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Vascular-related biological stress, DNA methylation, allostatic load and domain-specific cognition: an integrated machine learning and causal inference approach

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Abstract

Background Vascular disease in aging populations spans a wide range of disorders including strokes, circulation disorders and hypertension. As individuals age, vascular disorders co-occur and hence exert combined effects. In the present study we introduce vascular-related biological stress as a novel biomarker to capture the combined effects of vascular disease burden for more precision in early detection of cognitive changes in aging.

Objective to determine the role of vascular-related biological Stress, DNA methylation-based biological aging and Allostatic Load in the relationship between vascular disorders and major cognitive domains including global cognition, episodic memory and executive function in a representative sample of adults across the age span.

Methods The present study included participants from MIDUS refresher sample. Vascular-related biological stress included: BMI, Average blood pressure, sitting, Waist-hip ratio, Blood hemoglobin A1c percent, Blood dehydroepiandrosterone (ng/mL), Blood fasting insulin levels uIU/mL, Blood serum interleukin-8 (pg/mL), Blood serum interleukin-6 (pg/mL), Blood fasting glucose levels mg/dL and Blood fibrinogen (mg/dL). DNA methylation-based biological age measures included GrimAge2 that was constructed based on DNA methylation surrogate markers for select plasma proteins and smoking-pack years. Allostatic load scores were calculated based on biomarkers commonly used in allostatic load calculations: cortisol (urine), norepinephrine (urine), epinephrine (urine), dopamine (urine), glycosylated hemoglobin (HBA1C, blood), low density lipoprotein (LDL, blood), C-reactive protein (CRP, blood) dehydroepiandrosterone sulfate (DHEAS, blood), high-density lipoprotein (HDL, blood) and systolic blood pressure (average, sitting). Least Absolute Shrinkage and Selection Operator (LASSO) and response models (item and continuous) were used to calculate vascular-related biological stress and theta scores. Four-way decomposition modeling approach was used to calculate the natural direct and indirect effects in the relationship between vascular disease and major cognitive domains.

Results 550 individuals with data on biomarkers, DNA methylation and cognition assessments were included in the present study. Median age was 54 (range = 26, 78) with females representing 48% of the sample. In the relationship

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between vascular disease and cognition, the overall proportions mediated through vascular-related biological stress (item-response scale) were 0.60 ($P=0.01$); 1.1 ($P=0.308$); 0.53 ($P=0.002$) for global cognition, episodic memory and executive function respectively. The overall proportions mediated through DNA methylation (GrimAge2) were 0.27 ($P=0.002$); 0.39 ($P=0.102$); 0.20, ($P=0.002$) for global cognition, episodic memory and executive function respectively and 0.10 ($P=0.08$); 0.09 ($P=0.5$); 0.07 ($P=0.18$) through allostatic load (sum scores).

Conclusions Our findings suggest that vascular-related biological stress, DNA methylation and to some extent allostatic load mediate the effects of vascular disease on global cognition and executive function.

Keywords Age span, Biomarkers, Vascular, ADRD, Biological stress, DNA methylation, Allostatic load, Cognition, Episodic memory, Executive function

Introduction

Vascular disorders are considered among the most preventable precipitating factors of Alzheimer Disease and Related Dementias (ADRD) in aging [1]. Vascular disease in aging populations spans a wide range of disorders including strokes, circulation disorders and hypertension that share overlapping pathophysiological pathways. Previous studies often focused on individual rather than the combined vascular disease effects on cognition and ADRD and total effects in particular [2]. Hence, little is known about indirect pathways that contribute to cognition in aging.

Vascular disorders share common pathophysiological etiologies such as ischemia, inflammation and atherosclerosis [3–5] and hence their effects are less likely to occur in isolation [5]. In the present investigation "Vascular-Related Biological Stress" is introduced as a novel biomarker of the combined effects of vascular disease burden for more individual-level precision in capturing vascular-related biological stress in response to cumulative vascular disease burden and for early detection of cognitive changes in aging. Early identification of cognitive changes is key for timely intervention and prevention of progression to ADRD [6, 7].

Biomarkers measured in accessible tissues such as blood are essential for early detection and monitoring of at-risk individuals and the general population at large given their accessibility and cost-effectiveness [8, 9]. In addition to vascular-related biological stress, we assess DNA methylation [10] and Allostatic load [11] for a comprehensive assessment of health biomarkers and given their relatedness with healthy aging [12–14]. Delineating the effects of vascular disorders on cognition would yield important mechanistic insights on causal pathways through which such disorders progress to ADRD at the biological, physiological and molecular levels.

Our goal was to determine the contribution of each of vascular-related biological stress, DNA methylation and allostatic load in the relationship between vascular disorders and cognitive domains including global cognition, episodic memory and executive function in a representative sample of adults across the age span.

Methods

Population description

Midlife in the United States (MIDUS) is a national study of non-institutionalized adults in the United States with representation across the spectrum of aging in adults. The present study included participants from MIDUS refresher sample conducted in 2011 [15–17]. In addition, as recommended, a systematic search of MIDUS bibliography as of October 23 2024 was conducted to capture any related literature based on MIDUS refresher data and no publications were found addressing the present topic [18].

Vascular disease, Vascular-Related biological stress, DNA methylation and allostatic load

Theta scores were calculated for vascular disease and biological stress using item response theory (IRT) methods using R package Latent Trait Model "LTM" [19–21]. In a sensitivity analysis, continuous response model (CRM) was used to calculate biological stress on a continuous scale [21–24]. For IRT calculations of continuous biomarkers, each biomarker z-score was converted into three equal quantiles such that the first and second quantiles combined represent the low risk level and the highest quantile represent the high risk level. For calculation of theta on the continuous scale, raw continuous data of each biomarker was used to calculate theta using continuous response models [23, 24]. Advantages compared to sum scores' based approaches include that biomarkers are not assumed to be equally informative, since factors differ in their discrimination ability in relation the latent construct, their range of variability and unit change that would be clinically meaningful, hence item response approaches add improved precision through taking into consideration individual-specific levels of each factor, their difficulty and discrimination ability and the continuous variant of the approach provides more flexibility and granularity of information on a continuous scale [19, 21, 23, 24].

Vascular events were based on self-report [25]. Vascular scores were calculated based on the following events: heart disease, high blood pressure, circulation, clots,

transient ischemic attack, cholesterol disorders and diabetes mellitus. Diabetes mellitus in MIDUS refresher include both type I and type II. Vascular events constituting the vascular scores were determined a priori according to etiology and natural history in relation to vascular health [26–28]. Individuals who reported "borderline" in any of the vascular measures were not included.

Vascular-Related Biological Stress Scores biomarkers were selected using Least Absolute Shrinkage and Selection Operator (LASSO), a supervised machine learning modeling approach and supported by clinical relevance, biological plausibility, biomarker availability in the MIDUS refresher study, prior knowledge and correlations with vascular disorders [29–34]. All relevant available biomarkers were included without exclusions [35]. Lambda minimum was chosen through fitting a model with scaled predictors and 10-fold cross-validation. Then, the model was rerun with the best lambda and coefficients were obtained [29]. In addition to LASSO, reasonable model fit was verified based on Root Mean Square Error of Approximation (RMSEA) [36].

The following biomarkers were included: BMI, Average blood pressure, sitting, Waist-hip ratio, Blood hemoglobin A1c percent, Blood dehydroepiandrosterone (ng/mL), Blood fasting insulin levels uIU/mL, Blood serum interleukin-8 (pg/mL), Blood serum interleukin-6 (pg/mL), Blood fasting glucose levels mg/dL and Blood fibrinogen (mg/dL).

DNA methylation measures included GrimAge2 [37]. GrimAge was constructed based on DNA methylation surrogate markers for select plasma proteins (Adrenomedullin, beta 2-microglobulin, CD56, ceruloplasmin, cystatin C, EGF fibulin-like ECM protein 1, growth differentiation factor 15, leptin, myoglobin, plasminogen activator inhibitor 1, serum paraoxonase/arylesterase 1, and tissue inhibitor metalloproteinases 1) and smoking-pack years in a two-stage procedure. The updated version included in addition C-reactive protein (log) and hemoglobin A1C (log) [10].

Allostatic load scores were calculated using sum scores as a common approach for its calculation based on biomarkers reflecting key body interacting and adaptive systems including: CVD, metabolic, immunity/inflammation and neuroendocrine. Biomarkers included cortisol (urine), norepinephrine (urine), epinephrine (urine), dopamine (urine), glycosylated hemoglobin (HBA1C, blood), low density lipoprotein (LDL, blood), C-reactive protein (CRP, blood) dehydroepiandrosterone sulfate (DHEAS, blood), high-density lipoprotein (HDL, blood) and systolic blood pressure (average, sitting) [11].

The following biomarkers have been included in the analysis on a reverse scale: Blood dehydroepiandrosterone (DHEA) (ng/mL), dehydroepiandrosterone-sulfate (DHEA-S) (ug/dL) and high-density lipoprotein (HDL, blood) [11].

Global cognition, episodic memory and executive function

Global cognition was measured using the Brief Test of Adults Cognition by Telephone (BTACT), the global cognition composite included all of the BTACT tests except for the Stop and Go Switch Task (SGST) [15, 38, 39]. Episodic memory included Word List Immediate and Word List Delayed and Executive function was made of the remaining BTACT test variables, thus all forming subsets of the overall BTACT score [15]. The three variables were computed based on z scores of the respective tests. The executive function calculation included a task from the Stop and Go Test (SGST), hence phone type was accounted for given that executive function latency values vary as a function of phone type [15, 38, 39].

Statistical analysis

Median, minimum and maximum were used to summarize continuous variables and proportions were used to summarize categorical variables. Plots were used to visualize Item characteristic, item information and test information curves commonly used to visualize difficulty, discrimination and precision [19–21]. Correlations were assessed between vascular-related biological stress on both the item response and continuous scales, DNA methylation and allostatic load. Correlations between vascular disease scores, global cognition, episodic memory and executive function were also assessed. Missing biomarkers' values were replaced with the biomarker median value [40].

A four-way decomposition modelling approach was used to calculate the natural direct and indirect effects in the relationship between vascular disease and cognitive outcomes using the R Package CMA Verse [41–43]. Vascular-related Biological Stress, DNA methylation and allostatic load were assessed as mediators. Given that we are using four-way decomposition modelling approach, the overall proportion mediated and the four main decomposed effects were assessed for a more comprehensive picture of the direct and indirect effects including: CDE = Controlled Direct Effect (neither mediation nor interaction), INTREF = Reference Interaction (Interaction), INTMED = Mediated Interaction (Both mediation and interaction) and PNIE = Pure Natural Indirect Effect (mediation). Median values were used as reference mediator values. Models were adjusted for sex and chronological age. Standard errors were calculated using 1000 bootstrapped samples. Models and covariates were specified a priori for comparisons, with linear distribution for continuous variables and Poisson for count variables. In addition, Ramsey test (that formally tests omitted variables, incorrect functional form, simultaneous equation

problems and heteroskedasticity) was assessed for all exposure-mediator and exposure-outcome models. All exposure-outcome and exposure-mediator models with linear specification were verified to have P value > 0.05 [44]. Allostatic load models were further explored after adding interaction terms. All analyses were reviewed and discussed jointly by two investigators with statistical expertise.

Sensitivity analysis

Potential effects of measurement error of vascular disease burden were assessed in sensitivity analysis [41, 42, 45]. In a sensitivity analysis, continuous response model (CRM) was used to calculate biological stress on a continuous scale [21–24]. To further minimize possibility of reverse causality associations of exposure-outcome, exposure-mediator and mediator-outcome were assessed. In addition models were re-run after excluding individuals with biomarkers data collection date in 2016 and again after excluding both 2015 and 2016. Moreover, models were re-run with additional adjustment for education.

Results

Sample characteristics

550 individuals with complete data on biomarkers, DNA methylation and cognition assessments were included in the present study. Median age was 54 (range = 26, 78) with females representing 48% of the sample (Table 1).

Vascular disease diagnoses (ever) were as follows: 10% heart disease, 38% high blood pressure, 10% circulation disorders, 3.8% clots, 3.5% transient ischemic attack, 40% cholesterol disorders and 10% diabetes mellitus, with generally higher burden of vascular disease in older individuals (Supplementary Table 1).

Median DNA methylation age (GrimAge) was 62 years (range = 34, 92) denoting older biological age compared to the sample chronological age. A consistent inverse relationship was observed between vascular disease score and global cognition, episodic memory and executive function (Fig. 1). The correlation between DNA methylation and vascular-related biological stress (IRT) was 0.49, correlation between vascular-related biological stress (IRT) and allostatic load sum scores (SS) was 0.56 (supplementary Fig. 1).

Table 1 Sample characteristics

	Overall (N=550)
Age (y)*	
Median (Min, Max)	54.00 (26.00, 78.00)
Sex	
Male	286 (52.0%)
Female	264 (48.0%)
Vascular Disease Score (θ IR)	
Median (Min, Max)	-0.34 (-0.76, 2.77)
Vascular-related Biological Stress Score (θ IR)	
Median (Min, Max)	-0.07 (-1.20, 2.11)
Allostatic Load Score (Sum Scores)	
Median (Min, Max)	3.00 (0.00, 9.00)
DNA methylation (years)	
Median (Min, Max)	61.75 (34.60, 92.66)
Brief Test of Adult Cognition by Phone (z-scores)	
Median (Min, Max)	0.26 (-2.47, 2.73)
Episodic Memory (z-scores)	
Median (Min, Max)	0.03 (-2.39, 3.66)
Executive Function (z-scores)	
Median (Min, Max)	0.27 (-2.28, 2.53)

*Age at time of clinic visit

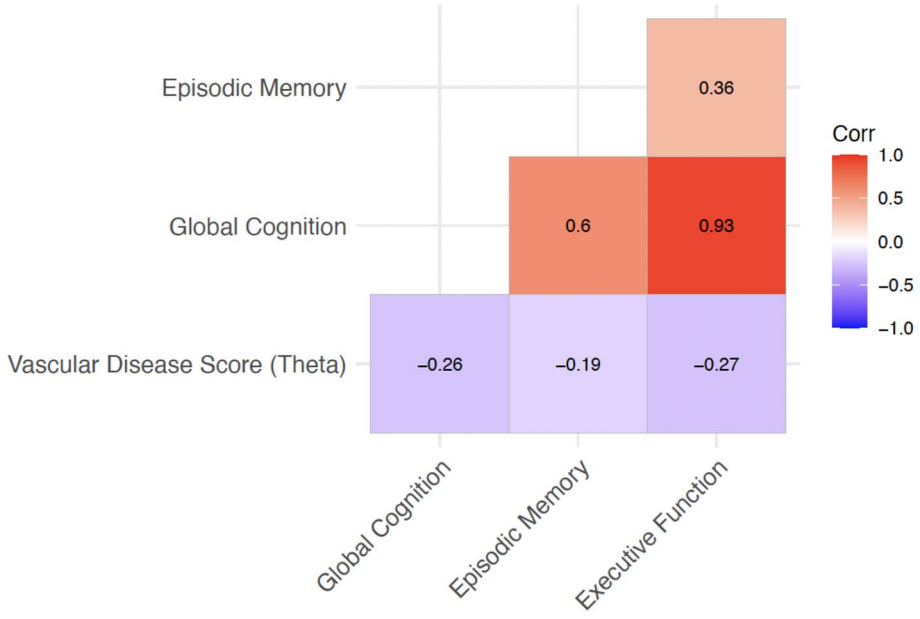

Fig. 1 Correlation between Vascular Disease score and Cognitive Domains. Vascular disease score represents vascular disease burden and cognitive domains represent global cognition, episodic memory and executive function

Median BTACT z-score was 0.2 (range = -2.4, 2.7), median episodic memory z-score = 0.03 (range = -2.4, 3.7) and executive function z-score = 0.2 (range = -2.3, 2.5) (Table 1).

Biomarkers included in the vascular-related biological stress and allostatic load scores are summarized in supplementary Fig. 2 and supplementary Tables 2, 3.

Four-way decomposition of the relationship between vascular disease and global cognition, executive function and episodic memory

Four-way decomposition of the relationship between vascular disease and cognition outcomes in relation to vascular-related biological stress (on the item response scale) was as follows: 1-Global cognition: overall proportion mediated = 0.60, P value = 0.01; 2-Episodic memory: overall proportion mediated = 1.13, P value = 0.308; 3-Executive memory: overall proportion mediated = 0.53, P value = 0.002 (Fig. 2, supplementary Table 4, supplementary Fig. 3).

Four-way decomposition of the relationship between vascular disease and cognition outcomes in relation to DNA methylation was as follows: 1-Global cognition: overall proportion mediated = 0.27, P value = 0.002; 2-Episodic memory: overall proportion mediated = 0.39, P value = 0.102; 3-Executive memory: overall proportion mediated = 0.20, P value = 0.002 (Fig. 2, supplementary Table 4).

Four-way decomposition of the relationship between vascular disease and cognition outcomes in relation to Allostatic load was as follows: 1-Global cognition: overall proportion mediated = 0.10, P value = 0.08; 2-Episodic memory: overall proportion mediated = 0.09, P value = 0.5; 3-Executive memory: overall proportion mediated = 0.07, P value = 0.186 (Fig. 2, supplementary Table 4). Extended four-way decomposition results and total effects (TE) in supplementary Tables 4 and 5.

Sensitivity analysis

We re-ran the models after assessment of 0.1 and 0.2 error margins in vascular disease measurement with global cognition. Overall proportion mediated through vascular-related biological stress remained significant with P value = 0.004 and 0.002 at both error levels respectively, and P value < 0.001 and P value = 0.004 at both error levels respectively for DNA methylation, while allostatic load was not signficant with P value = 0.07 and 0.05 at both error levels respectively. Comparable patterns were observed with vascular-related biological stress on the continuous scale (Supplementary Table 5). Main observations continued to be statistically significant after exclusion of individuals with biomarkers data collection date in 2016 and with executive function in relation to vascular-related biological stress (item response scale) overall proportion eliminated after excluding both 2015 and 2016. After adjustment for education, overall proportion mediated remained significant in the relationship between vascular disease and executive function in relation to vascular-related biological stress = 0.49,

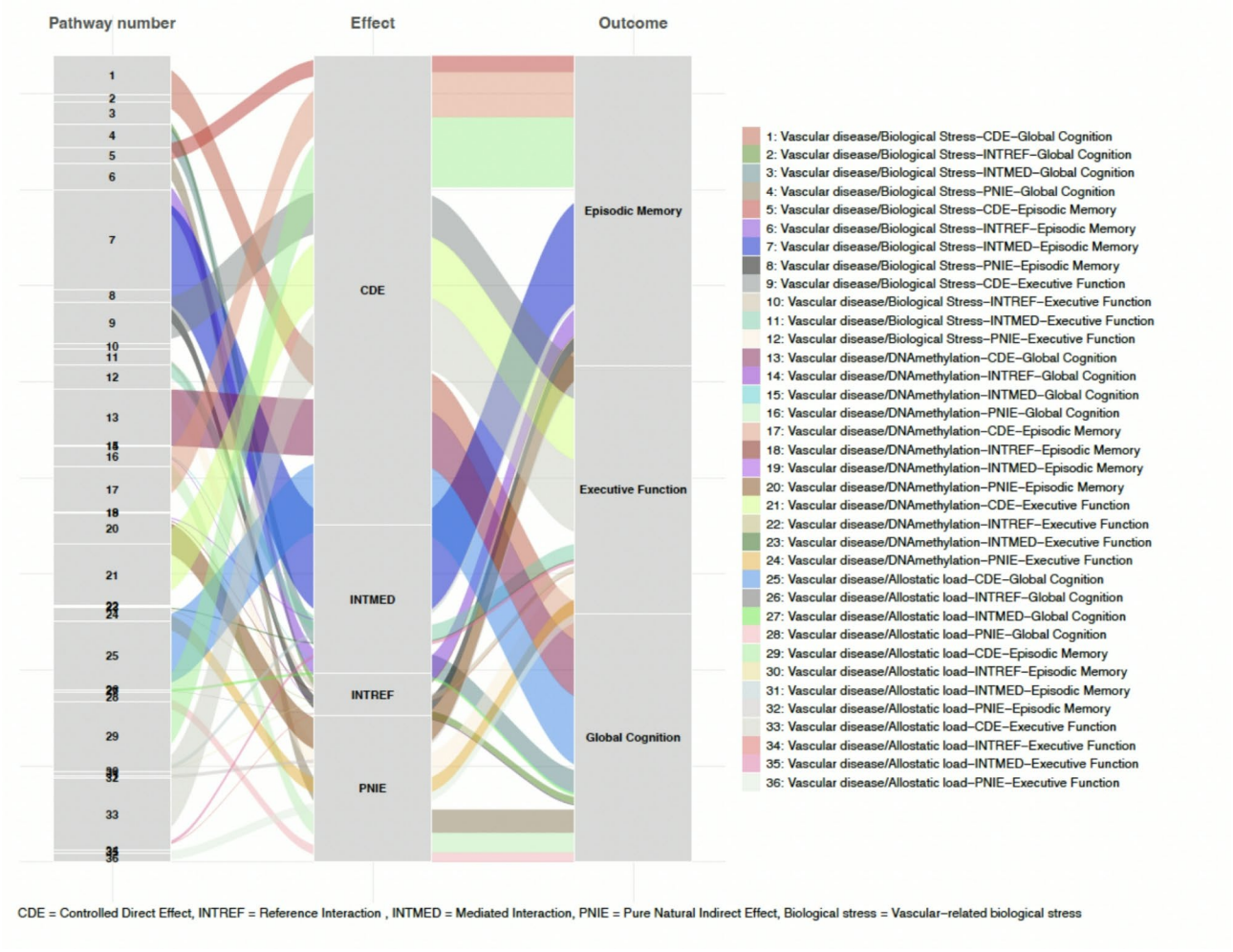

Fig. 2 Four-way decomposition of proportions of direct and indirect effects of vascular disease on Global Cognition, Episodic Memory and Executive Function in relation to vascular-related Biological Stress, DNA methylation and Allostatic load

P value = 0.04 and global cognition in relation to DNA methylation = 0.18, P value = 0.02.

Discussion

In the present investigation we established potentially causal links between vascular disease and key cognitive domains including global cognition, executive function and episodic memory. Findings of the present study suggest that vascular-related biological stress, DNA methylation and to some extent allostatic load mediate the effects of vascular disease on global cognition and executive function.

In the present investigation we introduced Vascular-Related Biological Stress as a novel biomarker towards more precision in capturing vascular-related biological stress in response to cumulative vascular disease burden at the individual level. Vascular-related biological stress included a set of well-defined biomarkers repesenting various body domains. Biomarkers in the vascular-related stress biological score are both key biomarkers of stress and are related to vascular disease burden, hence overall reflect vascular-related score of stress biological biomarkers [46, 47]. The findings support potential causal effects of vascular disease on cognition through biological stress and DNA methylation. In the present study, we opted to include GrimAge as a DNA methylation based measure of biological aging [10, 37]. GrimAge is based on surrogate markers for select plasma proteins that are closely reflective of aging in humans [10, 37]. DNA methylation reflects accumulated genetic memory in response to exposures [48]. Thus DNA methylation biomarkers likely captures individual specific information not only on an individual's response to trauma induced by vascular disease but also on an individual susceptibility to healthful survival versus progressing to morbid outcomes including cognitive changes with aging [48].

Our results suggest some indirect effects of vascular disease through allostatic load on cognition. Allostatic load was calculated using sum scores of commonly used biomarkers in allostatic load calculations [11]. Allostatic

load refers to accumulated allostasis in response to stress. At the physiological level, both vascular-related biological stress and allostatic systems allow wide adaptation to stress and hence provide opportunity for more precision compared to homeostatic systems [11, 49]. At the cellular level, allostatic effects could be reflected in physiological dysregulation and duration of dysregulation in comparison to a pre-stress state [11, 49].Allostasis effects are suggested to be associated with brain amyloid accumulation [50].

Previous work in MIDUS and NHANES reported relationships between increased stress and older biological age measured by allostatic load and biological age respectively with measures of functional capacity and life-course risk factors [51] suggesting possible relatedness between aging biology and stress biology. Previous studies reported variations in predictors of episodic memory compared to executive function. Previous evidence from MIDUS suggest associations between DNA methylation, inflammation and episodic memory and associations between metabolism and executive function [52, 53]. Allostatic load scores, cardiovascular biomarkers, ischemic stroke and small vessel disease are suggested to be associated with episodic memory and executive function [52, 54–56]. Early life adversity and domain-specific cognition have been suggested to be mediated by allostatic load among females [57]. Associations between physical activity and short-term episodic memory have been reported [58].

Our study has several limitations. First, vascular events were based on self-report and thus could be a potential source of recall bias. However, evidence suggests agreement between self-report and physician ascertained vascular events in hospital records [59]. In addition, potential measurement error was factored in a sensitivity analysis. Second, our analysis is restricted to individuals with available selected measures given our goal of detecting biological relationships, however the sample (age range = 26–78, female = 48%) is considered representative of the overall MIDUS refresher sample distribution. Lastly, as in the majority of observational studies, unmeasured confounding and reverse causality bias cannot be fully excluded. Relatedly, the present study was based on MIDUS refresher data, hence follow up data was not available. However vascular events were based on ever-exposures, hence reflects cumulative vascular disease burden. In addition, sensitivity analyses verified our main observations and exposure-mediator, mediator-outcome relationships were also confirmed.

In addition to DNA methylation and allostatic load, our novel measure of quantifying vascular-related Biological Stress has several important implications: First: a novel biomarker to detect potential causal contributions of cumulative vascular events that are common in aging, with higher precision tailored to each individual's state of health at the time of measurement; Second: new avenues to measure asymptomatic changes at the individual level that are independent of traditional diagnostic frameworks since the vascular-related biological stress scores were not based on disease cut-offs; Third: the feasibility of measuring these biological biomarkers open avenues for reproducibility and scalability for more equitable health care coverage. Lastly, the present proof-of-concept will hopefully provide a base for future developments towards more precision at both the individual level and across global settings.

In summary, we established links that could be potentially causal between vascular diseases and key cognitive domains in relation to a new biomarker vascular-related biological stress. Our findings suggest that vascular-related biological stress, DNA methylation and to some extent allostatic load mediate the effects of vascular disease on global cognition and executive function.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12883-025-04185-6.

Supplementary Material 1

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Author contributions

RW: research conception and execution, study design, statistical analysis, funding and writing the manuscript; RW & CM: statistical analyses review; RW, OW, HT, CM: reviewing the final drafts for intellectual content.

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Data availability

MIDUS refresher study data can be accessed by eligible researchers through MIDUS website: https://midus.wisc.edu

Declarations

Ethics approval and consent to participate

All participants included in the MIDUS-refresher study provided a written informed consent. MIDUS refresher was approved by the institutional review boards of Harvard University, Georgetown University, the University of California at Los Angeles, the University of Wisconsin institutional review boards, and the University of California at Los Angeles. All MIDUS refresher participants provided written informed consent. In addition, the present study is approved by the Institutional Review Board of Columbia University. All methods were carried out in accordance with relevant guidelines and

regulations. The present study has been conducted in accordance with STROBE guidelines for observational studies.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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References

1. O'Brien JT, Thomas A. Vascular dementia. Lancet. 2015;386:1698–706.
2. Pendlebury ST, Rothwell PM. Prevalence, incidence, and factors associated with pre-stroke and post-stroke dementia: a systematic review and meta-analysis. Lancet Neurol. 2009;8:1006–18.
3. Koene RJ, Prizment AE, Blaes A, Konety SH. Shared risk factors in cardiovascular disease and cancer. Circulation. 2016;133:1104–14.
4. Santos CY, Snyder PJ, Wu W-C, Zhang M, Echeverria A, Alber J. Pathophysiologic relationship between alzheimer's disease, cerebrovascular disease, and cardiovascular risk: A review and synthesis. Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring. 2017;7:69–87.
5. Hansson GK, Robertson A-KL, Söderberg-Nauclér C. Inflammation and atherosclerosis. Annu Rev Pathol Mech Dis. 2006;1:297–329.
6. Leifer BP. Early diagnosis of Alzheimer's disease: clinical and economic benefits. J Am Geriatr Soc. 2003;51:S281–8.
7. Ferreira PC, Zhang Y, Snitz B, Chang C-CH, Bellaver B, Jacobsen E, et al. Plasma biomarkers identify older adults at risk of Alzheimer's disease and related dementias in a real-world population-based cohort. Alzheimer's Dement. 2023;19:4507–19.
8. Waziry R, Gu Y, Boehme AK, Williams OA. Measures of aging biology in saliva and blood as novel biomarkers for stroke and heart disease in older adults. Neurology. 2023;101:e2355–63.
9. Waziry R, Gu Y, Williams O, Hägg S. Connections between cross-tissue and intra-tissue biomarkers of aging biology in older adults. Epigenetics Commun. 2023;3:7.
10. Lu AT, Binder AM, Zhang J, Yan Q, Reiner AP, Cox SR, et al. DNA methylation grimage version 2. Aging. 2022;14:9484.
11. McEwen BS. Protective and damaging effects of stress mediators. N Engl J Med. 1998;338:171–9.
12. Waziry R, Ryan C, Corcoran D, Huffman K, Kobor M, Kothari M, et al. Effect of long-term caloric restriction on DNA methylation measures of biological aging in healthy adults from the CALERIE trial. Nat Aging. 2023;3:248–57.
13. Li X, Ploner A, Wang Y, Magnusson PK, Reynolds C, Finkel D, et al. Longitudinal trajectories, correlations and mortality associations of nine biological ages across 20-years follow-up. Elife. 2020;9:e51507.
14. Guidi J, Lucente M, Sonino N, Fava GA. Allostatic load and its impact on health: A systematic review. Psychother Psychosom. 2020;90:11–27.
15. Ryff CD, Lachman ME et al. Midlife in the united states (MIDUS refresher 1): Cognitive project, 2011–2014. 2021.
16. Brim OG, Ryff CD, Kessler RC et al. The MIDUS National survey: an overview. How Healthy Are We. 2004;1–36.
17. Urban-Wojcik EJ, Lee S, Grupe DW, Quinlan L, Gresham L, Hammond A et al. Diversity of daily activities is associated with greater hippocampal volume. Cognitive, affective, & behavioral neuroscience. 2022;1–13.
18. MIDUS. Midlife in the united states publications (www.midus.wisc.edu/findings/index.php)
19. Kean J, Reilly J. Item response theory. Handbook for clinical research: Design, statistics and implementation. 2014;195–8.
20. Rizopoulos D, Ltm. Latent trait models under IRT. R package version 1.1-1. 2018.
21. Liu SH, Juster R-P, Dams-O'Connor K, Spicer J. Allostatic load scoring using item response theory. Compr Psychoneuroendocrinology. 2021;5:100025.
22. Zopluoglu C. EstCRM: an R package for Samejima's continuous IRT model. Appl Psychol Meas. 2012;36:149.
23. Wang T, Zeng L. Item parameter Estimation for a continuous response model using an EM algorithm. Appl Psychol Meas. 1998;22:333–44.
24. Samejima F. Homogeneous case of the continuous response model. Psychometrika. 1973;38:203–19.
25. Lein V. Midlife in the united States National study of health and well-being field report. Madison, WI: University of wisconsin survey center; 2015.
26. Yusuf S. Two decades of progress in preventing vascular disease. Lancet. 2002;360:2–3.
27. Madamanchi NR, Vendrov A, Runge MS. Oxidative stress and vascular disease. Arteriosclerosis, thrombosis, and vascular biology. 2005;25:29–38.
28. Alexander Y, Osto E, Schmidt-Trucksäss A, Shechter M, Trifunovic D, Duncker DJ, et al. Endothelial function in cardiovascular medicine: A consensus paper of the European society of cardiology working groups on atherosclerosis and vascular biology, aorta and peripheral vascular diseases, coronary pathophysiology and microcirculation, and thrombosis. Cardiovascular Res. 2021;117:29–42.
29. Tibshirani R. Regression shrinkage and selection via the Lasso. J Royal Stat Soc Ser B: Stat Methodol. 1996;58:267–88.
30. Hastie T, Qian J, Tay K. An introduction to Glmnet. CRAN R Repositary. 2021;5:1–35.
31. Waziry R, Gras L, Sedaghat S, Tiemeier H, Weverling GJ, Ghanbari M, et al. Quantification of biological age as a determinant of age-related diseases in the Rotterdam study: a structural equation modeling approach. Eur J Epidemiol. 2019;34:793–9.
32. Waziry R, Gu Y, Boehme AK, Williams OA. Measures of aging biology in saliva and blood as novel biomarkers for stroke and heart disease in older adults. Neurology. 2023. https://doi.org/10.1212/wnl.0000000000207909.
33. López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. Hallmarks of aging: an expanding universe. Cell. 2023.
34. Zou H. The adaptive Lasso and its oracle properties. J Am Stat Assoc. 2006;101:1418–29.
35. Thrampoulidis C, Abbasi E, Hassibi B. Lasso with non-linear measurements is equivalent to one with linear measurements. Adv Neural Inf Process Syst. 2015;28.
36. Chai T, Draxler RR. Root mean square error (RMSE) or mean absolute error (MAE)?–arguments against avoiding RMSE in the literature. Geosci Model Dev. 2014;7:1247–50.
37. Lu AT, Quach A, Wilson JG, Reiner AP, Aviv A, Raj K, et al. DNA methylation grimage strongly predicts lifespan and healthspan. Aging. 2019;11:303.
38. Lachman ME, Agrigoroaei S, Murphy C, Tun PA. Frequent cognitive activity compensates for education differences in episodic memory. Am J Geriatric Psychiatry. 2010;18:4–10.
39. Lachman ME, Agrigoroaei S, Tun PA, Weaver SL. Monitoring cognitive functioning: psychometric properties of the brief test of adult cognition by telephone. Assessment. 2014;21:404–17.
40. Berkelmans GF, Read SH, Gudbjörnsdottir S, Wild SH, Franzen S, Van Der Graaf Y, et al. Population median imputation was noninferior to complex approaches for imputing missing values in cardiovascular prediction models in clinical practice. J Clin Epidemiol. 2022;145:70–80.
41. Shi B, Choirat C, Coull BA, VanderWeele TJ, Valeri L. CMAverse: A suite of functions for reproducible causal mediation analyses. Epidemiology. 2021;32:e20–2.
42. VanderWeele TJ. A unification of mediation and interaction: A four-way decomposition. Epidemiol (Cambridge Mass). 2014;25:749.
43. Schafer JL. Multiple imputation: A primer. Stat Methods Med Res. 1999;8:3–15.
44. Ramsey JB. Tests for specification errors in classical linear least-squares regression analysis. J Royal Stat Soc Ser B: Stat Methodol. 1969;31:350–71.
45. Cook JR, Stefanski LA. Simulation-extrapolation Estimation in parametric measurement error models. J Am Stat Assoc. 1994;89:1314–28.
46. Beese S, Postma J, Graves JM. Allostatic load measurement: A systematic review of reviews, database inventory, and considerations for neighborhood research. Int J Environ Res Public Health. 2022;19:17006.
47. Mauss D, Li J, Schmidt B, Angerer P, Jarczok MN. Measuring allostatic load in the workforce: A systematic review. Ind Health. 2015;53:5–20.
48. Dor Y, Cedar H. Principles of DNA methylation and their implications for biology and medicine. Lancet. 2018;392:777–86.

49. Seeman TE, Singer BH, Rowe JW, Horwitz RI, McEwen BS. Price of adaptation—allostatic load and its health consequences: MacArthur studies of successful aging. Arch Intern Med. 1997;157:2259–68.
50. Adedeji DO, Holleman J, Juster R-P, Udeh-Momoh CT, Kåreholt I, Hagman G et al. Longitudinal study of alzheimer's disease biomarkers, allostatic load, and cognition among memory clinic patients. Brain, Behavior, & Immunity-Health. 2023;28:100592.
51. Hastings WJ, Almeida DM, Shalev I. Conceptual and analytical overlap between allostatic load and systemic biological aging measures: analyses from the National survey of midlife development in the united States. Journals Gerontology: Ser A. 2022;77:1179–88.
52. Karlamangla AS, Miller-Martinez D, Lachman ME, Tun PA, Koretz BK, Seeman TE. Biological correlates of adult cognition: midlife in the united States (MIDUS). Neurobiol Aging. 2014;35:387–94.
53. Sommerer Y, Dobricic V, Schilling M, Ohlei O, Bartrés-Faz D, Cattaneo G, et al. Epigenome-wide association study in peripheral tissues highlights DNA methylation profiles associated with episodic memory performance in humans. Biomedicines. 2022;10:2798.
54. Zahodne LB, Mayeda ER, Hohman TJ, Fletcher E, Racine AM, Gavett B, et al. The role of education in a vascular pathway to episodic memory: brain maintenance or cognitive reserve? Neurobiol Aging. 2019;84:109–18.
55. Schouten EA, Schiemanck SK, Brand N, Post MW. Long-term deficits in episodic memory after ischemic stroke: evaluation and prediction of verbal and visual memory performance based on lesion characteristics. J Stroke Cerebrovasc Dis. 2009;18:128–38.
56. Villeneuve S, Massoud F, Bocti C, Gauthier S, Belleville S. The nature of episodic memory deficits in MCI with and without vascular burden. Neuropsychologia. 2011;49:3027–35.
57. D'Amico D, Amestoy ME, Fiocco AJ. The mediating role of allostatic load in the relationship between early life adversity and cognitive function across the adult lifespan. Psychoneuroendocrinology. 2022;141:105761.
58. Huang O. Associations of light, leisure-time physical activity with short-term episodic memory in middle-aged and older Americans. Results from the MIDUS study; 2022.
59. Glymour MM, Avendano M. Can self-reported strokes be used to study stroke incidence and risk factors? Evidence from the health and retirement study. Stroke. 2009;40:873–9.

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