Article

Cerebral Metabolites on the Descending Limb of Acute Alcohol: A Preliminary ^1H MRS Study

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Abstract

Aims: Chronic alcohol use is associated with cerebral metabolite abnormalities, yet alcohol's acute effects on neurometabolism are not well understood. This preliminary study investigated cerebral metabolite changes *in vivo* on the descending limb of blood alcohol in healthy moderate drinkers.

Methods: In a pre/post design, participants ($N = 13$) completed magnetic resonance imaging (MRI) scans prior to and approximately 5 hours after consuming a moderate dose of alcohol (0.60 grams alcohol per kilogram of body weight). Magnetic resonance spectroscopy (^1H MRS) was used to quantify cerebral metabolites related to glutamatergic transmission (Glx) and neuroimmune activity (Cho, GSH, myo-inositol) in the thalamus and frontal white matter.

Results: Breath alcohol concentration (BrAC) peaked at $0.070 \pm 0.008\%$ (mean $\pm$ standard deviation) and averaged $0.025 \pm 0.011\%$ directly prior to the descending limb scan. In the thalamus, Glx/Cr and Cho/Cr were significantly elevated on the descending limb scan relative to baseline. BrAC area under the curve, an index of alcohol exposure during the session, was significantly, positively associated with levels of Glx/Cr, Cho/Cr and GSH/Cr in the thalamus. GSH/Cr on the descending limb was inversely correlated with subjective alcohol sedation.

Conclusions: This study offers preliminary evidence of alcohol-related increases in Glx/Cr, Cho/Cr and GSH/Cr on the descending limb of blood alcohol concentration. Findings add novel information to previous research on neurometabolic changes at peak blood alcohol in healthy individuals and during withdrawal in individuals with alcohol use disorder.

INTRODUCTION

Chronic heavy alcohol use is strongly linked to abnormalities in cerebral metabolite concentrations, but mechanisms are not well understood at present. Neuroimaging studies have used proton magnetic resonance spectroscopy (^1H MRS) to investigate neurometabolic changes associated with heavy drinking and/or alcohol use disorder (AUD). These studies commonly report lower N-acetylaspartate (NAA) and choline-containing compounds (Cho) in individuals with AUD [reviewed in (Zahr and Pfefferbaum, 2017)]. In

addition, elevated glutamate levels have been observed during acute alcohol withdrawal (Hermann *et al.*, 2012). While informative, such studies are not designed to capture the processes that lead to cerebral metabolite abnormalities observed with chronic alcohol exposure.

This ^1H MRS study is a preliminary investigation into alcohol-induced alterations in neurotransmission and neurometabolism. Chronic heavy drinking leads to allostasis, i.e. an aberrant shift in the 'set point' of neural systems (George *et al.*, 2012). More

specifically, dysregulation of glutamatergic homeostasis has been proposed as a key mechanism in development of substance use disorders (Kalivas, 2009). At the same time, neuroinflammation is linked to neurobiological and behavioral changes in heavy drinking. Seminal research by several groups has shown that alcohol activates the brain's glial cells, downstream effects of which include upregulated inflammatory signaling and increased proinflammatory cytokines in brain tissue (Blanco *et al.*, 2005; Crews *et al.*, 2013; Lippai *et al.*, 2013). In rodent models, neuroinflammatory signaling mediates the cognitive and behavioral deficits induced by chronic alcohol exposure (e.g. Pascual *et al.*, 2011).

Converging evidence across imaging modalities indicates that the brain's white matter and thalamic circuits are profoundly affected by alcohol use. Vulnerability of brain white matter to negative effects of alcohol use has been documented in numerous structural and diffusion-weighted MRI studies. AUD is associated with a global deficit in white matter volume (Monnig *et al.*, 2013). Diffusion-weighted MRI has linked heavy drinking to microstructural changes in the brain's white matter, which in turn are associated with cognitive and behavioral deficits (Pfefferbaum *et al.*, 2006). On a cellular level, heavy drinking may degrade white matter by inhibiting its lipid constituents, e.g. sphingolipids, according to a recent imaging mass spectrometry analysis of frontal white matter in postmortem human brain (de la Monte *et al.*, 2018). Subcortical structures such as the thalamus also show volume deficits in individuals with AUD (Grodin and Momenan, 2017), and functional MRI (fMRI) points to acute changes in thalamic activity under acute alcohol. One recent fMRI study reported that acute alcohol increased thalamus functional connectivity density (a measure of brain activity at rest) in healthy moderate drinkers, a change that was correlated with the subjective and motor effects of alcohol (Shokri-Kojori *et al.*, 2017). The researchers attributed the findings to metabolic upregulation, which appears consistent with a positron emission tomography (PET) study demonstrating that acute alcohol causes dopamine release in the thalamus (Leurquin-Sterk *et al.*, 2017). Thus, neuro-metabolism in white matter and thalamus may be uniquely affected by acute alcohol, warranting further investigation with ^1H MRS.

The current study investigated the neurometabolic profile of the descending limb of blood alcohol concentration using a pre/post design in healthy moderate drinkers. Using single voxel spectroscopy (SVS), we examined the changes in cerebral metabolites in the frontal white matter and thalamus of healthy individuals with no history of heavy drinking. Our primary hypotheses centered on cerebral metabolites associated with glutamatergic transmission (the summed peak of glutamate and glutamine, Glx) and neuroinflammatory processes (choline, Cho; myo-inositol, Ins). We hypothesized that, relative to baseline, Glx, Cho and Ins concentrations would increase on the descending limb of acute alcohol. We further hypothesized that cumulative exposure to alcohol during the experimental session, as reflected in breath alcohol concentration (BrAC) area under the curve (AUC), would predict increases in cerebral metabolite concentrations. Finally, we performed an exploratory analysis testing associations of cerebral metabolites with measures of subjective response to alcohol.

A small number of experimental studies have examined acute effects of moderate alcohol (peak blood alcohol concentration = 0.05–0.07 g/dL) in healthy humans using ^1H MRS. Biller and colleagues (2009) examined the acute effects of an oral alcohol dose (peak blood alcohol = 0.051 g/dL) in frontomedial and cerebellar regions. Compared to baseline, acute alcohol produced significant decreases in Cho, Ins, and creatine (Cr), with no change in NAA (Biller *et al.*,

2009). All metabolites had returned to baseline levels at 12 hours after alcohol consumption (Biller *et al.*, 2009). Gomez and colleagues (2012) assessed acute alcohol effects in occipital cortex using intravenous (IV) alcohol infusion to maintain BrAC at 0.06%. That study reported a significant increase in Ins and decreases in NAA and gamma-aminobutyric acid (GABA) relative to baseline (Gomez *et al.*, 2012). Glutamate and Cho decreased non-significantly, and Cr did not change (Gomez *et al.*, 2012). Using IV infusion to maintain a blood alcohol level of approximately 0.07 g/dL, Leurquin-Sterk and colleagues (2017) reported no change in glutamate, glutamine, or Glx in anterior cingulate cortex (Leurquin-Sterk *et al.*, 2017).

Methodological differences, including route of alcohol administration and voxel location, may account for mixed findings across studies. Taken together, however, these studies strongly suggest that acute neurometabolic effects of alcohol run counter to the effects observed during withdrawal and in abstinent individuals with AUD. An important next step in understanding the deleterious effects of alcohol on the brain is to characterize neurometabolic changes that occur directly after peak intoxication, under controlled conditions. Previous experimental studies have focused on the ascending limb or peak of blood alcohol, and clinical studies of acute alcohol withdrawal suffer from confounds such as anti-seizure medication use, differing lengths of abstinence and wide age ranges. Thus, assessing changes in cerebral metabolites during acute alcohol clearance in healthy young adults will help identify compensatory mechanisms that may contribute to pathology over time with chronic exposure.

In healthy moderate drinkers, we predicted that Glx would increase on the descending limb relative to baseline as a countervailing response to inhibition of glutamatergic activity during peak intoxication. Alcohol-induced glutamatergic inhibition has been demonstrated previously in animal models (Carboni *et al.*, 1993; Liu *et al.*, 2014). Neither Cho nor Ins is a specific marker of neuroinflammation, but both metabolites are elevated in a wide range of neuroinflammatory conditions (Chang *et al.*, 2013) and may reflect different facets of alcohol neurotoxicity. Ins is prevalent in astrocytes and functions as an organic osmolyte and a second messenger in several neurotransmitter systems (Rae, 2014). Elevated Ins has been reported in frontal white matter and thalamus of recently detoxified individuals with AUD (Schweinsburg *et al.*, 2000, 2001). However, the controlled experimental studies above reported mixed effects of acute moderate alcohol on Ins (Biller *et al.*, 2009; Gomez *et al.*, 2012). Because alcohol activates astrocytes *in vitro* (Blanco *et al.*, 2005), we predicted that Ins would increase on the descending limb of alcohol. Changes in Cho are thought to reflect cellular membrane synthesis and/or repair (Rae, 2014). Under acute alcohol, Cho concentration decreased in a frontomedial region that included gray and white matter (Biller *et al.*, 2009). Individuals in early abstinence from chronic heavy drinking showed low Cho concentrations in frontal white matter that increased with 1–3 months of abstinence (Ende *et al.*, 2005; Durazzo *et al.*, 2006). On the other hand, in observational studies of moderate and non-treatment-seeking heavy drinkers, Cho in frontal white matter correlated positively with recent amount of alcohol consumption (Ende *et al.*, 2006, 2013; Tunc-Skarka *et al.*, 2015). Moreover, in heavy drinkers, an episodic binge pattern was positively associated with Cho in the thalamus (Zahr *et al.*, 2016). These findings suggest complex dynamics of Cho concentrations in frontal white matter and thalamus in relation to moderate drinking, heavy drinking and abstinence. We predicted that Cho would increase on the descending limb as a cellular repair mechanism in the context of alcohol neurotoxicity. Finally, we

assessed glutathione (GSH), a major antioxidant prevalent in the brain's non-neuronal tissue (Rae and Williams, 2017), on an exploratory basis. Based on preclinical evidence that alcohol induces oxidative stress (Crews *et al.*, 2015), we considered that GSH levels may fluctuate in response to oxidative stress. No change in NAA was expected, as most prior research points to chronic heavy drinking as a necessary antecedent of low NAA levels.

For cerebral metabolites on which alcohol-related changes were expected, we investigated whether BrAC AUC significantly predicted metabolite concentrations. Given previous studies linking neural and subjective responses to alcohol (Bauer *et al.*, 2013; Cheng *et al.*, 2018), we tested correlations between cerebral metabolites and standardized measures of alcohol craving, alcohol-related stimulation and sedation, and anhedonia.

METHOD

Participants

Recruitment

Healthy adults were recruited from the Providence, RI, community via flyers and digital postings. Individuals interested in the study were pre-screened by phone and invited to the laboratory for an in-person screening session.

Screening and assessment

The in-person screening visit collected data on medical history, psychological symptoms, basic vital functions and demographic information in accordance with the eligibility criteria listed below. Participants were carefully screened for medical and psychiatric contraindications for alcohol administration. BrAC of 0.00% was confirmed at the start of the session. Urine samples were collected for drug testing (RediTest Alere iCup[®]) and pregnancy testing for females. The Timeline Followback Interview was used to gather data on alcohol and drug use in the previous 90 days (Sobell and Sobell, 1992). This protocol was approved by the Institutional Review Board of Brown University, and all participants provided written informed consent.

Inclusion criteria: (i) 21–45 years of age; (ii) able to speak and read English at 8th grade level or higher; (iii) self-reported alcohol use ≥ 0.60 g/kg at least once in the past year, to reduce risk of adverse response to alcohol; (iv) self-reported alcohol use at least once per month for the past six months; (v) body mass index in the 18.5–30 kg/m² range; and (vi) right-handed.

Exclusion criteria: (i) lifetime history of heavy drinking (i.e. monthly or more frequent heavy drinking episodes for a period of six months or more); (ii) more than three heavy drinking episodes [≥ 4 drinks for women, ≥ 5 drinks for men (Dawson, 2011)] in the past 90 days; (iii) currently seeking or ever having sought treatment or attended mutual help group (e.g. Alcoholics Anonymous) for alcohol/drug use, with the exception of smoking cessation; (iv) chronic disease requiring use of medication; (v) antibiotic use or probiotic use in the past 6 months; (vi) daily or near-daily use of non-steroidal anti-inflammatory drugs; (vii) chronic gastrointestinal disorder; (viii) positive urine toxicology for amphetamine, methamphetamine, cocaine, opioids, or benzodiazepines, or self-reported use of any of these drugs in past 30 days; (ix) positive screening for past 12-month drug use disorder [Drug Abuse Screening Test score > 2 (Skinner, 1982)]; (x) current major psychiatric disorder or clinically significant suicidality; (xi) history of fainting, weakness, infection, or psychological distress resulting from standard blood draw;

(xii) safety contraindication for MRI scan (e.g. metal implant, claustrophobia); (xiii) inability to abstain from tobacco use for 11 hours (i.e. before and during study sessions); (xiv) inability to abstain from cannabis use for 48 hours prior to sessions; (xv) for females, currently pregnant, nursing, or not using effective birth control if applicable. Some exclusion criteria pertain to elements of the protocol (e.g. blood collection) not discussed in this report.

Experimental protocol

The study used a within-subjects, pre-post design. Two MRI scans were collected on a single day, the first at baseline prior to alcohol administration, and the second on the descending limb of alcohol. Figure 1 shows an overview of the experimental session. Participants were instructed to abstain from alcohol for 48 hours and to abstain from food or drink for two hours prior to the start of the session. After the first MRI was collected, participants were given 0.60 grams alcohol per kilogram of body weight to target a peak blood alcohol level of 0.07 g/dL. The dose was reduced by 13% for female participants to account for sex differences in alcohol metabolism (Taylor *et al.*, 1996). The beverage was administered as vodka in the appropriate weight-adjusted amount mixed with seltzer water for a total volume of 500 ml. The beverage was divided into two doses, which were served five minutes apart, and each was consumed within one minute. This procedure was shown to increase blood alcohol at a rate of 0.001 g/dL/min (Fillmore and Vogel-Sprott, 1998). BrAC was measured at half-hour intervals using a handheld digital breath analyzer (Alco-Sensor[®]). Participants were allowed to drink water *ad libitum* throughout the protocol and were given a light meal approximately 3.5 hours after alcohol administration. Collection of the second MRI scan began 4–5.5 hours after alcohol administration, depending on scanner availability. Participants were transported home after procedures were completed and BrAC fell below 0.020%.

Measures of subjective response

During the experimental session, participants completed standardized assessments of alcohol-related stimulation and sedation, hedonic tone, and alcohol craving. The Brief Biphasic Alcohol Effects Scale (BBAES) is a 6-item scale of acute stimulant and sedative effects of alcohol (Martin *et al.*, 1993; Rueger and King, 2013). The Snaith Hamilton Pleasure Scale (SHAPS) is a 14-item measure of hedonic tone that primarily queries ability to take pleasure in sensory and social activities (Snaith *et al.*, 1995). A lower SHAPS score indicates lower capacity to experience pleasure, i.e. greater anhedonia. The SHAPS was adapted to query one's current state rather

Figure 1. Overview of experimental session. Tick marks represent half-hour intervals.

than the standard instructions referring to 'the past few days.' Alcohol craving was assessed using a visual analog scale that measured 100 mm, such that scores ranged from 0–100.

MRI structural and SVS acquisition

All MRI scans were collected on a Siemens 3 T PRISMA instrument with a 64-channel head coil. Whole-brain structural images were obtained with an axial gradient-echo MPRAGE, 3-D T1-weighted scan (TE/TR 3.02/1900 ms, flip angle=9°, slice thickness = 1 mm, FOV = 256 mm, 256×256 matrix, and 1×1 mm in-plane resolution).

SVS data were obtained with a 16×16×10 mm volume positioned in two regions of interest: (i) right hemisphere frontal white matter; (ii) right thalamus. Water-suppressed spectra were collected with a point-resolved spectroscopy sequence (PRESS) at TE/TR =

30/3000ms, bandwidth = 2500 Hz, 128 averages, NEX = 128. A non-water suppressed reference spectrum was collected with NEX = 16. The frontal white matter volume was placed by locating the anterior-most edge of the corpus callosum on axial view and placing the volume anterior and lateral to genu edge, avoiding gray matter. The thalamus volume was placed in axial view, aligning the interior edge with the midline of the brain. All voxels were placed by the first author. Figure 2 shows representative placements and spectra.

SVS processing and quantification

LCModel (Provencher, 1993) was used for pre- and post-processing of ¹H MRS data. Spectral quality was assessed using the criterion of Cramér-Rao lower bounds (CRLB) ≤20%. All spectra were examined visually by the research team and found to be satisfactory. Scan

Figure 2. Examples of SVS voxel placements and spectra. Top panel: Example of the placement of the thalamus voxel (left) and corresponding spectra processed by LCModel (right). Bottom panel: Example of the placement of the frontal white matter voxel (left) and corresponding spectra processed by LCModel (right). For both LCModel output images, the fitted spectrum (red) is superimposed on the original spectrum (black); the upper panel shows residuals, and the baseline is at bottom.

quality was assessed by checking statistics for full-width half maximum, signal-to-noise ratio, and shim line width. The five metabolites quantified were Glx (summed peak of glutamate and glutamine), Cho (summed peak of choline-containing compounds), Ins, GSH, and NAA (summed peak of N-acetyl-aspartate and N-acetylaspartylglutamate). Ratios of these metabolites relative to Cr (compound measure of creatine and phosphocreatine) were extracted from LCModel for analysis. FSL FAST v5.0 was used to segment T_1 -weighted images into cerebrospinal fluid (CSF), gray matter, and white matter (Zhang *et al.*, 2001). Tissue fractions for each SVS volume were calculated with the GannetCoRegister tool of the Gannet2.0 program, using the procedure described by Harris and colleagues (Edden *et al.*, 2014; Harris *et al.*, 2015). The CSF fraction in each voxel was used to calculate CSF-corrected metabolite values in order to correct for partial volume effects. All statistical analyses utilized these CSF-corrected values. Consistency of SVS placement between the two scans was assessed using the Dice coefficient, which quantifies the percentage of overlapping voxels on a scale ranging from 0% (no overlap) to 100% (perfect overlap).

Statistical analysis

Linear mixed models were used to assess change in cerebral metabolite concentrations from the baseline scan to the descending limb scan. Linear mixed models were implemented in SPSS v.24 using the MIXED command. Models accounted for the within-subjects design by including the correlation between repeated measures for each subject. The MIXED procedure calculates the degrees of freedom using the Satterthwaite equation, which is suitable for small samples and unstructured covariance structures. Models were specified using participant as the subject variable, timepoint as the repeated measure, an unstructured covariance matrix, and maximum likelihood as the estimation method. For all metabolites, Hedges g_{AV} was calculated as a measure of effect size (Hedges and Olkin, 1985; Lakens, 2013). Next, in a separate set of linear mixed models, we tested whether alcohol exposure during the experimental session (i.e. BrAC AUC) significantly predicted metabolite concentrations. BrAC AUC was calculated for each participant using the trapezoidal integration method. Accordingly, the BrAC AUC variable was all zeroes for the pre-alcohol scan and estimated AUC for the descending limb scan. Models were specified with participant as the subject variable, timepoint as the repeated measure, BrAC AUC as the predictor, an unstructured covariance matrix, and maximum likelihood as the estimation method. The Benjamini-Hochberg correction for multiple comparisons was applied to each set of hypothesis tests. The Benjamini-Hochberg procedure is a more powerful technique to control Type I error than the standard Bonferroni procedure (Benjamini and Hochberg, 1995). It uses the false discovery rate (FDR) and number of tests performed to adjust the critical p-value on a per-test basis. We chose a conservative FDR of 5% for linear mixed model analyses.

Associations between metabolite concentrations on the descending limb and subjective alcohol response were tested using bivariate Spearman correlations. Subjective response measures were as follows: (i) peak BBAES stimulation, which occurred 30 minutes after alcohol consumption; (ii) peak BBAES sedation score, which occurred 60 minutes after alcohol consumption; (iii) alcohol craving on the descending limb, which was assessed 180 minutes after alcohol consumption; and (iv) anhedonia on the descending limb, which was assessed 180 minutes post-consumption. As above, the Benjamini-Hochberg correction for multiple comparisons was

applied to correlations, except that we used a slightly less conservative FDR of 10% for these exploratory tests.

RESULTS

Participant characteristics

Table 1 shows participant characteristics. The healthy sample averaged 26.4 ± 2.8 (M $\pm$ SD) years of age and was 62% female. As required by eligibility criteria above, participants reported moderate alcohol intake over the 90 days preceding baseline assessment. Specifically, participants consumed an average of 3.3 ± 1.8 drinks per week and 1.8 ± 0.5 drinks per drinking day, and they reported drinking on slightly more than a quarter (27.2 ± 17.7) of days. All participants denied cigarette smoking.

BrAC

The alcohol administration procedure successfully raised BrAC to a peak value of $0.070 \pm 0.008\%$ 60 minutes after consumption. BrAC taken directly before the descending limb MRI scan averaged $0.025 \pm 0.011\%$, confirming that participants were on the descending limb of alcohol during the scan.

SVS scan quality

As shown in Table 2, values for full-width half maximum, signal-to-noise ratio, and shim line width were acceptable. Paired *t*-tests indicated that shim line width did not differ from baseline to descending limb scans in either frontal white matter ($P = 0.161$) or thalamus ($P = 0.636$). Dice coefficients, which represent percentage of voxel overlap within individuals for the two timepoints, equaled 56% for frontal white matter and 65% for thalamus.

Table 1. Participant characteristics ($N = 13$)

	Mean $\pm$ standard deviation or %
Age (years)	26.4 ± 2.8
Female gender	62%
Race	8% African-American; 8% more than one race; 15% Asian; 69% White
Ethnicity	15% Hispanic; 85% non-Hispanic
Average drinks per week	3.3 ± 1.8
Percent drinking days	27.2 ± 17.7
Average drinks per drinking day	1.8 ± 0.5
Smoker	0%
BBAES peak stimulation	4.1 ± 1.6
BBAES peak sedation	2.5 ± 2.1
SHAPS	34.2 ± 4.0
Alcohol craving	24.1 ± 28.2

Table 2. Quality metrics (M $\pm$ SD) for ^1H MRS scans ($n = 26$)

	Shim line width	Full-width half maximum	Signal-to-noise ratio
Frontal white matter	19.1 ± 1.8	0.046 ± 0.009	20.5 ± 3.0
Thalamus	19.1 ± 1.5	0.054 ± 0.012	11.0 ± 1.8

Change in cerebral metabolites on the descending limb of alcohol

Table 3 summarizes results for the CSF-corrected metabolite ratios. No metabolite data were excluded for exceeding the CRLB, except for GSH as noted in the table. Cho/Cr in the thalamus increased significantly on the descending limb of alcohol relative to baseline [$F(1,13) = 6.820$, $P = 0.022$; Fig. 3]. Cho/Cr in frontal white matter did not change significantly ($P = 0.440$). Glx/Cr increased significantly on the descending limb in the thalamus [$F(1,13) = 19.234$, $P = 0.001$; Fig. 3]. Increase in Glx/Cr in frontal white matter was not significant following correction for multiple comparisons [$F(1,13) = 4.763$, $P = 0.048$]. Ins/Cr did not change significantly in thalamus or frontal white matter (P 's > 0.370). The exploratory analysis of GSH/Cr showed an increase during the alcohol scan in the thalamus [$F(1,12.984) = 5.214$, $P = 0.040$; Fig. 3], but this finding did not survive correction for multiple comparisons. As expected, NAA/Cr did not differ from baseline to the alcohol scan in either voxel (P 's > 0.20).

Effect sizes

The effect size (see Table 3) was moderate for the significant increase in thalamus Cho/Cr ($g_{AV} = 0.66$) and large for the significant increase in thalamus Glx/Cr ($g_{AV} = 1.09$).

Change in cerebral metabolites as a function of breath alcohol concentration (BrAC) area under the curve (AUC)

Results of analyses using BrAC AUC as the predictor of cerebral metabolite changes were consistent with the pre/post differences above, with an additional finding for GSH. BrAC AUC significantly predicted increases in Cho/Cr [$F(1,13.145) = 7.431$, $P = 0.017$], Glx/Cr [$F(1,13.185) = 21.766$, $P = 0.0004$] and GSH/Cr [$F(1,13.140) = 6.622$, $P = 0.023$] in the thalamus. In frontal white matter, BrAC AUC predicted increases in Glx/Cr ($P = 0.030$) and GSH/Cr ($P = 0.044$), but these results did not remain significant after correction for multiple comparisons. BrAC AUC was not a significant predictor of Ins/Cr in either voxel (P 's > 0.20).

Associations of cerebral metabolite levels with subjective response to alcohol

Descriptive statistics for behavioral measures are shown in Table 1, and correlations between metabolites and behavioral measures are presented in Table 4. After correction for multiple comparisons, only one correlation was significant: GSH/Cr was negatively correlated with BBAES sedation [$\rho(8) = -0.800$, $P = 0.005$].

DISCUSSION

This study identified elevations in cerebral metabolites on the descending limb of blood alcohol in moderate drinking adults. Specifically, concentrations of Cho/Cr and Glx/Cr increased in the thalamus on the descending limb of alcohol relative to baseline. Moreover, participants' degree of alcohol exposure during the alcohol administration session, quantified by BrAC AUC, significantly predicted levels of Cho/Cr, Glx/Cr, and GSH/Cr in the thalamus on the descending limb. Although some cerebral metabolite changes were observed in frontal white matter, these effects were not significant following stringent correction for multiple comparisons. In this healthy sample, the observed increases in Cho/Cr and Glx/Cr likely reflect normal fluctuations as homeostasis is reestablished during alcohol clearance. With repeated alcohol exposure over time, however, such fluctuations may contribute to neuroadaptation and eventually to allostasis of neurotransmitter systems (George *et al.*, 2012). Thus, our findings provide valuable context for the neurometabolic dysregulation observed in individuals with AUD during acute withdrawal and early abstinence.

Heightened glutamatergic activity is a well-known aspect of alcohol withdrawal in individuals with physiological dependence. One MRS study of individuals with AUD in withdrawal, most of whom were still on the descending limb of blood alcohol, reported elevated glutamate levels in anterior cingulate cortex (Hermann *et al.*, 2012). Breath alcohol during the scan was positively correlated with glutamate levels (Hermann *et al.*, 2012). A subsequent study by the same group confirmed an elevated glutamate concentration in the bilateral anterior cingulate cortex in an overlapping sample of individuals undergoing alcohol withdrawal (Streit *et al.*, 2018). On the other

Table 3. Metabolite values^a for baseline and descending limb of alcohol

		Baseline	Descending limb	β (95% CI)	Correlation ^b	P-value	Hedges g_{AV}
Frontal white matter	Cho/Cr	0.353 $\pm$ 0.045	0.365 $\pm$ 0.053	0.011 (−0.019, 0.042)	0.414	0.440	0.23
	Ins/Cr	0.794 $\pm$ 0.116	0.832 $\pm$ 0.080	0.037 (−0.049, 0.123)	−0.142	0.372	0.36
	Glx/Cr	1.402 $\pm$ 0.125	1.529 $\pm$ 0.215	0.126 (0.001, 0.250)	0.265	0.048	0.68
	GSH/Cr ^c	0.349 $\pm$ 0.048	0.397 $\pm$ 0.088	0.046 (−0.002, 0.094)	0.369	0.060	0.63
	NAA/Cr	1.610 $\pm$ 0.113	1.573 $\pm$ 0.097	−0.039 (−0.128, 0.050)	−0.037	0.357	0.33
Thalamus	Cho/Cr	0.285 $\pm$ 0.036	0.311 $\pm$ 0.038	0.026 (0.005, 0.048)	0.476	0.022*	0.66
	Ins/Cr	0.693 $\pm$ 0.168	0.721 $\pm$ 0.117	0.027 (−0.038, 0.093)	0.738	0.379	0.18
	Glx/Cr	1.698 $\pm$ 0.158	1.880 $\pm$ 0.154	0.181 (0.092, 0.270)	0.510	0.001*	1.09
	GSH/Cr ^c	0.328 $\pm$ 0.047	0.394 $\pm$ 0.081	0.065 (0.004, 0.127)	−0.048	0.040	0.84
	NAA/Cr	1.562 $\pm$ 0.177	1.623 $\pm$ 0.143	0.060 (−0.037, 0.158)	0.447	0.206	0.35

Cho = summed peak of glycerophosphocholine and phosphocholine; CI = confidence interval; Cr = summed peak of creatine and phosphocreatine; g_{AV} = Hedge's g for correlated groups; Glx = summed peak of glutamate and glutamine; GSH = glutathione; Ins = myo-inositol; NAA = summed peak of N-acetylaspartate and N-acetylaspartylglutamate.

*Indicates significant p-value after Benjamini-Hochberg correction for multiple comparisons

^aBaseline and descending limb columns are observed values. Beta coefficients with 95% confidence intervals are the model-estimated mean difference between conditions, coded to represent the change from baseline at the descending limb.

^bCorrelations between repeated measures are provided to allow for calculation of alternative measures of effect size.

^cAfter applying quality control measures, n 's for GSH/Cr ranged from 9–13. For all other cells, $N = 13$.

Figure 3. Changes in cerebral metabolites in frontal white matter and thalamus from baseline to the descending limb of alcohol. Solid black lines represent mean values. Dashed gray lines represent individual participants. For participants missing GSH data at one timepoint, data are shown as gray diamonds. The * symbol indicates a significant difference between the two timepoints after correction for multiple tests.

Table 4. Correlations [ρ (P -value)] of metabolites with subjective response measures

	VAS craving	SHAPS anhedonia	BAES stimulation	BAES sedation
Frontal white matter				
Cho	-0.195 (0.523)	-0.282 (0.351)	-0.030 (0.922)	0.180 (0.556)
Ins	-0.033 (0.914)	-0.431 (0.142)	0.265 (0.381)	-0.161 (0.600)
Glx	-0.156 (0.611)	-0.597 (0.031)	-0.055 (0.858)	0.233 (0.444)
GSH	-0.033 (0.914)	0.050 (0.872)	-0.088 (0.774)	0.422 (0.151)
Thalamus				
Cho	-0.053 (0.864)	-0.160 (0.601)	0.564 (0.045)	-0.286 (0.344)
Ins	0.284 (0.347)	-0.635 (0.020)	0.025 (0.936)	-0.347 (0.246)
Glx	0.460 (0.114)	-0.254 (0.402)	0.160 (0.601)	-0.602 (0.029)
GSH ^a	0.644 (0.044)	0.335 (0.343)	0.482 (0.159)	-0.800 (0.005)*

* Significant correlation after correction for multiple comparisons.

^a $n = 10$ for GSH in the thalamus on the descending limb after quality control criteria were applied. For all other cells, $N = 13$.

hand, heavy drinkers with longer-term abstinence exhibited lower glutamate levels (Thoma *et al.*, 2011), and recent heavy drinking was negatively correlated with glutamate (Prisciandaro *et al.*, 2016). Under acute alcohol, glutamate decreased on the ascending limb and/or at peak blood alcohol in healthy humans, albeit non-significantly (Gomez *et al.*, 2012), and in rats (Liu *et al.*, 2014). To our knowledge, our study is the first to report an acute increase in Glx/Cr, a summed peak composed predominantly of glutamate (Goryawala *et al.*, 2016), on the descending limb of moderate alcohol in healthy individuals. The Glx/Cr increase observed in thalamus was positively associated with BrAC AUC. Taken together, the available evidence suggests that glutamate is suppressed during acute intoxication (whether following either moderate or heavy consumption) and elevated during alcohol clearance/withdrawal, and that chronic heavy use leads to neuroadaptation marked by suppressed glutamate levels. This trajectory remains speculative pending a comprehensive investigation of glutamate fluctuations throughout intoxication, withdrawal and abstinence.

Glutamate may function as a mediator both of neuroinflammatory response and of mesolimbic dopaminergic activation (Kalivas, 2009; Crews *et al.*, 2015). It is important to note that higher glutamate levels are not necessarily detrimental and that context is needed for interpretation. For example, whereas glutamate elevations during clinical alcohol withdrawal can be harmful, glutamate levels are positively correlated with cognitive performance in healthy aging (Zahr *et al.*, 2008). The current study cannot determine the functional significance of glutamatergic elevations in the thalamus on the descending limb. In contrast to some previous studies (Bauer *et al.*, 2013; Cheng *et al.*, 2018), we did not find an association of Glx/Cr with alcohol craving. This discrepancy may relate to differing voxel placement and craving measures, as well as our limited power to detect such associations in a small sample.

Several previous studies have reported that greater alcohol consumption predicts higher Cho during abstinence in both light and heavy drinkers (Ende *et al.*, 2006; Yeo *et al.*, 2013; Tunc-Skarka *et al.*, 2015) and in animal models of binge or chronic alcohol (Zahr *et al.*, 2009, 2010). The current study offers the first experimental evidence that an alcohol-related increase in Cho in healthy humans may begin as early as the descending limb of alcohol intoxication. Two previous ¹H MRS studies of acute alcohol in healthy humans reported that Cho decreased at ascending and/or peak blood alcohol concentration (Biller *et al.*, 2009; Gomez *et al.*, 2012). The increase in Cho that we observed on the descending limb is consistent with research showing that alcohol-related neurotoxicity elicits

compensatory neural responses *during*, and not solely *after*, alcohol exposure (Crews *et al.*, 2015). Because changes in Cho could ensue from any number of sources, however, these findings require further research.

Alcohol exposure during the session significantly predicted GSH increase in the thalamus. There has been some debate in the literature about the suitability of using a standard PRESS acquisition to measure GSH at physiological concentrations [(Sanaei Nezhad *et al.*, 2017); see Rae & Williams (2017) for review]. A recent study by Witjenburg and colleagues evaluated the performance of PRESS and three other methods for glutathione quantification. That study concluded that PRESS, implemented in a manner similar to that in our study, had excellent reproducibility and acceptable sensitivity for GSH measurement (Witjenburg *et al.*, 2019). Therefore, although our methods were not optimized for GSH detection, we consider our findings to be reliable. Given its role as a major antioxidant in the brain (Rae and Williams, 2017), the association of GSH with alcohol exposure could be interpreted as a response to acute oxidative stress, presumably from neurotoxic effects of alcohol. In terms of subjective effects, GSH/Cr in thalamus was negatively correlated with alcohol sedation. This finding was significant after correction for multiple comparisons but should be considered preliminary pending replication in a larger sample. Interestingly, in clinical research on chronic fatigue syndrome and major depressive disorder, cortical GSH was negatively correlated with fatigue and positively correlated with energy levels (Shungu *et al.*, 2012).

Contrary to hypotheses, we did not observe significant changes in Ins on the descending limb scan. Ins is a marker of astrocyte activation and also functions as a second messenger and organic osmolyte. Some previous studies have identified elevated Ins in abstinent individuals with AUD (Schweinsburg *et al.*, 2000, 2001), but findings are mixed overall, e.g. (Meyerhoff *et al.*, 2004). We did observe non-significant increases in Ins in both voxels, suggesting that Ins may be a useful marker of alcohol-related neurobiological processes but sample size limited our ability to detect changes.

This preliminary study has several important limitations, foremost of which are the small sample size and lack of a non-alcohol placebo control condition, which prevents us from definitively concluding that observed changes were due to alcohol consumption. With regard to alternative possibilities, diurnal fluctuations have been reported for many metabolites, including Glx and Cho (de Matos *et al.*, 2016; Spiegelhalter *et al.*, 2016; Al-Iedani *et al.*, 2018; Volk *et al.*, 2018; Arm *et al.*, 2019). There is relatively consistent evidence that Glx increases by roughly 6–8% during daytime hours,

with the greatest increase occurring between early morning and noon. Data on Cho are less consistent, with studies variously reporting no change, decrease, or increase throughout the day. Taking into account the magnitude and timecourse of diurnal changes in previous research, we conclude that our results for Glx and Cho are not better accounted for by diurnal effects, which were of a smaller magnitude, occurred over a longer period of time, and appeared to be due largely to early morning effects that did not align with our scan schedule. In addition, BrAC significantly predicted all cerebral metabolite changes, providing corroborating evidence that these changes were related to alcohol consumption. As noted above, our PRESS acquisition was not optimized for differentiation of glutamate, glutamine, and GSH. This limitation was addressed by using the combined peak of glutamate and glutamine (i.e. Glx) in statistical analyses and by considering GSH on an exploratory basis. Also as noted, Cho and Ins are not specific markers of inflammation and thus may index other factors impacting cellular turnover, glial cell activation, or oxidative stress. Ratios of metabolites to Cr were used because non-water suppressed data needed for quantification of water-referenced spectra were not collected for all participants. Finally, the degree of voxel overlap as quantified by the Dice coefficient was acceptable but modest. We recommend that future ^1H MRS studies on alcohol effects utilize automated placement options when possible and report voxel overlap statistics.

In conclusion, this study offers novel evidence that the cerebral metabolite profile of the descending limb of alcohol in healthy individuals may differ from effects previously described at peak intoxication in healthy individuals or during withdrawal in individuals with AUD. Metabolite changes during alcohol clearance were suggestive of heightened glutamatergic activity and cellular membrane turnover. Further inquiry into metabolite changes throughout the complete trajectory of blood alcohol concentration will advance understanding of alcohol-related neuropathology.

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CONFLICT OF INTEREST STATEMENT

None declared.

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