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Medical Conditions Linked to Atherosclerosis Are Associated With Magnified Cortical Thinning in Individuals With Alcohol Use Disorders

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Abstract

Aims: Magnetic resonance imaging (MRI) studies report widespread cortical thinning in individuals with alcohol use disorder (AUD), but did not consider potential effects of pro-atherogenic conditions such as hypertension, type 2 diabetes mellitus, hepatitis C seropositivity and hyperlipidemia on cortical thickness. The conditions are associated with regional cortical thinning in those without AUD. We predicted that individuals with concurrent AUD and pro-atherogenic conditions demonstrate the greatest regional cortical thinning in areas most vulnerable to decreased perfusion.
Methods: Treatment-seeking individuals with AUD ($n = 126$) and healthy controls (CON; $n = 49$) completed a 1.5 T MRI study. Regional cortical thickness was quantitated via FreeSurfer. Individuals with AUD and pro-atherogenic conditions (Atherogenic+), AUD without pro-atherogenic conditions (Atherogenic−) and CON were compared on regional cortical thickness.
Results: Individuals with AUD showed significant bilateral cortical thinning compared to CON, but Atherogenic+ demonstrated the most widespread and greatest magnitude of regional thinning, while Atherogenic− had reduced thickness primarily in anterior frontal and posterior parietal lobes. Atherogenic+ also showed a thinner cortex than Atherogenic− in lateral orbitofrontal and dorso/dorsolateral frontal cortex, mesial and lateral temporal and inferior parietal regions.
Conclusions: Our results demonstrate significant bilateral cortical thinning in individuals with AUD relative to CON, but the distribution and magnitude were influenced by comorbid pro-atherogenic conditions. The magnitude of cortical thinning in Atherogenic+ strongly corresponded to cortical watershed areas susceptible to decreased perfusion, which may result in morphometric abnormalities. The findings indicate that pro-atherogenic conditions may contribute to cortical thinning in those seeking treatment for AUD.

INTRODUCTION

Hypertension, type 2 diabetes mellitus, hepatitis C seropositivity and hyperlipidemia are common biomedical comorbidities in those with an alcohol use disorder (AUD), and individuals often concurrently experience two or more of the foregoing conditions (Mertens et al., 2005; Satre et al., 2007; Durazzo and Meyerhoff, 2017; Sullivan et al., 2018). The foregoing conditions are associated with abnormalities in brain morphometrics. In those without AUD, hypertension (Alosco et al., 2014), type 2 diabetes mellitus (Chen et al., 2017; Li

et al., 2018) and hepatitis C seropositivity (Hjerrild et al., 2016) are linked to significant reductions in cerebral cortical thickness. Hypertension is related to frontal cortical thinning and type 2 diabetes mellitus with cingulate and temporal thinning, whereas hepatitis C seropositivity is associated more prominently with anterior frontal and occipital thinning. The collective effects of these biomedical conditions on cortical thickness appear widespread in those without an AUD.

Additionally, hypertension, type 2 diabetes mellitus, hepatitis C seropositivity and hyperlipidemia are strongly associated with markedly increased risk for atherosclerosis (Adinolfi et al., 2014; Hurtubise et al., 2016). Cerebral atherosclerosis is robustly implicated in changes in arterial vasoreactivity and lumen viability that may lead to alterations in perfusion (Hurtubise et al., 2016). Decreased perfusion is associated with cortical thinning in those with hypertension and other cardiovascular diseases, and it is suggested as a mechanism promoting regional cortical thinning in these conditions (Alosco et al., 2013; Alosco et al., 2014). The paracentral lobule; precentral, middle and inferior frontal gyri; insula; and lateral temporal cortical regions appear to be particularly vulnerable to decreased perfusion secondary to compromised cerebrovascular integrity (see Payabvash et al., 2011; Durazzo et al., 2015 and references therein).

Several well-designed and executed studies have reported widespread cortical thinning in those with an AUD; however, these reports did not consider or assess for the effects of the pro-atherogenic conditions of hypertension, type 2 diabetes mellitus, hepatitis C seropositivity and hyperlipidemia on cortical thickness (Fortier et al., 2011; Bae et al., 2016; Wang et al., 2016; Uhlmann et al., 2019) or appeared to exclude participants for these conditions (Momenan et al., 2012; Grodin et al., 2017; Rolland et al., 2019; Tomasi et al., 2019). In our previous cross-sectional studies with this AUD cohort (Durazzo et al., 2011b; Durazzo et al., 2013), with a significantly smaller sample than the current study, medical conditions, including pro-atherogenic diseases, were not significantly associated with cortical thickness after correcting for multiple comparisons. Therefore, the associations of these common pro-atherogenic conditions with cortical thickness in individuals with an AUD are not clear.

Cortical thickness is purported to reflect the number and density of cells in a column (Rakic, 1988, 2008) and/or characterize the neuronal cell body size, the number of spines and synapses and the degree of myelination (Eickhoff et al., 2005; Fjell and Walhovd, 2010). Cortical thickness is phenotypically and genetically distinct from cortical surface area and volume measures (Panizzon et al., 2009; Kremen et al., 2010; Winkler et al., 2010). Cortical thickness may show greater sensitivity to neurodegenerative processes from various etiologies than cortical volumes (Hutton *et al.* 2009); consequently, this morphometric may increase the ability to detect more subtle structural abnormalities than volumes or density measures, and/or be differentially affected by alcohol/substance use disorders and associated comorbid conditions, compared to volume and surface area measures of the same brain regions (Durazzo et al., 2011b; Wang et al., 2016). Given the cerebral cortex is primarily composed of neuronal and glial tissues [i.e. astrocytes, oligodendrocytes and microglia (Pelvig et al., 2008)], and the ratio of glial cells to neurons is ~0.7:1 (von Bartheld, 2018), cortical thickness is a putative surrogate marker of the integrity of both cell types. Taken together, cortical thickness may serve as a proxy for regional cortical cytoarchitecture integrity (Makris et al., 2008; Durazzo et al., 2013).

Atherogenic cardiovascular disease and excessive alcohol consumption are associated with increased risk for major neurocognitive disorders (i.e. dementia) (Durazzo et al., 2014; Schwarzinger et al., 2018). The precise mechanism(s) by which heavy alcohol consumption promotes elevated dementia risk is not established, but regional brain atrophy is suggested as a contributing factor (Rehm et al., 2019). Given the widespread cortical thinning is observed in those with mild cognitive impairment (MCI) and Alzheimer disease (AD), particularly in inferior and mesial temporal and posterior parietal regions (Greene and Killiany, 2010; Li et al., 2011), better understanding the association of pro-atherogenic conditions in AUD with regional cortical thickness may assist in identifying biological mechanisms contributing to the increased risk of dementia in AUD.

The goal of this study was to assess the associations of combined effects of the pro-atherogenic conditions of hypertension, type 2 diabetes mellitus, hepatitis C seropositivity and hyperlipidemia on cortical thickness in those seeking treatment for AUD, in a considerably larger sample size than our previous research.

We hypothesize that:

1) Relative to healthy non-smoking controls (CON), individuals with AUD and the pro-atherogenic conditions of active hypertension, type 2 diabetes mellitus, hepatitis C seropositivity and/or hyperlipidemia (Atherogenic+) demonstrate widespread bilateral cortical thinning across all regions of interest.
2) Relative CON individuals with AUD, and negative for pro-atherogenic conditions (Atherogenic−), demonstrate bilateral cortical thinning across all regions of interest, but the magnitude is lower than Atherogenic+.
3) Based on the above review on cortical thickness in pro-atherogenic conditions and regions vulnerable to decreased perfusion, we predicted Atherogenic+ demonstrate the greatest thinning in bilateral superior middle and inferior frontal gyri, lateral temporal cortex and insula and inferior parietal cortex and supramarginal gyri compared to Atherogenic− and CON.
4) In AUD participants, the frequency of former and active cigarette smokers will be higher in Atherogenic+ compared to Atherogenic−.

Participants

AUD participants ($n = 126$) were recruited from the San Francisco VA Medical Center (SFVAMC) Substance Abuse Day Hospital and the San Francisco Kaiser Permanente Chemical Dependence Recovery outpatient treatment clinics. All AUD participants were actively in treatment at the time of study, and treatment duration was typically 14–35 days [for details on the treatment programs, see Durazzo et al., 2008]. Twenty individuals completed a SFVAMC-sponsored 14–21-day residential treatment program prior to entering the San Francisco VA outpatient program. CON ($n = 49$) were recruited from the local community. Participants were between 28 and 71 years of age and provided written informed consent prior to engaging in study procedures. Study procedures were approved by the University of California San Francisco and the SFVAMC and were in accordance with the Declaration of Helsinki.

Inclusion/exclusion criteria. Inclusion criteria for the AUD participants were fluency in English, DSM-IV diagnosis of alcohol dependence or abuse (all met criteria for dependence), average consumption of >150 standard alcohol-containing drinks (i.e. ~14 grams of pure ethanol) per month for at least 8 years prior to enrollment

for males and average consumption of >80 drinks per month for at least 6 years prior to enrollment for females. See Table 1 for group demographic and clinical data. AUD participant exclusion criteria were history of the following: dependence on any substance except nicotine in the 5 years immediately prior to study, any intravenous drug use in the 5 years prior to study, opioid agonist/replacement therapy, intrinsic cerebral masses, HIV seropositivity, cerebrovascular accident, arteriovenous and cavernous malformations, myocardial infarction, cerebral aneurysm, type 1 diabetes or use of insulin, COPD, non-alcohol-related seizures, significant exposure to established neurotoxins, Wernicke-Korsakoff syndrome, current delirium, demyelinating and neurodegenerative diseases, penetrating head injury or head injury resulting in loss of consciousness >10 minutes. Psychiatric exclusion criteria were history of bipolar disorder, cyclothymia, schizophrenia-spectrum disorders, obsessive–compulsive disorder, panic disorder and PTSD. Unipolar mood disorders (i.e. major depression, substance-induced mood disorder) were allowed, given their high prevalence in AUD (Grant et al., 2015). CON and AUD never smokers were lifetime never/non-smokers (i.e. never smoked cigarettes/tobacco products or consumed <40 cigarettes over lifetime, with no tobacco use within 10 years of study). CON were screened for any biomedical and psychiatric conditions known or suspected to influence brain neurobiology and neurocognition, including hypertension, diabetes, hepatitis C seropositivity and hyperlipidemia. Participants were breathalyzed and urine-tested for illicit substances before assessment, and no participant tested positive for substances at any assessment.

Clinical assessment

All participants completed the Structured Clinical Interview for DSM-IV Axis I Disorders, Version 2.0 (SCID-I/P) and semi-structured interviews for assessment of lifetime alcohol consumption (Lifetime Drinking History, LDH) and substance use (structured, in-house questionnaire assessing substance type and quantity and frequency of use). The average number of alcohol-containing drinks/month over 1 year prior to enrollment and average number of drinks/month over lifetime were calculated from the LDH. Participants also completed standardized self-report questionnaires assessing anxiety (State-Trait Anxiety Inventory, Trait form Y-2, STAI) and depressive (Beck Depression Inventory, BDI) symptomatology, as well as nicotine dependence by the Fagerstrom Test for Nicotine Dependence (FTND) (Pennington et al., 2013) for corresponding references to the above measures.

AUD participants who had medical record-verified pro-atherogenic conditions of hypertension, type 2 diabetes mellitus, hepatitis C virus antigen seropositivity and/or hyperlipidemia were assigned to the Atherogenic+ group ($n = 68$), and those without the above conditions were assigned to the Atherogenic− group ($n = 58$). No AUD participant had other biomedical conditions associated with atherosclerosis, other forms of vascular disease or conditions known or suspected to influence brain neurobiology. AUD participants seropositive for hepatitis C were not prescribed medications to manage active symptomatology at the time of the study. One AUD participant with type 2 diabetes was taking a sulfonylurea medication (glipizide). No AUD participant was seropositive for hepatitis B. AUD participants were considered positive for substance use disorder comorbidity if DSM-IV criteria were met for current or lifetime substance abuse or past dependence (>5 years prior to enrollment); most AUD participants with a comorbid substance use disorder met the criteria for past cocaine or methamphetamine abuse/dependence. AUD participants were considered positive for a psychiatric comorbidity if current or lifetime criteria for a unipolar mood or anxiety disorder were met, and most met the criteria for recurrent major depressive disorder. Seated blood pressure was obtained by medical staff, via automated sphygmomanometer, within 3 days of magnetic resonance scan for all AUD. The mean arterial pressure (diastolic BP + systolic/3) was calculated (Zahr et al., 2013). All AUD participants reported they were taking medications as prescribed by their health care providers.

Laboratory tests

Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma-glutamyltransferase (GGT) were obtained for AUD participants within 3 days of the magnetic resonance study to evaluate alcohol-related or other hepatocellular injury and serum prealbumin as indicator of nutritional status (Weinrebe *et al.*, 2002).

Magnetic resonance acquisition and processing

A volumetric magnetization-prepared rapid gradient-echo (MPRAGE) was acquired at 1.5 T with TR/TE/TI = 9.7/4/300 ms, 15° flip angle, 1×1 mm^2 in-plane resolution and 1.5-mm-thick coronal partitions oriented perpendicular to the main long axes of bilateral hippocampi as seen on sagittal scouts. See Gazdzinski and colleagues (Gazdzinski et al., 2005) for detailed MR acquisition methods. FreeSurfer (v4.5) volumetric segmentation and cortical surface reconstruction methods were used to obtain regional measures of cortical thickness (mm). Spatial normalization to the cortical surface template allowed automatic parcellation of the surface into 34 anatomical regions of interest per cortical hemisphere (Fischl *et al.*, 2004). Average cortical thickness was obtained for all 34 bilateral cortical regions and intracranial volume. Full FreeSurfer image processing details were previously described (Durazzo et al., 2011a; Durazzo et al., 2011b).

Statistical analyses

Participant characteristics. Comparisons among AUD subgroups and CON on clinical and demographic variables were conducted with multivariate analysis of variance, chi-square and Fisher's exact test, where appropriate. P-values < 0.05 were considered statistically significant for these comparisons.

Group comparisons of Atherogenic+, Atherogenic− and CON on regional cortical thickness. Comparisons between Atherogenic−, Atherogenic+ and CON were conducted with generalized linear modeling for each of the 34 cortical regions of interest. In preliminary analyses, the findings for group comparisons were highly congruent for left and right hemisphere regions of interests; therefore, the thickness values for each of the 34 cortical regions of interest represent the arithmetic average of the left and right hemisphere. In these comparisons, group (Atherogenic−, Atherogenic+ and CON) estimated intracranial volume (ICV), age, GGT and body mass index (BMI) served as predictors. The significant main effects for group ($P < 0.05$) were followed up with pairwise t-tests. In pairwise comparisons between Atherogenic− and Atherogenic+, in addition to age, GGT and BMI, lifetime average number of drinks/month, 1-year-average drinks/month, smoking status (never, former and active smoker) and ICV were also separately entered as covariates. In exploratory analyses, we tested for interactions between smoking status and alcohol consumption variables (i.e. lifetime average

Table 1. Group demographics and clinical variables

Measure	CON ($n = 49$)	Athero− ($n = 58$)	Athero+ ($n = 68$)	Group comparisons*
Age (years)	46 (9)	53 (9)	51 (8)	CON < Athero−, Athero+
Education (years)	16 (3)	14 (2)	14 (2)	CON > Athero−, Athero+
Males (%)	94	98	92	
White (%)	74	79	68	
Days abstinent	NA	33 (9)	33 (10)	
1-year-average drinks/month	13 (14)	415 (207)	414 (245)	CON < Athero−, Athero+
Lifetime average drinks/month	14 (16)	240 (121)	212 (138)	CON < Athero−, Athero+
Hypertension (%)	NA	NA	65	
Systolic blood pressure	NA	121 (6)	128 (9)	Athero− < Athero+
Diastolic blood pressure	NA	80 (4)	86 (7)	Athero− < Athero+
Mean arterial pressure	NA	94 (5)	100 (7)	Athero− < Athero+
Hepatitis C seropositivity (%)	NA	NA	35	
Hyperlipidemia (%)	NA	NA	19	
Type 2 diabetes (%)	NA	NA	5	
Body mass index	25 (4)	26 (4)	28 (5)	CON, Athero− < Athero+
Two or more concurrent medical conditions (%)	NA	NA	24	
Antihypertensive medication type (%):				
• Diuretic			62	
• Beta-blocker			29	
• Two or more antihypertensive medications	NA	NA	35	
Statin medication use (%)	NA	NA	12	
Never smoker (%)	NA	29	25	
Former smoker (%)	NA	9	13	
Active smoker (%)	NA	62	62	
FTND	NA	5 (2)	5 (2)	
Pack-years	NA	25 (18)		28 (18)
Gamma-glutamyltransferase (median; i.u.)	21	40	69	CON < Athero− < Athero+
Aspartate aminotransferase (median, i.u.)	25	28	30	
Alanine aminotransferase (median, i.u.)	23	32	30	
Prealbumin (mg/dl)	30 (5)	28 (7)	26 (8)	
Beck Depression Inventory	4 (3)	13 (9)	13 (9)	CON < Athero−, Athero+
STAI	33 (7)	47 (11)	45 (11)	CON < Athero−, Athero +
Any psychiatric comorbidity (%)	NA	40	41	
Mood disorders (%)	NA	38	40	
Substance use disorder comorbidity (%)	NA	24	27	
Antidepressant use (%)	NA	12	6	
Intracranial volume (cc)	1622 (200)	1577 (184)	1582 (170)	

Note: Mean (standard deviation), unless otherwise noted. Athero−, Atherogenic−; Athero+, Atherogenic +; CON, non-smoking light drinking controls; FTND, Fagerstrom Test for Nicotine Dependence; NA, not applicable; REL, relapsers; STAI, State-Trait Anxiety Inventory. *All listed group comparisons $P < 0.05$. Mean (SD). Gamma-glutamyltransferase, local normal range 7–64 institutional units (i.u.); aspartate aminotransferase, local normal range 5–35 i.u.; alanine aminotransferase, local normal range 7–56 i.u.; prealbumin local normal range 18–45 mg/dl.

and 1-year-average drinks/month) and AUD group (Atherogenic−, Atherogenic+); interactions were considered statistically significant at $P < 0.05$. Although we specified *a priori* predictions, we employed a modified Bonferroni procedure (Sankoh et al., 1997) adjusted significance level ($P = 0.05$) for pairwise comparisons (all two-tailed) for each region of interest. The adjusted significance level was based on the average intercorrelation among all 34 cortical regions of interest for all groups ($r = 0.56$) and the number of pairwise comparisons ($n = 3$) and produced an adjusted alpha $P = 0.01$ for pairwise comparisons among Atherogenic−, Atherogenic+ and CON. Effect sizes for mean group pairwise comparisons were calculated with Cohen's *d* (Cohen, 1988).

Associations between regional cortical thickness and antihypertensive and statin medication use in Atherogenic+. Associations of regional brain volumes with antihypertensive (yes vs. no, binary variable) and statin (yes vs. no, binary variable) medication use in Atherogenic+ were separately evaluated with multivariate multiple linear regression; smoking status (never, former and active smoker), age, GGT level and ICV were covariates. Associations were considered statistically significant at $P \leq 0.01$.

Associations between regional cortical thickness and blood pressure metrics in Atherogenic+ and Atherogenic−. Systolic, diastolic and mean arterial pressures were log10 transformed to address their skewed distributions, which resulted in acceptably symmetrical distributions for each measure. Associations of regional cortical thickness with log10 transformed systolic, diastolic, and mean arterial pressures were evaluated with multivariate multiple linear regression. AUD group (Atherogenic+, Atherogenic−) age, smoking status

(never, former and active smoker), GGT level and ICV served as covariates. Associations were considered statistically significant at $P \leq 0.01$.

RESULTS

Participant demographics and clinical measures

See Table 1 for group comparisons on demographic and clinical variables. Among the 24% of Atherogenic+ who had at least two pro-atherogenic conditions, 81% had concurrent hypertension and hepatitis C seropositivity. AUD active smokers had higher systolic, diastolic and mean arterial pressures than AUD never smokers (all $P < 0.02$), after adjustment for age and antihypertensive use. Among Atherogenic+ participants with hypertension, those taking antihypertensives had significantly lower systolic, diastolic and mean arterial pressures than those not using antihypertensive medications (all $P < 0.05$).

Regional cortical thickness comparisons of Atherogenic− and Atherogenic+ and CON

Group (Atherogenic−, Atherogenic+ and CON) was a significant predictor [$\chi^2_2 \geq 10.0$, $P \leq 0.009$] for all regions except the caudal anterior cingulate, transverse temporal, isthmus of cingulate gyri and pericalcarine region. Greater age [$\chi^2_1 \geq 5.0$, $P \leq 0.022$] was associated with a thinner cortex across groups in all regions except the pars orbitalis and middle temporal and superior temporal gyri. Greater ICV [$\chi^2_1 \geq 6.3$, $P \leq 0.011$] was related to thicker cortex only in the caudal and rostral anterior cingulate gyri, lateral orbitofrontal cortex and pericalcarine region. Greater BMI [$\chi^2_1 \geq 4.6$, $P \leq 0.032$] was associated with a thinner cortex in the transverse, middle temporal and inferior temporal gyri. GGT was not a significant predictor of thickness in any region (all $P > 0.10$). There were no significant interactions between AUD subgroup (Atherogenic+ vs. Atherogenic−) on alcohol consumption variables, smoking status, substance abuse and psychiatric comorbidities, age, BMI or GGT for any region of interest (all $P > 0.20$).

Pairwise comparisons indicated that Atherogenic+ had a thinner cortex than CON in all regions (all $P \leq 0.01$) except the caudal anterior cingulate, isthmus of cingulate and transverse temporal and pericalcarine regions, with generally large magnitude effect sizes (see Fig. 1a). Atherogenic− had a thinner cortex than CON in 23 of 34 regions (all $P \leq 0.01$), with moderate-to-large effect sizes; the greatest magnitude differences between Atherogenic− and CON were apparent in the anterior frontal and posterior parietal cortices (see Fig. 1b). Atherogenic+ showed a thinner cortex than Atherogenic− in 15 of 34 regions (all $P \leq 0.01$), with moderate-to-large effect sizes; differences between these groups were most apparent in the orbitofrontal and dorsal/dorsolateral frontal lobes and mesial and lateral temporal and inferior parietal regions (see Fig. 1c). Lifetime average number of drinks/month and 1-year-average drinks/month were not significant predictors in pairwise comparisons between Atherogenic+ and Atherogenic− (all $P > 0.20$).

Examination of mean regional thickness values in individuals with two or more pro-atherogenic conditions indicated that these participants had a thinner cortex across regions of interest, compared to those with only one condition in Atherogenic+. However, these differences were not statistically different, likely due to the small sample size of individuals with two or more pro-atherogenic conditions ($n = 16$).

Associations between regional cortical thickness and blood pressure metrics in Atherogenic+ and Atherogenic−

There were no significant associations between thickness in any region and blood pressure metrics and statin medication use in the combined group of Atherogenic+ and Atherogenic−.

Associations between regional cortical thickness and antihypertensive and statin medication use in Atherogenic+

There were no significant associations between thickness in any region and antihypertensive and statin medication use in Atherogenic+.

DISCUSSION

In this study, AUD treatment-seeking individuals demonstrated significant bilateral cortical thinning compared to CON, but the distribution and magnitude of the morphometric abnormalities were influenced by pro-atherogenic biomedical conditions in the AUD group. The Atherogenic+ group showed large magnitude bilateral thickness reductions compared to CON in 30 of 34 (88%) cortical regions, indicating widespread cortical thinning. Atherogenic− showed a thinner cortex than CON in 23 of 34 (68%) bilateral regions, with the greatest magnitude of thinning apparent in the anterior frontal and posterior parietal lobes. Atherogenic+ demonstrated a thinner cortex than Atherogenic− in 15 of 34 (44%) bilateral regions, with lateral orbitofrontal and dorsal/dorsolateral frontal lobes and mesial and lateral temporal and inferior parietal regions showing the largest magnitude differences between these groups. The cortical thinning observed in the AUD subgroups was not mediated or moderated by BMI, age, markers of hepatocellular injury or alcohol consumption.

Classic neuropathological studies have identified cortical watershed areas that are vulnerable to the effects of decreased cerebral blood flow or perfusion pressure. They are located at the intersection of fields of two non-anastomosing arterial systems situated between regions irrigated by the anterior cerebral artery and middle cerebral artery (anterior watershed zone, AWZ) and posterior cerebral artery and middle cerebral artery (posterior watershed zone, PWZ) (Momjian-Mayor and Baron, 2005; Mangla et al., 2011). The cortical thinning in both Atherogenic− and Atherogenic+ compared to CON showed a considerable overlap with the AWS and PWS. However, the magnitude (as reflected in effect sizes) of thinning in the lateral orbitofrontal cortex, insula, superior and middle frontal gyri and inferior and middle temporal gyri, corresponding to the AWZ, in Atherogenic+ relative to CON was substantially greater than the extent of thinning in Atherogenic− compared to CON in the same regions. Similarly, the magnitude of thinning in the inferior and superior parietal regions, associated with the PWZ, in Atherogenic+ relative to CON was substantially greater than the level of thinning in Atherogenic− compared to CON in those regions. The strongest pattern of thinning apparent in Atherogenic+ compared to Atherogenic− also shows a strong correspondence to the AWZ and PWZ (Fig. 1c). Atherogenic+ also showed significant thinning compared to CON and Atherogenic− in mesial temporal regions including the entorhinal and parahippocampal cortices and fusiform gyri. These regions are not typically associated with watershed zones because of the collateral irrigation by the middle and

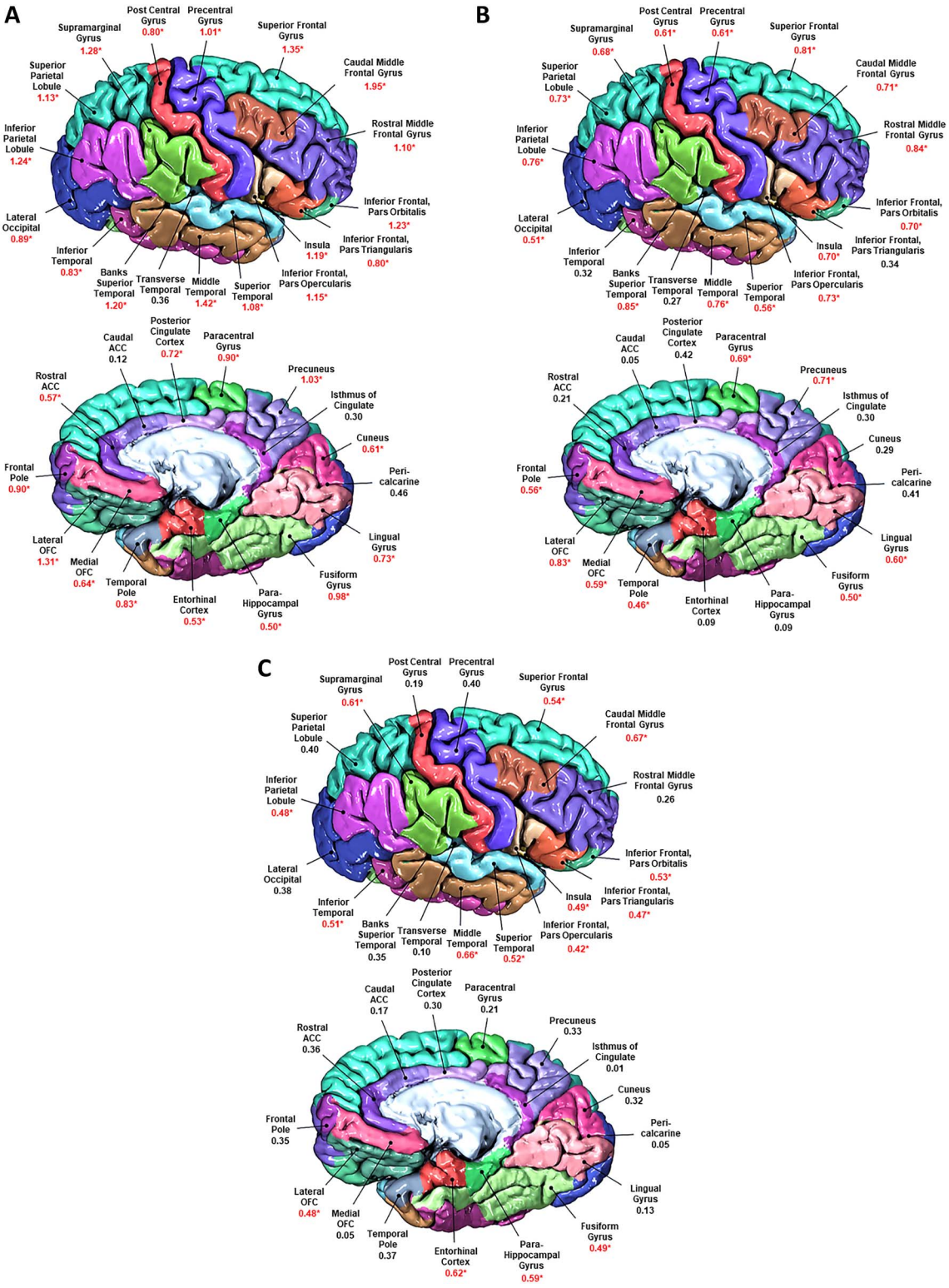

Fig. 1. (**a**) Region of interest comparison for Atherogenic+ versus CON. Values for each region of interest represent effect sizes. Effect sizes in red font with asterisks indicate statistically a thinner cortex in Atherogenic+ (all $P \leq 0.01$). (**b**) Region of interest comparison for Atherogenic− versus CON. Values for each region of interest represent effect sizes. Effect sizes in red font with asterisks indicate statistically a thinner cortex in Atherogenic− (all $P \leq 0.01$). (**c**) Region of interest comparison for Atherogenic− versus Atherogenic+. Values for each region of interest represent effect sizes. Effect sizes in red font with asterisks indicate statistically a thinner cortex in Atherogenic+ (all $P \leq 0.01$).

posterior cerebral arteries (Mangla et al., 2011), but show substantial thinning in MCI and AD (Greene and Killiany, 2010; Li et al., 2011). The entorhinal and parahippocampal cortices demonstrate higher metabolic activity relative to the surrounding cortical regions; correspondingly, greater metabolic demand may render these regions more vulnerable to atherosclerotic-related perfusion deficits (Stranahan and Mattson, 2010; Kivisaari et al., 2013). The above bilateral findings indicate potentially compromised arterial/arteriole integrity and/or altered hemodynamics in Atherogenic+. The overall pattern of cortical thinning for the Atherogenic+ and Atherogenic− in this study is consistent with the previous AUD research, but findings from this study suggest that pro-atherogenic conditions appear to exacerbate regional cortical thinning in this AUD cohort.

Hypertension and hepatitis C seropositivity were the most frequent conditions in Atherogenic+ and likely made the greatest contribution to the regional thinning observed relative to CON and Atherogenic−. Seventy-three percent (73%) of Atherogenic+ with hypertension were taking an antihypertensive, which is higher than the national average of ∼50% (Go et al., 2013). However, antihypertensive use at the time of study was not associated with cortical thickness in any region in this study, and evidence for protective effects of antihypertensives on regional brain structure is mixed (see Alosco et al., 2014 and references therein). Additionally, blood pressure measurements were not related to the thickness in any region in AUD. Previous studies reported blood pressure metrics were related to brain volumes in non-AUD samples (Zahr et al., 2013; Gonzalez et al., 2015); however, the current study only obtained a single blood pressure measurement, and multiple measurements over multiple days are required to accurately quantitate blood pressure (Whelton et al., 2018). Consequently, the lack of association between our single blood pressure metrics and regional cortical volumes may be, at least partially, attributable to the accuracy of the single blood pressure measurement. Surprisingly, the percentage of never, former and active cigarette smokers were not significantly different between Atherogenic+ and Atherogenic−. However, across AUD groups, active smokers had higher blood pressure metrics than never smokers (data not shown), which is fully congruent with active cigarette smoking as an established risk factor for hypertension (US Department of Health and Human Services, 2004). No Atherogenic+ with hepatitis C seropositivity was taking interferon or other medication to manage active symptomatology. Congruent with findings from the current study, individuals without an AUD, who were not actively using medications to treat hepatitis C symptomatology, showed significant anterior frontal cortical thinning compared to a non-seropositive group (Hjerrild et al., 2016).

Hypertension, type 2 diabetes mellitus, hepatitis C seropositivity and hyperlipidemia are suggested to promote proinflammatory conditions that may directly contribute to altered arterial integrity and function through atherosclerotic-related stenosis and/or altered hemodynamic regulation (Savoia and Schiffrin, 2007; Varbo et al., 2013; Hjerrild et al., 2016). Additionally, the hazardous level of alcohol consumption in AUD and chronic cigarette smoking are associated with proinflammatory conditions (likely mediated by increased oxidative stress) in multiple organs, including the vascular system and/or specifically on regional brain parenchyma (i.e. functional cellular tissue) (Crews et al., 2006; Durazzo et al., 2014; Gonzalez-Reimers et al., 2014). Although the level of alcohol consumption in this cohort was not related to regional thickness, the heavy alcohol consumption, combined with the other proinflammatory conditions in Atherogenic+, may be associated with increased oxidative stress leading to altered integrity of cerebral vasculature and/or brain parenchyma.

This study has limitations that may influence the generalizability of the results. The AUD participants were comprised largely of US Armed Services Veterans. We were unable to reliably obtain the date of illness onset or duration for the majority of participants in the Atherogenic+ group. Additionally, hepatitis C RNA viral load and fasting glucose and serum lipid levels were not obtained in this sample near time of study. Illness duration and quantitative measures of hepatitis RNA viral load, glucose and serum lipid levels may be related to the level of thinning apparent in the Atherogenic+ group. Accurate quantitation of the aforementioned measures may reveal specific information on the association between disease burden and cortical thickness in Atherogenic+. The small number of female participants precluded examination of sex effects. Blood pressure was obtained once prior to magnetic resonance scanning of the AUD participants and at least two blood pressure measurements on two or more separate occasions is the standard for accurate quantitation (Whelton et al., 2018; Muntner et al., 2019). Given the sample size of Atherogenic+, we were not able to determine the potential individual contributions of hypertension, hepatitis C seropositivity, type 2 diabetes and hyperlipidemia to regional cortical thickness. Additionally, premorbid factors (e.g. genetic risk or resiliency factors) and comorbid factors were not assessed in this study (e.g. diet/nutrition, exercise and subclinical hepatic, pulmonary, cardiac or cerebrovascular dysfunction), which may have affected cortical thickness in the AUD groups.

The pattern of cortical thinning results for the AUD participants in this study is consistent with previous research, but new evidence here indicates that pro-atherogenic conditions in AUD may further exacerbate regional cortical thinning, particularly in lateral and mesial temporal and posterior parietal regions that demonstrate marked thinning in MCI and AD; atrophic changes in these regions during middle age may contribute to the increased risk of AD in those with AUD. Larger-scale studies are necessary to determine the specific associations of hypertension, hepatitis C, diabetes and hyperlipidemia with cortical thickness in AUD. These findings also reinforce AUD is a heterogeneous disorder that is characterized by multiple comorbid conditions that can adversely affect brain neurobiology and function.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest or disclosures to report.

REFERENCES

Adinolfi LE, Zampino R, Restivo L, *et al.* (2014) Chronic hepatitis C virus infection and atherosclerosis: Clinical impact and mechanisms. *World J Gastroenterol* **20**:3410–7.

Alosco ML, Gunstad J, Jerskey BA, *et al.* (2013) The adverse effects of reduced cerebral perfusion on cognition and brain structure in older adults with cardiovascular disease. *Brain Behav* **3**:626–36.

Alosco ML, Gunstad J, Xu X, *et al.* (2014) The impact of hypertension on cerebral perfusion and cortical thickness in older adults. *JASH* **8**: 561–70.

Bae S, Kang I, Lee BC, *et al.* (2016) Prefrontal cortical thickness deficit in detoxified alcohol-dependent patients. *Experimental Neurobiol* **25**:333–41.

Chen Z, Zang X, Liu M, *et al.* (2017) Abnormal alterations of cortical thickness in 16 patients with type 2 diabetes mellitus: A pilot MRI study. *Chinese Medical Sciences Journal = Chung-kuo i hsueh k'o hsueh tsa chih* **32**:75–2.

Cohen J (1988) *Statistical Power Analysis for the Behavioral Sciences*. Hillsdale, NJLawrence Erlbaum Associates.

Crews FT, Bechara R, Brown LA, *et al.* (2006) Cytokines and alcohol. *Alcohol Clin Exp Res* **30**:720–30.

Durazzo TC, Gazdzinski S, Yeh PH, *et al.* (2008) Combined neuroimaging, neurocognitive and psychiatric factors to predict alcohol consumption following treatment for alcohol dependence. *Alcohol Alcohol* **43**:683–91.

Durazzo TC, Mattsson N, Weiner MW, *et al.* (2014) Smoking and increased Alzheimer's disease risk: A review of potential mechanisms. *Alzheimers Dement* **10**:S122–S45.

Durazzo TC, Meyerhoff DJ (2017) Psychiatric, demographic, and brain morphological predictors of relapse after treatment for an alcohol use disorder. *Alcohol Clin Exp Res* **41**:107–16.

Durazzo TC, Meyerhoff DJ, Murray DE (2015) Comparison of regional brain perfusion levels in chronically smoking and non-smoking adults. *Int J Environ Res Public Health* **12**:8198–213.

Durazzo TC, Mon A, Gazdzinski S, *et al.* (2011a) Chronic cigarette smoking in alcohol dependence: Associations with cortical thickness and N-acetylaspartate levels in the extended brain reward system. *Addict Biol* **18**:379–91.

Durazzo TC, Mon A, Gazdzinski S, *et al.* (2013) Chronic cigarette smoking in alcohol dependence: Associations with cortical thickness and N-acetylaspartate levels in the extended brain reward system. *Addict Biol* **18**:379–91.

Durazzo TC, Tosun D, Buckley S, *et al.* (2011b) Cortical thickness, surface area, and volume of the brain reward system in alcohol dependence: Relationships to relapse and extended abstinence. *Alcohol Clin Exp Res* **35**:1187–200.

Eickhoff S, Walters NB, Schleicher A, *et al.* (2005) High-resolution MRI reflects myeloarchitecture and cytoarchitecture of human cerebral cortex. *Hum Brain Mapp* **24**:206–15.

Fischl B, Destrieux C, Halgren E, *et al.* (2004) Automatic parcellation of the human cerebral cortex. *CerebCortex* **14**:11–22.

Fjell AM, Walhovd KB (2010) Structural brain changes in aging: Courses, causes and cognitive consequences. *Rev Neurosci* **21**:187–221.

Fortier CB, Leritz EC, Salat DH, *et al.* (2011) Reduced cortical thickness in abstinent alcoholics and association with alcoholic behavior. *Alcohol Clin Exp Res* **35**:2193–201.

Gazdzinski S, Durazzo TC, Studholme C, *et al.* (2005) Quantitative brain MRI in alcohol dependence: Preliminary evidence for effects of concurrent chronic cigarette smoking on regional brain volumes. *Alcohol Clin Exp Res* **29**:1484–95.

Go AS, Mozaffarian D, Roger VL, *et al.* (2013) Executive summary: Heart disease and stroke statistics–2013 update: A report from the American Heart Association. *Circulation* **127**:143–52.

Gonzalez-Reimers E, Santolaria-Fernandez F, Martin-Gonzalez MC, *et al.* (2014) Alcoholism: A systemic proinflammatory condition. *World J Gastroenterol* **20**:14660–71.

Gonzalez CE, Pacheco J, Beason-Held LL, *et al.* (2015) Longitudinal changes in cortical thinning associated with hypertension. *J Hypertens* **33**: 1242–8.

Grant BF, Goldstein RB, Saha TD, *et al.* (2015) Epidemiology of DSM-5 alcohol use disorder: Results from the National Epidemiologic Survey on alcohol and related conditions III. *JAMA Psychiat* **72**:757–66.

Greene SJ, Killiany RJ (2010) Subregions of the inferior parietal lobule are affected in the progression to Alzheimer's disease. *Neurobiol Aging* **31**:1304–11.

Grodin EN, Cortes CR, Spagnolo PA, *et al.* (2017) Structural deficits in salience network regions are associated with increased impulsivity and compulsivity in alcohol dependence. *Drug Alcohol Depend* **179**:100–8.

Hjerrild S, Renvillard SG, Leutscher P, *et al.* (2016) Reduced cerebral cortical thickness in non-cirrhotic patients with hepatitis C. *Metab Brain Dis* **31**:311–9.

Hutton C, Draganski B, Ashburner J, *et al.* (2009) A comparison between voxel-based cortical thickness and voxel-based morphometry in normal aging. *Neuroimage* **48**:371–80.

Hurtubise J, McLellan K, Durr K, *et al.* (2016) The different facets of Dyslipidemia and hypertension in atherosclerosis. *Curr Atheroscler Rep* **18**:82.

Kivisaari SL, PA, Taylor KI (2013) The Perirhinal, Entorhinal, and Parahippocampal Cortices and Hippocampus: An Overview of Functional Anatomy and Protocol for Their Segmentation in MR Images. In OJs SU (ed). *fMRI Basics and Clinical Applications (2nd edition)*. Berlin, HeidelbergSpringer, 239–67.

Kremen WS, Prom-Wormley E, Panizzon MS, *et al.* (2010) Genetic and environmental influences on the size of specific brain regions in midlife: The VETSA MRI study. *NeuroImage* **49**:1213–23.

Li C, Li C, Yang Q, *et al.* (2018) Cortical thickness contributes to cognitive heterogeneity in patients with type 2 diabetes mellitus. *Medicine* **97**:e10858.

Li Y, Wang Y, Wu G, *et al.* (2011) Discriminant analysis of longitudinal cortical thickness changes in Alzheimer's disease using dynamic and network features. *Neurobiol Aging* **33**:e15–30. doi: 10.1016/j.neurobiolaging.2010.11.008.

Makris N, Gasic GP, Kennedy DN, *et al.* (2008) Cortical thickness abnormalities in cocaine addiction–a reflection of both drug use and a pre-existing disposition to drug abuse? *Neuron* **60**:174–88.

Mangla R, Kolar B, Almast J, *et al.* (2011) Border zone infarcts: Pathophysiologic and imaging characteristics. *Radiographics: A Review Publication of the Radiological Society of North America, Inc* **31**:1201–14.

Mertens JR, Weisner C, Ray GT, *et al.* (2005) Hazardous drinkers and drug users in HMO primary care: Prevalence, medical conditions, and costs. *Alcohol Clin Exp Res* **29**:989–98.

Momenan R, Steckler LE, Saad ZS, *et al.* (2012) Effects of alcohol dependence on cortical thickness as determined by magnetic resonance imaging. *Psychiatry Res* **204**:101–11.

Momjian-Mayor I, Baron JC (2005) The pathophysiology of watershed infarction in internal carotid artery disease: Review of cerebral perfusion studies. *Stroke* **36**:567–77.

Muntner P, Shimbo D, Carey RM, *et al.* (2019) Measurement of blood pressure in humans: A scientific statement from the American Heart Association. *Hypertension (Dallas, Tex: 1979)* **73**:e35–66.

Panizzon MS, Fennema-Notestine C, Eyler LT, *et al.* (2009) Distinct genetic influences on cortical surface area and cortical thickness. *Cerebral cortex (New York, NY: 1991)* **19**:2728–35.

Payabvash S, Souza LC, Wang Y, *et al.* (2011) Regional ischemic vulnerability of the brain to hypoperfusion: The need for location specific computed tomography perfusion thresholds in acute stroke patients. *Stroke* **42**:1255–60.

Pelvig DP, Pakkenberg H, Stark AK, *et al.* (2008) Neocortical glial cell numbers in human brains. *Neurobiol Aging* **29**:1754–62.

Pennington DL, Durazzo TC, Schmidt T, *et al.* (2013) The effects of chronic cigarette smoking on cognitive recovery during early abstinence from alcohol. *Alc Clin Exp Research* **37**:1220–7.

Rakic P (1988) Specification of cerebral cortical areas. *Science* **241**:170–6.

Rakic P (2008) Confusing cortical columns. *Proc Natl Acad Sci U S A* **105**:12099–100.

Rehm J, Hasan OSM, Black SE, *et al.* (2019) Alcohol use and dementia: A systematic scoping review. *Alzheimers Res Ther* **11**:1.

Rolland B, Dricot L, Creupelandt C, *et al.* (2019) Respective influence of current alcohol consumption and duration of heavy drinking on brain morphological alterations in alcohol use disorder. *Addict Biol* **25**:e12751. doi: 10.1111/adb.12751.

Sankoh AJ, Huque MF, Dubey SD (1997) Some comments on frequently used multiple endpoint adjustment methods in clinical trials. *Stat Med* **16**:2529–42.

Satre DD, Gordon NP, Weisner C (2007) Alcohol consumption, medical conditions, and health behavior in older adults. *Am J Health Behav* **31**:238–48.

Savoia C, Schiffrin EL (2007) Vascular inflammation in hypertension and diabetes: Molecular mechanisms and therapeutic interventions. *Clinical Science (London, England: 1979)* **112**:375–84.

Schwarzinger M, Pollock BG, Hasan OSM, *et al.* (2018) Contribution of alcohol use disorders to the burden of dementia in France 2008-13: A nationwide retrospective cohort study. *Lancet Public Health* **3**: e124–e32.

Stranahan AM, Mattson MP (2010) Selective vulnerability of neurons in layer II of the entorhinal cortex during aging and Alzheimer's disease. *Neural Plast* **2010**:108190.

Sullivan EV, Zahr NM, Sassoon SA, *et al.* (2018) The role of aging, drug dependence, and hepatitis C comorbidity in alcoholism cortical compromise. *JAMA Psychiat* **75**:474–83.

Tomasi DG, Wiers CE, Shokri-Kojori E, *et al.* (2019) Association between reduced brain glucose metabolism and cortical thickness in alcoholics: Evidence of neurotoxicity. *Int J Neuropsychopharmacol* **22**:548–59.

Uhlmann A, Bandelow B, Stein DJ, *et al.* (2019) Grey matter structural differences in alcohol-dependent individuals with and without comorbid depression/anxiety-an MRI study. *Eur Arch Psychiatry Clin Neurosci* **269**:285–94.

Varbo A, Benn M, Tybjaerg-Hansen A, *et al.* (2013) Elevated remnant cholesterol causes both low-grade inflammation and ischemic heart disease, whereas elevated low-density lipoprotein cholesterol causes ischemic heart disease without inflammation. *Circulation* **128**: 1298–309.

US Department of Health and Human Services (2004) The health consequences of smoking: A report of the surgeon general. *Ctr Dis Control Prev* . http://www.ncbi.nlm.nih.gov/pubmed/20669512 (2 February 2020, date last accessed).

von Bartheld CS (2018) Myths and truths about the cellular composition of the human brain: A review of influential concepts. *J Chem Neuroanat* **93**:2–15.

Wang GY, Demirakca T, van Eijk J, *et al.* (2016) Longitudinal mapping of Gyral and Sulcal patterns of cortical thickness and brain volume regain during early alcohol abstinence. *Eur Addict Res* **22**:80–9.

Weinrebe W, Graf-Gruss R, Schwabe R, *et al.* (2002) The two-factor method-a new approach to categorizing the clinical stages of malnutrition in geriatric patients. *J Am Geriatr Soc* **50**:2105–7.

Whelton PK, Carey RM, Aronow WS, *et al.* (2018) 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA guideline for the prevention, detection, evaluation, and Management of High Blood Pressure in adults: A report of the American College of Cardiology/American Heart Association task force on clinical practice guidelines. *Circulation* **138**:e484–594.

Winkler AM, Kochunov P, Blangero J, *et al.* (2010) Cortical thickness or grey matter volume? The importance of selecting the phenotype for imaging genetics studies. *NeuroImage* **53**:1135–46.

Zahr NM, Mayer D, Rohlfing T, *et al.* (2013) In vivo glutamate measured with magnetic resonance spectroscopy: Behavioral correlates in aging. *Neurobiol Aging* **34**:1265–76.