GABA levels.

Heterogeneity is a common concern in meta-analyses. In the current work, 35.8% (24/69) of the main models showed moderate to substantial heterogeneity determined by  $I^2$  (Table 3). This type of data heterogeneity could be based in variations of alcohol- and control group inclusion criteria, demographics, MRS parameters, and MRS quality metrics. Heterogeneity is an important limitation to consider when interpreting findings within this meta-analysis, particularly within models with small number of effects ( $k < 10$ ). Sub-group analyses and meta-regressions were included to probe

**Table 3.** Meta-regression results: Demographics.

Metabolite	Abstinence (Days)					Age (Years)					% Male					
	K	β	Z	P	R <sup>2</sup>	K	β	Z	P	R <sup>2</sup>	K	β	Z	P	R <sup>2</sup>	
ACC																
Glu	15	-0.004	-2.10	<b>0.036</b>	0.26	15	0.001	0.06	0.952	0.00	15	-0.005	-0.73	0.466	0.00	
NAA	14	0.001	-0.21	0.836	0.00	14	-0.011	-0.75	0.455	0.00	14	0.001	0.06	0.955	0.00	
Cho	13	-0.000	-0.02	0.983	0.00	13	-0.015	-1.04	0.298	0.00	13	-0.019	-2.73	<b>0.006</b>	0.68	
Cr	11	0.000	0.00	0.996	0.00	11	-0.161	-0.93	0.350	0.00	11	-0.016	-1.61	0.101	0.23	
ml	10	0.001	0.71	0.481	0.00	10	0.003	0.15	0.880	0.00	10	-0.017	-1.41	0.159	0.27	
FRONTAL GM																
NAA	15	-0.001	-0.30	0.761	0.00	15	0.0151	0.56	0.578	0.00	14	0.007	1.09	0.274	0.00	
Cho	15	-0.004	-0.51	0.608	0.00	15	-0.005	-0.19	0.852	0.00	14	0.000	0.07	0.947	0.00	
Cr	11	-0.008	-0.95	0.343	0.00	11	0.012	0.45	0.656	0.00	11	0.001	0.17	0.864	0.00	
FRONTAL WM																
NAA	17	0.005	2.00	<b>0.045</b>	0.21	17	0.006	0.21	0.836	0.00	17	-0.005	-0.95	0.345	0.05	
Cho	16	-0.000	-0.06	0.955	0.00	16	-0.024	-0.90	0.368	0.00	16	0.000	0.08	0.935	0.00	
Cr	14	-0.004	-0.50	0.617	0.00	14	-0.022	-1.04	0.280	0.05	14	-0.004	-0.98	0.329	0.02	
ml	13	0.001	0.22	0.827	0.00	13	-0.029	-1.37	0.171	0.44	13	0.000	0.01	0.993	0.00	
CEREBELLUM																
NAA	12	0.004	0.24	0.809	0.00	12	0.128	2.57	<b>0.010</b>	0.38	12	0.017	1.78	0.074	0.10	
Cho	11	0.021	1.28	0.200	0.08	11	0.153	4.15	<b>0.000</b>	0.95	12	0.029	4.64	<b>0.000</b>	1.00	

All other VOIs and metabolites did not reach the inclusion criteria of  $\geq 10$  effects. Bold = significant ( $p < 0.05$ ), *Italicized* = trending ( $p > 0.05$  but  $< 0.10$ ).

at potential sources of demographic and MRS-specific heterogeneity across the models.

Most MRS studies included in this meta-analysis were within treatment-seeking individuals (74.4%), a group known to differ in demographic, clinical, and alcohol consumption variables from their treatment-naïve counterparts [27–30]. The sub-group analysis found an effect of treatment-seeking status on frontal WM NAA effects (lower levels in studies of treatment-seekers), and the remove-one sensitivity analysis also indicated a significant thalamic NAA model for studies in treatment-seekers only. Whereas most AUD research has been conducted with individuals seeking treatment [27, 28], the majority of individuals with problematic drinking do not seek treatment [86]. Relying on treatment-seeking status as a proxy for alcohol use severity is also limited, which is further complicated by the variety of healthcare systems, treatment options, and diagnosis criteria available in different countries. This creates a problem of generalizability of research findings to the much larger untreated AUD population and future studies might consider this disparity appropriately.

Meta-regressions were conducted to explore other potential sources demographic heterogeneity, including abstinence duration and age. Some longitudinal studies in treatment-seekers have noted corresponding changes in brain metabolites after variable lengths of abstinence [48, 50, 56, 57, 87]. Abstinence duration does not appear to be consistently related to differences in metabolite levels between alcohol and control groups based on the current meta-regressions, yet there were two significant models of note. First, as previously discussed, there was a negative association between abstinence duration and Glu in the ACC (longer abstinence, lower levels of Glu in alcohol groups). Second, there was a significant positive effect of abstinence duration on frontal WM NAA study effect sizes (longer abstinence, higher levels of NAA in alcohol groups v controls). Thus, the meta-regressions were able to highlight metabolites that are potentially sensitive to abstinence based on the current studies available. Studies providing increased granularity of abstinence duration will help to clarify this finding, as both models became insignificant when the study with the longest duration was removed. There was also an age effect on cerebellar NAA and Cho study effect sizes. While the age range was very narrow (average age 41–53), the age association might suggest specific cerebellar vulnerability in younger individuals who misuse alcohol. Future properly powered longitudinal studies will be able to provide a more specific timeframe of abstinence-related changes in different populations and better address the importance of alcohol-related alterations across the lifespan.

We also investigated the impact of MRS quantification type in a sub-group analysis and of MRS-specific TE and data quality (COV) in meta-regressions. MRS metabolite levels can be expressed as absolute concentrations [88–90] or relative concentrations (e.g. ratio-to-Cr or to-water); the latter assumes Cr or water levels as a stable internal concentration for reference [35]. Almost one-third of the included studies used ratio-to-Cr when reporting metabolite levels. The ratio-to-Cr approach, representing an internal calibration in attempts to reduce variance from uncontrolled sources of signal variability, works well when the Cr signals are invariant. If alcohol affects regional Cr levels, normalization to Cr may lead to erroneous interpretation of metabolite levels, calling into question this quantification method in this population. Indeed, we found that absolute Cr levels in alcohol-users were significantly altered in several brain regions investigated. These findings suggest that future studies should, if possible, avoid using ratio-to-Cr methods within alcohol misuse and AUD populations and be cautious of results using it may lead to erroneous conclusions.

Our results, and those of other [36, 44], show that TE influences effect size, making it a critical issue when designing new clinical studies. MRS-specific TE parameters specifically influenced GM study effects in the ACC (Cr and Glu), frontal GM (NAA and Cho), and cerebellum (Cho) (note: these models used log-transformed

**Table 4.** Meta-regression results: MRS specific characteristics.

Metabolite	logTE (ms)					COV: groups combined				
	K	$\beta$	Z	p	R <sup>2</sup>	K	$\beta$	Z	p	R <sup>2</sup>
ACC										
Glu	12	1.397	3.17	<b>0.002</b>	0.66	14	0.304	0.12	0.904	0.00
NAA	13	0.450	0.59	0.556	0.00	12	-1.999	-0.46	0.646	0.00
Cho	12	0.557	1.09	0.275	0.08	11	-2.203	-0.69	0.490	0.00
Cr	10	1.135	2.07	<b>0.039</b>	0.42	11	-3.808	-1.40	0.162	0.23
ml	-	-	-	-	-	-	-	-	-	-
FRONTAL GM										
NAA	14	-1.073	-2.86	<b>0.004</b>	0.29	15	1.917	0.48	0.628	0.00
Cho	15	-0.713	-2.86	<b>0.004</b>	0.77	15	-0.370	-0.10	0.922	0.00
Cr	11	-0.398	-1.40	0.163	0.34	11	2.920	0.81	0.415	0.00
FRONTAL WM										
NAA	17	0.423	0.96	0.338	0.00	14	5.886	1.57	0.117	0.36
Cho	16	0.472	0.26	0.798	0.00	13	-3.169	-0.61	0.541	0.00
Cr	14	0.072	0.20	0.839	0.00	13	5.758	1.69	<i>0.091</i>	0.59
ml	13	1.539	1.40	0.162	0.53	10	1.179	1.21	0.226	0.15
CEREBELLUM										
NAA	12	-0.753	-1.87	<i>0.062</i>	0.10	12	-1.152	-0.34	0.736	0.00
Cho	12	-0.898	-2.84	<b>0.005</b>	0.68	12	0.127	0.05	0.957	0.00

All other VOIs and metabolites did not reach the inclusion criteria of  $\geq 10$  effect.

Bold = significant ( $p < 0.05$ ), *Italicized* = trending ( $p > 0.05$  but  $< 0.10$ ).

TE and all except ACC Cr meta-regressions were replicated with the raw TE). The effects of TE were consistently positive in the ACC (longer TE associated with higher levels of metabolites in alcohol groups) and consistently negative in the frontal GM and cerebellum (longer TE associated with lower levels of metabolites in alcohol groups). While the number of effects for some metabolites with relatively long TEs were limiting, the findings likely reflect the ability to measure peak areas in the presence of overlapping resonances. Imaging techniques and user expertise improve over time; therefore, it is important for future work to use the expert consensus recommendations for MRS sequence parameters, processing techniques, and data reporting to standardize the field and reduce heterogeneity among studies [36, 37, 82].

There are limitations to the current work. First, several variables of interest, such as additional data quality metrics (e.g., spectral linewidth, metabolite CRLB, or signal-to-noise ratio) and alcohol intake (better characterization of alcohol use severity on metabolite levels across the brain) were not able to be included in sub-group or meta-regressions due to the inconsistency in reporting across studies. Field strength was also not able to be assessed due to the majority of studies being either 1.5 T or 3 T, limiting the ability to run sub-group analyses or meta-regressions. Second, most of the studies available were comprised of older and overwhelmingly male populations, making both the percent male and the age meta-regressions limited in scope. Significant findings for both age and SABV indicates the need for future studies to be mindful of age and SABV when recruiting, as these factors could impact the generalizability of findings. Other important variables, such as race/ethnicity, or other substance use were rarely reported. Third, there were various processing pipelines and software used, which were not always described well enough to ensure reproducibility. As with other imaging techniques, the effects of software and various modeling choices is becoming increasingly more apparent within MRS data [91–93]. Fourth, there was not a “true” control for each model as the inclusion criteria

varied from study to study; thus, some studies allowed alcohol consumption within the control group at a low frequency and quantity while others did not. Lastly, a limitation of MRS is that signals are derived and averaged over large voxels within the brain with varying tissue composition, precluding the ability to understand what is happening at a cellular level. The type of voxel (i.e., gray or white matter) was determined based on the description within the study, yet the actual percentage of various tissues were rarely reporting limiting the ability to cross check the VOI name with actual tissue composition. More studies are needed to better understand regionally specific mechanisms associated with alcohol specific MRS findings to optimize the translation value, as well as to investigate if “normalization” of metabolite levels corresponds with a significant treatment effect and improved AUD symptomology. These limitations highlight the need for standardization in data reporting; inclusion of participants across sex, age, and race/ethnicity to better generalize results; and further research to translate the MRS-derived findings to neurobiological mechanisms to support treatment development and assessment for AUD.

## CONCLUSION

Above and beyond individual studies, this meta-analysis provides formalized evidence that MRS can help identify the brain location(s) and types of metabolite(s) impacted by alcohol misuse and AUD for the first time. Future studies can leverage this new information to investigate specific pharmacological or other (e.g., behavioral, neurostimulation) treatments that might directly or indirectly effectively target the observed meta-neurometabolic disturbances. Region-specific alterations were detected for certain metabolites, with most significant models in cortical or subcortical GM. Specifically, lower levels of NAA and Cho were the most pervasive alcohol-related alterations throughout brain regions. Lower NAA levels likely indicate a decrease in neuronal (GM) and axonal (WM) viability or function, and changes in Cho metabolites may indicate

altered cerebral cell synthesis and membrane turnover related to alcohol misuse. Thus, having been shown to be vulnerable to chronic alcohol use, these metabolites may constitute important targets for treatment or markers of treatment efficacy. Models for Glu/Gln/Glx were not significant, potentially reflecting the highly dynamic behavior of glutamatergic tone with treatment status and duration of abstinence and/or technical challenges in reliably measuring these metabolites [44, 81]. In addition, the few GABA studies conducted showed significantly lower levels of GABA within the ACC, suggesting that GABA-ergic treatment targets may continue to be of interest for future clinical trials. Overall, beyond surveying the extant literature, summarizing the chronic brain effects of AUD and highlighting specific needs for future studies, this work exemplifies the importance and potential translational value of using neuroscience-based approaches toward the development of new treatments for individuals with neuropsychiatric disorders.

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### AUTHOR CONTRIBUTIONS

Conceptualization: AEK, BDB, LMS. Methodology: AEK, BDB, LL, DJM, LMS. Software: AEK, BDB. Formal Analysis: AEK. Data Curation: AEK, BDB. Writing—Original Draft: AEK, BDB, LMS. Writing—Review & Editing: All authors. Visualization: AEK, BDB, RG. Supervision: LMS.

### COMPETING INTERESTS

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### ADDITIONAL INFORMATION

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