Title

Soluble Epoxide Hydrolase-Derived Linoleic Acid Oxylipins in Serum Are Associated with Periventricular White Matter Hyperintensities and Vascular Cognitive Impairment

Journal

Translational Stroke Research, 10(5)

ISSN

1868-4483

Authors

Yu, Di
Hennebelle, Marie
Sahlas, Demetrios J
et al.

Publication Date

2019-10-01

DOI

10.1007/s12975-018-0672-5

Peer reviewed

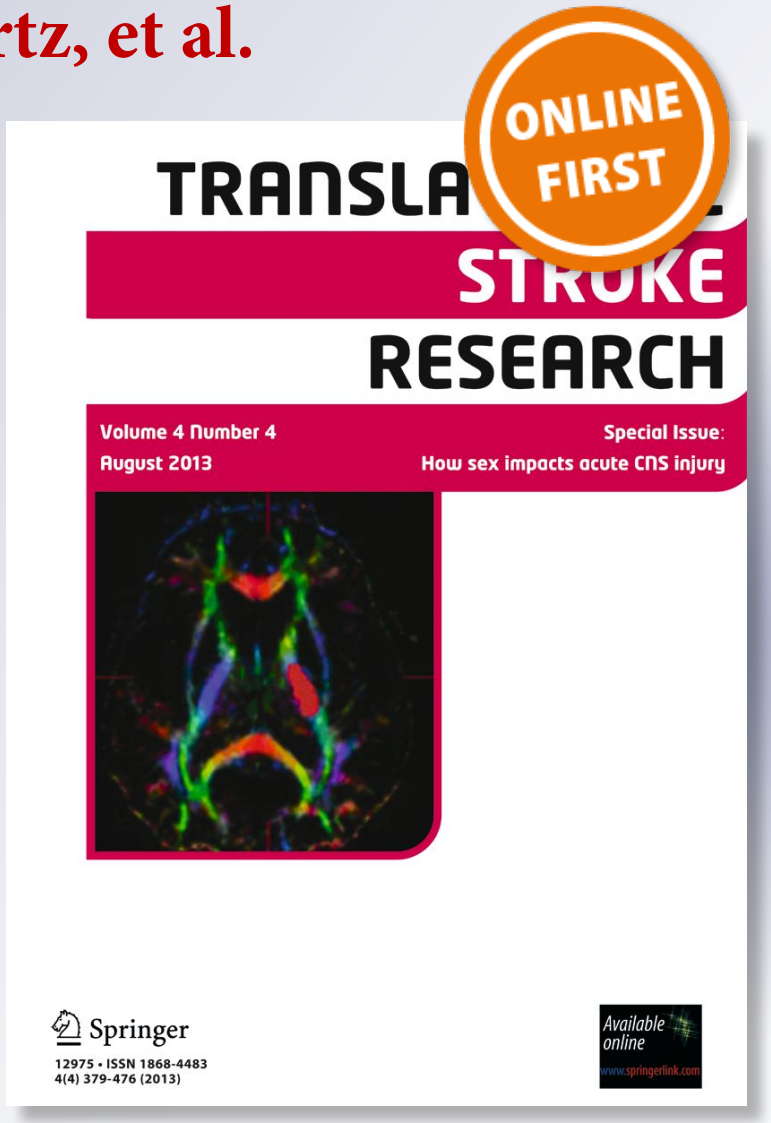
Soluble Epoxide Hydrolase-Derived Linoleic Acid Oxylipins in Serum Are Associated with Periventricular White Matter Hyperintensities and Vascular Cognitive Impairment

Di Yu, Marie Hennebelle, Demetrios J. Sahlas, Joel Ramirez, Fuqiang Gao, Mario Masellis, Hugo Cogo-Moreira, Richard H. Swartz, et al.

Translational Stroke Research

ISSN 1868-4483

Transl. Stroke Res.
DOI 10.1007/s12975-018-0672-5



Springer

Translational Stroke Research
https://doi.org/10.1007/s12975-018-0672-5

ORIGINAL ARTICLE

Soluble Epoxide Hydrolase-Derived Linoleic Acid Oxylipins in Serum Are Associated with Periventricular White Matter Hyperintensities and Vascular Cognitive Impairment

Di Yu[1,2,3,4] · Marie Hennebelle[5] · Demetrios J. Sahlas[6] · Joel Ramirez[1] · Fuqiang Gao[1] · Mario Masellis[1,7] · Hugo Cogo-Moreira[8,9] · Richard H. Swartz[1,7] · Nathan Herrmann[1] · Pak Cheung Chan[10,11] · Jacqueline A. Pettersen[12] · Donald T. Stuss[1,7,13] · Sandra E. Black[1,3,4,7] · Ameer Y. Taha[5] · Walter Swardfager[1,2,3,4,14]

Received: 23 May 2018 / Revised: 15 October 2018 / Accepted: 18 October 2018

Abstract

White matter hyperintensities (WMH) are presumed to indicate subcortical ischemic vascular disease but their underlying pathobiology remains incompletely understood. The soluble epoxide hydrolase (sEH) enzyme converts anti-inflammatory and vasoactive cytochrome p450-derived polyunsaturated fatty acid epoxides into their less active corresponding diol species. Under the hypothesis that the activity of sEH might be associated with subcortical ischemic vascular disease and vascular cognitive impairment, this study aimed to compare the relative abundance of sEH substrates and products in peripheral blood between patients with extensive WMH (discovered due to transient ischemic attack; $n = 29$) and healthy elderly with minimal WMH ($n = 25$). The concentration of 12,13-DiHOME (a sEH-derived linoleic acid metabolite), and the ratio of 12,13-DiHOME to its sEH substrate, 12,13-EpOME, were elevated in the extensive WMH group ($F_{1,53} = 5.9$, $p = 0.019$), as was the 9,10-DiHOME/9,10-EpOME ratio ($F_{1,53} = 5.4$, $p = 0.024$). The 12,13-DiHOME/12,13-EpOME ratio was associated with poorer performance on a composite score derived from tests of psychomotor processing speed, attention, and executive function ($\beta = -0.473$, $p = 0.001$, adjusted $r^2 = 0.213$), but not with a composite verbal memory score. In a mediation model, periventricular WMH (but not deep WMH), explained 37% of the effect of the 12,13-DiHOME/12,13-EpOME ratio on the speed/attention/executive function composite score (indirect effect = − 0.50, 95% bootstrap confidence interval [− 0.99, − 0.17] Z-score units). Serum oxylipin changes consistent with higher sEH activity were markers of vascular cognitive impairment, and this association was partly explained by injury to the periventricular subcortical white matter.

Keywords Small vessel disease · Vascular cognitive impairment · Oxylipins · Soluble epoxide hydrolase · Linoleic acid

Ameer Y. Taha and Walter Swardfager contributed equally to this work.

✉ Walter Swardfager
w.swardfager@utoronto.ca

[1] Hurvitz Brain Sciences Program, Sunnybrook Research Institute, Toronto, Canada

[2] Department of Pharmacology & Toxicology, University of Toronto, Toronto, Canada

[3] Canadian Partnership for Stroke Recovery, Sunnybrook Research Institute, Toronto, Canada

[4] LC Campbell Cognitive Neurology Unit, Sunnybrook Research Institute, Toronto, Canada

[5] Department of Food Science and Technology, College of Agriculture and Environmental Sciences, University of California, Davis, CA, USA

[6] Department of Medicine (Neurology Division), McMaster University, Hamilton, Canada

[7] Department of Medicine (Neurology Division), University of Toronto, Toronto, Canada

[8] Departamento Psiquiatria, Universidade Federal de São Paulo, São Paulo, Brazil

[9] Department of Education and Psychology: Division of Methods and Evaluation, Freie Universität Berlin, Berlin, Germany

[10] Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Canada

[11] Department of Laboratory Medicine and Molecular Diagnostics, Sunnybrook Health Sciences Centre, Toronto, Canada

[12] Department of Medicine (Neurology Division) and the Northern Medical Program, University of British Columbia, Vancouver, Canada

[13] Department of Psychology, University of Toronto, Toronto, Canada

[14] University Health Network Toronto Rehabilitation Institute, Toronto, Canada

Published online: 15 November 2018

Introduction

Subcortical ischemic vascular disease (SIVD), also known as cerebral small vessel disease, refers to pathological abnormalities of the penetrating cerebral arterioles, capillaries, and venules. SIVD is highly prevalent in patients with dementia, but it is also found in cognitively normal individuals with advancing age. SIVD contributes to a spectrum of vascular cognitive impairment (VCI), most commonly involving deficits in psychomotor processing speed, attention, and executive function, and it is one of the most common co-contributors to dementia with mixed pathologies. [1] Currently, magnetic resonance imaging (MRI) features are used to infer SIVD, including periventricular and deep (i.e. superficial) white matter hyperintensities (WMH) on T2-weighted or fluid-attenuated inversion recovery sequences. [2] However, the relationships between vascular risk factors, WMH, and VCI can be highly variable. [3] Therefore, biomarkers that could identify SIVD, and the corresponding risk of decline in cognitive function, are a matter of clinical importance but they remain lacking.

Oxylipins are bioactive lipid species generated from the oxidation of polyunsaturated fatty acids, known to play a myriad of physiological and pathophysiological roles. Oxylipins can be generated non-enzymatically, or enzymatically via cyclooxygenases, lipoxygenases, and cytochrome P450 monooxygenases. [4] The cytochrome P450-derived eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) epoxides are essential regulators of inflammatory resolution pathways, [5, 6] whereas arachidonic acid (AA)-derived epoxide species (epoxyeicosatrienoic acids; EETs) have vasodilatory effects that regulate blood flow. [7, 8] The fatty acid epoxides are inactivated by soluble epoxide hydrolase (sEH), which catalyzes their conversion into less active or inactive diol species, some of which can be cytotoxic. [9–11] The roles of the linoleic acid (LA)-derived oxylipins are not as well understood; however, the pro-toxins leukotoxin (9,10-EpOME) and isoleukotoxin (12,13-EpOME), as well as their sEH metabolites (9,10-DiHOME and 12,13-DiHOME), can influence physiological functions via their effects on PPARɣ receptors, [12] and the diols in particular exhibit cytotoxic, immunological, and pro-oxidative effects. [13, 14]

Recent studies in animal models suggest effectiveness of sEH inhibition in ameliorating the detrimental consequences of experimental cerebral ischemia. [15] In humans, an autopsy study revealed an elevation of sEH products in the brain tissue of VCI patients, as well as increased sEH expression in the vascular endothelial cells of the affected small vessels. [16] Therefore, sEH activity may be associated with SIVD, and the resultant structural and cognitive abnormalities, but the relationships between these abnormalities and intravascular oxylipin concentrations have not been assessed in living humans.

The present study examined sEH-related oxylipins in serum from a cohort carefully selected to have either extensive or minimal SIVD based on their WMH burden. We hypothesized that polyunsaturated fatty acid diol/epoxide ratios, reflective of sEH activity, would be elevated in people with extensive SIVD. We further hypothesized that diol/epoxide ratios would be related to the extent of WMH, and to deficits in the cognitive domains most commonly involved in VCI.

Methods

Participants

This was a cross-sectional study designed to investigate the characteristics and effects of SIVD by recruiting patients of a stroke prevention clinic ($n = 29$) and healthy elderly controls ($n = 25$) between 2007 and 2012, into strata of extensive and minimal WMH burden observed on 3.0 T MRI. A group with at least moderate WMH, defined as periventricular WMH extending at least 5 mm from the ventricular border consistent with Fazekas Periventricular Hyperintensity Scale [17] score ≥ 2, or ≥ 4 focal lesions ≥ 5 mm in diameter, was recruited within 3 months of a transient ischemic attack without residual physical symptoms. A comparison group of healthy elderly controls 50–85 years of age was recruited who had ≤ 3 focal hyperintensities none of which were ≥ 3 mm in diameter.

All participants in both groups were fluent in English and had standardized Mini Mental State Examination (sMMSE) score > 19. Participants who had other neurological disorders, cortical infarcts, or cortical hyperintensities (i.e., > 3 cortical hyperintense foci, or any cortical lesion > 3 mm in diameter) visible on 3.0 T MRI were excluded from each group. Participants with Alzheimer's disease or cortical stroke were excluded in order to determine relationships between the diol/epoxide ratios and SIVD specifically. Possible participants with any unstable medical condition or history of neurological or psychiatric disorder, beyond mild depressive symptoms, were excluded. The study was approved by local research ethics boards, and all participants provided written informed consent.

Blood Collection and Oxylipin Extraction

Blood was drawn at a convenient time of day without prior fasting into an SST Vacutainer™ and spun at 10,000*g*, and serum was separated and stored at − 80 °C until the time of analysis, in November, 2017. Assays were conducted blind to clinical diagnoses. Free (unesterified) oxylipins were extracted by solid phase extraction (SPE). The free fraction was examined because it is thought to be the bioactive pool. [18]

Free oxylipins were quantified by adding 10 μL surrogate standard solution, and 600 uL of extraction buffer (250 uM EDTA in water: 0.002% BHT in methanol (4:1) with 0.01% acetic acid) to 200 μL serum. The surrogate standard solution

contained 2 μM of d11–11(12)-EpETrE and d11–14,15-DiHETrE in methanol (i.e., 20 pmol of each deuterated oxylipin per sample). Samples were vortexed and centrifuged 10 min at 15,871×*g* at 0 °C. The supernatant was poured into a Sep-Pak tC18 1-cc column (Waters, Milford, MA, USA), pre-washed with 1.5 mL methanol, and equilibrated with 1.5 mL 20% methanol. The column was then washed with 1.5 mL 20% methanol followed by 1.5 mL hexanes. Oxylipins were eluted using 2 mL methanol. Samples were dried under nitrogen, reconstituted in 100 uL methanol, and filtered before analysis by LC-MS/MS.

Oxylipin Quantification

A total of 24 oxylipins were analyzed by ultra-high-pressure liquid chromatography tandem mass spectrometry (UPLC-MS/MS) as previously described, [19, 20] on an Agilent 1290 Infinity (Agilent Corporation, Palo Alto, CA, USA) UHPLC system connected to a 6460 QqQ tandem mass spectrometer (Agilent Technologies, Palo Alto, CA, USA) equipped with an electrospray ionization source (Jet Stream technology). Oxylipins were separated on an Agilent 2.1 × 150 mm Eclipse Plus C18 column with a 1.8-μm particle size. Standards obtained from Cayman Chemicals (Ann Arbor, MI, USA) were used to generate the standard curve for each oxylipin.

The autosampler temperature was kept at 4 °C and the column at 45 °C. The mobile phase A contained 0.1% acetic acid in ultrapure water and the mobile phase B contained acetonitrile/methanol/acetic acid (84/16/0.1). Gradient elution was performed as follows: solvent B was increased from 35 to 40% from 0 to 3 min, to 48% from 3 to 4 min, to 60% from 4 to 10 min, to 70% from 10 to 20 min, to 85% from 20 to 24 min, and to 99% from 24.5 to 24.6 min. Solvent B was then held at 99% for 4 min, decreased to 35% from 26 to 26.1 min, and held at 35% for 1.9 min. The flow rate started at 0.3 mL/min. It was decreased to 0.25 mL/min after 3 min, held at 0.25 mL/min for 21.6 min, increased to 0.35 mL/min at 24.6 min, and finally decreased to 0.3 mL/min at 27.3 min. The total run time was 28 min per sample. The instrument was operated in negative electrospray ionization mode and used optimized multiple reaction monitoring conditions of the parent and fragmentation product ion to measure each oxylipin. [20] Peaks were quantified according to external standard curves and corrected for the surrogate standard recovery using MassHunter Wokstation–Quantitative Analysis (Agilent Technologies, Palo Alto, CA, USA).

Magnetic Resonance Imaging

Transcranial MRI was performed at 3.0 T on a Discovery MR750 (General Electric). The parameters of the T1 acquisition were as follows: axial 3D FSPGR EDR IR Prep: TE = 3.2 ms, TR = 8.1 ms, NEX = 1, flip angle = 8°, FOV = 22 cm, matrix = 256 × 192, slice thickness = 1.0 mm. The interleaved proton density and T2-weighted parameters were as follows: axial 2D FSE-XL, EDR, fat sat. ASSET: TE = 11.1 and 90 ms, TR = 2500 ms, NEX = 1, flip angle = 90°, FOV = 22 cm, matrix = 128 × 96, slice thickness = 3 mm. The T2 fluid-attenuated inversion recovery parameters were as follows: axial T2FLAIR, EDR FAST: TE = 140 ms, TR = 9700 ms, NEX = 1, FOV = 22 cm, matrix = 256 × 192, slice thickness = 3.0 mm. The T2* gradient echo (GRE) parameters were as follows: TE = 20 ms, TR = 350 ms, flip angle = 20°, FOV = 22 cm, matrix = 256 × 160, slice thickness = 3 mm.

Image processing was done using Lesion Explorer (sabre.brainlab.ca), which is a comprehensive semi-automated segmentation procedure to obtain regional brain volumes. [21–23] The reliability of the method has been previously established (global ICC = .99; regional ICC = .98; similarity index = .97; scan-rescan reliability for all tissue types: ICC > .9) and it has been validated extensively against kNN (ICC = .97), the age-related white matter change (r = .86), and Cholinergic Pathways Hyperintensities Scale (r = .97). [22, 23] Prior to tissue segmentation, skull and non-brain tissue were removed using Brain-Sizer. The segmentation uses co-registered T1 and interleaved PD/T2 images to identify normal-appearing gray matter, normal-appearing white matter, cerebrospinal fluid, WMH, and lacunar infarcts, based on fitting voxel intensities to 4 Gaussian curves. [22] Compatible with neuroimaging consensus standards for the study of cerebral small vessel disease, [2] WMH were segmented using PD/T2 sequences and further segmented from lacunar-like infarcts within the hyperintensities using the T1 segmentation. Skull and dura were removed using Brain-Sizer.

Cognitive Assessments

Cognitive assessments were administered by clinicians or trained researchers under the supervision of a neuropsychologist. Psychomotor processing speed, attention, and executive function, were assessed using the Digit Symbol Substitution Test (DSST), Trail Making Test Part B (TMT-B), Stroop Color-Word Interference Test, and FAS Verbal Fluency Test. Because each of these domains are affected in VCI, [24] we had no reason a priori to choose one particular domain or test; therefore, these results were combined into a single composite Z-score for analysis. A verbal memory composite Z-score was computed from four California Verbal Learning Test, 2nd Edition (CVLT-II) measures: acquisition, short delayed free recall, long-delayed free recall, and recall discriminability. These tests were chosen based on consensus recommendations by the National Institutes of Neurological Disorders and Stroke–Canadian Stroke Network for a standardized assessment of VCI. [24]

Statistical Analyses

Categorical data were summarized using counts and percentages while continuous data were summarized using means and standard deviations. Between-groups differences participant characteristics were assessed using Student's t or chi-square tests.

Oxylipin data were first inspected based on the detectability of each oxylipin. Those detectable in fewer than 50% of the participants were considered as categorical variables (detectable vs. not detectable) for analysis. Those that could be detected in more than 50% of the participants were log-transformed to correct skewed distributions and they were examined as continuous variables. The concentration or detectability of each individual oxylipin was compared between groups through a Student's t or a chi-square test, respectively. For pairs of diols and epoxides that were detected in most samples, diol/epoxide ratios were calculated and used as surrogate markers of sEH activity for hypothesis testing [25, 26].

Multivariate analysis of covariance (MANCOVA) was used to examine the association between SIVD (fixed factor) and the ratios of diols to their respective epoxides (dependent variable), with age and sex as covariates. Participant characteristics found to differ significantly between groups were included in post hoc models. These analyses were performed in SPSS (version 23) and Bonferroni adjusted for multiple comparisons.

Two unit–weighted cognitive composite Z-scores were computed, one from the 4 tests of psychomotor processing speed/attention/executive function, and the other from 4 measures of verbal memory from the CVLT-II. The composite scores were compared with diol/epoxide ratios using linear regression, in the whole group and in SIVD and non-SIVD participants separately post hoc, controlling for age and sex. The relationships between the diol/epoxide ratios and the composite psychomotor processing speed/attention/executive function score were Bonferroni corrected for the number of oxylipin ratios tested. Demographic characteristics found to differ significantly between groups were included in post hoc linear regression models. Relationships with individual cognitive test results were explored post hoc to determine if some tests might be more sensitive than others.

To test the hypothesis that the relationships between serum oxylipin ratios and cognitive performance were mediated by WMH burden, mediation models were used. The indirect effects were considered significant if the 95% bias-corrected bootstrap confidence intervals for the products in the coefficients of the indirect path did not cross zero under 10,000 permutations. Covariates (age and sex) were regressed at once on the mediators and the outcome. These analyses were performed using the PROCESS Macro (version 2.16.3) for SPSS. [27]

Results

Participant Characteristics

The characteristics of the whole group ($n = 54$) and of the SIVD ($n = 29$) and non-SIVD ($n = 25$) subgroups are presented in Table 1. The subgroups were similar in age ($p = 0.96$), gender ($p = 0.75$), and total years of education ($p = 0.24$). Mean MMSE scores, the proportion with hypertension, and proportions using concomitant antihypertensives ($p = 0.033$) and antithrombotics ($p = 0.012$) differed significantly between the groups. As per the intended cohort design, participants in the extensive SIVD subgroup had larger volumes of both deep ($t_{1,53} = 3.66$, $p = 0.001$) and periventricular WMH ($t_{1,53} = 5.27$, $p < 0.001$) compared to healthy elderly controls. Performance on all cognitive tests was poorer in the SIVD group compared to the non-SIVD group, although all mean scores were within the "normal" range (Table 2).

Serum Oxylipin Concentrations

The sEH-related oxylipins examined are summarized in Table 3, along with their observed detectabilities and serum concentrations. Out of 24 oxylipins, 8 species were detectable in more than 50% of the subjects, including four LA-derived species (2 epoxides and 2 diols), two AA-derived diols, one EPA-derived diol, and one DHA-derived epoxide (Table 2). One EPA-derived diol was absent in the entire group (Table 3).

The concentrations of 12,13-DiHOME (sEH-derived LA metabolite; $t_{1,53} = 2.08$, $p = 0.043$) were found to be significantly higher in SIVD patients than controls. The cytochrome p450-derived EPA metabolite 11(12)-EpETE was less likely to be detected in the SIVD subgroup ($\chi^2_{1,53} = 6.41$, $p = 0.011$).

Associations Between Diol/Epoxide Ratios and SIVD

Two ratios could be generated from the oxylipins that were detectable in more than 50% of the cohort: 9,10-DiHOME/9,10-EpOME, and 12,13-DiHOME/12,13-EpOME. Both ratios were generated from LA-derived epoxides and diols. The mean ± standard deviation 9,10-DiHOME/9,10-EpOME ratios were 3.37 ± 2.19 in SIVD and 2.1 ± 1.63 in healthy controls, and the mean ± standard deviation 12,13-DiHOME/12,13-EpOME ratios were 0.81 ± 0.41 in SIVD and 0.58 ± 0.34 in healthy controls.

In a MANCOVA model with SIVD as an independent variable, controlling for age and sex, SIVD was associated with higher 12,13-DiHOME/12,13-EpOME ratio ($F_{1,53} = 5.9$, $p = 0.019$) and higher 9,10-DiHOME/9,10-EpOME ($F_{1,53} = 5.4$, $p = 0.024$) ratio. These associations remained significant after adjustment for multiple comparisons ($p < 0.025$). Age was also associated with higher 12,13-DiHOME/12,13-EpOME

Table 1 Participant characteristics

Characteristic	Total ($n = 54$)	Extensive SIVD ($n = 29$)	Healthy control ($n = 25$)	t or chi-square	p
Demographics					
Age (years)	71.7 ± 9.6	71.8 ± 11.0	71.7 ± 7.9	0.06	.957
Gender (male)	46%	48%	44%	0.09	.753
Education (years)	14.9 ± 3.3	14.4 ± 3.2	15.4 ± 3.3	− 1.19	.240
sMMSE (score)	28.3 ± 1.8	27.8 ± 2.2	28.8 ± 1.0	− 2.11	.040*
Vascular factors					
Hypertension	57%	75%	39%	6.18	.013*
Hyperlipidemia	42%	54%	29%	3.60	.165
Coronary artery disease	19%	29%	8%	5.02	.081
Type 2 diabetes	6%	10%	0%	3.20	.074
Smoking status					
Never	57%	50%	65%		
Quit	36%	42%	31%	1.18	.556
Current	6%	8%	4%		
Concomitant medications					
Antihypertensives	56%	69%	40%	4.56	.033*
Antithrombotics	46%	62%	28%	6.27	.012*
Lipid-lowering agents	43%	52%	32%	2.14	.144
PUFA supplements	7%	3%	12%	1.43	.232
Anticoagulants	6%	10%	0%	2.74	.098
WMH volumes (cc)					
Deep	1.69 ± 2.2	2.55 ± 2.5	0.53 ± 0.5	3.66	.001*
Periventricular	18.3 ± 20.6	29.0 ± 21.6	3.91 ± 3.6	5.27	< .001*

($F_{1,53} = 9.6$, $p = 0.003$) and 9,10-DiHOME/9,10-EpOME ratios ($F_{1,53} = 5.0$, $p = 0.031$).

SIVD remained associated with the 12,13-DiHOME/12,13-EpOME and 9,10-DiHOME/9,10-EpOME ratios in post hoc models, controlling use of antithrombotic ($F_{1,53} = 5.1$, $p = 0.029$ and $F_{1,53} = 5.4$, $p = 0.025$, respectively) and antihypertensive ($F_{1,53} = 4.4$, $p = 0.042$ and $F_{1,53} = 5.5$, $p = 0.023$, respectively) medications, and in a post hoc model that excluded patients using a PUFA supplement ($F_{1,49} = 5.1$, $p = 0.029$ and $F_{1,49} = 5.4$, $p = 0.025$, respectively). Trends in the 12,13-DiHOME/12,13-EpOME ($F_{1,53} = 3.5$, $p = 0.068$) and 9,10-DiHOME/9,10-EpOME ($F_{1,53} = 3.7$, $p = 0.061$) ratios persisted in models controlling for hypertension. In those models, neither the 9,10-DiHOME/9,10-EpOME ratio ($F_{1,53} = 0.01$, $p = 0.929$) nor the 12,13-DiHOME/12,13-EpOME ratio ($F_{1,53} = 0.2$, $p = 0.537$) was associated with hypertension.

Associations Between the LA-Derived Diol/Epoxide Ratio and Cognitive Performance

In linear regression models controlling for age and sex, the psychomotor processing speed/attention/executive function score was associated with the 12,13-DiHOME/12,13-EpOME ratio ($\beta = -0.473$, $p = 0.001$, adjusted $r^2 = 0.213$ Fig. 1a) in the entire group (Bonferroni adjusted $p < .025$), and in the subgroup of participants with extensive SIVD ($\beta = -0.471$, $p = 0.020$; Fig. 1b) but not in the non-SIVD subgroup ($\beta = -0.207$, $p = 0.322$; Fig. 1b). These relationships persisted when excluding participants using PUFA supplements from the whole group ($\beta = -0.457$ $p = 0.002$) and from the SIVD subgroup ($\beta = -0.442$, $p = 0.039$). The psychomotor processing speed/attention/executive function score was not associated with 9,10-DiHOME/9,10-EpOME ratio in the whole group ($\beta = -0.214$, $p = 0.132$; Fig. 1c) or in the SIVD subgroup ($\beta = -0.093$, $p = 0.634$; Fig. 1d).

In linear regression models controlling for age and sex, the memory composite score was not associated with either the 12,13-DiHOME/12,13-EpOME ratio ($\beta = -0.239$, $p = 0.115$, Fig. 2a) or the 9,10-DiHOME/9,10-EpOME ratio ($\beta = -0.032$, $p = 0.831$, Fig. 2b). No relationships between the two oxylipin ratios and memory were detected in the SIVD subgroup ($\beta = -0.168$, $p = 0.471$, Fig 2c and $\beta = 0.092$, $p = 0.665$, Fig 2d respectively).

Examining individual cognitive scores in linear regression models post hoc, the 12,13-DiHOME/12,13-EpOME ratio was associated with Z-scores in the SIVD group (Fig. 3), including TMT-B ($\beta = -0.528$, $p = 0.008$; Fig. 3a) and Stroop Color-Word Interference Test ($\beta = -0.417$, $p = 0.030$; Fig. 3b), but not DSST ($\beta = -0.420$, $p = 0.053$; Fig. 3c) and

Table 2 Cognitive outcomes

Cognitive assessments		Total ($n = 54$)	Extensive SIVD ($n = 29$)	Healthy control ($n = 25$)	t	p
Psychomotor processing speed, attention, and executive function						
Digit Symbol Substitution Test	Seconds	54.1 ± 16.2	45.3 ± 13.5	64.0 ± 12.9		
	Z-score	0.3 ± 1.0	− 0.3 ± 0.8	0.9 ± 0.7	− 5.79	< .001*
Stroop Color-Word Interference Test	Seconds	35.8 ± 18.3	43.7 ± 21.6	26.9 ± 6.5		
	Z-score	0.2 ± 1.1	− 0.3 ± 1.0	0.8 ± 0.8	− 4.47	< .001*
Trail Making Test Part B	Seconds	110.4 ± 67.4	139.8 ± 77.0	76.2 ± 29.1		
	Z-score	− 0.1 ± 1.1	− 0.6 ± 0.9	0.5 ± 0.9	− 4.59	< .001*
FAS Verbal Fluency Test	Words	40.7 ± 13.7	8.3 ± 3.1	46.1 ± 11.5		
	Z-score	0.3 ± 1.1	− 0.1 ± 1.2	0.7 ± 0.8	− 2.79	.007*
Composite	*Z*-score	0.0 ± 1.0	− 0.6 ± 0.9	0.6 ± 0.7	− 5.45	< .001*
Verbal memory						
CVLT-II—Acquisition	Words	45.8 ± 12.6	41.4 ± 13.2	51.0 ± 9.7		
	Z-score	0.5 ± 1.3	0.0 ± 1.4	1.0 ± 1.0	− 3.05	.004*
CVLT-II—Short delay free recall	Words	9.1 ± 4.0	7.7 ± 4.4	10.6 ± 2.9		
	Z-score	0.2 ± 1.3	− 0.3 ± 1.4	0.8 ± 0.8	− 3.39	.001*
CVLT-II—Long delay free recall	Words	9.4 ± 4.1	8.0 ± 4.5	10.9 ± 3.0		
	Z-score	0.1 ± 1.3	− 0.3 ± 1.4	0.5 ± 0.9	− 2.63	.011*
CVLT-II—Recall discriminability	Words	2.8 ± 0.8	2.5 ± 0.9	3.2 ± 0.6		
	Z-score	0.2 ± 1.0	0.2 ± 1.0	0.6 ± 0.8	− 3.22	.003*
Composite	*Z*-score	0.0 ± 1.0	− 0.4 ± 1.1	0.4 ± 0.7	− 3.27	.002*

* *Z*-scores were used for between-group comparisons

FAS ($\beta = -0.240$, $p = 0.294$; Fig. 3d) scores. No significant associations were seen in the non-SIVD group ($p > .05$).

Mediation Effect of White Matter Hyperintensity Volume on Cognitive Performance

In a mediation model (Fig. 4), an indirect effect of 12,13-DiHOME/12,13-EpOME on the psychomotor processing speed/attention/executive composite score mediated by periventricular WMH volume was found (path a × b = − 0.50, 95% bootstrap confidence interval [− 0.99, − 0.17] *Z*-score units). Controlling for this indirect effect, the direct effect of the 12,13-DiHOME/12,13-EpOME ratio on the speed/attention/executive composite score was also significant (path e' coefficient = − 0.87, 95% bootstrap confidence interval [− 1.63, − 0.10] Z-score units). The model showed a significant relationship between the 12,13-DiHOME/12,13-EpOME ratio and periventricular WMH volume (path a coefficient = 0.77, SE = 0.24, $p = 0.021$), and approximately 37.0% of the effect of the 12,13-DiHOME/12,13-EpOME ratio on the composite speed/attention/executive score was explained by periventricular WMH volume.

The 12,13-DiHOME/12,13-EpOME ratio was not related to deep WMH volumes (path c coefficient = 0.09, SE = 0.26, $p = 0.73$), and no mediation effect of deep WMH volumes on the psychomotor processing speed/attention/executive composite score was observed (path c × d = − 0.003, 95% bootstrap confidence interval [− 0.15, 0.08]).

In a post hoc model including hypertension as a covariate, the indirect effect of the 12,13-DiHOME/12,13-EpOME ratio on the composite score mediated by periventricular WMH volume remained significant (path a × b = − 0.61, 95% CI [− 1.35, − 0.17]Z-score units), but the direct effect did not (path e' coefficient = − 0.48, 95% CI [− 1.33, 0.37]Z-score units).

Discussion

The findings indicate potential for sEH-related oxylipins as novel peripheral biomarkers related to small vessel disease; specifically, SIVD was associated with the ratios of sEH-derived diols 12,13-DiHOME and 9,10-DiHOME to their respective parent epoxides, 12,13-EpOME and 9,10-EpOME. These findings are consistent with the hypothesized increase in systemic sEH activity. The results in vivo support the previously reported elevation of sEH products post-mortem in the cerebral vasculature and brain tissue of patients with VCI. [16] Since the present study excluded patients with evidence of cortical stroke, the findings here identify a relationship between sEH activity and subcortical ischemic vasculopathy specifically.

Table 3 Concentrations of 24 sEH-related oxylipins assessed

Oxylipins	Abbreviation	Total detectability	Extensive SIVD; median (IQR) nM or % detectable	Healthy control; median (IQR) nM or % detectable	*t* or chi-square	*p*
Linoleic acid (LA) metabolites						
9(10)-Epoxyoctadecamonoenoic acid	9,10-EpOME	100%	1.5 (2.7)	1.8 (2.1)	−1.11	.274
12(13)-Epoxyoctadecamonoenoic acid	12,13-EpOME	100%	12.1 (13.5)	14.1 (15.9)	−0.30	.763
9,10-Dihydroxyoctadecamonoenoic acid	9,10-DiHOME	100%	4.7 (5.1)	3.6 (4.6)	1.38	.172
12,13-Dihydroxyoctadecamonoenoic acid	12,13-DiHOME	100%	9.6 (9.2)	6.3 (6.3)	2.08	.043*
Arachidonic Acid (AA) metabolites						
5(6)-Epoxyeicosatrienoic acid	5,6-EpETrE	12%	17%	16%	0.02	.903
8(9)-Epoxyeicosatrienoic acid	8,9-EpETrE	16%	10%	24%	1.80	.179
11(12)-Epoxyeicosatrienoic acid	11,12-EpETrE	14%	17%	16%	0.02	.903
14(15)-Epoxyeicosatrienoic acid	14,15-EpETrE	9%	7%	20%	2.04	.153
5,6-Dihydroxyeicosatrienoic acid	5,6-DiHETrE	7%	14%	4%	1.53	.216
8,9-Dihydroxyeicosatrienoic acid	8,9-DiHETrE	37%	41%	36%	0.16	.686
11,12-Dihydroxyeicosatrienoic acid	11,12-DiHETrE	100%	0.3 (0.2)	0.4 (0.3)	−0.11	.913
14,15-Dihydroxyeicosatrienoic acid	14,15-DiHETrE	100%	0.6 (0.2)	0.6 (0.3)	−0.06	.955
Eicosapentaenoic acid (EPA) metabolites						
8(9)-Epoxyeicosateteaenoic acid	8,9-EpETE	5%	7%	8%	0.02	.877
11(12)-Epoxyeicosatetraenoic acid	11,12-EpETE	10%	3%	28%	6.41	.011*
14(15)-Epoxyeicosateteaenoic acid	14,15-EpETE	7%	7%	8%	0.02	.877
17(18)-Epoxyeicosateteaenoic acid	17,18-EpETE	1%	0%	4%	1.18	.277
5,6-Dihydroxyeicosatetraenoic acid	5,6-DiHETE	0%	0%	0%		
14,15-Dihydroxyeicosatetraenoic acid	14,15-DiHETE	8%	14%	0%	3.72	.054
17,18-Dihydroxyeicosatetraenoic acid	17,18-DiHETE	84%	7.2 (3.9)	4.9 (5.6)	1.93	.060
Docosahexaenoic acid (DHA) metabolites						
7(8)-Epoxydocosapentaenoic acid	7,8-EpDPE	1%	0%	4%	1.18	.277
10(11)-Epoxydocosapentaenoic acid	10,11-EpDPE	53%	0.1 (0.2)	0.2 (0.2)	−0.46	.647
13(14)-Epoxydocosapentaenoic acid	13,14-EpDPE	3%	3%	4%	0.01	.915
16(17)-Epoxydocosapentaenoic acid	16,17-EpDPE	7%	7%	8%	0.02	.877
19(20)-Epoxydocosapentaenoic acid	19,20-EpDPE	22%	21%	16%	0.20	.658

Concentrations were log-transformed prior to statistical testing

*$p < .05$ is considered as statistically significant

The present results provide correlative evidence of sEH involvement in SIVD, but they cannot speak to a causal or mechanistic role per se. However, recent mechanistic studies have identified a harmful role of a DHA-derived diol in the progression of diabetic retinopathy, indicating that sEH can cause small vessel damage directly. [10] Furthermore, in rats, expression of sEH was increased in cerebral white matter after experimental ischemia, and sEH inhibitors alleviated axonal injury and demyelination, [9] which are observed in WMH territory in human imaging pathology studies. [28] Human pathology studies have indicated that the etiologies of WMH are heterogeneous, involving variably demyelination, arteriolosclerosis, axonal injury, venous collagenosis, reactive astrogliosis, and damage to the ependyma that lines the ventricles. [28] The correlation identified here between the 12,13-DiHOME/12,13-EpOME ratio and periventricular WMH ($B = 0.77$, $p = .021$) may be of interest because sEH is expressed highly in the ependymal cells of the choroid plexus, [28] and ependymal damage has been associated with periventricular WMH on autopsy. Further studies to characterize the oxylipin composition of brain tissue affected by periventricular WMH post-mortem, and assess sEH activity directly therein, are warranted.

The serum oxylipins found to differ most consistently between the groups were LA metabolites. Shifts in LA metabolism in the post-ischemic brain were described recently in a rat model. [29] In that study, an LA oxylipin was found to regulate neurotransmission, and the cytochrome p450-derived LA

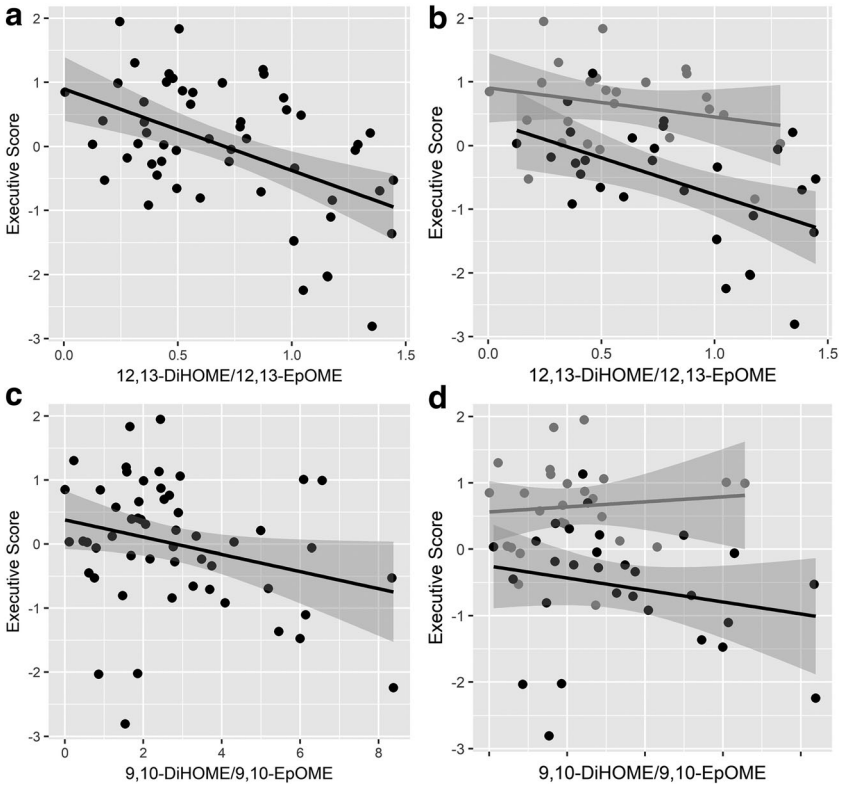


Fig. 1 Associations between the 12,13-DiHOME/12,13-EpOME ratio and a composite *Z*-score derived from four tests of psychomotor processing speed, attention, and executive function in **a** the entire group, and **b** broken down by subgroups of subcortical ischemic vascular disease (SIVD; black) patients and normal controls (minimal SIVD; gray); and associations between the 9,10-DiHOME/9,10-EpOME ratio and the same composite score in **c** the entire group, and **d** the subgroups (extensive SIVD: black; minimal SIVD: gray)

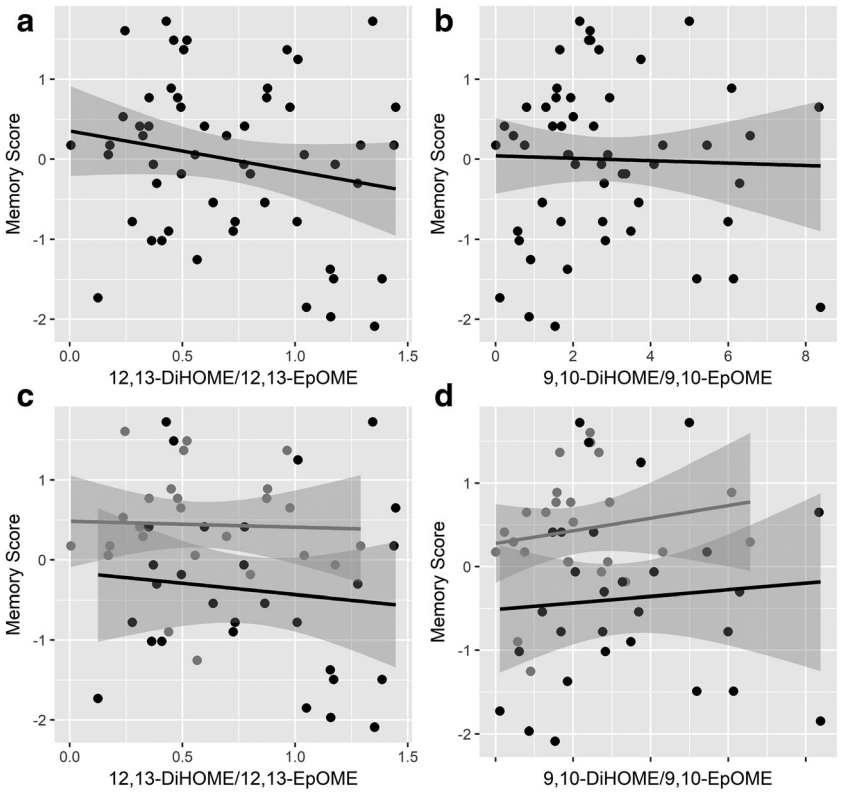


Fig. 2 Associations between **a** the 12,13-DiHOME/12,13-EpOME ratio and a composite Z-score derived from four California Verbal Learning Test, 2nd Ed. memory measures in the entire group **b** the 9,10-DiHOME/9,10-EpOME ratio and the composite memory score in the entire group **c** the 12,13-DiHOME/12,13-EpOME ratio and the composite memory score in the extensive SIVD (black) and normal control (minimal SIVD: gray) subgroups and **d** the 9,10-DiHOME/9,10-EpOME ratio and the composite memory score in the subgroups (extensive SIVD: black; minimal SIVD: gray)

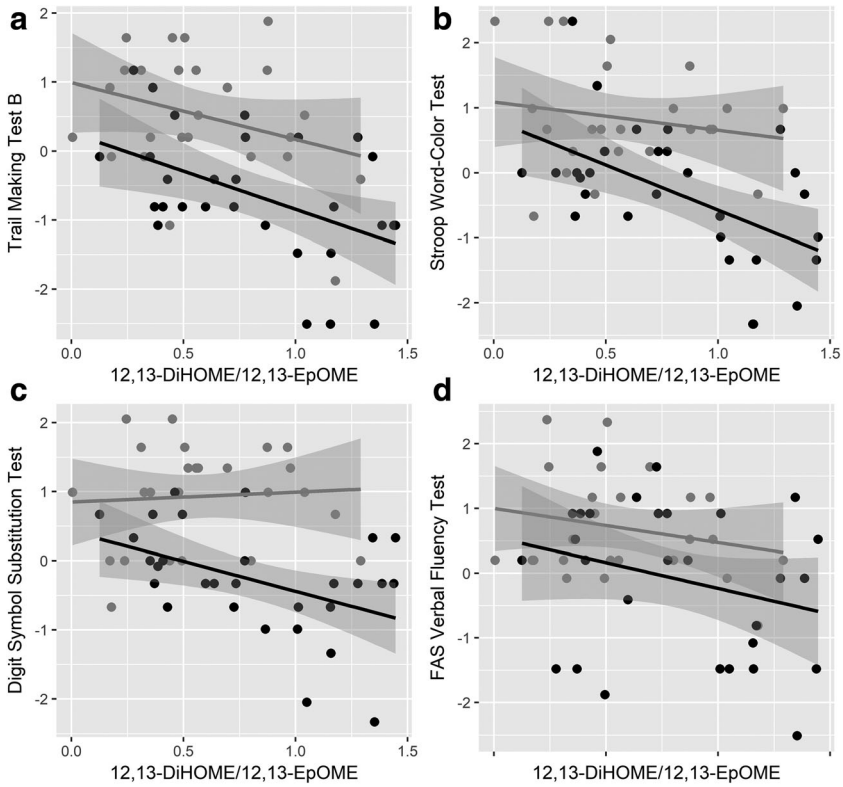


Fig. 3 Exploratory associations between the 12,13-DiHOME/12,13-EpOME ratio and four tests of psychomotor processing speed, attention, and executive function in the subcortical ischemic vascular disease (SIVD; black) and normal control (minimal SIVD; gray) subgroups; **a** Trail Making Test B, **b** Stroop Color-Word Interference task, **c** Digit Symbol Substitution Test, and **d** The FAS Verbal Fluency Test

oxylipins were found to increase in the hippocampus and cerebellum following experimental CO_2-induced ischemia. The latter effect was thought to represent an endogenous adaptive response since some cytochrome p450-derived species are neuroprotective and anti-inflammatory. On the other hand, sEH activity was also increased in cerebral ischemia models, producing diol products, which are less active and possibly cytotoxic. [9] The present data suggest that a shift in LA metabolism also occurs systemically in people with ischemic vascular disease, with a particular relative overproduction of the diol species; however, the specificity to the LA metabolites may reflect the greater abundance of LA in peripheral blood. It is notable that an EPA-derived epoxide was significantly less likely to be detected in SIVD, but it was detectable in only 10% of participants overall. The LA metabolite ratios are indirect measures of epoxide hydrolase activity, and hydrolysis of other fatty acid epoxides, which were more difficult to detect peripherally, may be relevant in SIVD.

A higher 12,13-DiHOME/12,13-EpOME ratio was associated with poorer psychomotor processing speed/attention/

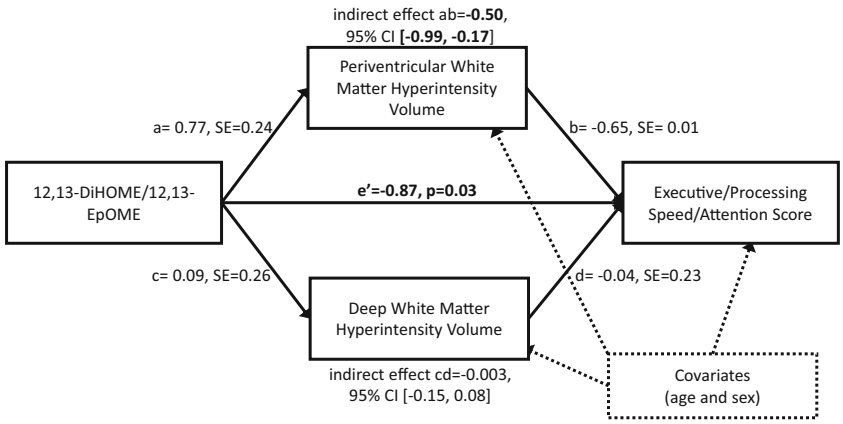


Fig. 4 Mediation model showing a direct relationship between the 12,13-DiHOME/12,13-EpOME ratio (e′) and the composite psychomotor processing speed/attention/executive function *Z*-score, in addition to an indirect relationship mediated by the volume of periventricular white matter hyperintensities (ab)

executive function scores, explaining 21.3% of their variance. In post hoc exploratory analyses, the individual tests most closely associated with the 12,13-DiHOME/12,13-EpOME ratio were the TMT-B and Stroop tasks, which are the most heavily dependent on set-shifting aspects of executive function. The association within the SIVD subgroup suggests a dose relationship beyond the difference observed between healthy elderly and those with symptomatic SIVD. These cognitive findings are consistent with a profile of VCI, which affects executive domains most prominently, while having smaller and more variable effects on memory [30, 31].

In path analysis, a relationship between the 12,13-DiHOME/12,13-EpOME ratio and VCI was mediated by periventricular WMH volumes; however, there was also a direct effect independent of white matter changes observable on T2-weighted structural MRI. Although speculative, it is possible that sEH activity might interfere with functional and/or reactive hyperemia because the AA-derived sEH substrates (EETs) are vasodilatory mediators released at the neurovascular unit. [8] Studies involving fMRI and other imaging modalities (e.g., diffusion weighted imaging, arterial spin labelling) might be useful to assess associations between sEH activity and the disruption of functional connectivity, cerebral blood flow, or more subtle structural changes in ischemic white matter. Regardless, because the relationships between WMH and cognition can be highly variable, there is a need for biologically based SIVD markers that can explain variance in cognitive decline.

As a potential limitation, the cross-sectional observational nature of this study does not permit causal inferences. Since sEH-related oxylipins have not been examined previously in relation to VCI in living people, and a relatively small sample size was available here, further studies are necessary to confirm the relationship between sEH LA metabolism, SIVD, and VCI, and to disentangle their effects from those of specific vascular risk factors; however, the present results are consistent with a previous preliminary report of an association between lower concentrations of a sEH substrate (a DHA-derived epoxide in that study) and greater WMH volumes in 37 cognitively intact people with hypertension over 55 years of age. [32] As another limitation, blood for this study was taken at a convenient time; however, while fasting morning blood tests might have improved precision, the signals detected appeared to be robust to time of day and possible postprandial effects. The detectability of many oxylipin species has also limited the scope of the current investigation, with the LA metabolites more frequently detected than those of other PUFAs, which might also be relevant but present at lower concentrations in peripheral blood; in SIVD, the concentrations or proportions detectable of AA- and EPA-derived diols were numerically but not significantly higher, and those of other AA and EPA epoxides were numerically but not significantly lower, suggesting the need to explore these potential differences in larger studies. As a further limitation, the potential pathophysiological relevance of oxylipin concentrations in serum remains to be understood, as do their relationships with oxylipin concentrations in the cerebrospinal fluid, and in the interstitial fluid of the perivascular spaces between the brain parenchyma and the cerebral vasculature, which might be relevant. Finally, the oxylipin ratios are indirect measures, offering corollary evidence supporting a previous report of increased sEH expression in patients with VCI post-mortem [16].

In conclusion, a shift in polyunsaturated fatty acid metabolism in peripheral blood consistent with systemic sEH activity was associated with the presence and extent of WMH observed on MRI. A shift in LA metabolism was associated with poorer executive function, particularly in those with symptomatic SIVD, which was partly related to the extent of periventricular WMH. These results, if subsequently validated, suggest a novel peripheral blood biomarker of subcortical ischemic vasculopathy and the related vascular cognitive impairment. Taken together with mechanistic studies in animals and observations post-mortem in people with vascular dementia, the results suggest further investigation of sEH as a potential target for intervention in subcortical VCI.

Acknowledgements WS gratefully acknowledges support from the Alzheimer's Association (US) & Brain Canada, The Canadian Partnership for Stroke Recovery, Sunnybrook Health Sciences Centre Department of Psychiatry, Sunnybrook Research Institute Hurvitz Brain Sciences Program, the University of Toronto Department of Pharmacology & Toxicology, and the Centre for Collaborative Drug Research. RHS is grateful to acknowledge support from the Heart and Stroke Foundation New Investigator Award, the Ontario Brain Institute's Ontario Neurodegenerative Disease Research Initiative (ONDRI), the Departments of Medicine at the University of Toronto and Sunnybrook Health Sciences Centre, the Canadian Partnership for Stroke Recovery, and partial support from the Brill Chair in Neurology at Sunnybrook (Chair holder SEB).

The authors would like to thank Dr. Alan Moody for his contributions to the study, including data collection. The authors would also like to thank Maisha Khan for her contributions to the study, including helping with the samples.

Dr. Yurika Otoki is thanked for her contributions to the oxylipin method development.

Funding This study is funded by the Alzheimer's Association (US) and Brain Canada (AARG501466). JR & SEB received financial and salary support from the Fondation Leducq, Canadian Institutes of Health Research (#125740 & #13129), Heart & Stroke Foundation Canadian Partnership for Stroke Recovery, Hurvitz Brain Sciences Research program at Sunnybrook Research Institute and the Linda C. Campbell Foundation. JR additionally received partial funding from the Canadian Vascular Network and the ONDRI. SEB received financial and salary support from the Sunnybrook Research Institute, Sunnybrook Health Sciences Centre Department of Medicine and the Brill Chair Neurology, University of Toronto.

Compliance with Ethical Standards

Conflict of Interest DY declares that she has no conflict of interest.

HCM declares that he has no conflict of interest.

RHS declares grant and personnel funding from the Heart and Stroke Foundation, CIHR, Ontario Brain Institute, Sunnybrook Health Sciences Centre and the University of Toronto, Department of Medicine.

NH declares research support from Lundbeck and consultant fees from Astellas, Merck & Lilly.

PCC declares that he has no conflict of interest.

JP declares that she has no conflict of interest.

DTS declares that he has no conflict of interest. .

SEB declares institutional grants from Pfizer, GE Healthcare, Eli Lilly, Cognoptix, Biogen Idec, and personal honoraria from Novartis, Merck, Pfizer, Eli Lilly, and Medscape/Biogen.

AT declares that he has no conflict of interest.

WS declares that he has no conflict of interest.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

References

1. Pantoni L. Cerebral small vessel disease: from pathogenesis and clinical characteristics to therapeutic challenges. Lancet Neurol. 2010;9:689–701. https://doi.org/10.1016/S1474-4422(10)70104-6.
2. Wardlaw JM, Smith C, Dichgans M. Mechanisms of sporadic cerebral small vessel disease: insights from neuroimaging. Lancet Neurol. 2013;12:483–97. https://doi.org/10.1016/S1474-4422(13)70060-7.
3. Wardlaw JM, Valdés Hernández MC, Muñoz-Maniega S. What are white matter hyperintensities made of? Relevance to vascular cognitive impairment. J Am Heart Assoc. 2015;4:001140. https://doi.org/10.1161/JAHA.114.001140.
4. Swardfager W, Hennebelle M, Yu D, Hammock BD, Levitt AJ, Hashimoto K, et al. Metabolic/inflammatory/vascular comorbidity in psychiatric disorders; soluble epoxide hydrolase (sEH) as a possible new target. Neurosci Biobehav Rev. 2018;87:56–66. https://doi.org/10.1016/j.neubiorev.2018.01.010.
5. Gabbs M, Leng S, Devassy JG, Monirujjaman M, Aukema HM. Advances in our understanding of oxylipins derived from dietary PUFAs. Adv Nutr. 2015;6:513–40. https://doi.org/10.3945/an.114.007732.
6. Serhan CN, Chiang N, Van Dyke TE. Resolving inflammation: dual anti-inflammatory and pro-resolution lipid mediators. Nat Rev Immunol. 2008;8:349–61. https://doi.org/10.1038/nri2294.
7. Davis CM, Liu X, Alkayed NJ. Cytochrome P450 eicosanoids in cerebrovascular function and disease. Pharmacol Ther. 2017;179:31–46. https://doi.org/10.1016/j.pharmthera.2017.05.004.
8. Kisler K, Nelson AR, Montagne A, Zlokovic BV. Cerebral blood flow regulation and neurovascular dysfunction in Alzheimer disease. Nat Rev Neurosci. 2017;18:419–34. https://doi.org/10.1038/nrn.2017.48.
9. Chen Y, Tian H, Yao E, Tian Y, Zhang H, Xu L, et al. Soluble epoxide hydrolase inhibition promotes white matter integrity and long-term functional recovery after chronic hypoperfusion in mice. Sci Rep. 2017;7:7758. https://doi.org/10.1038/s41598-017-08227-z.
10. Hu J, Dziumbla S, Lin J, Bibli S-I, Zukunft S, de Mos J, et al. Inhibition of soluble epoxide hydrolase prevents diabetic retinopathy. Nature. 2017;552:248–52. https://doi.org/10.1038/nature25013.
11. Moghaddam MF, Grant DF, Cheek JM, Greene JF, Williamson KC, Hammock BD. Bioactivation of leukotoxins to their toxic diols by epoxide hydrolase. Nat Med. 1997;3:562–6.
12. Lecka-Czernik B, Moerman EJ, Grant DF, Lehmann JM, Manolagas SC, Jilka RL. Divergent effects of selective peroxisome proliferator-activated receptor-gamma 2 ligands on adipocyte versus osteoblast differentiation. Endocrinology. 2002;143:2376–84. https://doi.org/10.1210/endo.143.6.8834.
13. Sisemore MF, Zheng J, Yang JC, Thompson DA, Plopper CG, Cortopassi GA, et al. Cellular characterization of leukotoxin diol-induced mitochondrial dysfunction. Arch Biochem Biophys. 2001;392:32–7. https://doi.org/10.1006/abbi.2001.2434.
14. Viswanathan S, Hammock BD, Newman JW, Meerarani P, Toborek M, Hennig B. Involvement of CYP 2C9 in mediating the proinflammatory effects of linoleic acid in vascular endothelial cells. J Am Coll Nutr. 2003;22:502–10.
15. Chang L-H, Lin H-C, Huang S-S, Chen I-C, Chu K-W, Chih C-L, et al. Blockade of soluble epoxide hydrolase attenuates post-ischemic neuronal hyperexcitation and confers resilience against stroke with TrkB activation. Sci Rep. 2018;8:118. https://doi.org/10.1038/s41598-017-18558-6.
16. Nelson JW, Young JM, Borkar RN, Woltjer RL, Quinn JF, Silbert LC, et al. Role of soluble epoxide hydrolase in age-related vascular cognitive decline. Prostaglandins Other Lipid Mediat. 2014;113–115:30–7. https://doi.org/10.1016/j.prostaglandins.2014.09.003.
17. Fazekas F, Chawluk J, Alavi A, Hurtig H, Zimmerman R. MR signal abnormalities at 1.5 T in Alzheimer's dementia and normal aging. Am J Roentgenol. 1987;149:351–6. https://doi.org/10.2214/ajr.149.2.351.
18. Obinata H, Hattori T, Nakane S, Tatei K, Izumi T. Identification of 9-hydroxyoctadecadienoic acid and other oxidized free fatty acids as ligands of the G protein-coupled receptor G2A. J Biol Chem. 2005;280:40676–83. https://doi.org/10.1074/jbc.M507787200.
19. Hennebelle M, Otoki Y, Yang J, Hammock BD, Levitt AJ, Taha AY, et al. Altered soluble epoxide hydrolase-derived oxylipins in patients with seasonal major depression: an exploratory study. Psychiatry Res. 2017;252:94–101. https://doi.org/10.1016/j.psychres.2017.02.056.
20. Yang J, Schmelzer K, Georgi K, Hammock BD. Quantitative profiling method for oxylipin metabolome by liquid chromatography electrospray ionization tandem mass spectrometry. Anal Chem. 2009;81:8085–93. https://doi.org/10.1021/ac901282n.
21. Gibson E, Gao F, Black SE, Lobaugh NJ. Automatic segmentation of white matter hyperintensities in the elderly using FLAIR images at 3T. J Magn Reson Imaging. 2010;31:1311–22. https://doi.org/10.1002/jmri.22004.
22. Ramirez J, Gibson E, Quddus A, Lobaugh NJ, Feinstein A, Levine B, et al. Lesion explorer: a comprehensive segmentation and parcellation package to obtain regional volumetrics for subcortical hyperintensities and intracranial tissue. NeuroImage. 2011;54:963–73. https://doi.org/10.1016/j.neuroimage.2010.09.013.
23. Ramirez J, Scott CJ, Black SE. A short-term scan-rescan reliability test measuring brain tissue and subcortical hyperintensity volumetrics obtained using the lesion explorer structural MRI processing pipeline. Brain Topogr. 2013;26(1):35–8. https://doi.org/10.1007/s10548-012-0228-z.
24. Hachinski V, Iadecola C, Petersen RC, Breteler MM, Nyenhuis DL, Black SE, et al. National Institute of Neurological Disorders and Stroke-Canadian Stroke Network vascular cognitive impairment harmonization standards. Stroke. 2006;37:2220–41. https://doi.org/10.1161/01.STR.0000237236.88823.47.
25. Ulu A, Davis BB, Tsai H-J, Kim I-H, Morisseau C, Inceoglu B, et al. Soluble epoxide hydrolase inhibitors reduce the development of atherosclerosis in apolipoprotein e-knockout mouse model. J Cardiovasc Pharmacol. 2008;52:314–23. https://doi.org/10.1097/FJC.0b013e318185fa3c.
26. Hanif A, Edin ML, Zeldin DC, Morisseau C, Nayeem MA. Effect of soluble epoxide hydrolase on the modulation of coronary

reactive hyperemia: role of oxylipins and PPARγ. PLoS One. 2016;11(9):e0162147. https://doi.org/10.1371/journal.pone.0162147.

27. Preacher KJ, Hayes AF. SPSS and SAS procedures for estimating indirect effects in simple mediation models. Behav Res Methods Instrum Comput. 2004;36:717–31.
28. Sura P, Sura R, Enayetallah AE, Grant DF. Distribution and expression of soluble epoxide hydrolase in human brain. J Histochem Cytochem. 2008;56:551–9. https://doi.org/10.1369/jhc.2008.950659.
29. Hennebelle M, Zhang Z, Metherel AH, Kitson AP, Otoki Y, Richardson CE, et al. Linoleic acid participates in the response to ischemic brain injury through oxidized metabolites that regulate neurotransmission. Sci Rep. 2017;7:4342. https://doi.org/10.1038/s41598-017-02914-7.
30. Santiago C, Herrmann N, Swardfager W, Saleem M, Oh PI, Black SE, et al. White matter microstructural integrity is associated with executive function and processing speed in older adults with coronary artery disease. Am J Geriatr Psychiatry. 2015;23:754–63. https://doi.org/10.1016/j.jagp.2014.09.008.
31. Swardfager W, Cogo-Moreira H, Masellis M, Ramirez J, Herrmann N, Edwards JD, et al. The effect of white matter hyperintensities on verbal memory. Neurology. 2018;90:e673–e82. https://doi.org/10.1212/WNL.0000000000004983.
32. Shinto L, Lahna D, Murchison CF, Dodge H, Hagen K, Quinn JF, et al. Plasma Omega-6 derived metabolite is associated with an "unfavorable" MRI profile and an Omega-3 derived metabolite is associated with a "favorable" MRI profile in a cohort of cognitively Normal hypertensive young-old. Alzheimers Dement. 2017;13:P1515–P6. https://doi.org/10.1016/j.jalz.2017.07.636.