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Changes in the serum metabolite profile correlate with decreased brain gray matter volume in moderate-to-heavy drinking young adults

Noora Heikkinen [a, b, *, 1], Olli Kärkkäinen [c, d, 1], Eila Laukkanen [e, f], Virve Kekkonen [e], Outi Kaarre [b, e], Petri Kivimäki [b], Mervi Könönen [a, g], Vidya Velagapudi [h], Jatin Nandania [h], Soili M. Lehto [i, j, k], Eini Niskanen [l], Ritva Vanninen [a], Tommi Tolmunen [f, g]

[a] Department of Clinical Radiology, Kuopio University Hospital, Puijonlaaksontie 2, 70210 Kuopio, Finland
[b] Doctoral Programme of Clinical Research, School of Medicine, University of Eastern Finland, Yliopistonranta 1, 70210 Kuopio, Finland
[c] Institute of Public Health and Clinical Nutrition, University of Eastern Finland, Yliopistonranta 1, 70210 Kuopio, Finland
[d] Department of Forensic Psychiatry, University of Eastern Finland, Niuvanniemi Hospital, Niuvankuja 65, 70240 Kuopio, Finland
[e] Department of Adolescent Psychiatry, Kuopio University Hospital, Puijonlaaksontie 2, 70210 Kuopio, Finland
[f] Department of Psychiatry, School of Medicine, University of Eastern Finland, Yliopistonranta 1, 70210 Kuopio, Finland
[g] Department of Clinical Neurophysiology, Kuopio University Hospital, Puijonlaaksontie 2, 70210 Kuopio, Finland
[h] Metabolomics Unit, Institute for Molecular Medicine Finland FIMM, University of Helsinki, Tukholmankatu 8, 00270 Helsinki, Finland
[i] Department of Psychology and Logopedics, Faculty of Medicine, University of Helsinki, Haartmaninkatu 8, 00014 Helsinki, Finland
[j] Institute of Clinical Medicine, University of Eastern Finland, Yliopistonranta 1, 70210 Kuopio, Finland
[k] Department of Psychiatry, Kuopio University Hospital, Puijonlaaksontie 2, 70210 Kuopio, Finland
[l] Department of Applied Physics, University of Eastern Finland, Yliopistonranta 1, 70210 Kuopio, Finland

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ABSTRACT

Our aim was to analyze metabolite profile changes in serum associated with moderate-to-heavy consumption of alcohol in young adults and to evaluate whether these changes are connected to reduced brain gray matter volumes. These study population consisted of young adults with a 10-year history of moderate-to-heavy alcohol consumption (n = 35) and light-drinking controls (n = 27). We used the targeted liquid chromatography mass spectrometry method to measure concentrations of metabolites in serum, and 3.0 T magnetic resonance imaging to assess brain gray matter volumes. Alterations in amino acid and energy metabolism were observed in the moderate-to-heavy drinking young adults when compared to the controls. After correction for multiple testing, the group of moderate-to-heavy drinking young adults had increased serum concentrations of 1-methylhistamine ($p = 0.001$, $d = 0.82$) when compared to the controls. Furthermore, concentrations of 1-methylhistamine ($r = -0.48$, $p = 0.004$) and creatine ($r = -0.52$, $p = 0.001$) were negatively correlated with the brain gray matter volumes in the females. Overall, our results show association between moderate-to-heavy use of alcohol and altered metabolite profile in young adults as well as suggesting that some of these changes could be associated with the reduced brain gray matter volume.

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Introduction

Alcohol use dramatically increases during late adolescence. In one study, approximately one in five 12th graders (17- to 18-year-olds) in the United States reported heavy episodic (binge) drinking in the previous 2 weeks (Johnston, O'Malley, Miech, Bachman, & Schulenberg, 2016). Heavy episodic drinking is suggested to be especially detrimental to adolescent brain development. An early onset of alcohol use is associated with increased alcohol intake during adulthood, and has been considered one of the leading risk factors for developing alcohol use disorder later in life (DeWit, Adlaf, Offord, & Ogborne, 2000). Drinking during adolescence

* Corresponding author. Department of Clinical Radiology, Kuopio University Hospital, P.O. Box 100, FI-70029, Puijonlaaksontie 2, 70210 Kuopio, Finland. Tel.: +358 50 345 6088.
E-mail address: noora.heikkinen@uef.fi (N. Heikkinen).
[1] Equal contribution.

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appears to prime the brain for alcohol use disorder (Kyzar, Floreani, Teppen, & Pandey, 2016).

Neuroimaging studies have shown that heavy alcohol consumption has been connected with a smaller gray matter volume in adults (Momenan et al., 2012) as well as in adolescents (Ewing, Sakhardande, & Blakemore, 2014; Heikkinen et al., 2017; Squeglia & Gray, 2016). However, in pathology studies, heavy alcohol use has not been associated with significant loss in gray matter volumes but rather with white matter atrophy and focal neuronal loss (de la Monte & Kril, 2014). At the same time, changes in amino acid and energy metabolism, such as decreased amounts of glutamine and citrulline, have been reported in association with alcohol consumption (Gika & Wilson, 2014; Jaremek et al., 2013; Lehikoinen et al., 2018; Wurtz et al., 2016). Moreover, similar changes in metabolic processes have been reported in the brain tissue of rodents after alcohol exposure (Meinhardt et al., 2015).

Changes in metabolic processes and in brain structure are likely to be linked. However, to the best of our knowledge, there have been no previous studies combining brain morphometry and metabolic profile analysis in humans. Such information could help us to better understand the mechanisms that underlie alcohol-related decreases in brain gray matter volume, as well as to help identify individuals at high risk of developing brain damage due to alcohol consumption. In this study, we compared moderate-to-heavy drinking and light-drinking young adults, and investigated 1) differences in metabolic profiles and 2) correlations between metabolic profiles and gray matter volumes. Previously, we have reported detailed descriptions of changes in brain morphometry and cortical activity in the same subjects (Heikkinen et al., 2017; Kaarre et al., 2018).

Materials and methods

This study formed part of the Adolescents and Alcohol study, which is focused on Finnish adolescent health and alcohol use. Prior to participating in the study, written informed consent was obtained from all participants. Permission for the study was provided by the Ethics Committee of Kuopio University Hospital and the University of Eastern Finland, the Finnish National Supervisory Authority for Welfare and Health, and the Finnish Ministry of Social Affairs and Health. The baseline and follow-up study settings have been described in detail elsewhere (Kekkonen et al., 2015).

The original cohort was gathered in 2004–2005, when the participants were aged 13–17 years (n = 4127) (time point 1). All the participants were asked to complete a questionnaire concerning their family, hobbies, lifestyle, and substance use. The questionnaire included the Alcohol Use Disorders Identification Test (AUDIT), a structured questionnaire originally designed by the World Health Organization. In this study, we used a shortened version of the AUDIT known as AUDIT-C containing only three questions measuring alcohol consumption. The questions were: “How often do you have a drink containing alcohol?”, “How many drinks containing alcohol do you have on a typical day when you are drinking?”, and “How often do you have six or more drinks on one occasion?” The maximum score for each question was 4, amounting to a maximum total score of 12. The same questionnaire was sent for completion in 2010–2011 (n = 797) (time point 2). The participants in this study were selected from this group in 2013–2015 (time point 3).

Moderate-to-heavy alcohol use was defined as an AUDIT-C score of 4 or more in males and 3 or more in females (Reinert & Allen, 2007). The light-drinking controls had low AUDIT-C scores (i.e., maximum 2) at both time points, and reported no binge drinking episodes. Alcohol users were listed in descending order based on AUDIT-C scores firstly at time point 2 and secondly at time point 1. Participants were contacted in this descending order. Age-, gender- and education-matched light-drinking controls were recruited in parallel to match the moderate-to-heavy alcohol users. Exclusion criteria were a history of a head injury requiring medical treatment, neurological illness, severe mental disorder, metal or implanted devices in the body contraindicating magnetic resonance imaging (MRI), regular use of other intoxicating substances, and pregnancy.

Altogether, 40 moderate-to-heavy drinking subjects and 40 light-drinking controls were recruited. Of the 80 participants, one moderate-to-heavy drinking participant did not complete the MRI scanning due to technical problems, and the images of another moderate-to-heavy drinking participant, as well as two controls, were excluded due to congenital structural abnormalities (i.e., focal cortical dysplasia, a large cyst, enlarged ventricles). Fourteen subjects were excluded due to an unsuitable AUDIT-C score at time point 3: 5 light-drinking control participants due to binge drinking episodes but no regular alcohol use, 6 light-drinking controls due to an AUDIT-C score of more than 2, and 3 moderate-to-heavy drinkers due to an AUDIT-C score of less than the cut-off point at one of the time points. Altogether, 35 moderate-to-heavy drinking (20 females and 15 males) and 27 light-drinking participants (15 females and 12 males) were included in the analysis.

Psychiatric assessment

All participants were interviewed at the last time point using a structured clinical interview for DSM-IV axis I and II psychiatric disorders (SCID-I and SCID-II) (First, Gibbon, Spitzer, Williams, & Benjamin, 1997; First, Spitzer, Gibbon, & Williams, 1996). The interviews were conducted by specialists in adolescent psychiatry trained to undertake SCID interviews.

Metabolic profiling analysis

Venous blood samples were obtained via venipuncture by a trained nurse to 5-mL serum separating tubes, then left to stand for 30 min, and centrifuged at 2500 $\times$ *g* for 10 min to separate the serum. Serum samples were frozen at −80 °C until analyzed. The sample collection was carried out according to the routine protocol in the accredited medical laboratory of Kuopio University Hospital. The samples were shipped to a metabolomics unit at the Institute of Molecular Medicine Finland (FIMM) in Helsinki, Finland, to be analyzed. Metabolomic analysis of the samples was performed using liquid chromatography–mass spectrometry.

Metabolomics analysis was done to determine concentration of 100 polar metabolites using LCMS. All the measured metabolites with corresponding multiple reaction monitoring (MRM) conditions were given in supplementary files published elsewhere (Kolho, Pessia, Jaakkola, de Vos, & Velagapudi, 2017). A strict quality control system is followed during the sample analysis. All the samples were double randomized before samples extraction and before injecting in to the LCMS. To ensure the reproducibility and integrity of the method during runs, QC samples (normal pooled human serum) were incorporated to a batch of samples and run after every 10th experimental sample, and a blank sample was run after every fifth run.

Details of the extraction method and instrument and analytical conditions were described in a publication by Roman-Garcia et al. (2014). Briefly, 10 μL of labeled internal standard mixture was added to the 100-μL samples, and the samples were allowed to equilibrate with the internal standards. A total of 400 μL of extraction solvent (1% formic acid in acetonitrile) was added and the collected supernatant was dispensed into an OstroTM 96-well plate (Waters Corporation, Milford, Massachusetts, United States) and then filtered by applying a vacuum at a delta pressure of

300–400 mbar for 2.5 min on a Hamilton robot's vacuum station. After this, 5 μL of filtered sample extract was injected into an Acquity UPLC system coupled to a Xevo® TQ-S triple quadrupole mass spectrometer (Waters Corporation, Milford, Massachusetts, United States), which was operated in both positive and negative polarities with a polarity switching time of 20 ms for metabolite separation and quantification. The Multiple Reaction Monitoring (MRM) acquisition mode was selected for the quantification of metabolites. MassLynx 4.1 software was used for data acquisition, data handling, and instrument control. The data were processed using TargetLynx software.

Before statistical analysis of the data, analytical results were manually checked to ensure peak shape and integration of peak for each metabolite for all the samples. To ensure reproducibility of the method, concentration, retention time and peak shape of each metabolite of QC samples were compared with the validation data. Also, %CV of concentration of QCs sampled within the run were calculated for each metabolite. Concentration values for each metabolite for all the samples were also checked for below lower limit of quantification (BLLOQ). Finally, concentration values for each metabolite were converted to μmol/L by dividing obtained concertation from TagetLynx software with respective molecular weight of the metabolites for statistical analysis.

For metabolomics analysis, all the metabolite standards were purchased from Sigma-Aldrich (St. Louis, Missouri, United States). Internal standards were ordered from Cambridge Isotope Laboratory. Inc., United States. LC–MS grade solvents, 2-propanol, acetonitrile, and methanol (HiPerSolv) were obtained from VWR International (Helsinki, Finland). Analytical grade chemicals formic acid, ammonium formate, and ammonium hydroxide were procured from Sigma-Aldrich (St. Louis, Missouri, United States). Deionized water (18 MΩ cm at 25 °C) used for solution preparation was made using Milli-Q water purification system (Bamstead EASYpure RoDi ultrapure water purification system, Thermo Scientific, Ohio, United States). Whole blood from normal humans was procured from the Finnish Red Cross blood service (Helsinki, Finland) from which serum was prepared and aliquoted (350 μL) into the Eppendorf tubes and stored at −80 °C. These serum samples were used as QCs during metabolomics analysis.

MR image acquisition and analysis

Participants underwent 3-T MRI of the brain (Philips Achieva 3.0T TX, Philips, Netherlands). A T1-weighted 3D TFE sequence (TR 8.24 ms, TE 3.82 ms, flip angle 8°, FOV 240, 190 contiguous sagittal slices with 0.94 × 0.94 × 1.0 mm voxels) as well as T2-weighted and FLAIR sequences were acquired. An experienced neuroradiologist evaluated all structural images for any abnormalities.

Gray matter segments were acquired using the VBM8 toolbox (http://www.dbm.neuro.uni-jena.de/vbm/vbm8/) in SPM8 (Wellcome Department of Imaging Neuroscience, London, England; http://www.fil.ion.ucl.ac.uk/spm) running under Matlab R2007b (Mathworks Inc., Natick, Massachusett, United States). The T1-weighted images were normalized into the MRI space and segmented into gray matter, white matter, and cerebrospinal fluid, and modulated with non-linear components only to retain their original volume. Finally, the segments were smoothed with an 8-mm FWHM Gaussian kernel. The volumes of whole brain gray matter segments were retained for statistical analyses.

Statistics

Metabolic profiling was performed using univariate and multivariate analysis. For univariate analysis, we calculated *p*-values (Student's *t* test, α level = 0.05) and effects sizes (Cohen's *d*) for comparisons between moderate-to-heavy- and light-drinking subjects for each measured metabolite. Principal component analysis (PCA) was used to analyze overall variance among all the subjects. The number of principal components needed to explain 95% of variance in the data was used to adjust the α level for multiple testing correction in the metabolic profiling analysis (Bonferroni's method). Furthermore, we used partial least-squares discriminant analysis (PLS-DA) to identify the variables explaining most of the variation between moderate-to-heavy drinking subjects and light-drinking controls. For the PLS-DA, variables were normalized by standard deviation (z score) and mean centered before analysis. Variable importance in the projection (VIP) values is reported for each variable. Metabolites with a VIP value above 1.5, a *d* value larger than 0.5 (or smaller than −0.5), and a *p* value below 0.05 when comparing moderate-to-heavy- and light-drinking participants were selected for correlation analysis against the gray matter volumes. This was done to include metabolites associated with alcohol use and exclude metabolites associated with gender, because metabolic profiles differ between males and females, and males, on average, tend to have larger gray matter volumes than females. Pearson's correlation coefficient was used to measure the strength of associations. We used SPSS (IBM Corporation, version 21) to perform univariate and correlation analyses, SIMCA (Umetrics, version 14.0.0.1359) to perform multivariate analyses, and Prism (GraphPad Software Inc., version 5.03) to create figures.

Results

We identified 100 metabolites from the samples (Table 1). Concentrations of several metabolites were different between moderate-to-heavy drinking subjects and light-drinking controls. The mean concentrations, VIP, *d*, and *p* values for all measured metabolites are provided in Table 1. Due to the correlative nature of metabolites in serum samples, 38 principal components were required for explaining 95% of variance in the present metabolic profiling data. Therefore, Bonferroni's adjustment for multiple testing reduces the α level for t-statistics to 0.0013. Using this adjustment in the group-wise comparison, only the increased concentrations of 1-methylhistamine in the moderate-to-heavy drinking subjects ($p = 0.0011$), when compared to the controls, can be considered a statistically significant finding in the present analysis (Table 1).

The mean abundance and standard deviations of the metabolites are separately presented for males and females in Table 1. The observed higher concentrations of creatine and arginine in the moderate-to-heavy drinking subjects are mainly due to elevated concentrations in the moderate-to-heavy drinking females (Fig. 1, Table 1) when compared to the light-drinking controls. There were no statistically significant differences between the study groups in body weight (controls: 73.3 ± 15.2 kg [mean ± SD]; cases: 72.4 ± 15.2; $p = 0.436$) or BMI (controls: 23.7 ± 4.0; cases: 24.9 ± 3.8; $p = 0.294$).

The moderate-to-heavy drinking subjects had decreased brain gray matter volumes (675.7 ± 51.2 mL [mean ± SD]; $p = 0.007$) when compared to the light-drinking controls (712.3 ± 51.4 mL). A more detailed analysis of the morphometry analysis has been published elsewhere (Heikkinen et al., 2017). Furthermore, eight variables with a VIP value above 1.5, a *d* value larger than 0.5 (or smaller than −0.5), and a *p* value below 0.05 (Table 1) were chosen for Pearson's correlation analysis between gray matter volume and metabolite abundance in the whole participant group. For the correlation analysis, we adjusted the α level to 0.0063 to account for multiple testing (Bonferroni's adjustment). Significant inverse correlations were observed between gray matter volume and both

Table 1
Metabolite concentrations (μM) in serum samples (alphabetical order)

Compound	All controls		All alcohol users		*t*-test (all subjects)		PLS-DA	Female controls		Female alcohol users		*t*-test (female)		PLS-DA	Male controls		Male alcohol users		*t*-test (male)		PLS-DA
	Mean	SD	Mean	SD	*p*	*d*	VIP	Mean	SD	Mean	SD	*p*	*d*	VIP	Mean	SD	Mean	SD	*p*	*d*	VIP
1-Methylhistamine	0.0091	0.0023	0.0122	0.0043	**0.001***	**0.82**	**2.33**	0.0093	0.0023	0.0128	0.0043	0.010	0.87	1.76	0.0088	0.0022	0.0115	0.0041	0.055	0.75	1.65
2-Aminoisobutyric acid	3.08	0.99	3.95	1.46	**0.011**	**0.65**	**1.88**	2.95	1.06	3.98	1.42	0.029	0.75	1.50	3.23	0.86	3.92	1.51	0.192	0.52	1.10
2-Deoxyuridine	0.671	0.270	0.682	0.224	0.868	0.04	0.45	0.586	0.253	0.664	0.248	0.383	0.31	0.61	0.777	0.253	0.705	0.184	0.420	−0.33	0.71
3-Hydroxyanthranilic acid	0.084	0.039	0.097	0.051	0.263	0.29	0.98	0.081	0.044	0.103	0.062	0.266	0.39	0.90	0.087	0.031	0.090	0.030	0.821	0.09	0.34
3-OH-DL-Kynurinine	0.142	0.055	0.137	0.043	0.683	−0.11	0.83	0.149	0.066	0.137	0.043	0.532	−0.22	1.28	0.135	0.038	0.138	0.044	0.848	0.08	0.63
4-Pyridoxic acid	0.019	0.023	0.026	0.050	0.523	0.17	0.54	0.021	0.028	0.015	0.013	0.377	−0.31	0.62	0.016	0.014	0.040	0.073	0.281	0.43	0.91
5-Hydroxyindole-3-acetic acid	0.099	0.108	0.103	0.107	0.884	0.04	0.39	0.088	0.062	0.122	0.135	0.386	0.31	0.62	0.112	0.146	0.077	0.033	0.396	−0.34	0.94
Acetoacetic acid	252.96	182.59	255.25	222.29	0.966	0.01	0.82	271.86	218.93	217.29	257.53	0.526	−0.22	1.02	229.34	118.72	305.87	149.62	0.177	0.54	1.38
Acetylcarnitine	7.61	3.15	7.02	2.68	0.442	−0.20	0.70	8.27	3.85	6.86	2.70	0.223	−0.43	0.85	6.77	1.61	7.24	2.64	0.607	0.21	1.01
Adenine	0.0011	0.0005	0.0011	0.0005	0.668	0.11	0.39	0.0010	0.0004	0.0014	0.0006	0.063	0.64	1.30	0.0012	0.0006	0.0008	0.0003	0.106	−0.64	1.33
Adenosine	0.49	0.90	0.47	0.72	0.943	−0.02	0.12	0.393	0.746	0.529	0.849	0.636	0.17	0.33	0.606	1.041	0.399	0.487	0.516	−0.26	0.55
Alanine	433.71	120.08	435.44	125.14	0.957	0.01	0.82	423.24	130.62	460.92	143.51	0.444	0.27	1.28	446.79	103.96	401.46	84.04	0.239	−0.47	1.32
Allantoin	1.22	0.69	1.30	0.96	0.709	0.10	0.58	1.37	0.80	1.25	1.00	0.708	−0.13	0.34	1.03	0.45	1.37	0.89	0.249	0.46	1.49
Aminoadipic acid	3.94	0.93	3.44	0.92	**0.041**	**−0.52**	**1.68**	3.74	0.94	3.36	1.03	0.298	−0.37	1.50	4.20	0.85	3.54	0.74	0.048	−0.77	1.60
AMP	0.21	0.66	0.13	0.31	0.529	−0.16	0.71	0.129	0.368	0.187	0.403	0.670	0.15	0.62	0.321	0.892	0.059	0.073	0.286	−0.43	0.90
Arginine	111.96	17.53	124.35	21.14	**0.019**	**0.60**	**1.77**	109.90	20.80	129.01	24.60	0.024	0.77	1.55	114.55	11.77	118.14	12.96	0.481	0.29	1.37
Asparagine	62.56	19.94	72.16	27.24	0.135	0.39	1.36	57.92	20.00	78.47	31.72	0.040	0.70	1.44	68.36	18.28	63.74	16.30	0.511	−0.27	1.06
Aspartate	6.97	2.00	7.71	2.63	0.235	0.31	1.04	7.76	2.20	8.00	3.03	0.803	0.09	0.71	5.97	1.10	7.31	1.90	0.047	0.78	1.63
Asymmetric dimethylarginine	1.97	0.47	1.99	0.47	0.859	0.05	0.20	1.91	0.39	2.03	0.50	0.449	0.27	0.59	2.04	0.54	1.93	0.40	0.564	−0.23	0.50
Betaine	28.68	12.24	31.90	14.95	0.376	0.23	0.71	26.71	12.66	28.71	15.21	0.692	0.14	0.76	31.14	11.23	36.14	13.46	0.330	0.39	1.01
cAMP	0.010	0.005	0.010	0.004	0.948	−0.02	0.75	0.010	0.004	0.009	0.004	0.517	−0.23	0.51	0.010	0.006	0.011	0.003	0.602	0.21	0.45
Carnitine	34.56	6.26	35.77	7.56	0.511	0.17	0.60	33.20	6.16	35.28	7.88	0.417	0.29	0.74	36.27	5.96	36.43	7.06	0.952	0.02	0.05
Carnosine	0.302	0.045	0.315	0.050	0.280	0.28	1.18	0.294	0.048	0.306	0.044	0.474	0.25	0.52	0.312	0.037	0.328	0.054	0.393	0.34	1.05
Chenodeoxycholic acid	59.66	34.64	62.68	42.78	0.770	0.08	0.51	53.87	30.13	50.78	32.91	0.784	−0.10	0.69	66.91	38.36	78.56	48.85	0.521	0.26	0.58
Choline	18.21	6.04	21.00	7.34	0.121	0.40	1.28	17.29	6.11	21.83	8.76	0.104	0.56	1.19	19.36	5.76	19.89	4.62	0.801	0.10	0.57
Citrulline	18.30	4.17	19.28	5.24	0.437	0.20	0.94	17.89	5.10	18.92	6.19	0.615	0.18	1.15	18.81	2.48	19.75	3.54	0.457	0.30	0.74
Cotinine	0.014	0.054	0.372	0.572	**0.002**	**0.77**	**2.55**	0.003	0.002	0.328	0.545	0.032	0.74	1.95	0.028	0.078	0.430	0.600	0.036	0.82	1.69
Creatine	35.30	18.67	59.22	36.72	**0.004**	**0.74**	**2.17**	39.86	20.42	79.02	35.89	0.001	1.09	2.17	29.61	14.29	32.83	14.49	0.584	0.22	0.98
Creatinine	107.08	13.19	100.74	19.29	0.155	−0.37	1.10	100.57	12.52	89.78	13.06	0.023	−0.78	1.67	115.22	8.74	115.36	16.35	0.979	0.01	0.12
Cystathionine	0.090	0.222	0.060	0.085	0.482	−0.18	0.91	0.120	0.294	0.075	0.110	0.544	−0.21	0.97	0.052	0.021	0.040	0.013	0.114	−0.63	1.31
Cytidine	0.232	0.089	0.208	0.076	0.262	−0.29	0.98	0.252	0.109	0.196	0.077	0.091	−0.59	1.18	0.208	0.045	0.225	0.072	0.497	0.28	1.22
Cytosine	0.026	0.017	0.039	0.031	0.055	0.49	1.40	0.031	0.018	0.039	0.029	0.361	0.32	0.64	0.019	0.013	0.039	0.035	0.081	0.69	1.62
Decanoylcarnitine	0.152	0.117	0.146	0.131	0.851	−0.05	0.78	0.170	0.138	0.124	0.153	0.373	−0.31	1.02	0.130	0.077	0.176	0.086	0.173	0.54	1.39
Deoxycytidine	5.87	1.74	5.95	1.68	0.873	0.04	0.41	5.63	1.73	5.74	1.66	0.856	0.06	0.18	6.18	1.71	6.22	1.68	0.951	0.03	0.52
D-Glucuronic acid	0.192	0.140	0.246	0.129	0.124	0.40	1.18	0.192	0.138	0.254	0.148	0.225	0.42	1.30	0.192	0.143	0.235	0.097	0.374	0.36	0.82
Dimethylglycine	3.88	1.41	4.19	1.30	0.384	0.23	0.71	4.01	1.73	4.13	1.53	0.845	0.07	0.49	3.71	0.81	4.27	0.91	0.122	0.61	1.37
D-Ribose-5-phosphate	2.15	0.94	3.07	3.56	0.203	0.33	1.03	2.12	1.07	3.85	4.45	0.162	0.49	1.04	2.19	0.74	2.04	1.12	0.694	−0.16	0.34
Folic acid	0.015	0.005	0.016	0.005	0.608	0.13	0.99	0.015	0.006	0.017	0.004	0.194	0.45	0.97	0.015	0.004	0.013	0.005	0.487	−0.28	0.71
GABA	0.321	0.106	0.308	0.081	0.563	−0.15	0.68	0.357	0.095	0.295	0.082	0.055	−0.66	1.54	0.277	0.101	0.324	0.076	0.205	0.51	1.11
Gamma-Glutamylcysteine	0.546	0.204	0.643	0.957	0.611	0.13	0.53	0.556	0.235	0.696	1.253	0.681	0.15	0.33	0.532	0.157	0.572	0.182	0.575	0.23	0.56
Glutamine	885.78	205.95	817.70	225.11	0.233	−0.31	1.19	857.07	228.58	820.18	291.51	0.696	−0.14	1.57	921.68	166.75	814.39	70.10	0.040	−0.80	1.67
Glutathione	0.151	0.099	0.155	0.121	0.896	0.03	0.50	0.165	0.105	0.184	0.141	0.681	0.15	0.57	0.133	0.089	0.116	0.070	0.597	−0.21	0.47
Glyceraldehyde	92.42	20.12	104.87	29.92	0.072	0.46	1.58	89.98	21.73	111.93	34.09	0.042	0.70	1.54	95.48	17.41	95.45	19.62	0.998	0.00	0.89
Glycine	247.50	82.45	214.27	71.69	0.101	−0.42	1.52	241.70	94.73	199.84	85.82	0.194	−0.45	1.13	254.74	63.11	233.51	39.01	0.312	−0.41	0.86
Glycocholic acid	0.683	0.331	0.694	0.307	0.900	0.03	0.60	0.611	0.271	0.732	0.327	0.269	0.39	0.94	0.774	0.375	0.644	0.269	0.324	−0.40	0.88
Guanidinoacetic acid	1.53	0.54	1.59	0.61	0.665	0.11	0.41	1.46	0.57	1.59	0.73	0.588	0.19	0.80	1.61	0.49	1.60	0.39	0.944	−0.03	0.10
Guanosine	2.01	1.57	2.18	1.78	0.690	0.10	1.04	2.11	1.81	2.43	1.59	0.585	0.19	0.68	1.88	1.19	1.85	1.96	0.961	−0.02	0.05
Hexanoylcarnitine	0.039	0.036	0.038	0.028	0.878	−0.04	0.59	0.046	0.046	0.035	0.033	0.459	−0.26	0.79	0.031	0.012	0.041	0.019	0.140	0.59	1.36
Hippuric acid	7.68	6.60	7.43	6.22	0.883	−0.04	0.34	8.06	6.45	9.02	7.40	0.701	0.14	0.36	7.20	6.75	5.32	3.08	0.364	−0.37	0.76
Histidine	77.50	11.87	77.27	11.82	0.939	−0.02	0.83	74.91	13.11	79.18	11.03	0.318	0.35	1.02	80.74	9.13	74.72	12.34	0.187	−0.53	1.21
Homocysteine	189.67	60.54	195.75	76.19	0.739	0.09	0.79	186.80	54.12	185.00	89.32	0.947	−0.02	0.53	193.26	67.55	210.09	50.50	0.483	0.28	0.69
Homogentisic acid	1.25	0.90	1.35	0.91	0.670	0.11	0.84	1.43	1.09	1.07	0.91	0.319	−0.35	0.88	1.03	0.51	1.72	0.76	0.015	0.93	1.95
Homoserine	0.770	0.062	0.763	0.083	0.713	−0.10	0.46	0.772	0.077	0.766	0.095	0.842	−0.07	0.54	0.767	0.035	0.759	0.064	0.687	−0.16	0.35

Hydroxyproline	8.73	16.55	8.79	7.57	0.985	0.00	0.35	5.09	4.68	9.07	8.98	0.138	0.52	1.18	13.28	23.49	8.42	5.07	0.460	−0.30	0.62
Hypoxanthine	14.49	2.96	13.87	3.87	0.499	−0.18	0.52	13.90	2.59	13.87	4.49	0.985	−0.01	0.37	15.23	3.21	13.87	2.86	0.273	−0.44	0.92
IMP	0.536	0.232	0.519	0.177	0.749	−0.08	0.85	0.435	0.183	0.544	0.194	0.110	0.56	1.12	0.663	0.225	0.486	0.145	0.025	−0.86	1.82
Inosine	3.85	1.60	3.96	2.37	0.829	0.06	0.73	3.72	1.63	4.23	2.20	0.461	0.26	0.92	4.01	1.54	3.60	2.54	0.642	−0.19	0.40
Isobutyrylcarnitine	0.442	0.177	0.440	0.201	0.967	−0.01	0.39	0.430	0.155	0.414	0.181	0.792	−0.09	0.54	0.457	0.201	0.475	0.221	0.841	0.08	0.34
Isoleucine	86.03	31.84	93.04	30.07	0.386	0.23	1.02	74.64	34.06	85.30	21.78	0.282	0.38	1.23	100.26	21.60	103.37	35.93	0.802	0.10	0.88
Isovalerylcarnitine	0.189	0.075	0.199	0.067	0.616	0.13	0.77	0.165	0.077	0.196	0.068	0.231	0.42	0.88	0.220	0.060	0.202	0.064	0.489	−0.28	0.60
Kynurenic acid	0.030	0.010	0.031	0.015	0.771	0.08	0.99	0.027	0.010	0.027	0.014	0.995	0.00	0.06	0.034	0.008	0.037	0.013	0.583	0.22	0.59
L-5-Hydroxytryptophan	0.034	0.014	0.037	0.014	0.344	0.25	0.82	0.034	0.012	0.039	0.015	0.317	0.35	0.69	0.034	0.016	0.036	0.012	0.797	0.10	0.41
Leucine	215.30	84.77	220.70	84.57	0.807	0.06	0.73	183.38	86.05	198.15	71.22	0.594	0.19	0.60	255.21	63.61	250.77	91.34	0.892	−0.06	0.73
L-Glutamic acid	19.84	11.03	26.71	10.80	**0.019**	**0.60**	**1.75**	16.00	8.78	23.80	10.82	0.033	0.73	1.76	24.64	11.65	30.58	9.47	0.172	0.54	1.17
L-Kynurenine	1.37	0.26	1.36	0.23	0.858	−0.05	0.44	1.35	0.26	1.36	0.22	0.906	0.04	0.14	1.41	0.25	1.37	0.24	0.707	−0.15	0.41
L-Methionine	31.12	8.21	32.56	9.20	0.533	0.16	1.14	28.30	7.76	33.13	9.13	0.118	0.54	1.51	34.66	7.34	31.79	9.25	0.408	−0.33	1.19
Lysine	219.93	47.31	238.42	50.82	0.155	0.37	1.28	207.10	49.62	244.42	48.46	0.038	0.71	1.54	235.98	38.66	230.42	52.76	0.771	−0.12	1.03
Myoinositol	12.74	1.82	13.08	2.17	0.525	0.17	0.79	12.79	2.05	13.45	2.51	0.422	0.28	1.16	12.68	1.47	12.58	1.47	0.860	−0.07	0.20
NAD	0.0048	0.0017	0.0055	0.0024	0.196	0.34	0.98	0.0049	0.0018	0.0056	0.0028	0.435	0.28	0.60	0.0046	0.0015	0.0053	0.0014	0.220	0.49	1.05
Neopterin	0.0034	0.0015	0.0035	0.0022	0.818	0.06	0.96	0.0037	0.0015	0.0039	0.0025	0.818	0.08	0.37	0.0029	0.0014	0.0029	0.0015	0.988	0.01	0.04
Niacinamide	0.161	0.112	0.176	0.104	0.601	0.14	0.57	0.166	0.140	0.169	0.109	0.933	0.03	0.60	0.156	0.059	0.185	0.097	0.388	0.35	0.81
Nicotinic acid	0.624	0.252	0.655	0.183	0.591	0.14	0.56	0.530	0.234	0.620	0.189	0.236	0.42	0.97	0.741	0.222	0.701	0.162	0.606	−0.21	0.54
Normetanephrine	0.0007	0.0003	0.0007	0.0003	0.841	0.05	0.35	0.0007	0.0004	0.0007	0.0003	0.478	−0.25	0.50	0.0006	0.0002	0.0007	0.0003	0.200	0.51	1.11
Octanoylcarnitine	0.113	0.105	0.111	0.110	0.951	−0.02	0.66	0.131	0.131	0.097	0.131	0.473	−0.25	0.90	0.090	0.047	0.129	0.067	0.113	0.63	1.48
Ornithine	54.00	13.78	55.95	15.39	0.611	0.13	0.96	51.27	15.85	54.63	16.58	0.561	0.21	1.04	57.41	9.61	57.72	13.43	0.949	0.03	0.81
Orotic acid	2.22	0.43	2.45	0.85	0.209	0.33	1.11	2.15	0.34	2.39	0.80	0.301	0.36	0.81	2.31	0.52	2.53	0.91	0.460	0.30	0.63
Pantothenic acid	0.280	0.109	0.298	0.114	0.545	0.16	0.45	0.271	0.102	0.299	0.116	0.487	0.25	0.51	0.292	0.117	0.297	0.111	0.900	0.05	0.18
Phenylalanine	64.91	12.93	69.81	12.41	0.142	0.38	1.32	62.83	13.79	70.88	10.91	0.070	0.63	1.29	67.51	11.24	68.38	14.04	0.868	0.07	1.04
Phosphoethanolamine	2.09	0.56	2.15	0.71	0.700	0.10	0.36	2.08	0.56	2.08	0.70	0.979	−0.01	0.32	2.09	0.56	2.25	0.73	0.548	0.24	0.73
Proline	242.34	77.93	230.49	76.46	0.557	−0.15	1.07	222.40	84.33	222.15	76.47	0.993	0.00	1.21	267.27	60.47	241.62	75.00	0.364	−0.37	1.17
Propionylcarnitine	0.388	0.126	0.388	0.120	0.994	0.00	0.52	0.381	0.128	0.380	0.121	0.980	−0.01	0.41	0.396	0.122	0.399	0.118	0.957	0.02	0.60
Pyridoxine	0.0023	0.0004	0.0025	0.0005	0.307	0.27	0.78	0.0023	0.0004	0.0024	0.0006	0.542	0.22	0.61	0.0024	0.0003	0.0025	0.0002	0.309	0.41	0.85
Serine	144.54	30.98	135.21	27.02	0.218	−0.32	1.19	140.29	28.06	140.96	28.24	0.946	0.02	0.64	149.86	33.53	127.55	23.20	0.061	−0.73	1.58
Sorbitol	46.74	51.99	63.79	86.84	0.378	0.23	1.07	66.70	62.03	59.58	93.75	0.806	−0.09	0.50	21.78	12.31	69.40	76.31	0.050	0.77	1.91
Spermidine	0.162	0.040	0.154	0.065	0.588	−0.14	1.12	0.157	0.046	0.137	0.073	0.379	−0.31	0.72	0.168	0.030	0.176	0.045	0.610	0.21	0.49
Succinate	8.96	2.33	7.46	1.92	**0.008**	**−0.67**	**2.01**	9.24	2.37	7.62	2.18	0.050	−0.68	1.63	8.60	2.22	7.24	1.48	0.078	−0.69	1.46
Sucrose	0.904	0.359	0.828	0.413	0.459	−0.19	0.55	0.919	0.382	0.755	0.403	0.243	−0.41	0.84	0.885	0.328	0.926	0.406	0.786	0.11	0.24
Symmetric dimethylarginine	3.60	0.89	3.38	0.68	0.299	−0.27	0.83	3.58	0.77	3.25	0.63	0.191	−0.46	0.95	3.62	1.02	3.56	0.70	0.864	−0.07	0.15
Taurine	207.30	77.22	240.39	177.59	0.377	0.23	0.67	219.22	83.03	201.83	95.72	0.589	−0.19	0.38	192.39	66.32	291.81	238.22	0.189	0.52	1.18
Taurochenodeoxycholic acid	0.447	0.422	0.557	0.515	0.377	0.23	0.79	0.444	0.426	0.677	0.593	0.220	0.43	0.86	0.450	0.418	0.398	0.324	0.731	−0.14	0.33
Taurocholic acid	0.367	0.150	0.349	0.114	0.584	−0.14	0.66	0.398	0.187	0.337	0.116	0.259	−0.40	0.81	0.329	0.067	0.364	0.109	0.358	0.37	1.23
Threonine	124.62	30.11	129.66	39.85	0.592	0.14	0.63	121.26	32.01	136.07	43.76	0.290	0.37	0.74	128.81	26.97	121.12	31.99	0.528	−0.26	0.87
Trimethylamine-N-oxide	2.25	1.50	3.71	5.80	0.215	0.32	0.91	2.01	1.21	4.42	7.37	0.232	0.42	0.84	2.55	1.74	2.76	2.12	0.793	0.11	0.43
Tryptophan	44.16	5.94	45.16	5.76	0.510	0.17	0.93	42.88	6.53	46.68	5.28	0.074	0.62	1.31	45.75	4.64	43.15	5.74	0.231	−0.48	1.06
Tyrosine	58.30	14.60	64.15	17.58	0.174	0.35	1.34	58.27	16.89	66.86	18.96	0.186	0.46	1.59	58.35	11.10	60.55	14.80	0.684	0.17	1.21
UDP-glucose	1.90	0.47	1.71	0.74	0.272	−0.29	0.95	1.78	0.50	1.60	0.90	0.483	−0.25	0.53	2.04	0.39	1.87	0.40	0.297	−0.42	1.03
Uracil	0.260	0.187	0.244	0.200	0.747	−0.08	0.65	0.276	0.226	0.213	0.232	0.440	−0.27	0.86	0.241	0.118	0.285	0.135	0.396	0.34	1.31
Valine	432.47	131.67	442.31	130.02	0.773	0.08	0.67	391.89	146.32	414.33	99.11	0.603	0.18	0.55	483.19	87.26	479.62	154.63	0.946	−0.03	0.74
Xanthine	1.504	0.500	1.552	0.985	0.823	0.06	0.41	1.50	0.55	1.70	1.24	0.567	0.20	0.77	1.51	0.43	1.35	0.36	0.319	−0.40	0.83
Xanthosine	0.057	0.017	0.063	0.022	0.206	0.33	0.96	0.057	0.018	0.064	0.022	0.315	0.35	0.77	0.056	0.016	0.062	0.023	0.467	0.29	0.72

d, Cohen's *d* effect size; *p*, *p*-value; PLS-DA, partial least squares discriminant analysis; SD, standard deviation; VIP, variable importance in the projection; *, significant at the adjusted α level of 0.0013; Compounds in bold were used in the correlation analyses.

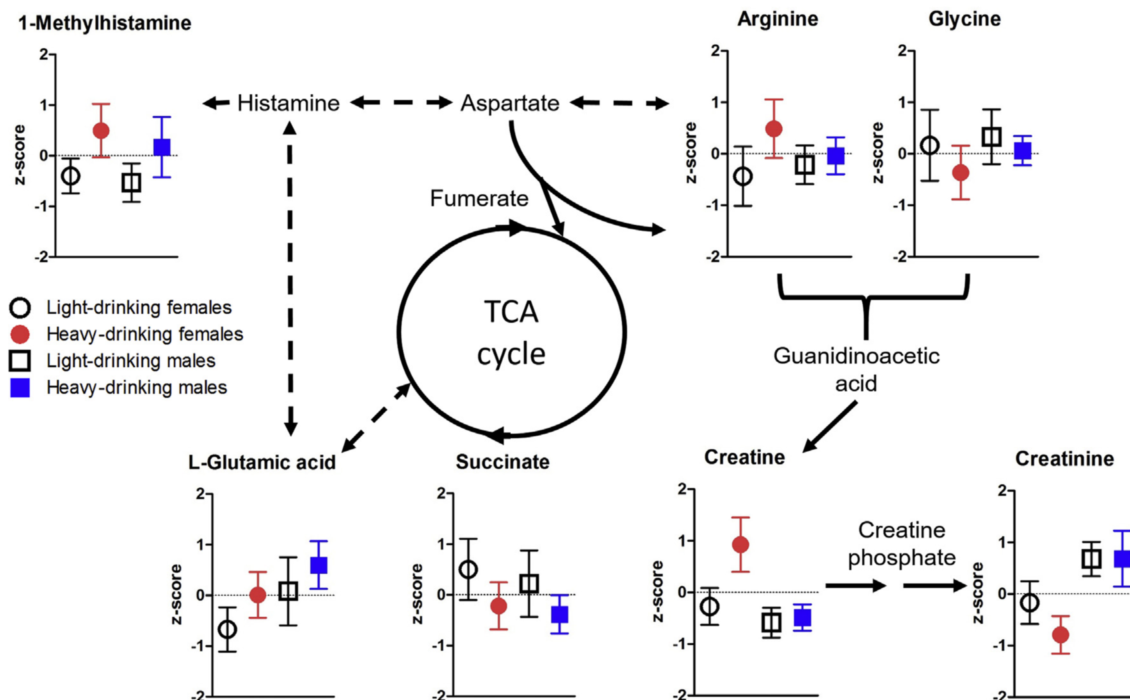

Fig. 1. Simplified metabolic pathway describing key findings from the metabolic profiling analysis. Moderate-to-heavy use of alcohol is associated with alterations in amino acid, energy, and creatine metabolism in young adults. Results are presented as mean z scores with 95% confidence intervals. Metabolite concentrations in serum are shown for moderate-to-heavy drinking and light-drinking participants (colored and white symbols, respectively), separately for females and males (circles and squares, respectively). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

creatine ($r = -0.582$, $p < 0.0001$, Fig. 2) and 1-methylhistamine ($r = -0.346$, $p = 0.0059$, Fig. 2). Considering genders separately, these correlations were significant in females ($r = -0.52$, $p = 0.0012$; and $r = -0.48$, $p = 0.0038$; respectively) but not in males ($r = -0.25$, $p = 0.216$; and $r = -0.20$, $p = 0.316$, respectively).

Discussion

The present results from metabolic profile analysis indicate that moderate-to-heavy alcohol consumption in young adults is associated with alterations in the amino acid and energy metabolism (Fig. 1, Table 1), which is in agreement with previous reports from humans (Jaremek et al., 2013; Lehikoinen et al., 2018; Wurtz et al., 2016) and animal models (Meinhardt et al., 2015). Before adjusting for multiple testing, elevated concentrations of 1-methylhistamine and L-glutamic acid, as well as lower succinate concentrations, were observed in both female and male moderate-to-heavy drinkers when compared to the light-drinking controls, indicating that moderate-to-heavy alcohol consumption is associated with common alterations in amino acid and energy metabolism in young people. Elevated concentrations of arginine and creatine compared to controls were only observed in the moderate-to-heavy drinking females, suggesting that changes in urea and creatine metabolism are more pronounced in females (Fig. 1). However, after adjusting for multiple testing, only elevated 1-methylhistamine concentrations remained as a statistically significant difference between the study groups. Furthermore, inverse correlations were recorded between brain gray matter volume and serum concentrations of 1-methylhistamine and creatine only in females (Fig. 2).

Elevated serum 1-methylhistamine concentrations could be a proxy for neuroinflammatory processes in the brain, which could explain our findings of a negative correlation between 1-methylhistamine concentrations and gray matter volume (Fig. 2). Serum 1-methylhistamine concentrations correlate with histamine release in the brain, because histamine is inactivated in the brain by histamine 1-methyltransferase (EC 2.1.1.8) and converted into 1-methylhistamine, in contrast to peripheral organs, where oxidation by diamino-oxidase is more common (Haas & Panula, 2003; Lintunen et al., 2001). Therefore, the elevated concentrations of 1-methylhistamine in the serum samples of moderate-to-heavy drinking subjects could be due to increased histamine concentration in the brain. This is in agreement with previous reports of elevated concentrations of both histamine and 1-methylhistamine in post mortem samples of cirrhotic alcoholics who died in hepatic coma and in alcohol-preferring rats (Lintunen et al., 2001; Lozeva, Tuomisto, Tarhanen, & Butterworth, 2003). Alcohol has been found to have direct effects on the brain histamine concentration by modulating histamine metabolism (Zimatkin & Anichtchik, 1999). Histamine release in the brain is part of the neuroimmune response, which has been considered to play an important part in alcohol-induced brain damage (Crews & Vetreno, 2014). Moreover, histamine modulates the function of brain systems (e.g. dopamine and glutamate systems) that are important for behaviors related to alcohol consumption (e.g., impulse control) (Jin & Panula, 2005; Volkow, Wang, Fowler, & Tomasi, 2012). In fact, antagonism of the histamine system has been proposed as a new treatment option to reduce alcohol consumption (Nuutinen, Vanhanen, Maki, & Panula, 2012). Overall, the present results of elevated 1-methylhistamine concentrations in moderate-to-heavy drinking young adults could be associated with priming of the brain toward the development of alcohol use disorder in later life (DeWit et al., 2000).

In the present study, serum creatine concentrations were higher in the moderate-to-heavy drinking females when compared to controls, and creatine concentrations were inversely correlated with the brain gray matter volume in females (Fig. 2, Table 1). Creatine kinase uses creatine to form phosphocreatine, which is used to form adenosine triphosphate (ATP) in organs with high energy demands (Joncquel-Chevalier Curt, Voicu, Fontaine, & et al.,

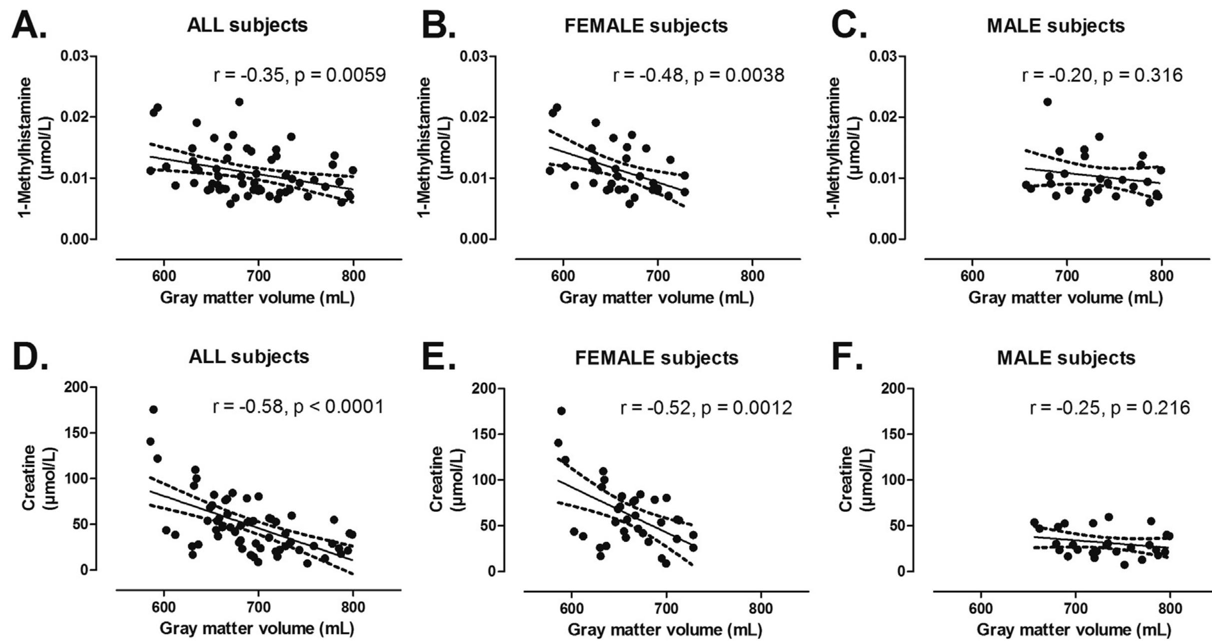

Fig. 2. Correlations between 1-methylhistamine, creatine, and brain gray matter volume. When comparing data from all study subjects, significant correlations were observed between gray matter volume and serum 1-methylhistamine concentrations (A), and gray matter volume and serum creatine concentrations (D). When considering genders separately, these correlations were robust in females, as shown in B and E. In male subjects, these correlations were not significant (C and F).

2015). Creatine is synthesized from glycine and arginine via the synthesis of guanidinoacetate by arginine–glycine amidinotransferase (EC:2.1.4.1), followed by methylation of guanidinoacetate by guanidinoacetate N-methyltransferase (EC:2.1.1.2). The elevated concentrations of creatine could be due to the increased synthesis and/or reduced uptake of creatine. Altered creatine synthesis is supported by the notion that moderate-to-heavy drinking females also had a trend toward higher concentrations of arginine and lower concentrations of glycine (Fig. 1). Elevated amounts of glutamate and creatine have previously been reported in the brains of a heterogenic group of participants with varying alcohol use from heavy use to diagnosed AUD in a magnetic resonance spectroscopy study (Yeo et al., 2013). Lower concentrations of creatine in the brain, measured with magnetic resonance spectroscopy, have been thought to be linked to neuronal damage in patients with a diagnosed alcohol use disorder, and it has therefore also been hypothesized that an elevated concentration of creatine could be an adaptive mechanism to alcohol consumption in recreational and binge-drinking individuals (Tunc-Skarka, Weber-Fahr, & Ende, 2015). Our results corroborate those of Tunc-Skarka et al.'s. The inverse correlation between serum creatine concentrations and brain gray matter volumes in females could be due to alcohol-induced changes in energy metabolism (Figs. 1 & 2).

Additionally, in the present study, we observed a trend of higher concentrations of L-glutamic acid in the moderate-to-heavy drinking young adults when compared to controls (Table 1, Fig. 1). These results are in agreement with previous research showing that chronic alcohol consumption is associated with elevated glutamate concentrations in serum and cerebrospinal fluid, and with alterations in the glutamatergic system in the brain (Holmes, Spanagel, & Krystal, 2013; Kärkkäinen et al., 2013; Laukkanen et al., 2015; Lehikoinen et al., 2018). High baseline serum glutamate concentrations have also been considered as a possible biomarker for the efficacy of acamprosate in the treatment of subjects with alcohol use disorder (Nam et al., 2015). Elevated glutamate concentrations are thought to cause cell apoptosis, swelling, and death by the mechanism of excitotoxicity (Leibowitz, Boyko, Shapira, & Zlotnik, 2012). However, in the present study, we did not observe a significant correlation between serum glutamate concentrations and gray matter volume (Table 1). This could be due to the statistical power of the present analysis, which only enabled the detection of large effects.

Reduced glutamine concentrations have been associated with alcohol consumption (Lehikoinen et al., 2018; Würtz et al., 2016). Furthermore, the glutamate/glutamine ratio has also been suggested as the biomarker for alcohol-caused liver injury (Harada et al., 2016). In the present study, although we did not observe a significant difference between all moderate-to-heavy and light-drinking young adults ($p = 0.233$, $d = -0.31$), we did observe a trend toward lower concentrations in moderate-to-heavy drinking males compared to light-drinking young adults ($p = 0.040$, $d = 0.80$), but not in females ($p = 0.696$, $d = -0.14$; Fig. 1; Table 1). This could be due to sex-related differences, e.g. in the concentrations of gonadal steroids, as well as exposure-related issues such as the time and amount of alcohol consumed because glutamine concentrations appear to decrease in a relatively linear manner in relation to the amount consumed (Würtz et al., 2016).

The trend toward lower succinate concentrations in the moderate-to-heavy drinking participants could be due to alcohol-induced alterations in mitochondrial function, glycolysis, and pentose phosphate pathways (Meinhardt et al., 2015). Furthermore, altered function of the closely related urea cycle, and arginine metabolism in particular, have also previously been reported in abstinent alcoholics possibly reflecting permanent liver damage (Hasselblatt et al., 2006). Moreover, 2-aminoisobutyric acid is a product of pyrimidine metabolism (Crumpler, Dent, Harris, & Westall, 1951). Therefore, the trend toward elevated concentrations of 2-aminoisobutyric acid in the moderate-to-heavy drinking participants could be due to increased metabolism of pyrimidines, possibly reflecting changes seen in the components of amino acid and energy metabolism discussed above. Finally, aminoadipic acid is a gliatoxic agent and can influence, for example, GABAergic function, and could therefore influence alcohol-induced neuropathology (Park, Jo, Zheng, Patel, & Stern, 2009). Interestingly, our

laboratory detected altered GABAergic activity in the brains of the same group of moderate-to-heavy drinking participants in an earlier study (Kaarre et al., 2018).

In the present study, moderate-to-heavy drinking subjects had higher concentrations of the nicotine metabolite cotinine (Table 1). Many of the moderate-to-heavy drinking subjects (16 out of 35) used tobacco, while only 2 of the 27 light-drinking controls used tobacco. This is a common limitation of clinical studies on alcohol use, since most persons with heavy alcohol consumption also use tobacco (Kalman, Morissette, & George, 2005). Therefore, some of the changes seen in the metabolic profiles of the moderate-to-heavy drinking subjects could be due to the use of tobacco or the combined effect of alcohol and tobacco use. It has been determined in animal models that some components of tobacco smoke (e.g., nicotine-derived nitrosamine ketone) can influence alcohol-induced neuropathology, especially in the white matter of pre-frontal parts of the brain (Tong et al., 2015).

In the present study we measured metabolite concentrations only in the periphery. This can be considered a limitation, as there is variation between the metabolites on how well serum concentrations reflect the brain concentrations. For some metabolites, these associations are more linear. For example, 1-methylhistamine is mainly produced in the brain, but its serum concentration has been considered to reflect the concentration in the brain (Haas & Panula, 2003; Lintunen et al., 2001). For some other metabolites, which are mainly produced in peripheral organs, such as creatine (Joncquel-Chevalier Curt et al., 2015), the connection between serum and brain metabolite concentrations is more complex. However, even in these cases peripheral changes in metabolite concentrations can serve as important biomarkers. For example, there is preliminary evidence that serum metabolite concentrations could be used to predict acamprosate treatment outcomes although the mechanisms of action are based on the central neurotransmitter affinity (Hinton et al., 2017; Nam et al., 2015).

Other limitations of the present study include the relatively low number of subjects for the metabolic profiling analysis. However, in the MRI analysis, the groups were reasonably sized. In practice, this means that we were only able to observe differences between the moderate-to-heavy drinking subjects and light-drinking controls with a large effects size. Because of the limited sample size, we did not investigate correlations between alcohol use associated changes in specific brain regions (Heikkinen et al., 2017) and metabolite concentrations, which should be done in future studies with larger cohorts. Moreover, the family history of the participants was not recorded. Some of the metabolic and/or brain structural differences could be due to hereditary factors. Although there was no significant difference between the subjects and controls in BMI, recording other possible co-factors such as exercise routines, sleep patterns and stress levels of the participants would have strengthened the results of the study. The strengths of the study include the monitoring of alcohol consumption by the participants during adolescence, as well as the collection of venous blood samples following overnight fasting, thus controlling for the effects of diet on metabolites with a rapid conversion rate. Both 1-methylhistamine and creatine have a half-life of 3 h or less (Belic et al., 1999; Deldicque et al., 2008).

In conclusion, the moderate-to-heavy drinking young adults, who had also been drinking for at least 10 years during their adolescence, had an altered metabolic profile when compared to light-drinking controls. We observed significant inverse correlations between brain gray matter volume and serum concentrations of 1-methylhistamine and creatine in young females. This finding may reflect the neurotoxic and neuromodulatory effects of alcohol on the brain. Furthermore, moderate-to-heavy alcohol consumption appears to have a larger effect on the metabolic profiles and brain gray matter volumes of female subjects when compared to males. Further research is warranted to explain the mechanism and possible long-term consequences of these metabolic and structural changes. Future research should look into the possibility that changes in serum metabolic profiles (in combination with measures of alcohol consumption such as AUDIT) could be used to identify young adults at risk of developing alcohol-induced decreases in brain gray matter.

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