Vascular Risk Factor Burden Correlates With Cerebrovascular Reactivity But Not Resting State Coactivation in the Default Mode Network

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Purpose: White matter hyperintensities (WMH) are prevalent among older adults and are often associated with cognitive decline and increased risk of stroke and dementia. Vascular risk factors (VRFs) are linked to WMH, yet the impact of multiple VRFs on gray matter function is still unclear. The goal of this study was to test for associations between the number of VRFs and cerebrovascular reactivity (CVR) and resting state (RS) coactivation among individuals with WMH.

Materials and Methods: Twenty-nine participants with suspected WMH were grouped based on the number of VRFs (subgroups: 0, 1, or ≥2). CVR and RS coactivation were measured with blood oxygenation level-dependent (BOLD) imaging on a 3T magnetic resonance imaging (MRI) system during hypercapnia and rest, respectively. Default-mode (DMN), sensory-motor, and medial-visual networks, generated using independent component analysis of RS-BOLD, were selected as networks of interest (NOIs). CVR-BOLD was analyzed using two methods: 1) a model-based approach using CO2 traces, and 2) a dual-regression (DR) approach using NOIs as spatial inputs. Average CVR and RS coactivations within NOIs were compared between VRF subgroups. A secondary analysis investigated the correlation between CVR and RS coactivation.

Results: VRF subgroup differences were detected using DR-based CVR in the DMN (F20,255.17, P = 0.015) but not the model-based CVR nor RS coactivation. DR-based CVR was correlated with RS coactivation in the DMN (r² = 0.28, P = 0.006) but not the sensory-motor nor medial-visual NOIs.

Conclusion: In individuals with WMH, CVR in the DMN was inversely associated with the number of VRFs and correlated with RS coactivation.

Cerebral Small Vessel Disease (SVD) is associated with impaired vascular regulation and is considered a major risk factor for stroke and dementia.1 Currently available clinical imaging techniques are able to probe SVD indirectly by identifying parenchyma lesions such as white matter hyperintensities (WMH) of presumed vascular origin.2 WMH lesions tend to occur in periventricular and deep white matter regions, and are thought to be caused by demyelination and/or microvascular ischemic events.2,3 WMH lesion volume is associated with cognitive decline, in particular executive function, processing speed, and memory domains4,5 as well as a reported doubling the risk of dementia1,3 and tripling the risk of stroke.3 Recent studies linked WMH to impaired brain hemodynamics, ie, reduced cerebral blood flow and cerebrovascular reactivity5,6 and alteration of cognitive networks, ie, reduced resting state connectivity.7

Systemic vascular risk factors (VRFs) such as hypertension, diabetes, and hypercholesterolemia are thought to contribute to the development of SVD pathology. Several early studies showed an increase in WMH prevalence in individuals with type 2 diabetes and hypertension relative to controls,\textsuperscript{8,9} while others reported only a modest effect of VRF on WMH volume.\textsuperscript{10} Reports on the association of hypercholesterolemia and WMH are also controversial.\textsuperscript{8,11} VRFs contribute to endothelial dysfunction by decreasing the availability and/or activity of the vasodilatory agent nitric oxide,\textsuperscript{12} impacting vascular function throughout the body, including the brain.\textsuperscript{13–15} The relationship between VRFs and WMH is an active area of research and in the current study we hypothesize that individuals with WMH and multiple VRFs will have altered vascular and neuronal functions relative to individuals with WMH and few or no VRFs. We investigated two gray matter measures: cerebrovascular reactivity (CVR), assessed by inhalation of a carbon dioxide-enriched gas mixture, and resting state coactivation (RS coactivation), measured as the correlation of spontaneous brain activity between brain regions. These two techniques demonstrated a wide clinical utility\textsuperscript{14–16} and are well suited for assessing the impact of WMH on the function of remote gray matter regions.

Our primary objective was to assess the contribution of VRFs to RS coactivation and CVR in three relevant brain networks: the DMN, sensory-motor, and the medial-visual networks. The DMN is chosen because of its sensitivity to cognitive decline, particularly in the episodic and working memory domains pertaining to VRF and WMH impact.\textsuperscript{1,16,17} The sensory-motor and medial-visual networks were chosen because they are highly vascularized primary sensory regions that may be sensitive to vascular disturbances, as shown in previous VRFs studies.\textsuperscript{14,18} A secondary analysis examines the correlation between RS-BOLD and CVR-BOLD within the same networks.

### Materials and Methods

#### Participants

Twenty-nine elderly adults participated in this study. Participants were recruited to the study if they were suspected of having WMH based on the presence of one or more of the following criteria: 1) the individual reporting a memory complaint to their Sunnybrook neurologist, 2) evidence of WMH findings on a previous magnetic resonance imaging (MRI) that was independent of the current study, 3) a history of VRFs. Exclusion criteria were: cortical infarcts, age <50, genetic SVD, and known severe carotid stenosis. Individuals were also excluded if they had contraindications to MRI. Cognitive status was obtained from medical charts. The presence of VRFs (hypertension, diabetes, and hypercholesterolemia) was ascertained based on self-report as corroborated by medical notes and current medications. Participants were assigned to one of three subgroups based on the number of VRFs (i.e, 0, 1, ≥2). Detailed information on VRF characteristics for each subgroup is included in Table 1. Participants completed a Montreal cognitive assessment (MoCA)\textsuperscript{19} and an MRI session that included a resting state (RS) scan and a CO\textsubscript{2} inhalation challenge. Written informed consent was obtained from all participants. The study was approved by the Sunnybrook Research Ethics Board.

#### MRI

MRI was performed on a 3T Philips Achieva system using a body coil for transmission and an 8-channel receiver head coil. CO\textsubscript{2}
inhalation challenge\textsuperscript{20,21} was used as a measure of CVR. $T_1$-weighted blood oxygenation level-dependent (BOLD) echo-planar imaging was performed throughout the CO\textsubscript{2} inhalation epochs with the following parameters: TR/TE = 2000/30 msec, matrix = $64 \times 64$, voxel size = $3.6 \times 2.9 \times 3$ mm\textsuperscript{3}, number of slices = 40, flip angle = 90\textdegree, first four dynamics discarded. BOLD acquisitions for RS and CVR were identical except for scan durations (RS: 6 min 8 sec, CVR: 8 min 38 sec). $T_1$-weighted images were acquired for image registration and segmenting gray matter, TR/TE/TI = 9.5/2.3/1400 msec, matrix = $256 \times 164$, voxel size = $1 \times 1 \times 1.2$ mm\textsuperscript{3}, number of slices = 140, flip angle = 8\textdegree, and scan duration = 8 minutes 56 seconds. Fluid attenuation inversion recovery (FLAIR) images were acquired to determine the WMH volume with the following parameters: TR/TE/TI = 9000/125/2800 msec, matrix = $240 \times 217$, voxel size = $1 \times 1.1 \times 3$ mm\textsuperscript{3}, number of slices = 52, scan duration = 4 minutes 48 seconds.

**Hypercapnia Challenge**

Moderate hypercapnia was induced through administration of a gas mixture using a RespirAct breathing circuit with participants breathing via a tight-fitting mask (Thornhill Research, Toronto, Canada). RespirAct was implemented in accordance with previously described reports\textsuperscript{20,21} that involved increasing end-tidal partial CO\textsubscript{2} pressure (P\textsubscript{ET}CO\textsubscript{2}) levels by 10 mmHg while keeping P\textsubscript{ET}O\textsubscript{2} levels constant. Elevated CO\textsubscript{2} levels were delivered 45 seconds after the start of scanning for a period of 45 seconds, followed by 90 seconds of normocapnia. The second CO\textsubscript{2} period was 2 minutes in duration followed by 3 minutes 30 seconds of normocapnia. CO\textsubscript{2} values were measured using a gas analyzer at the exhalation.

**Image Analysis**

**VASCULAR RISK FACTORS SUBGROUP COMPARISON.** Resting state coactivation analysis. Imaging data were analyzed using tools available through the FMRIB Software Library (FSL, v. 4.1, http://fsl.fmrib.ox.ac.uk/). Preprocessing of RS-BOLD images included motion correction, spatial smoothing with a Gaussian kernel of 5mm FWHM, slice-time correction and a bandpass filter. Following preprocessing and registration, DR was carried out on the CVR-BOLD data using the RS-BOLD NOIs as spatial inputs. Extracted CVR time-series are specific for each NOI and were subsequently used in the second linear model (Fig. 1). The results of the DR were CVR patterns corresponding to RSNs, which were tabulated as a %BOLD change value as in Eq.(1) and referred to as DR-based CVR.

Cerebrovascular reactivity analysis: Dual-regression. CVR-BOLD image processing was the same as RS-BOLD except a temporal high-pass filter with 250-sec cutoff was used instead of a bandpass filter. Following preprocessing and registration, DR was carried out on the CVR-BOLD data using the RS-BOLD NOIs as spatial inputs. Extracted CVR time-series are specific for each NOI and were subsequently used in the second linear model (Fig. 1).

Cerebrovascular reactivity analysis: Model-based. CVR was also computed using the P\textsubscript{ET}CO\textsubscript{2} trace in a general linear model. Similar to the DR-based CVR, the resulting maps were converted to variance-normalized %BOLD change and are further referred to as model-based CVR.

**RSN Coactivation vs. CVR**

Pearson’s correlations between average RS coactivation and average DR-based CVR were calculated for each NOI using SPSS statistical software, v. 21 (IBM, IMB SPSS Statistics for Mac, Armonk, NY).

**Contribution of Gray Matter Segmentation and WMH Lesion Volumes to NOIs**

$T_1$-weighted images were segmented using FSL FAST\textsuperscript{29} to create gray matter (GM) partial volume maps, which were registered to group template space and thresholded to only include voxels with ≥50% GM contribution. NOI masks (DMN, sensory-motor, and medial-visual) generated during group ICA were used to calculate percentage of GM in each network by dividing GM volume within NOI by the total NOI volume.
FLAIR images were processed using in-house software, i.e., a two-class fuzzy C-means clustering technique, to segment voxels as WMH. The segmentation was confirmed by visual inspection and the total volume of the segmented WMH regions was calculated after normalizing intracranial volume to the average head size of 1300 ml. A WMH probability map was generated using the binary WMH masks for all participants, as a means to compare the WMH locations and the NOIs. The WMH masks were combined into a single probability map for visualization. In addition to a qualitative comparison of WMH distribution and NOI locations, a percentage of WMH in each NOI was calculated by dividing WMH volume within NOI by the total NOI volume.

**Statistical Analyses**

An analysis of covariance (ANCOVA) was used to test for an effect of VRF subgroup on RS coactivation, DR-based CVR and model-based CVR measures. VRF subgroup comparisons were carried out for each NOI with age and GM percentage covariates using SPSS statistical software, v. 21 (IBM, IMB SPSS Statistics for Mac). Values of $P < 0.05$ were considered statistically significant. Post-hoc analyses were rerun after removing the potential outlier effects from participants with a probable Alzheimer’s disease (AD) diagnosis. In addition, the contribution of motion to the VRF subgroup differences was examined using analysis of variance on relative mean motion during RS-BOLD and CVR-BOLD (obtained from the motion correction step).
Results

Demographics

Four participants were excluded due to head motion greater than 1.5 mm during the RS scan. The remaining 25 participants were assigned to VRF subgroups depending on their number of risk factors. VRF subgroups did not differ significantly in WMH volumes, age, gender ratio, and MoCA scores (see Table 1).

Vascular Risk Factors Subgroup Comparison

NOIs used for VRF subgroup comparisons are shown in Fig. 2: sensory-motor NOI included postcentral and precentral gyri as well as regions in parietal operculum cortex and superior temporal gyrus; medial-visual NOI consisted of occipital pole, bilateral lingual gyri, intracalcarine, and cuneal cortices; and DMN NOI encompassed precuneus, posterior cingulate, and paracingulate gyri; bilateral: hippocampus, angular, postcentral gyri, as well as middle temporal and medial frontal regions.

Group comparison of the RS coactivation showed no significant differences in any of the three networks (DMN: \( P = 0.31 \); sensory-motor: \( P = 0.44 \); medial-visual: \( P = 0.88 \)), as shown in Fig. 3a. DR-based CVR group differences were significant after adjustment for age and GM percentage in the DMN \( (F_{20,2} = 5.17, P = 0.015) \) but not sensory-motor \( (P = 0.16) \) nor medial-visual networks \( (P = 0.13) \), as presented in Fig. 3b. These DMN findings remained significant after correction for multiple comparisons \( (P_{\text{corrected}} = 0.05/3 = 0.017) \) and after excluding four participants with AD \( (F_{16,2} = 6.22, P = 0.01) \). A post-hoc analysis demonstrated that DR-based CVR in the DMN in VRF \( \geq 2 \) group was significantly reduced compared to participants in VRF 0 group \( (P = 0.025) \) and VRF 1 group \( (P = 0.007) \). No significant difference was detected between VRF 0 and VRF 1 groups.

No significant group differences were detected when using the model-based analysis of the CVR data (DMN: \( P = 0.15 \); sensory-motor: \( P = 0.15 \); medial-visual: \( P = 0.32 \), data not shown).

RS Coactivation vs. CVR

Significant correlation between RS coactivation and DR-based CVR was detected in the DMN \( (r^2 = 0.28, P = 0.006) \) but not in sensory-motor nor medial-visual NOIs \( (P > 0.05) \), as shown in Fig. 4.

Influence of WMH and Head Motion

Figure 5 shows a probability map of WMH distribution in the current population. Contribution of WMH to NOIs was minimal (DMN: \( 0–1.2\% \); sensory-motor: \( 0–2.9\% \); medial-visual: \( 0–4.6\% \), percentage refers to the proportion of NOI occupied by WMH). Head motion during RS-BOLD and...
CVR-BOLD scans was not significantly different between the VRF subgroups ($P = 0.73$ and $P = 0.10$, respectively).

**Discussion**

In this cohort of older adults at risk for stroke and cognitive decline, the number of VRFs did not contribute to the WMH volume but was associated with decreased regional CVR. Individuals with two or more VRFs had greater CVR impairment in the DMN than those without VRFs or with only one. VRF subgroup differences were detected using the data-driven approach as opposed to the model-based approach that used the PETCO$_2$ traces. Although the number of VRFs did not have a direct effect on average RS coactivation within the NOIs, we did observe a correlation between average RS coactivation and DR-based CVR within the DMN.

A hypercapnia CVR paradigm has previously been used to detect vascular impairment in type 2 diabetes, hypertension, and hypercholesterolemia studies. A decrease in global and regional CVR was also seen in individuals with high WMH volumes. The results of the current study add to this literature by identifying regions vulnerable to an increasing number of VRFs in the presence of WMH. Our findings of CVR impairment in the DMN are in part supported by the results of a study by Last et al showing that the presence of multiple VRFs, ie, type 2 diabetes and hypertension, associates with decreased CVR in the temporal lobe relative to diabetes alone.

Regions showing significant vulnerability to VRFs were those associated with DMN. Similar regions showed a decrease in glucose metabolism on FDG-PET scans of AD and mild cognitive impairment (MCI) cohorts and were strongly associated with characteristic cognitive decline in these groups. Increased WMH volume is a common neuroimaging finding in AD and MCI patients. Vascular damage associated with SVD may directly contribute to AD pathology by reducing amyloid-$\beta$ (A$\beta$) clearance. Grimmer et al detected a positive correlation between WMH volume and amyloid deposition over time in parieto-occipital regions. VRFs are also known to increase the risk and accelerate the onset of AD, as well as other dementias, mediated by both vascular and metabolic changes. For example, peripheral hyperinsulinemia, a hallmark of type 2 diabetes, indirectly promotes A$\beta$ accumulation and neurofibrillary tangles formation by downregulating the enzymes responsible for their clearance. Furthermore, evidence from several longitudinal studies suggest that mid-life hypertension and high cholesterol levels are associated with increased risk of dementia later in life.

In this study we saw no correlations between MoCA scores and RS coactivation or CVR brain measures, which could be because MoCA is designed to obtain a global measure of cognitive status. More specialized tests targeting
DMN functions should be used in the future to examine the link between cognition and CVR/RS coactivation in this network.

Whereas CVR changes have been reported in the sensory-motor network as a function of the adult lifespan, we did not detect a significant association with VRF sub-grouping in this network. CVR differences were also previously observed in several regions of the visual network, namely, bilateral lingual gyrus and cuneal cortex, in individuals with hypertension and type 2 diabetes compared to hypertension only. In the current study, CVR was averaged across a larger area of visual cortex, which may have reduced group differences and weakened potential associations. Furthermore, CVR variability within the VRF sub-groups was higher in sensory-motor and medial-visual NOIs compared to DMN, which may also explain the lack of significant findings and suggests an impact of other factors on CVR in these networks that were not accounted for in the model.

In the secondary analysis a positive correlation was detected between CVR and RS coactivation in the DMN. This is consistent with previously reported associations between whole brain CVR and RS coactivation. A study examining resting state cerebral blood flow (CBF) also showed a significant correlation between baseline CBF and functional connectivity in the DMN, further supporting the importance of examining the vascular integrity of functional networks. The authors of the study suggested that the close correlation between CBF and RS coactivation in the DMN might be due to a tight coupling between metabolic and hemodynamic mechanisms required to support regulation of states of consciousness.

CVR group differences were detected using a dual-regression approach but not when the global $P_{ET}CO_2$ signal was used as an input in a single-regression (model-based approach). Dual-regression has the advantage of accounting for regional variation in brain CO$_2$ response, which can contribute to improved sensitivity. Others suggested that this is an important methodological consideration and identified anatomical location and/or vascular territories as potential sources of CO$_2$ response variance across the brain. The dual-regression approach, used in this study, further advances our understanding of cerebrovascular function by demonstrating that there is a close correspondence between regions with similar vascular response and functional RSNs.

This cross-sectional study has both limitations and strengths. Although the sample size was small, we implemented the CO$_2$-based CVR in a clinical population and developed a novel method of analysis that was able to incorporate a priori networks of interest and was found to have additional sensitivity to VRF status when compared to the model-based method. Future confirmation of this approach in a larger population is needed. Next, the inclusion criteria specified a potential for WMH, which was found to range from 0.1–70.6 mL. Future studies are needed to establish if these results generalize to populations that are healthy and otherwise free of WMH. There is value in considering continuous measures of VRFs, such as HbA1C or systolic blood pressure readings, but in the current study we employed a more parsimonious approach of a categorical score, one that has been used by others in a similar research context. We note also that only one participant had three VRFs, thus our choice of the third category of ≥2.

In conclusion, this study examined the effects of VRFs on CVR and RS coactivation in individuals with WMH. CVR was reduced in the DMN in the group with ≥2 VRFs. We saw no significant effect of the number of VRFs on RS coactivation, although a positive correlation between CVR and RS coactivation in the DMN suggests a link between the two metrics. Future work could incorporate longitudinal data to gain further insight on the effect of CVR on RS coactivation and its potential use as an imaging biomarker.

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References


