

RESEARCH ARTICLE

MRS demonstrates elevated brain glutathione in vascular mild cognitive impairment compared to cognitively normal coronary artery disease controls

Jinghan Jenny Chen^{1,2} | Yejin Kang^{1,2} | Damien Gallagher^{1,3} | Nathan Herrmann^{1,3} |
 Kate Survilla¹ | Danielle Vieira¹ | Ethan Mah^{1,2} | Simon J. Graham^{1,4} | Alex Kiss⁵ |
 Sandra E. Black^{1,4,6,7,8} | Joel Ramirez^{1,4,6,7} | Paul Oh⁹ | Susan Marzolini⁹ |
 Katherine A. Zukotynski^{1,6,10} | Phillip H. Kuo¹¹ | Krista L. Lanctôt^{1,2,3,9} 

¹Hurvitz Brain Sciences Program, Sunnybrook Research Institute, Toronto, Ontario, Canada

²Department of Pharmacology & Toxicology, University of Toronto, Toronto, Ontario, Canada

³Department of Psychiatry, University of Toronto, Toronto, Ontario, Canada

⁴Department of Medical Biophysics, University of Toronto, Toronto, Ontario, Canada

⁵Department of Research Design and Biostatistics, Sunnybrook Research Institute, Toronto, Ontario, Canada

⁶Dr. Sandra Black Centre for Brain Resilience and Recovery, LC Campbell Cognitive Neurology, Sunnybrook Hospital, Toronto, Ontario, Canada

⁷Graduate Department of Psychological Clinical Science, University of Toronto Scarborough, Scarborough, Ontario, Canada

⁸Neurology Division, Department of Medicine, Sunnybrook Health Sciences Centre, University of Toronto, Toronto, Canada

⁹KITE Toronto Rehabilitation Institute, Toronto Rehabilitation Institute-University Health Network, Toronto, Ontario, Canada

¹⁰Department of Medical Imaging, McMaster University, Hamilton, Ontario, Canada

¹¹Department of Radiology, City of Hope National Medical Center, Duarte, California, USA

Correspondence

Krista L. Lanctôt, Hurvitz Brain Sciences Program, Sunnybrook Health Sciences Centre, 2075 Bayview Avenue, Room FG 21, Toronto, ON M4N 3M5, Canada.
 Email: Krista.Lanctot@sunnybrook.ca

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Abstract

INTRODUCTION: Oxidative stress (OS) is implicated in dementia. While elevated peripheral OS biomarkers were observed in vascular mild cognitive impairment (vMCI), the role of central antioxidants remains unclear. We assessed levels of the major brain antioxidant glutathione (GSH) in vMCI compared to cognitively normal coronary artery disease (CAD) controls (CN).

METHODS: In vivo tissue-corrected GSH in the anterior cingulate cortex (ACC) and occipital cortex (OC) were quantified in persons with vMCI and CN using MEscher-GARwood Point RESolved magnetic resonance Spectroscopy.

RESULTS: Among participants (vMCI, $n = 22$, age [mean \pm SD] = 67.4 ± 7.3 ; CN, $n = 21$, age = 66.7 ± 7.8), ACC-GSH (i.u. \pm SD) was higher in vMCI (4.42 ± 0.59) versus CN (3.72 ± 1.01) ($Z = -2.5$, $p = .01$), even after controlling for age and sex (B [SE] = 0.74 [0.26], $p = .007$). Increased ACC-GSH correlated with poorer executive function (EF) (B [SE] = -0.31 [0.14], $p = .04$). OC-GSH showed no effect.

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DISCUSSION: Higher ACC-GSH in vMCI may reflect a compensatory response to OS. ACC-GSH was negatively correlated with EF, suggesting a linkage between regional brain antioxidants and disease-relevant cognitive domains.

KEYWORDS

antioxidant, brain biomarker, glutathione, MEGA-PRESS, mild cognitive impairment, vascular cognitive impairment

Highlights

- Brain GSH was measured in vascular MCI and matched controls using MEGA-PRESS.
- In contrast to GSH deficits in AD, anterior cingulate GSH was elevated in vMCI.
- Brain GSH was correlated with disease-relevant cognitive domains in vMCI.
- The GSH antioxidant system may be etiologically implicated in vMCI.

1 | BACKGROUND

The global prevalence of dementia is projected to increase to over 150 million cases by 2050,¹ representing a significant burden to global healthcare systems. Although Alzheimer's disease (AD) dementia is the most common form, vascular dementia (VD) is widely considered the second most common, accounting for approximately 20% of cases.² Additionally, between 46% and 70% of clinically diagnosed AD dementia cases also exhibit cerebrovascular changes such as cerebral amyloid angiopathy, microinfarcts, or small vessel disease, that is, showing "mixed" etiology.³ The 2016 Rush Memory and Aging Project reported that among those with AD dementia, 56% had infarcts and 78% had vascular pathology.⁴ Thus, vascular contributions play an important role in dementia.

Patients with cerebrovascular risk factors, such as coronary artery disease (CAD), obesity, diabetes, hypertension, and hyperlipidemia have an increased risk of developing dementia, including AD dementia.^{5–7} The literature suggests that vascular risk factors contribute to dysfunction of the neurovascular unit (NVU),⁵ a collection of neurons, glial cells, and vascular cells that regulate cerebral blood flow. Dysfunction of the NVU may result in reduced cerebral blood flow, leading to neuroinflammation, oxidative stress (OS), and eventually neurodegeneration.^{8,9}

One of the critical pathophysiological processes in the brain that is exacerbated by vascular risk factors is OS.^{8,9} The brain's high metabolic demand makes it especially vulnerable to oxidative damage, which is now thought to contribute to neurodegenerative diseases, including AD and vascular-related cognitive impairment (VCI).^{10–12} OS results from an imbalance between the production of reactive oxygen species (ROS) and the brain's antioxidant defenses.¹² Alterations in these defenses are implicated in neuronal damage and synaptic dysfunction, both of which are early signs of cognitive decline.^{13,14} Among the primary antioxidant systems, glutathione (GSH) stands out as a key player in maintaining cellular redox homeostasis. As the brain's most abundant antioxidant,¹⁴ GSH directly neutralizes ROS and helps maintain the integrity of cellular structures.¹⁵

GSH depletion is thought to contribute to OS-induced neuronal death and loss in several neurodegenerative diseases, including Parkinson's disease,¹⁶ AD,¹⁷ and amyotrophic lateral sclerosis,¹⁴ suggesting that targeting GSH may offer a potential strategy for preventing neurodegeneration. However, data from *post mortem* studies on GSH levels in AD have yielded mixed results,^{18,19} and the precise role of GSH in early disease stages remains unclear. In vivo assessment of GSH has been conducted using brain magnetic resonance spectroscopy (MRS) and peripheral blood measurements.^{20–22} Brain GSH levels have been shown to be significantly reduced in AD and MCI patients compared to healthy controls, as demonstrated by a meta-analysis of MRS studies using MEScher-GARwood Point RESolved Spectroscopy (MEGA-PRESS) techniques.²³ Those in vivo measurements provide more reliable insights into ongoing neuro-metabolic processes compared to *post mortem* studies.^{24,25} However, there is still a notable gap in the literature, as no MRS studies have yet explored brain GSH levels during the early stages of VCI.

In this study, we investigated cross-sectional in vivo brain GSH as a biomarker of disease in individuals with vascular MCI (vMCI) compared to cognitively normal CAD controls. We hypothesized that consistent with previous findings of elevated OS and antioxidant depletion in neurodegenerative diseases such as AD,^{11,26} brain GSH would be similarly decreased in MCI of vascular etiology.

2 | MATERIALS AND METHODS

2.1 | Study design and participants

Consecutive consenting participants from a CAD patient population starting a cardiac rehabilitation (CR) program at the University Health Network Toronto Rehabilitation Institute (UHN-TRI) were screened. Participants were between 55 and 85 years old and were able to read and communicate fluently in English. All participants in the study had stable CAD, which was defined by one or more of the following: a history of myocardial infarction, coronary angiographic evidence of

greater than 50% stenosis in at least one major coronary artery, a prior revascularization procedure, or having two or more vascular risk factors such as hypertension, dyslipidemia, diabetes, sedentary lifestyle, smoking, obesity, and/or sleep apnea. Patients were excluded from the study if they had a clinical history of stroke, epilepsy, current uncontrolled medical illnesses such as uncontrolled asthma, diabetes, hypercholesterolemia, severe hypo/hypertension, other significant acute medical illnesses affecting liver, renal, and/or lung function, substance abuse, uncontrolled hypothyroidism, a current neurological condition or major psychiatric condition such as bipolar disorder, schizophrenia, or a previously diagnosed neurodegenerative illness including dementia of any subtype. Participants were also excluded if they had contraindications to magnetic resonance imaging (MRI) or MRS (e.g., claustrophobia, ferromagnetic medical implants), were concurrently participating in a pharmacological study, or were taking any supplements containing N-acetylcysteine (NAC) that could have affected GSH values.

Participants were initially screened based on clinical inclusion/exclusion criteria. Next, they received cognitive screening for categorization as cognitively normal CAD control or vMCI based on cognitive performance (see Section 2.2 for specific details). vMCI participants were matched to cognitively normal CAD controls by age (± 5 years) and sex; education matching was not implemented due to the available participant sample. Controls were recruited from the same CAD population at UHN-TRI-CR. These participants scored ≥ 28 on the Montreal Cognitive Assessment (MoCA) or did not meet criteria for modest deficits (1 standard deviation [SD] below population norm) in specific cognitive domains.

MoCA was collected for all participants (and a subgroup completed a longer neuropsychological battery; see Section 2.2 for specific details), as well as brain MRI and MRS, and demographic/clinical information (age, sex, education, smoking history, body mass index [BMI], physical assessment data, psychiatric and neurological history, clinical comorbidities, and concomitant medications). The vMCI participants were characterized with positron emission tomography (PET) using the brain radiotracer ^{18}F -florbetaben to assess amyloid beta positivity. The resulting PET images were interpreted independently by two physicians certified in both radiology and nuclear medicine who were blinded to patient clinical characteristics. Each ^{18}F -florbetaben PET scan was rated according to the US FDA NEURACEQ label²⁷ as positive or negative for amyloid deposition, with final interpretation based on clinical consensus. vMCI patients who were amyloid positive but had cerebrovascular pathology were still included in the study. Written informed consent was freely obtained from all participants prior to study enrollment. All study procedures were approved by the Research Ethics Boards (REB) of Sunnybrook Health Sciences Centre and UHN.

2.2 | Cognitive testing

All participants were screened using the MoCA²⁸ as a measure of global cognition. The MoCA includes an executive function (EF) component, which is more sensitive to subtle cognitive changes compared

RESEARCH IN CONTEXT

1. **Systematic review:** A meta-analysis reported lower In vivo brain antioxidant levels of GSH in AD and MCI, using MEGA-PRESS. However, it is unknown whether similar changes occur in MCI secondary to vascular disease (vMCI).
2. **Interpretation:** In contrast to the GSH decline reported in AD, our findings indicated that those with vMCI exhibited a region-specific increase in brain GSH in the ACC. GSH was correlated with cognitive performance associated with the same region, suggesting a link between regional brain antioxidants and disease-relevant cognitive domains.
3. **Future directions:** The GSH antioxidant system may be etiologically implicated in the evolution of vMCI, and brain GSH levels may differ according to etiology, brain region, and disease stage. Clarifying brain antioxidant changes across the spectrum of disease severity in different etiologies will help with the planning of personalized preventive strategies.

to the Mini-Mental State Examination.^{28,29} Although typically a MoCA score of 26 or higher is considered normal,²⁸ a more conservative threshold of 28 was chosen, given that UNH-TRI CR's patient population has historically been well educated.^{30,31} This decision was supported by recently published MoCA norms stratified by both age and education, suggesting that cut-offs between 27 and 28 are appropriate in individuals with ≥ 16 years of education.³² For the current study, those who scored ≥ 28 were assumed to be cognitively normal, whereas those who scored < 28 on the MoCA received additional neuropsychological testing using the National Institute of Neurological Disorders and Stroke-Canadian Stroke Network (NINDS-CSN) 60-min standardized battery.³³

The NINDS-CSN 60-min standardized battery was used to evaluate domain-specific cognitive performance.³³ Cognitive impairment was defined as at least 1 SD below population norm (e.g., age- and education-matched) in EF, verbal memory, visual memory, processing speed, or working memory^{34,35} based on established consensus criteria.³⁶ Working memory (Digit Span), EF (Trail Making Test Part B, semantic fluency, phonemic fluency), processing speed (digit-symbol coding), and visuospatial and verbal memory (Rey Complex Figure Test, Hopkins Verbal Learning Test-Revised) were assessed. Domain-specific composite z-scores were calculated as the mean of each subscale's performance compared to age-matched population norms. Years of education were not accounted for by the normative scores in most of the domain-specific scales. Individuals who did not score at least 1 SD below norms for all assessed cognitive domains in the NINDS-CSN 60-min battery, even if they scored < 28 on the MoCA, were also deemed cognitively normal CAD controls.

2.3 | Anatomical brain MRI

Anatomical MRI was performed on a research-dedicated 3T system (Prisma, Siemens Healthineers, Erlangen, Germany) using the standardized Canadian Dementia Imaging Protocol,³⁷ which included high-resolution 3D T1, a Proton Density (PD)/T2-weighted sequence, and fluid attenuated inversion recovery (FLAIR). Anatomical MRI scans were reviewed and rated by a single radiologist who was blinded to the clinical characteristics of participants. "Probable" vMCI was defined as having cognitive deficits (as previously described) in addition to having "diffuse, subcortical cerebrovascular disease" with (1) the presence of two or more silent brain infarcts in supratentorial locations and/or (2) extensive white matter disease defined as a score of ≥ 2 on the Age-Related White Matter Changes (ARWMC) scale.³⁸ Participants who exhibited modest deficits in the aforementioned cognitive domains, with a clinical profile consistent with vascular etiology (i.e., cognitive impairment and significant comorbid vascular risk factors), and evidence of focal white matter lesions (ARWMC scale score of 1) on neuroimaging were classified as "possible vMCI," supporting an increased likelihood of impairment due to underlying vascular disease.^{36,39,40}

Brain tissue and white matter lesion measures were obtained using Semiautomatic Brain Region Extraction (SABRE),⁴¹ and FLAIR or proton density (PD)/T2 images were used to identify white matter hyperintensities (WMHs) using one of two semiautomatic protocols (Fuzzy Lesion Extractor [FLEX]⁴² or Lesion Explorer⁴³), with manual selection editing to obtain reliable segmentation outputs by trained personnel who were blinded to clinical information and followed a standardized protocol. The final output of the neuroimaging pipeline provided comprehensive volumetrics that included normal appearing gray and white matter, ventricular cerebrospinal fluid (vCSF) and sulcal CSF (sCSF), and measures of cerebral small vessel disease: periventricular and deep (p/dWMHs), lacunes, and MRI-visible perivascular spaces.⁴⁴ Of note, dWMHs are usually focal hyperintensities, farther away from the ventricles and toward the cortical surface.^{44,45}

2.4 | Brain MRS

Proton MRS data of brain GSH concentrations were obtained in the anterior cingulate cortex (ACC) and the occipital cortex (OC) at 3T using the Siemens Magnetom Prisma and a high-resolution 20-channel HE1-4 head coil with a voxel size of $32 \times 30 \times 28$ mm (see Figure 1 for sample ACC and OC voxel placement).

2.4.1 | MRS acquisition parameters

The MEGA-PRESS pulse sequence was used with repetition time (TR) = 2600 ms and echo time (TE) = 68.00 ms. The excitation and the refocusing pulses were applied at 90° and 180°, respectively. There were 160 difference-edited averages of paired "on" and "off"

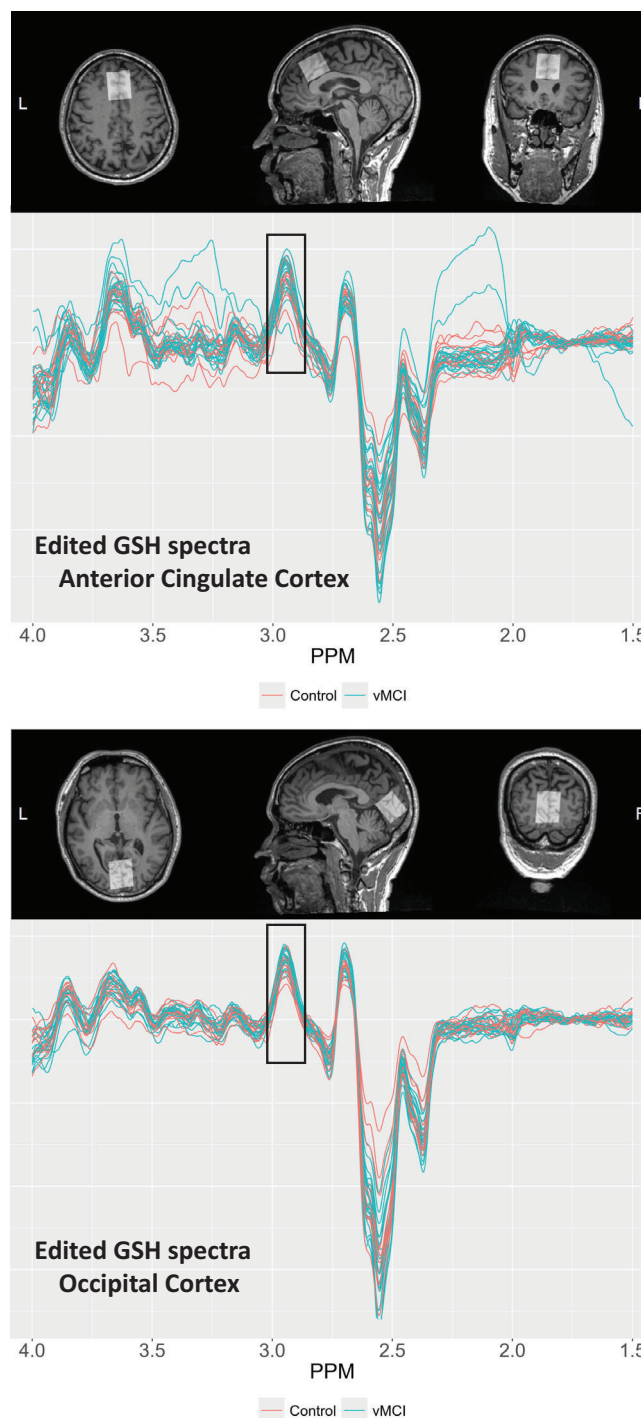


FIGURE 1 Sample MRS voxel locations in ACC (top) and OC (bottom) and GSH-edited spectra line plots for all acquired scans for ACC (top) and OC (bottom), prior to quality control based on fit error and signal-to-noise metrics. Line plots were colored by vMCI (teal) and cognitively normal CAD controls (orange), with the GSH peak denoted by the black rectangle. Voxel placements were rendered using Gannet and SPM12, line plots rendered using Gannet and R. ACC, anterior cingulate cortex; CAD, coronary artery disease; GSH, glutathione; MRS, magnetic resonance spectroscopy; OC, occipital cortex; vMCI, vascular mild cognitive impairment.

acquisitions, which were used to detect GSH with an appropriate signal-to-noise ratio (SNR). During the “on” acquisition, a Shinnar–Le Roux inversion pulse was applied for editing at 4.56 ppm, with a bandwidth of 75.00 Hz, targeted to a α -CH resonance J-coupled to the desired cysteinyl β protons of GSH at 2.95 ppm. The editing pulse was applied at 8.00 ppm during the “off” acquisition, well outside the spectral window of interest. Water suppression was performed during on and off acquisitions using the VARIable Power and Optimized Relaxation delays (VAPOR) method,⁴⁶ including outer volume suppression (OVS) pulses in three spatial dimensions to improve localization of the targeted brain region. Data from the ACC were acquired first for 14 min and 26 s (including 12 averages without water suppression, for use as a concentration reference), followed by the OC for the same duration. Voxels were shimmed using the “fastestmap” advanced gradient echo shim protocol built into the Siemens Magnetom Prisma system.

2.4.2 | MRS analysis

The resulting spectra were processed, fitted, and quantified using the Gannet package (version 3.1.5)⁴⁷ in MATLAB (version R2020a, MathWorks), according to package documentation. Frequency and phase correction were applied prior to fitting using default package settings. GSH was fitted using the non-linear, five-Gaussian model, with fit parameters optimized using the least-squares Levenberg–Marquardt algorithm based on the difference spectra and water reference to give the institutional unit (i.u.) concentration of GSH (see Figure 1 for the cohort of GSH-edited spectral line graphs by brain location and group). Institutional units were used as metabolite measures were considered to be pseudo-absolute in the Gannet package. SPM12 (version 7771)⁴⁸ and Gannet were used to co-register and segment each voxel using the T1-weighted Magnetization Prepared Rapid Gradient Echo (MPRAGE) images. Gannet applied correction for partial volume effects due to CSF, white, and gray matter tissue content using the Gasparovic et al. method⁴⁹ to obtain tissue-corrected GSH concentrations (Figure 2). Additionally, creatine-normalized GSH values (GSH/tCr) were also calculated using Gannet.

2.4.3 | MRS data quality

The linewidth (mean [SD]) at full width half maximum for GSH (FWHM_{GSH}) was 10.4 (1.95) Hz in the ACC and 10.3 (0.69) Hz in the OC, whereas $\text{FWHM}_{\text{water}}$ was 8.21 (0.82) Hz in the ACC and 8.75 (0.81) Hz in the OC, and NAA's linewidth (FWHM_{NAA}) was 8.10 (0.83) Hz in the ACC and 8.10 (0.46) Hz in the OC. The SNR for GSH (SNR_{GSH}) was 17.3 (8.32) in the ACC and 13.9 (3.35) in the OC. Gannet reports a fit error metric to assess data modeling quality, defined as the combined error of the GSH and water reference model fits, calculated from the standard deviations of the model fit divided by the model amplitude, expressed as a percentage and normalized to the water

reference signal.⁴⁷ The quality of GSH spectra modeling was quantitatively assessed through fit error of the model and SNR: GSH spectra with a high fit error ($\geq 20\%$) and extreme SNR_{GSH} (more than 2 SD from the mean SNR_{GSH} for that voxel), indicative of poor scan quality and poor model fit, were excluded.^{47,50}

2.5 | Statistical analyses

All statistical analyses and data visualization were conducted in R (version 4.4.0) using the packages (Table 1) ggplot2, dplyr, and car. Continuous variables were summarized in mean (SD) format, and categorical variables were summarized as a count number (n) and percentage (%). Between-group differences were assessed for continuous variables using ANOVA or Welch's ANOVA (when unequal variance was observed between groups). Where appropriate, chi-squared or Fisher's exact tests were used to evaluate qualitative variables. If between-group differences in demographic or clinical factors were found, they were controlled for as appropriate. Summary and between-group comparative statistics were carried out for demographic, physical, and clinical characteristics (brain tissue and WMH volumes, cognitive measures). As these were meant to be descriptive of participant characteristics rather than measuring study primary outcomes, multiple comparisons were not adjusted for, and between-group differences were considered significant at $\alpha < 0.05$ (two-tailed).

For the main analysis, brain GSH distributions in each group were checked for normality using a Shapiro–Wilk test, and groups were compared with a cross-sectional ANOVA if normally distributed and a non-parametric permutation test if skewed. As GSH was acquired in two regions of interest (ROIs), the ACC and OC, the classic threshold value $\alpha < 0.05$ for a two-tailed test was divided by two (i.e., $\alpha < 0.025$) to provide a Bonferroni correction for the main outcome.

A planned post hoc analysis for the main outcome was conducted using analysis of covariance (ANCOVA) if normally distributed and a generalized linear model (GLM) with a Gaussian distribution if skewed to assess whether brain GSH remained different between diagnostic groups after controlling for confounders. Sex differences have been reported in rodents, healthy humans, and AD,^{51,52} but these results are conflicting on whether GSH concentration and related enzymes are higher in males or females. Literature in *post mortem* brain tissue has suggested that brain GSH concentrations *In vivo* show age-related decreases in the frontal cortex and hippocampus, with more stability in the OC.⁵³ Age-related changes in the GSH redox system have also been reported.⁵⁴ Thus, age and sex were chosen a priori as covariates in this post hoc analysis; this relationship is expressed as

$$\text{Brain GSH} \sim \text{vMCI status (a factor variable)} + \text{age} + \text{sex} \quad (1)$$

Additional exploratory post hoc analyses were conducted to assess the contributions of other demographic or clinical characteristics that were significantly different between vMCI and cognitively normal CAD

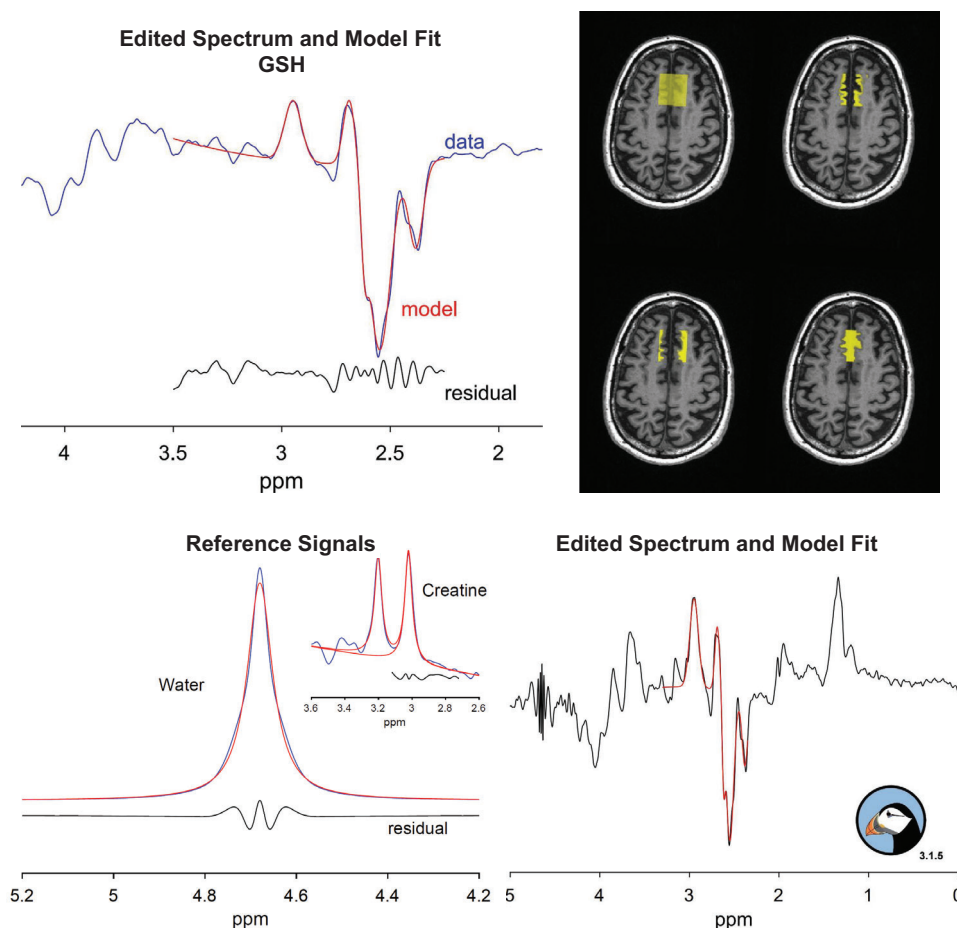


FIGURE 2 Outputs from a representative ACC GSH scan. Top left: GSH-edited spectral line plot with edited data (blue) and fitted curve (red); the peak at 2.95 ppm corresponds to GSH. Bottom left: spectral line plots and fitted curves for reference molecules (water and creatine) of the same scan; GSH levels were estimated relative to water reference. Top right: tissue segmentation (white, gray, and cerebrospinal fluid volume) of corresponding ACC voxel from anatomical T1 images using SPM12 and Gannet. Bottom right: GSH spectrum after tissue correction. Images were rendered using Gannet. ACC, anterior cingulate cortex; GSH, glutathione.

participants, in the format

$$\text{Brain GSH} \sim \text{vMCI status (a factor variable)} + \text{group differences} \quad (2)$$

An exploratory analysis was also conducted to investigate the relationship between brain GSH and cognition, controlling for vMCI diagnosis. For this analysis, normative cognitive performance scores were already age-corrected, so other relevant characteristics that were significantly different between vMCI and cognitively normal CAD participants were controlled on this basis (e.g., education), expressed as

$$\text{Cognitive domain composite } z\text{-score} \sim \text{brain GSH} + \text{vMCI status} + \text{YEd} \quad (3)$$

The exploratory analyses were conducted for the purpose of generating future hypotheses and thus did not include correction for multiple comparisons. Exploratory findings were considered significant at $\alpha < 0.05$ (two-tailed). For all regression models, regression coefficients were reported as unstandardized beta coefficients (β) to allow for the interpretation of variables in terms of their original units.

3 | RESULTS

3.1 | Participant characteristics

Participant recruitment is summarized in Figure 3. Of note, in CAD controls, 15 participants with MoCA scores ≥ 28 did not complete the NINDS-CSN 60-min battery. Additionally, one vMCI participant withdrew consent prior to MRS data collection and was excluded. Of the vMCI, two participants tested amyloid positive. Both had a clinical diagnosis of vMCI with concomitant white matter disease confirmed on neuroimaging and were thus included.

Demographic data are summarized in Table 1. CAD control and vMCI participants were well matched in most aspects, including age, sex, ethnicity, smoking status, and full-time employment status. The patient population was predominantly male. Participants were highly educated; CAD controls had more years of education than persons with vMCI (mean [SD], control = 18.3 [2.9]; vMCI = 15.3 [3.4], $F_{(1,40.3)} = 9.2$, $p = .004$). In addition, despite the fact that all participants were recruited from the same CR program, a higher proportion of vMCI

TABLE 1 Demographic, clinical, and cognitive characteristics of participants enrolled in the study.

	CAD control (n = 21)	vMCI (n = 22)	Significance
Demographic characteristics			
Age, years	66.7 (7.8)	67.4 (7.3)	$F_{(1,41)} = 0.09, p = .76$
Male (%)	18 (86%)	16 (73%)	$\chi^2 (1, N = 42) = 0.45, p = 0.50$
White (%)	16 (67%)	12 (54%)	$\chi^2 (3, N = 42) = 3.9, p = .27$
Asian (%)	1 (5%)	5 (23%)	
South Asian (%)	3 (14%)	1 (5%)	
Black (%)	3 (14%)	4 (18%)	
History of smoking (tobacco) (%)	9 (43%)	6 (27%)	Fisher's exact test, $p = .20$
Years of education	18.3 (2.9)	15.3 (3.4)	$F_{(1,40.3)} = 9.2, p = .004$
Full-time employed (%)	7 (33%)	10 (46%)	$\chi^2 (1, N = 42) = 0.66, p = .42$
Drinks per week, number	2.5 (2.9)	2.1 (4.2)	$F_{(1,37.8)} = 0.12, p = .73$
Physical/clinical characteristics			
BMI, kg/m ²	28.7 (4.9)	25.8 (5.4)	$F_{(1,40.9)} = 3.3, p = .08$
Systolic BP, mmHg	119 (16.0)	130 (15.7)	$F_{(1,41)} = 6.0, p = .02$
Diastolic BP, mmHg	72.0 (8.7)	79.2 (8.4)	$F_{(1,41)} = 7.7, p = .01$
Heart rate, b·min ⁻¹	62.1 (8.6)	63.6 (8.3)	$F_{(1,40.7)} = 0.4, p = .56$
PP, mmHg	46.5 (14.0)	51.2 (14.6)	$F_{(1,41)} = 1.1, p = .29$
MAP, mmHg	87.5 (9.60)	96.3 (9.08)	$F_{(1,41)} = 9.5, p = .004$
Waist circumference, cm	98.3 (11.1)	93.1 (12.2)	$F_{(1,34)} = 1.8, p = .19$
Body fat, %	31.9 (8.1)	27.3 (6.7)	$F_{(1,26)} = 1.3, p = .26$
VO ₂ peak, ml/kg/min	21.8 (6.1)	28.0 (9.9)	$F_{(1,33)} = 1.3, p = .27$
CES-D, score	6.2 (4.4)	7.5 (5.5)	$F_{(1,34.8)} = 1.1, p = .29$
Vascular risk factors			
Hypertension (%)	8 (38%)	16 (73%)	$\chi^2 (1, N = 43) = 6.7, p = .01$
Diabetes (%)	2 (9.5%)	6 (27%)	Fisher's exact test, $p = .10$
Dyslipidemia (%)	8 (38%)	15 (68%)	$\chi^2 (1, N = 43) = 2.8, p = .1$
Obesity, BMI ≥ 30 (%)	6 (29%)	2 (9%)	Fisher's exact test, $p = .13$
Current smoker (%)	0 (0%)	1 (5%)	Fisher's exact test, $p = 1.0$
Number of vascular risk factors	1.1 (1.2)	1.8 (1.3)	$F_{(1,41)} = 3.1, p = .09$
Concomitant medications			
Aspirin	13 (62%)	18 (81%)	Fisher's exact test, $p = .19$
Other antiplatelet medications	17 (81%)	11 (50%)	Fisher's exact test, $p = .06$
Statins	19 (91%)	20 (91%)	Fisher's exact test, $p = 1.0$
Thiazide diuretics	2 (10%)	1 (5%)	Fisher's exact test, $p = .61$
Calcium channel blockers	2 (10%)	5 (23%)	Fisher's exact test, $p = .41$
Systemic beta blockers	10 (48%)	14 (64%)	Fisher's exact test, $p = .36$
ACE inhibitors	9 (43%)	7 (32%)	Fisher's exact test, $p = .54$
Antidiabetics	1 (5%)	6 (27%)	Fisher's exact test, $p = .09$
Thyroid hormone	3 (14%)	1 (5%)	Fisher's exact test, $p = .34$
Supplements ^a	8 (38%)	9 (41%)	Fisher's exact test, $p = 1.0$
Cognitive performance			
Global cognition			
MoCA	27.7 (1.20)	23.0 (1.95)	$F_{(1, 35.1)} = 90, p < .001$

(Continues)

TABLE 1 (Continued)

	CAD control (n = 21)	vMCI (n = 22)	Significance
Domain composite z-scores	(n = 6) ^b	(n = 22)	
Executive function	0.78 (0.50)	−0.39 (0.50)	$F_{(1,26)} = 25.9, p < .001$
Verbal memory	0.56 (0.69)	−0.87 (0.92)	$F_{(1,26)} = 12.5, p = .002$
Visual memory	0.31 (0.87)	−0.17 (0.84)	$F_{(1,26)} = 1.5, p = .2$
Working memory	0.48 (0.59)	−0.21 (0.63)	$F_{(1,26)} = 5.9, p = .02$
Processing speed	0.24 (0.14)	−0.07 (0.66)	$F_{(1,25.6)} = 4.2, p = .05$

Note: Values reported as count (percentage) or as mean (SD). Fisher's exact test was used when counts of categorical variables were ≤ 5 and matched to a 2×2 contingency table.

Statistically significant values ($p < 0.05$) are bolded.

Abbreviations: ACE, angiotensin-converting enzyme; b·min^{−1}, beats per minute; BMI, body mass index; BP, blood pressure; CAD, coronary artery disease; CESD, Center for Epidemiological Studies Depression questionnaire; MAP, mean arterial pressure; MoCA, Montreal Cognitive Assessment; PP, pulse pressure; SD, standard deviation; vMCI, vascular mild cognitive impairment; VO₂, volume of oxygen uptake.

^aSupplements included glucosamine and chondroitin, vitamins (multivitamin formulations, B12, C, and D), probiotics, iron, magnesium, and calcium.

^b15 control participants scored ≥ 28 on MoCA and subsequently did not complete the NINDS-CSN 60-min battery.

participants had comorbid hypertension and corresponding higher mean systolic blood pressure (sBP), diastolic blood pressure (dBP), and mean arterial pressure (MAP) (Table 1).

3.1.1 | Concomitant medications

vMCI participants and cognitively normal CAD controls did not differ in their use of concomitant medications (Table 1). While a trending proportion of CAD control participants were taking antiplatelet medications compared to vMCI participants ($p = .054$), all study participants (100% in both groups) were taking medications with antiplatelet effects (aspirin and/or other antiplatelet medications). Despite previously mentioned differences in BP, a similar proportion of CAD controls and vMCI participants were taking medications with antihypertensive effects (such as thiazide diuretics, calcium channel blockers, and angiotensin-converting enzyme [ACE] inhibitors) (Table 1).

3.1.2 | Cognition in vMCI and cognitively normal CAD controls

As expected, the MoCA score was lower in vMCI participants compared to that of cognitively healthy CAD controls (Table 1). Compared to CAD controls who completed the full NINDS-CSN 60-min battery ($n = 6$), vMCI participants had significantly poorer performance in EF, verbal memory, and working memory.

3.1.3 | Brain white matter changes

There were no group differences in the proportion of participants in each category of the ARWMC (Table 2). In the vMCI group, there were greater ventricular CSF and WMH volumes compared to CAD controls, specifically the pWMH volumes (a trend toward greater dWMH volume was also observed). There were no differences in the volume

of lacunes or MRI-visible perivascular spaces between CAD controls and vMCI participants. Tissue composition analysis revealed no significant differences in gray matter, white matter, or CSF fractions between vMCI patients and cognitively normal CAD controls in either the ACC or OC voxels (Table 2).

3.2 | Main outcome: brain GSH differences in vMCI

Two ACC-GSH and two OC-GSH were excluded due to fit error >20% or extreme SNR values, and one OC-GSH spectrum was excluded due to scan file corruption (Figure 3). All were from vMCI participants, one of whom was amyloid-positive. Tissue-corrected ACC-GSH values were found to be higher in vMCI participants compared to CAD controls (Figure 4, Table 3). OC-GSH values were not different between vMCI and CAD control participants.

3.2.1 | Post hoc analysis controlling for literature confounders

The GLM model controlling for age and sex indicated that ACC-GSH was higher in the vMCI group by 0.74 i.u. relative to the CAD control group ($\beta_{\text{vMCI status}} [\text{SE}] = 0.74 [0.26], p = .007$).

3.2.2 | Exploring between-group contributions to brain GSH

A post hoc GLM model of brain ACC-GSH by vMCI status explored the contributions of group differences in years of education and MAP, expressed as

$$\text{Brain GSH} \sim \text{vMCI status (a factor variable)} + \text{YEd} + \text{MAP} \quad (4)$$

The model indicates that MAP was also significantly associated with ACC-GSH, controlling for diagnosis and YEd, and every 1 mmHg

TABLE 2 Brain tissue volumes and white matter ratings for cognitively normal CAD control and vMCI participants.

	CAD control (n = 21)	vMCI (n = 22)	Significance
ARWMC score, count (%)			
1	15 (71%)	15 (68%)	$\chi^2 (2, N = 43) = 1.9, p = .38$
2	2 (10%)	5 (23%)	
3	4 (19%)	2 (9%)	
Whole brain volumetrics, mean (SD)			
Normal-appearing WM, cm ³	422.0 (64.2)	426.0 (79.4)	$F_{(1,41)} = 0.03, p = .88$
Normal-appearing GM, cm ³	553.0 (49.2)	551.0 (65.8)	$F_{(1,41)} = 0.01, p = .92$
sCSF, cm ³	237.0 (52.8)	232.0 (64.1)	$F_{(1,40.2)} = 0.08, p = .77$
vCSF, cm ³	43.2 (25.3)	151.0 (226.0)	$F_{(1,21.6)} = 4.9, p = .04$
pWMH, cm ³	8.84 (13.70)	50.60 (82.60)	$F_{(1,22.2)} = 5.4, p = .03$
dWMH, cm ³	0.58 (0.92)	10.50 (23.40)	$F_{(1,21)} = 3.9, p = .06$
pLACN, mm ³	20.7 (70.7)	1660 (4640)	$F_{(1,21)} = 2.8, p = .11$
dLACN, mm ³	5.00 (13.6)	351 (1100)	$F_{(1,21)} = 2.2, p = .15$
PVS, mm ³	59.7 (88.0)	114 (367)	$F_{(1,23.5)} = 0.46, p = .51$
ACC voxel tissue fraction, mean (SD)			
ACC voxel GM fraction	0.438 (0.035)	0.427 (0.038)	$F_{(1,39)} = 0.98, p = .33$
ACC voxel WM fraction	0.290 (0.058)	0.315 (0.054)	$F_{(1,39)} = 1.99, p = .17$
ACC voxel CSF fraction	0.272 (0.064)	0.258 (0.072)	$F_{(1,37.9)} = 0.41, p = .53$
OC voxel tissue fraction, mean (SD)			
OC voxel GM fraction	0.526 (0.050)	0.530 (0.047)	$F_{(1,38)} = 0.09, p = .77$
OC voxel WM fraction	0.295 (0.041)	0.312 (0.042)	$F_{(1,38)} = 1.81, p = .19$
OC voxel CSF fraction	0.180 (0.064)	0.157 (0.059)	$F_{(1,38)} = 1.32, p = .26$

Statistically significant values ($p < 0.05$) are bolded.

Abbreviations: ACC, anterior cingulate cortex; ARWMC, age-related white matter change; CAD, coronary artery disease; dLACN, deep LACN; dWMH, deep WMH; GM, gray matter; OC, occipital cortex. Chi-squared with Yates's correction, Welch's corrected ANOVA, or ANOVA was used as appropriate.; pLACN, periventricular lacunes; PVS, perivascular spaces; pWMH, periventricular white matter hyperintensities; sCSF, sulcal cerebrospinal fluid; SD, standard deviation; vCSF, ventricular CSF; vMCI, vascular mild cognitive impairment; WM, white matter.

increase in MAP was correlated with a 0.03 i.u. increase in ACC-GSH ($\beta_{\text{MAP}} [\text{SE}] = 0.03 [0.01], p = .02$).

Analogous post hoc modeling was conducted for OC-GSH using ANCOVA, but models were not significant. Thus, vMCI status was determined not to be associated with OC-GSH values.

3.3 | Post hoc creatine-referenced GSH

Consistent with tissue-corrected results, ACC-GSH/tCr values were significantly higher in vMCI patients compared to CAD controls – $F_{(1,30.8)} = 4.4, p = .04$. This difference remained significant after adjusting for age and sex ($\beta [\text{SE}] = 0.0068 [0.0029], p = .028$).

3.4 | Exploring brain GSH and cognition

Domain-specific composite z-scores are shown in Table 1. Exploratory ANCOVA models were used to assess the relationship between domain-specific composite z-scores by brain GSH.

Controlling for diagnosis (vMCI status) and years of education ($F_{(3,22)} = 11.2, p < .001$), elevated ACC-GSH was associated with decreased EF composite score ($B_{\text{ACC-GSH}} [\text{SE}] = -0.31 [0.14], p = .04$). Holding vMCI status and years of education constant, each unit of increase in ACC-GSH was associated with a lower EF performance by 0.31 z-scores. There were no interactions between diagnosis and ACC-GSH with respect to the EF composite score. Controlling for diagnosis and years of education, other cognitive domains (verbal memory, verbal memory, visual memory, working memory, and processing speed) were not associated with ACC-GSH values.

4 | DISCUSSION

While higher peripheral biomarkers of OS have been described in vMCI, the role of central antioxidants has not been characterized in persons with vMCI. This study used MRS to compare In vivo brain GSH in cognitively normal CAD controls and vMCI participants, finding that tissue-corrected ACC-GSH values were higher in vMCI, even when controlling for potential confounders (age and sex). In

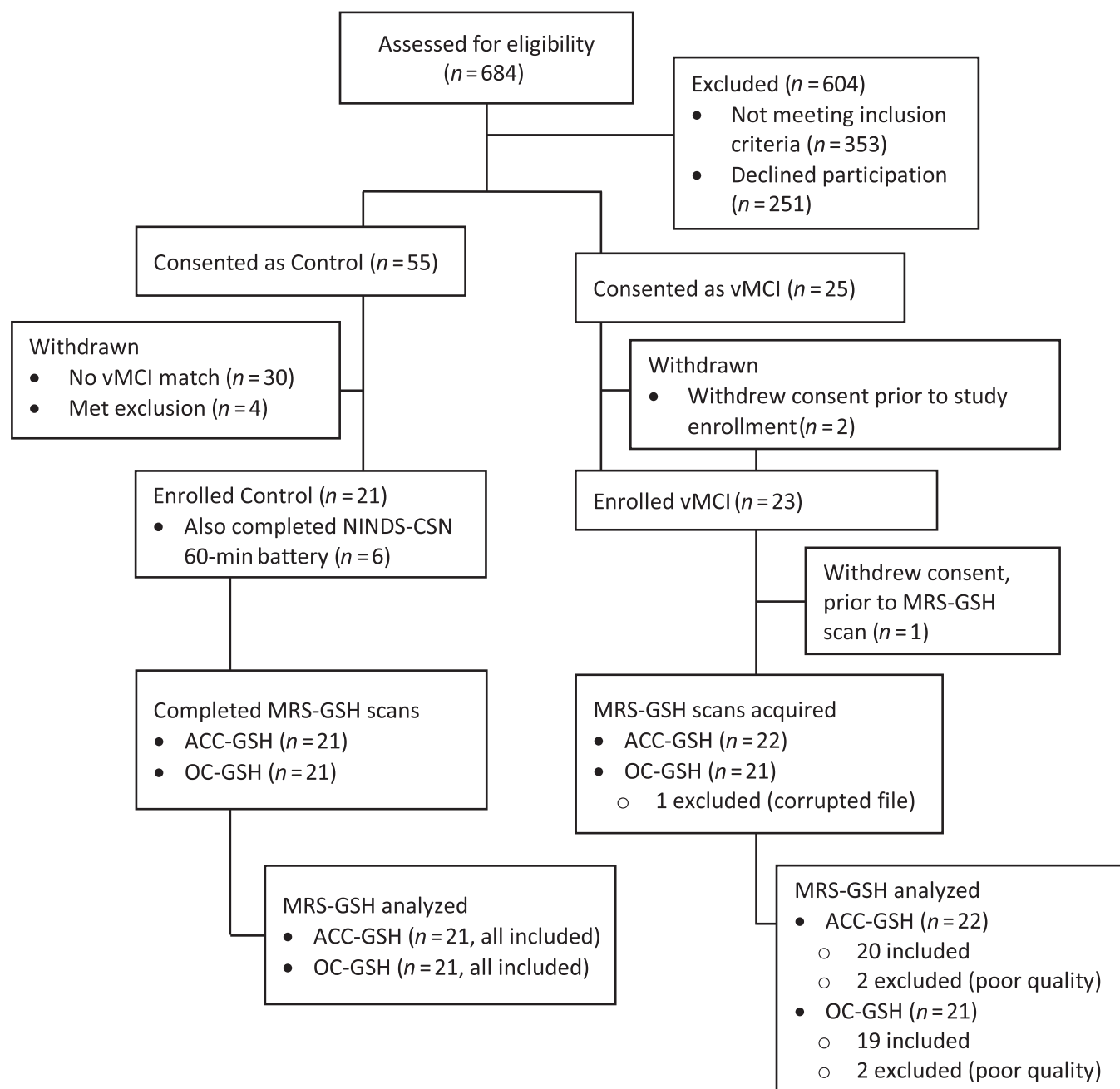


FIGURE 3 Flow chart of participant recruitment; note that one vMCI participant withdrew consent after study enrollment. Of all enrolled vMCI participants, 22 completed the study, one vMCI withdrew consent after enrollment but prior to MRS data collection and, thus, was excluded from the analysis. After consent, participants received cognitive screening for categorization as cognitively healthy CAD control or vMCI based on cognitive performance. Of note, in CAD controls, 15 participants had MoCA scores ≥ 28 and subsequently did not complete the NINDS-CSN 60-min battery. ACC, anterior cingulate cortex; CAD, coronary artery disease; GSH, glutathione; MoCA, Montreal Cognitive Assessment; MRS, magnetic resonance spectroscopy; NINDS-CSN, National Institute of Neurological Disorders and Stroke-Canadian Stroke Network; OC, occipital cortex; vMCI, vascular mild cognitive impairment.

contrast, no differences in OC-GSH values were found between groups. These findings were unexpected, as central GSH was hypothesized to decrease in the vMCI group due to increased OS,³⁰ similar to reports of antioxidant depletion in AD.^{17,25,55–57} The contrast between increased central GSH in vMCI and decreased central GSH in ADD suggests that there may be different pathophysiological mechanisms at play in these disease phenotypes and stages.

This finding may indicate that ACC-GSH increases early in the disease process as part of a compensatory antioxidant response in cerebrovascular disease. In support of this, we previously observed elevated peripheral OS markers in possible vMCI patients, indicative of OS challenge.³⁰ Compensatory increases in antioxidant activity in the early stages of CAD without cognitive impairments have also been reported.^{58,59} As our finding differs from previously reported

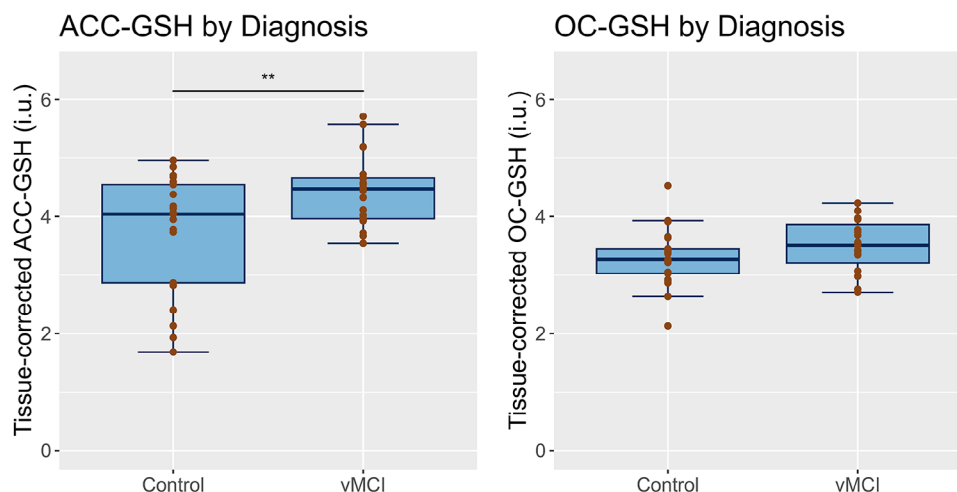


FIGURE 4 Box-and-whisker plots denoting median, interquartile range, minimum, and maximum of tissue-corrected GSH in ACC (left) and OC (right) in cognitively normal CAD control ($n = 21$) and vMCI ($n = 19$ to 20) participants. Each included observation was represented as an individual point; $**p = .01$. There were no differences between OC-GSH in vMCI versus CAD controls. ACC, anterior cingulate cortex; OC, occipital cortex; GSH, glutathione; i.u., institutional units; vMCI, vascular mild cognitive impairment.

TABLE 3 Tissue-corrected brain GSH of enrolled participants.

Tissue-corrected brain GSH	CAD control $n = 21$	vMCI $n = 20$ (ACC) $n = 19$ (OC)	Significance
Anterior cingulate cortex (ACC)	3.72 (1.01)	4.42 (0.59)	$Z = -2.5, p = .01$
Occipital cortex (OC)	3.29 (0.50)	3.43 (0.45)	$F_{(1, 38)} = 0.8, p = .37$

Note: Values are reported as mean (SD) in i.u. after tissue correction. A non-parametric permutation test was used for ACC-GSH, and Welch's ANOVA was used for OC-GSH.

Statistically significant values ($p < 0.05$) are bolded.

Abbreviations: ACC, anterior cingulate cortex; CAD, coronary artery disease; GSH, glutathione; i.u., institutional units; SD, standard deviation; vMCI, vascular mild cognitive impairment.

decreases in AD versus controls as measured by MEGA-PRESS,^{55,60} it is possible that the upregulation of brain GSH in vMCI may eventually decline as cognitive impairment worsens. In the very early stages of disease there may be differences in how antioxidant defense mechanisms are mobilized among those with frank cerebrovascular disease as compared to neurodegenerative disease.

The convergence of results between the water-scaled and creatine-referenced analyses strengthens confidence in the observed GSH alterations. This consistency is particularly notable given that peripheral creatine has been reported to be elevated in individuals with hypertension.^{61,62} Although all participants in this study had a history of CAD, the vMCI group had a higher proportion with comorbid hypertension and elevated MAP, which may lead to an underestimation of GSH/tCr ratios in this group. Therefore, the observed group differences in ACC-GSH levels are likely attributable to true disease-related changes, rather than measurement confounds or tissue composition effects.

Whereas the ACC-GSH value was elevated in vMCI participants compared to cognitively normal CAD controls, no significant differences were found for OC-GSH. OS and the production of ROS are not distributed equally through the brain,⁶³ so it may be that the occipital regions have lower ROS production and less OS. Certain brain regions,

such as the prefrontal cortex, hippocampus, amygdala, and cerebellar granular cells, are more vulnerable to OS.¹² With that in mind, the connections of the ACC to the prefrontal cortex and amygdala may make it more sensitive to OS challenge. In this study of vMCI participants with mild disease severity, elevated ACC-GSH may reflect a relatively intact ability to respond to OS challenges in the ACC.

Exploratory analyses found that elevated ACC-GSH was associated with poorer EF composite score, after controlling for vMCI diagnosis and years of education. Functional MRI studies have linked brain activity of the dorsal ACC to executive functioning (such as cognitive flexibility, task-switching, and fluency).^{64–67} For our study, the EF composite was calculated from the mean of Trail Making Test B, phonemic fluency, and semantic fluency z-scores. In tasks that involve the ACC, the association between ACC-GSH values and EF composite score is consistent with a link between regional brain GSH value and cognitive functions related to that brain region.

4.1 | Strengths and limitations

This study had several strengths. Whereas multiple studies have characterized in vivo brain GSH in AD and MCI populations,^{23,25,55–57,60}

brain GSH in vascular cognitive impairment has not been reported. Vascular contributions to dementia are increasingly recognized, and more than half of clinically diagnosed AD dementia participants also exhibit cerebrovascular pathology.⁶⁸ Another strength of the present study is that vMCI participants underwent amyloid PET imaging. Clinical findings indicated that two participants likely had mixed disease etiology, consisting of amyloid positivity in addition to cerebrovascular pathology. The remaining 20 vMCI participants were amyloid-negative, supporting vascular disease as the primary etiology. This observation further highlights the importance of mixed disease, even in the early and very mild stages of cognitive impairment. Global brain tissue volumetric analysis supported our vMCI categorization: probable and possible vMCI participants as a group had greater global ventricular CSF and pWMH volumes compared to controls – indicative of greater vascular brain pathology burden and atrophy consistent with cerebrovascular disease-related impairment.^{69,70} Another strength was that demographic characteristics between cognitively healthy CAD control participants and vMCI participants were well matched, with the exception of years of education, which was high in both groups and controlled for as a covariate in subsequent analysis. Previous studies examining brain GSH in AD have typically compared it to healthy controls without significant vascular diseases.^{55–57} In contrast, our study compared vMCI patients to cognitively healthy CAD patients who share similar vascular disease profiles and age ranges. This allows for a comparison between groups where only cognition differs, as the vascular component is present in both groups. vMCI participants had significantly poorer performance in global cognition, EF, verbal memory, and working memory compared to CAD controls, which was reflective of the target patient population.³⁶

There were some limitations to the study and the possible analysis. While the GSH measured here mostly came from reduced GSH in the brain, oxidized GSH (GSSG) may have contributed somewhat to this signal. *Post mortem* studies showed decreased GSH:GSSG ratio and higher OS markers in several neurodegenerative disorders,¹² and GSH:GSSG ratio in normal conditions has typically been around 100:1,^{71,72} although in vitro studies using motor neurons have shown that this ratio can fluctuate down to 20:1.⁷³ A study of PRESS and MEGA-PRESS protocols with a degrading “phantom” GSH showed that MEGA-PRESS (used here) appeared to be more specific to reduced GSH.⁷⁴ However, it was not possible in the current study to measure in vivo GSSG as a specific molecule of interest. A ratio of reduced:oxidized GSH would give additional insight into the redox status of that voxel. Additionally, although it is currently not feasible to measure in vivo levels of central OS markers in patients, future studies should also consider assessing potential relationships between peripheral OS markers and central GSH levels. The sample size was also small in this study, limiting the generalizability of findings and reducing the number of potential confounders that could be controlled for in analyses. Lastly, there was high variability in the GSH estimates, particularly in the ACC of the cognitively healthy CAD control group. While this can reflect scan quality, which is frequently lower in frontal regions, the measured SNR values suggest that scan quality was within acceptable limits. While variability was expected in our CAD control group due to their

cardiovascular comorbidities, future studies could include a cognitively normal control group without significant cardiovascular comorbidities – thus characterizing the full spectrum of brain GSH values in this age group.

4.2 | Conclusion and future directions

GSH, the primary antioxidant in the brain, and its depletion play an important role in OS-mediated neurodegeneration. However, GSH had not been characterized by individuals with vMCI, who are known to have high OS.³⁰ Contrary to GSH depletion seen in neurodegenerative disease, cross-sectional analysis of brain GSH in vMCI patients versus cognitively normal controls with CAD showed upregulation of GSH in vMCI. GSH values in the ACC region were significantly higher in vMCI than those of CAD controls, suggesting a region-specific and disease-specific change in brain antioxidant response. It is possible that as cerebrovascular disease progresses, the brain GSH antioxidant response may eventually fail and exhibit depletion similar to that seen in ADD.

This study provides increased understanding of antioxidant changes as a potential mechanism of disease pathology in individuals with vMCI. Future studies should clarify disease-specific alterations in the antioxidant response across the spectrum of disease severity to facilitate planning and personalization of antioxidant therapies, which hold promise but have shown variable efficacy in clinical studies to date.^{75–77}

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CONFLICT OF INTEREST STATEMENT

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J.J.C., D.G., N.H., K.A.S., D.V., E.M., Y.K., S.E.B., and J.R., S.J.G., P.O., and K.A.Z. declare that they have no conflict of interest. Author disclosures are available in the [Supporting Information](#).

CONSENT STATEMENT

All human participants provided voluntary informed consent to the study.

ORCID

Krista L. Lanctôt  <https://orcid.org/0000-0001-7024-6637>

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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