

Proteomic Indicators of Health Predict Alzheimer's Disease Biomarker Levels and Dementia Risk

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Objective: Few studies have comprehensively examined how health and disease risk influence Alzheimer's disease (AD) biomarkers. The present study examined the association of 14 protein-based health indicators with plasma and neuroimaging biomarkers of AD and neurodegeneration.

Methods: In 706 cognitively normal adults, we examined whether 14 protein-based health indices (ie, SomaSignal® tests) were associated with concurrently measured plasma-based biomarkers of AD pathology (amyloid- β [$A\beta$]_{42/40}, tau phosphorylated at threonine-181 [pTau-181]), neuronal injury (neurofilament light chain [NfL]), and reactive astrogliosis (glial fibrillary acidic protein [GFAP]), brain volume, and cortical $A\beta$ and tau. In a separate cohort (n = 11,285), we examined whether protein-based health indicators associated with neurodegeneration also predict 25-year dementia risk.

Results: Greater protein-based risk for cardiovascular disease, heart failure mortality, and kidney disease was associated with lower $A\beta$ _{42/40} and higher pTau-181, NfL, and GFAP levels, even in individuals without cardiovascular or kidney disease. Proteomic indicators of body fat percentage, lean body mass, and visceral fat were associated with pTau-181, NfL, and GFAP, whereas resting energy rate was negatively associated with NfL and GFAP. Together, these health indicators predicted 12, 31, 50, and 33% of plasma $A\beta$ _{42/40}, pTau-181, NfL, and GFAP levels, respectively. Only protein-based measures of cardiovascular risk were associated with reduced regional brain volumes; these measures predicted 25-year dementia risk, even among those without clinically defined cardiovascular disease.

Interpretation: Subclinical peripheral health may influence AD and neurodegenerative disease processes and relevant biomarker levels, particularly NfL. Cardiovascular health, even in the absence of clinically defined disease, plays a central role in brain aging and dementia.

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Alzheimer's disease (AD) is the most common neurodegenerative disease and is characterized by the presence of amyloid- β (A β) plaques, tau-containing neurofibrillary tangles, neurodegeneration, and inflammatory processes.^{1,2} Prior studies suggest that peripheral disease, such as hypertension, obesity, ischemic heart disease, kidney disease, and liver disease, are associated with cognitive decline and risk of AD.^{3–5} Although the relationship between suboptimal health and increased risk for cognitive impairment and AD/dementia has been demonstrated, less is known about how peripheral health factors influence AD pathology and nonspecific features of AD, such as neuronal damage and astrogliosis. A more precise understanding of how peripheral health, disease, and disease risk differentially relate to discrete aspects of AD pathology could inform our understanding of AD biology and identify effective strategies for prevention and therapeutic interventions.

Numerous studies have examined the relationship between individual health factors and molecular biomarkers of AD and neurodegenerative disease, yet a comprehensive understanding of how health factors may influence AD and neurodegenerative processes remains elusive. For example, the relationship between vascular risk factors and cortical A β and tau—measured using positron emission tomography (PET)—has been inconsistent, with some^{6,7} but not all^{8,9} studies demonstrating a positive association between cardiovascular disease and cortical A β and tau. Given their low cost and high degree of accessibility, blood-based measures of A β , phosphorylated tau (pTau), neuronal damage (eg, neurofilament light chain [NFL]), and reactive astrogliosis (ie, glial fibrillary acidic protein [GFAP]) provide the opportunity to examine the relationship between peripheral health and AD and neurodegeneration at a larger scale.^{10–12} Recent studies have demonstrated that hypertension, ischemic heart disease, and diabetes were associated with higher concentrations of plasma A β_{40} and A β_{42} .¹⁰ A separate study found that chronic kidney disease (CKD), hypertension, and diabetes were associated with elevated A β_{40} , A β_{42} , and total tau, whereas only CKD and diabetes were associated with NFL.¹³

The advent of large-scale proteomics has made it possible to conduct a comprehensive health examination through measurement of thousands of circulating plasma proteins. Protein-based indicators have been developed and validated against gold standard measurements of health, disease, and disease risk and therefore provide a broad and dynamic picture of health across multiple organ systems in a scalable and consistent manner.^{14,15} By directly assessing proteomic signatures of health and

disease, these tools allow for detection and quantification of subthreshold disease and disease risk in individuals without clinically defined disease. Additionally, this approach to health measurement provides enhanced sensitivity, allowing for classification across the full spectrum from healthy to severe disease, and removes measurement error related to self- or informant-report. Using the proteome-based health indicators developed by SomaLogic,^{14,15} the current study cross-sectionally examined the association between 14 protein-based health indicators and plasma measures of A $\beta_{42/40}$, pTau-181, NFL, and GFAP, cortical A β and tau, and neurodegeneration using data from the Baltimore Longitudinal Study of Aging (BLSA). Furthermore, using data from the Atherosclerosis Risk in Communities (ARIC) study, we examined whether proteomic indicators of health associated with AD/neurodegeneration biomarkers also predicted 25-year dementia risk.

Patients and Methods

Participant Characteristics

The current analysis used data from the BLSA and ARIC cohort studies. The BLSA is a longitudinal aging study of community-dwelling adults that began in 1958.¹⁶ BLSA participant recruitment and enrollment procedures have been described elsewhere.^{17–19} We used BLSA data from a subset of cognitively unimpaired participants ($n = 706$) from the BLSA neuroimaging substudy. Inclusion and exclusion criteria for neuroimaging have been previously described.¹⁹ From 1994 to 2005, participants completed visits biannually, unless they were enrolled in the neuroimaging substudy, where visits occurred annually. After 2005, study visits were completed as follows: participants <60 years of age were seen every 4 years, participants 60 to 79 years of age were seen biennially, and participants ≥ 80 years of age were seen annually. Study visits included brain magnetic resonance imaging (MRI), neuropsychological testing, health and physical assessments, and a blood draw. Plasma proteomics was conducted using blood collected at the time of first 3T MRI, or for a smaller subset of participants, at the time of first PET scan or Alzheimer's Disease and Related Dementias (ADRD) biomarker measurement. The present study was cross-sectional in design and used ADRD biomarker and neuroimaging data from visits concurrent with the first proteomic measurement. An exception was analyses using tau PET imaging data, which included nonconcurrent tau PET scans (due to the small sample size of concurrent tau PET scans). Participants provided written informed consent during each visit. The BLSA protocol was approved by

the institutional review board (IRB) of the National Institute of Environmental Health Science, National Institutes of Health (03AG0325). The BLSA PET substudy has ongoing approval from the IRB of the Johns Hopkins Medical Institutions (NA_00051793 and IRB00047185).

The ARIC study is an ongoing study that enrolled 15,792 community-dwelling adults aged 45 to 64 years between 1987 and 1989 from 4 sites across the United States: Washington County, Maryland; Forsyth County, North Carolina; Jackson, Mississippi; and Minneapolis suburbs, Minnesota.²⁰ Participants completed visits every 3 years until visit 4. After a 15-year gap, participants returned for visit 5 from 2011 to 2013 and visit 6 from 2016 to 2017. Participants completed brief cognitive assessments during visits 2 and 4, and comprehensive cognitive and functional assessments were completed during visits 5 and 6 (Fig S1). Proteomics was conducted using blood collected at visit 3 (1993–1995). Participants provided written informed consent during each study visit. If participants were deemed to lack capacity, a proxy provided written informed consent. IRBs at each participating center approved the study.

Protein Measurement and Proteomic Indices of Health (SomaSignal Test)

Plasma samples were collected from BLSA participants after an overnight fast at the National Institute on Aging (NIA) Clinical Research Unit at Harbor Hospital, Baltimore, Maryland.²¹ Aliquots (0.5 μ l volumes) of plasma samples were stored at -80°C . Plasma samples were assayed using SomaScan's Multiplexed Proteomic Technology version 4.1 platform (SomaLogic Inc., Boulder, CO), which quantified 7,288 human protein measurements using modified aptamers, which act as protein-binding reagents with defined shapes and unique nucleotide sequences that may be identified and quantified using DNA technology techniques.^{22,23} Intra- and inter-run coefficients of variation are approximately 5% and median intraclass coefficients are approximately 90% for the SomaScan[®] platform.^{24–27}

Fourteen protein-based health indicators (SomaSignal tests [SSTs]) were derived for each participant (SomaLogic Inc, Boulder, CO^{14,15}). SST algorithms were derived based on gold standard measures of health, modifiable behaviors, and incident disease risk measured in 13 independent cohort studies. Machine learning algorithms were used to determine which plasma protein combinations best predicted 14 distinct metrics of health; these algorithms were calibrated, validated, and used to define a set of 14 SSTs (Fig 1, Table S1). Detailed methods for SST development have been described previously.^{14,15} Proteins included in each SST are listed in Table S2. Within the BLSA, 1806 plasma samples, which

included repeat measurement, were assessed, 7 of which were removed for poor sample quality. Of the remaining 1,799 samples, 102 were blind duplicates used for quality control analyses. In total, we derived SSTs on 1,697 samples ($n = 1,331$ unique participants).²⁶ For our cross-sectional analyses, we selected SST measurements that were derived concurrently with plasma ADRD biomarkers. Participant selection is detailed in Figure S2. Mean coefficients of variation in the duplicate samples for all 14 SSTs ranged from 0.9 to 13.1% (Table S3). Several SST kidney function ($n = 51$) and visceral fat ($n = 22$) measurements were beyond the range of the cohorts used for SST development; these values were therefore imputed. SST values were then \log_2 transformed, and values that were beyond ± 5 standard deviations (SD) from the mean were winsorized. Validation of each SST was performed using relevant clinical measures from the BLSA (Table S4).

Plasma samples were collected during ARIC visit 3 (1993–1995) using the aptamer-based SomaScan multiplexed version 4 modified platform (SomaLogic Inc., Boulder, CO) to quantify 4,712 unique proteins using modified aptamers.^{22,23} Plasma samples were frozen at -80°C and shipped on dry ice to the ARIC laboratory, where they remained frozen until aliquoted. Median interassay coefficients of variation (relative scale) for SOMAmers for ARIC visit 3 were reported to be 6.3%.²³ Prior studies have described ARIC protein measurement and quality control procedures.^{23,28} Data from 11,701 participants were available at ARIC visit 3. Participants were excluded for missing plasma samples, prevalent dementia, plasma samples that fell outside the normalization scale factor, and samples with $>5\%$ of SOMAmer measurements exceeding 6 median absolute deviations from the median (outliers). A total of 11,285 participants were included in the present study (Figure S1).

AD and Neurodegenerative Disease Biomarkers

Plasma ADRD biomarkers were measured from BLSA blood samples collected concurrently with the initial 3T MRI or (for a subset of participants) the first amyloid PET scan. Amyloid ($\text{A}\beta_{40}$, $\text{A}\beta_{42}$), NfL, and GFAP (see Fig 1) were measured on the Single Molecule Array (Simoa) HD-X instrument using the Neurology 4-Plex E assay (Quanterix Corporation, Billerica, MA). pTau-181 was measured using the Simoa pTau181 version 2 assay on the HD-X Instrument (Quanterix Corporation). Intra-assay coefficients of variation were as follows: $\text{A}\beta_{40}$, 2.8; $\text{A}\beta_{42}$, 1.9; GFAP, 5.0; NfL, 5.1; and pTau-181, 4.4.²⁹ GFAP, NfL, and pTau-181 were non-normally distributed and \log_2 transformed prior to analyses.

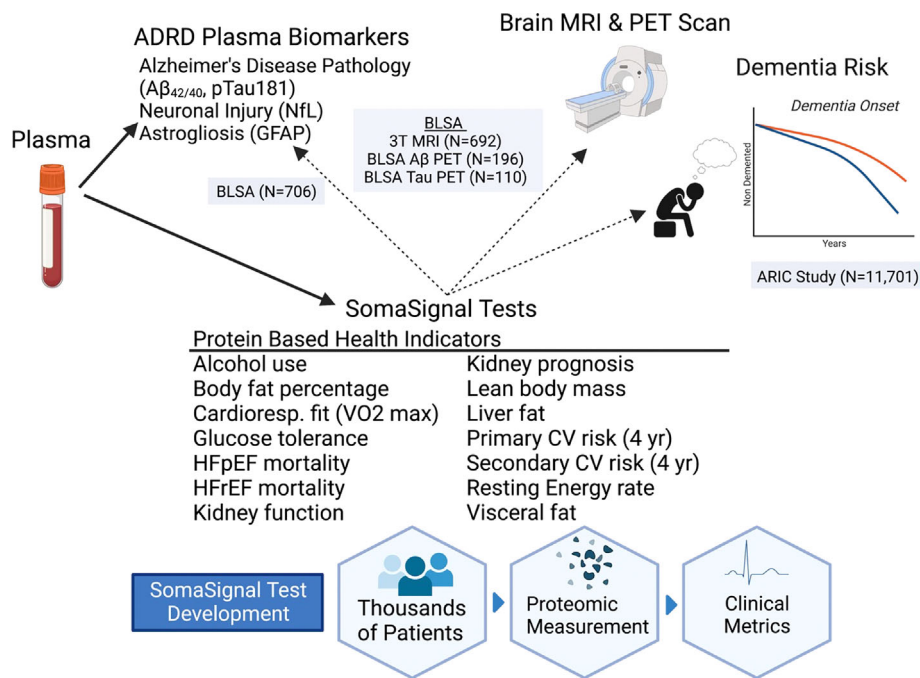


FIGURE 1: Study design. The current study examines the relationship between plasma protein-based health indicators (SomaSignal tests) and plasma Alzheimer's Disease and Related Dementias (ADRD) biomarkers, magnetic resonance imaging (MRI)-defined brain volume, positron emission tomography (PET)-defined amyloid and tau, and dementia risk. The 14 facets of health, disease, and disease risk captured by the SomaSignal tests are listed. Aβ = amyloid-β; ARIC = Atherosclerosis Risk in Communities study; BLSA = Baltimore Longitudinal Study of Aging; CV = cardiovascular risk; GFAP = glial fibrillary acidic protein; HFpEF = heart failure with preserved ejection fraction; HFrEF = heart failure with reduced ejection fraction; NfL = neurofilament light chain; pTau181 = tau phosphorylated at threonine-181; VO₂ max = Cardiorespiratory maximal oxygen consumption. Figure created with BioRender.com. [Color figure can be viewed at www.annalsofneurology.org]

MRI Acquisition, Preprocessing, and Spatial Pattern of Abnormality for Recognition of Early Alzheimer's Disease Scores

Participants enrolled in the BLSA Neuroimaging Substudy¹⁹ completed scans on a 1.5T (General Electric [GE] Signa) or 3T (Philips Achieva) MRI scanner beginning in either 1994 or 2008 to 2010, respectively.^{19,30} High-resolution volumetric spoiled gradient recalled acquisition in a steady state scans (1.5T: repetition time [TR] = 35 milliseconds, echo time [TE] = 5 milliseconds, flip angle = 45°, image matrix = 256 × 256 × 124 voxels, voxel size = 0.94 × 0.94 × 1.50mm³) were acquired on a GE Signa scanner. T1-weighted magnetization-prepared rapid gradient echo scans (3T: TR = 6.8 milliseconds, TE = 3.2 milliseconds, flip angle = 8°, image matrix = 256 × 256 × 170, voxel size = 1.0 × 1.0 × 1.2mm³) were acquired on a 3T Philips Achieva scanner. Preprocessing steps and harmonization procedures have been described previously.^{19,30,31} Within the present study (n = 692), 674 participants underwent 3T MRI, whereas 18 underwent 1.5T MRI. MUSE (Multi-atlas Region Segmentation Utilizing Ensembles) anatomical labeling was used to create RAVENS maps,^{30,32,33} which quantify the regional tissue densities and are used to determine AD-like brain patterns.^{19,34} Spatial

Pattern of Abnormality for Recognition of Early Alzheimer's Disease (SPARE-AD) scores were generated using high-dimensional pattern classification approaches^{34,35} to quantify the extent of brain atrophy in an AD-like pattern and distinguish brains of individuals with AD from cognitively unimpaired individuals. The SPARE-AD scores have demonstrated classification accuracy of 52.3%.³⁶ Higher SPARE-AD scores reflect greater AD-like brain atrophy.

PET Imaging

Beginning in 2005, a subset of participants in the BLSA Neuroimaging Substudy underwent ¹¹C-Pittsburgh compound-B (PiB) PET neuroimaging to measure cortical amyloid. PiB PET scans were acquired over 70 minutes on either a GE Advance (image matrix = 128 × 128 × 35 slices, voxel size = 2.0 × 2.0 × 4.25mm³, 4.5mm full-width at half-maximum [FWHM] at the center of the field of view) or a Siemens High Resolution Research Tomograph (HRRT; image matrix = 256 × 256 × 207, voxel size = 1.22 × 1.22 × 1.22mm³, 2.5mm FWHM at the center of the field of view) scanner following an intravenous bolus injection of approximately 555MBq of radiotracer PiB. Full details of PiB data acquisition and processing have been described previously.⁸ Distribution volume ratios (DVRs)

were computed using cerebellar gray matter as a reference volume and averaging DVR values across several cortical regions to reflect mean cortical A β levels. Participants were divided into A β positive (PiB+) and A β negative (PiB-) groups based on their mean cortical DVR using a Gaussian mixture model threshold of 1.064.

Beginning in 2016, a subset of participants in the BLSA Neuroimaging Substudy underwent ^{18}F -florbetapir (FTP) PET neuroimaging to measure cortical tau. FTP scans were acquired over 30 minutes on a Siemens HRRT scanner (image matrix = $256 \times 256 \times 107$, voxel size = $1.22 \times 1.22 \times 1.22\text{mm}^3$, 2.5mm FWHM at the center of the field of view), 75 minutes after an intravenous bolus injection of approximately 370MBq of radio-tracer FTP. Tau acquisition and preprocessing methods have been described elsewhere.⁸ Partial volume corrected images were used to compute standardized uptake value ratio (SUVR) images using the inferior cerebellar gray matter as a reference region. Average bilateral standardized SUVRs in the entorhinal cortex and inferior temporal gyrus (ITG) were computed. There was one outlier for the ITG, which was truncated to $z = 5.0$ for all analyses.

ARIC Dementia Ascertainment

Dementia was adjudicated in the ARIC study in the years following SST measurement based on the NIA-Alzheimer's Association workgroups and the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition,^{37,38} and ascertained through 2017 for the present study. Dementia surveillance methods have been described previously.^{6,39} Dementia was ascertained using data from comprehensive neuropsychological and functional assessments, and informant interview from visits 5 and 6. Prior to and following visit 5, dementia was ascertained using the International Classification of Diseases, Ninth Revision and diagnostic codes from death certificates. Between visits 5 and 6, dementia was also identified using a 6-item screener and the Ascertain Dementia 8-Item Informant Questionnaire administered via phone annually.

Covariates

Age, sex (male/female), race (White/non-White), and education (BLSA: total years; ARIC: categories of less than high school/high school, GED, or vocational school/at least some college) were self-reported by participants. Because race and study site were confounded, a race-center variable was used for ARIC analyses. *APOE* ϵ 4 genotyping was completed using polymerase chain reaction plus restriction isotyping with the type IIP enzyme Hhai or the Taqman method.⁴⁰ *APOE* ϵ 4 was defined as 0 ϵ 4 alleles versus ≥ 1 ϵ 4 alleles.

Statistical Analyses

SST indices, plasma biomarkers, mean cortical DVR, regional SUVR, and SPARE-AD values were standardized (mean = 0, SD = 1) within the current sample for linear regression analyses to facilitate comparability of effect estimates. For each SST measure, separate unadjusted and adjusted linear regression analyses were completed to determine the association between SST proteomic indices of health (predictor variables) and continuous ADRD biomarkers (outcome variables). Age, sex, race, education, and *APOE* ϵ 4 were included as covariates in all adjusted analyses. For tau PET analyses, PiB status (PiB+/PiB-) was also included as a covariate. Logistic regression was used to relate SST indices to PiB status (coding: PiB+: 1, PiB-: 0). Spearman correlations were completed independently for both PiB+ and PiB- participants to determine whether SST indices correlated with mean cortical DVR values within amyloid-positive and amyloid-negative subgroups. Based on the findings linking SST indices to SPARE-AD, secondary mediation analyses were completed to determine whether AD pathology, astrogliosis, and neuronal injury (as measured by plasma biomarkers) mediated the association between SST health indices (predictor variables) and SPARE-AD-defined brain atrophy (outcome variable). The PROCESS macro,⁴¹ model 4, was used for simple and parallel mediation analyses. Although this method relies on ordinary least squares regression assumptions, it does not require that causal assumptions be met.⁴² Ninety-five percent confidence intervals for indirect effects were estimated using 10,000 bootstrapped samples. Time to event analyses, including Kaplan-Meier and Cox proportional hazards models, were completed to determine whether select proteomic indicators of health were associated with incident dementia. SPSS version 28, R version 4.1.1, and SAS version 9.4 were used for statistical analyses. A significance level of $p < 0.05$ was used for analyses. A false discovery rate approach was used to correct for multiple comparisons.

Results

Participant Characteristics

Characteristics for the present study are presented in the Table (BLSA) and Table S5 (ARIC). A total of 706 BLSA participants were included in the primary analyses. All BLSA participants were cognitively unimpaired at the time of biomarker measurement. BLSA participants had lower prevalence of heart ischemic disease (8.5%) and chronic kidney disease (23.7%) than that observed in older adults (age ≥ 65 years) in the United States (heart disease, 18.3%; chronic kidney disease, 33.7%),^{43,44} whereas the prevalence of heart failure (8.1%) was on par with that observed in

TABLE. Baltimore Longitudinal Study of Aging Participant Characteristics

Characteristic	Participants with A β ₄₂ /A β ₄₀ , NfL, GFAP, n = 706 ^a	Participants with pTau-181, n = 566 ^b	Participants with A β PET, n = 196 ^c	Participants with Tau PET, n = 110 ^d	Participants with SPARE-AD, n = 692 ^e
Demographic variables					
Age, yr	68.33 \pm 13.44	66.99 \pm 13.94	75.88 \pm 8.29	77.20 \pm 8.30	68.34 \pm 13.58
Female	382 (54.1%)	308 (54.4%)	104 (53.1%)	63 (57.3%)	377 (54.5%)
White race	476 (67.4%)	363 (64.1%)	153 (78.1%)	87 (79.1%)	463 (66.9%)
Non-White race	230 (32.6%)	203 (35.9%)	43 (21.9%)	23 (20.9%)	229 (33.1%)
Education, yr	17.11 \pm 2.54	17.06 \pm 2.57	17.14 \pm 2.13	17.52 \pm 2.13	17.10 \pm 2.53
Clinical variables					
Hypertension	351 (49.7%)	285 (50.4%)	123 (62.8%)	51 (46.4%)	354 (51.2%)
Diabetes mellitus	77 (10.9%)	55 (9.7%)	26 (13.3%)	14 (12.7%)	72 (10.4%)
Obesity	136 (23.6%)	105 (23.5%)	52 (28.0%)	24 (25.0%)	131 (23.9%)
Heart ischemic disease	60 (8.5%)	47 (8.3%)	18 (9.2%)	8 (7.3%)	51 (7.4%)
Congestive heart failure	57 (8.1%)	44 (7.8%)	18 (9.2%)	5 (4.5%)	56 (8.1%)
Stroke	65 (9.2%)	38 (6.7%)	26 (13.3%)	11 (10%)	60 (8.7%)
COPD	111 (15.7%)	82 (14.5%)	34 (17.3%)	14 (12.7%)	110 (15.9%)
CKD	167 (23.7%)	121 (21.4%)	57 (29.1%)	24 (21.8%)	157 (22.7%)
<i>APOE</i> ϵ 4 alleles					
0 ϵ 4 alleles	516 (73.1%)	417 (73.7%)	137 (69.9%)	76 (69.1%)	506 (73.1%)
1–2 ϵ 4 alleles	190 (26.9%)	149 (26.3%)	59 (30.1%)	34 (30.9%)	186 (26.9%)

Note: Data are presented as either mean \pm standard deviation or n (%). Obesity reflects body mass index \geq 30.

Abbreviations: A β = amyloid- β ; CKD = chronic kidney disease; COPD = chronic obstructive pulmonary disease; GFAP = glial fibrillary acidic protein; NfL = neurofilament light chain; PET = positron emission tomography; pTau-181 = tau phosphorylated at threonine-181; SPARE-AD = Spatial Pattern of Abnormality for Recognition of Early Alzheimer's Disease.

^an = 129 participants missing obesity.

^bn = 119 participants missing obesity.

^cn = 10 participants missing obesity.

^dn = 14 participants missing obesity.

^en = 143 participants missing obesity.

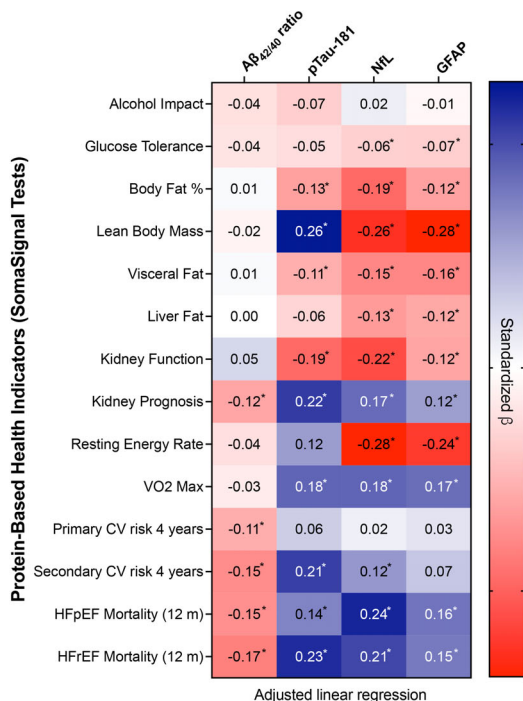
the US population.⁴⁵ Bivariate correlations among proteomic indicators of health are presented in Table S6.

Proteomic Indicators of Health and AD and Neurodegenerative Disease Biomarkers

The adjusted associations between proteomic health indicators and plasma ADRD biomarkers are presented in Figure 2A. After adjusting for age, sex, race, education, and *APOE* ϵ 4 status, we found consistent and strong

associations of higher kidney disease, secondary (not primary) cardiovascular disease risk, and heart failure prognosis risk (with both preserved [HFpEF] and reduced [HFrEF] ejection fraction) with levels of plasma ADRD biomarkers indicative of greater AD pathology and neurodegenerative burden. Notably, the kidney disease risk associations and cardiovascular disease risk and heart failure prognosis associations remained largely similar when participants with evidence of kidney dysfunction (estimated

A Associations of proteomic indicators of health with ADRD biomarkers



B Relative contribution of proteomic indicators of health to ADRD biomarkers

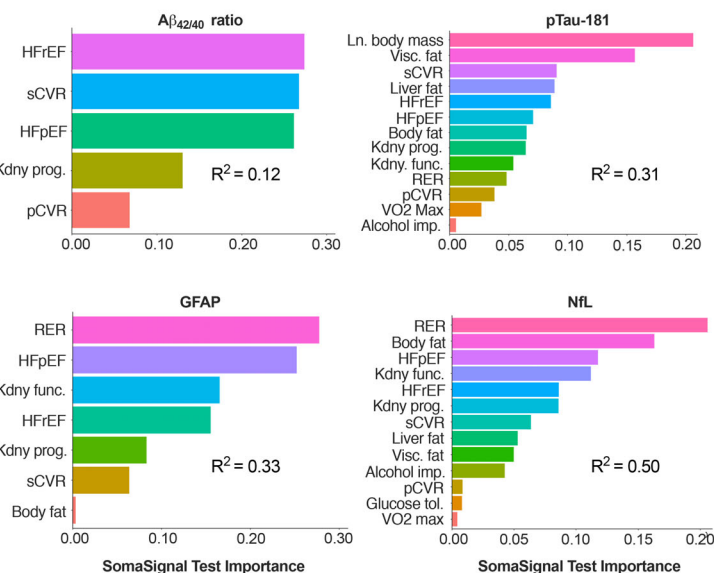


FIGURE 2: Association of proteomic indicators of health with plasma Alzheimer's disease and neurodegeneration biomarkers. (A) Standardized estimates from the covariate-adjusted (adjusted for age, sex, race, education [years], and APOE ϵ 4 genotype) linear regression analyses examining the association between SomaSignal test proteomic indicators of health (see Table S1 for descriptions) and Alzheimer's Disease and Related Dementias (ADRD). Estimates are presented in each cell and represent the standard deviation difference in biomarker level per standard deviation increase in SomaSignal test value. *False discovery rate adjusted $p < 0.05$. Analyses included $n = 706$ participants for amyloid- β (A β)_{42/40}, neurofilament light chain (NfL), and glial fibrillary acidic protein (GFAP), and $n = 566$ for tau phosphorylated at threonine-181 (pTau-181). (B) Relative contribution of each proteomic indicator of health to ADRD biomarkers generated using lasso regression models. Only SomaSignal test contributions with an absolute value greater than zero were included. Importance was generated by first summing the absolute value of the standardized betas of each health indicator, then dividing the absolute value of each beta by the total sum of betas. Alcohol imp = alcohol impact; CV = cardiovascular risk; HFpEF = heart failure with preserved ejection fraction; HFrEF = heart failure with reduced ejection fraction; Kdny func = kidney function; Kdny prog = kidney prognosis; Ln body mass = lean body mass; pCVR = primary cardiovascular risk; RER = resting energy rate; sCVR = secondary cardiovascular risk; VO2 Max = cardiorespiratory maximal oxygen consumption; Visc fat = visceral fat. [Color figure can be viewed at www.annalsofneurology.org]

glomerular filtration rate < 60 ; Table S7) and cardiovascular disease (ie, heart ischemic disease, congestive heart failure, or stroke; Table S8) were excluded. We observed several other relationships of note. Specifically, higher body fat percentage and visceral fat were associated with lower pTau-181, NfL, and GFAP, whereas greater lean body mass was associated with higher pTau-181 and lower NfL and GFAP. Surprisingly, higher maximal oxygen consumption was associated with higher pTau-181, NfL, and GFAP.

Using lasso regression, we conducted post hoc analyses to determine the combined and relative contribution of all 14 proteomic indicators to the prediction of plasma ADRD biomarker levels. Together, proteomic indicators of health explained approximately 12% of variance in A β _{42/40} ($R^2 = 0.12$), 30% of variance in pTau-181 ($R^2 = 0.31$) and GFAP ($R^2 = 0.33$), and 50% of variance

in NfL ($R^2 = 0.50$). The relative contribution of individual health indicators is displayed in Figure 2B.

Proteomic Indicators of Health and Cortical A β and Tau

We next examined the association between proteomic health indicators and cortical A β , as measured using PiB PET imaging ($n = 196$, 29% A β -positive). After adjusting for demographic factors, only the proteomic indicator of poor prognosis in HFpEF was associated with A β -positive status; however, this relationship was no longer significant after correcting for multiple comparisons (Fig 3A, Table S9). We next stratified participants by PiB PET-defined A β status and examined the relationship between proteomic indicators of health and amyloid level. Among A β -positive participants, primary cardiovascular risk, and poor prognosis in HFpEF were positively correlated with

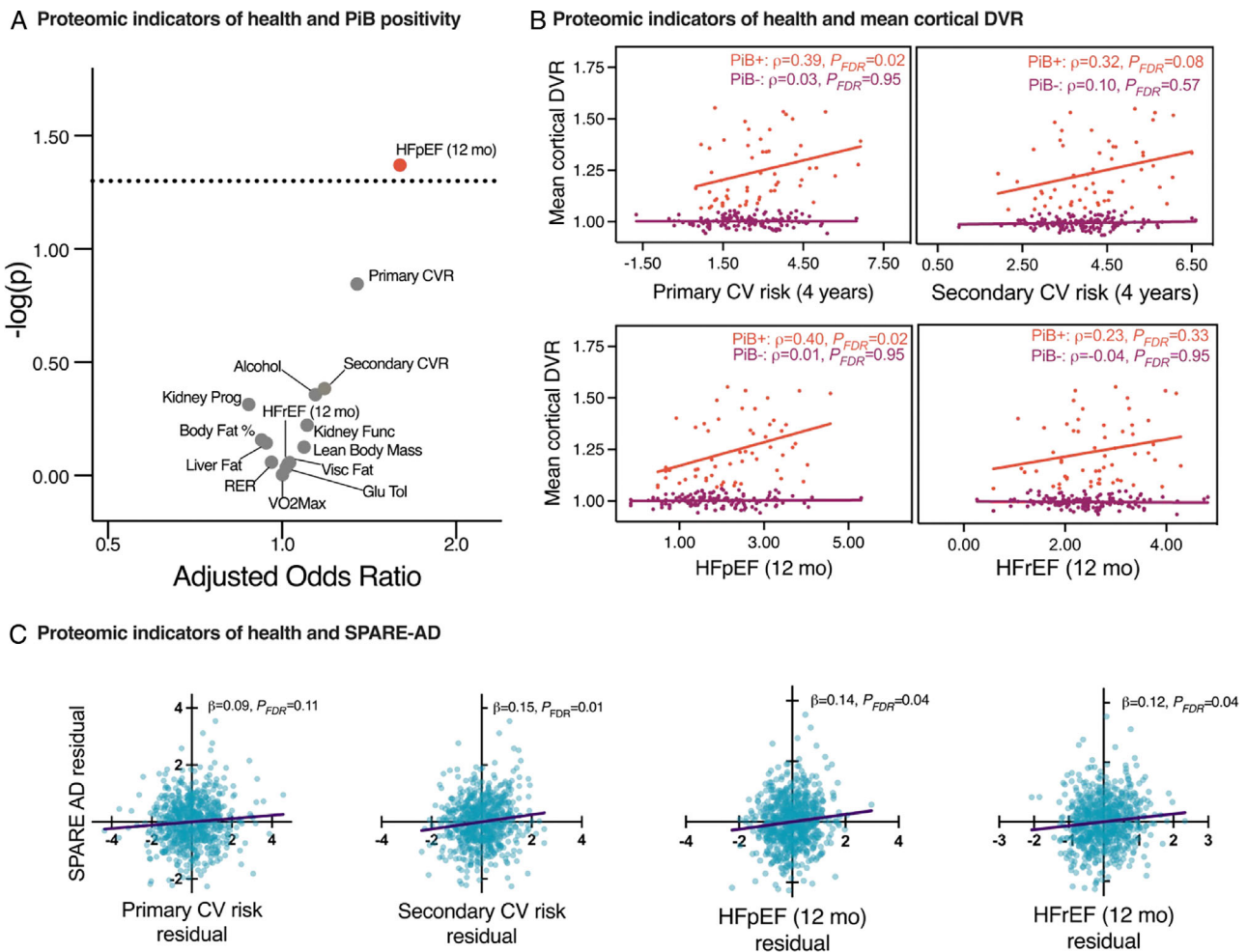


FIGURE 3: Association of proteomic health indicators with cortical amyloid- β (A β ; 11 C-Pittsburgh compound-B [PiB]) and brain atrophy. (A) Adjusted odds ratios for the relationship between proteomic health indicators and elevated A β (PiB). Odds ratios were derived from logistic regression analyses adjusted for age, sex, race, education, and APOE4 genotype. Odds ratios represent the difference in odds per standard deviation increase in SomaSignal test. Orange data points are significantly associated with elevated PiB. Elevated PiB status was defined using a distribution volume ratio (DVR) of 1.064 as a threshold. The dotted line reflects $p_{uncorrected} = 0.05$. (B) Spearman correlations of the relationship among proteomic indicators of cardiovascular risk and poor heart failure prognosis and mean cortical DVR for participants who are PiB positive (orange) and PiB negative (purple). Elevated PiB status was defined using a DVR of 1.064 as a threshold. (C) Linear regression analyses of the relationship among proteomic indicators of cardiovascular risk, poor heart failure prognosis, and Spatial Pattern of Abnormality for Recognition of Early Alzheimer's Disease (SPARE-AD). The residual values reflect each variable of interest with variation due to covariates (age, sex, race, education, and APOE4 status) removed; β reflects the standardized beta values from linear regression analyses. p_{FDR} = false discovery rate-adjusted p values corrected at a 0.05 threshold. (CVR) = (cardiovascular) risk; Func = function; Glu Tol = glucose tolerance; HFpEF = heart failure with preserved ejection fraction; HFpEF = heart failure with reduced ejection fraction; Prog = prognosis; REr = resting energy rate; VO2Max = cardiorespiratory maximal oxygen consumption; Visc = visceral. [Color figure can be viewed at www.annalsofneurology.org]

higher cortical A β (see Fig 3B, Table S9). Only the association of poor HFpEF prognosis with cortical A β remained after adjusting for demographic factors (Table S10). There were no significant associations among PiB-negative participants (Table S9).

Next, we examined the association between each of these indices and cortical tau (FTP PET), as measured in the entorhinal cortex and ITG. These regions were chosen because they represent both early (entorhinal) and

middle (ITG) stages of cortical tau propagation in AD. Although there were several nominal associations of body composition and cardiometabolic factors with cortical tau, no associations survived multiple comparison correction (Table S11; unadjusted results in Table S12).

Proteomic Indicators of Health and Brain Volume

Among the 14 proteomic health indicators, only secondary cardiovascular risk and poorer prognosis in HFpEF

and HFrEF were associated with greater AD-like patterns of brain atrophy (SPARE-AD score) after adjusting for demographic characteristics (see Fig 3C; full results in Table S13).

For proteomic indicators of health that were associated with AD-like atrophy, we performed mediation analyses to determine whether AD pathology, neuronal injury, and astrogliosis (for which ADRD plasma biomarkers are a proxy) represent the mechanism through which peripheral health relates to AD-like brain atrophy. As displayed in Figure 4, neuronal injury (NFL) and astrogliosis (GFAP) partially mediated the association between secondary cardiovascular risk and brain atrophy (NFL accounted for 8% and GFAP accounted for 7% of the relationship), whereas the association between heart failure prognosis and brain atrophy was partially mediated by pTau-181 and astrogliosis (10–12%). See Table S14 for full mediation results.

Given that GFAP and pTau-181 were identified as mediators in several mediation models and represent distinct neuropathological processes, we conducted post hoc parallel mediation analyses. These findings suggest that GFAP and pTau-181 mediated the association between proteomic indicators of heart failure prognosis (approximately 16%), secondary cardiovascular risk (13%), and brain atrophy (see Fig 4). However, within parallel mediation models, pTau-181 and GFAP were no longer

significant independent mediators of the heart failure prognosis/secondary cardiovascular risk–brain atrophy relationships.

Proteomic Indicators of Health and Dementia Risk

Given that greater primary and secondary cardiovascular risk and poorer heart failure prognosis were consistently associated with markers of AD and neurodegeneration, we examined whether these same protein-based measures predicted 25-year incident dementia risk using data from the ARIC study ($n = 11,285$, mean age \pm SD = 60.2 ± 5.7 years; Table S5). There were 2,063 of 11,285 (18.3%) incident dementia cases over a median follow-up time of 21.1 years (interquartile range = 14.2–23.2). The cumulative incidence rate was 10.1 dementia cases per 1,000 person-years. After adjusting for age, sex, race-center, education, and *APOE* ϵ 4, higher primary and secondary cardiovascular risk, as well as poorer HFpEF and HFrEF prognosis were associated with increased dementia risk (Fig 5, Table S15). Specifically, for every 1 SD increase in primary and secondary cardiovascular risk, there was a 10% (hazard ratio [HR] = 1.10, 95% confidence interval [CI] = 1.06–1.14) and 19% (HR = 1.19, 95% CI = 1.13–1.24) increase in dementia risk, respectively. For every 1 SD in poorer HFpEF and HFrEF prognosis, there was an 11% (HR = 1.11, 95% CI = 1.06–1.15) and 17% (HR = 1.17, 95% CI = 1.12–1.23)

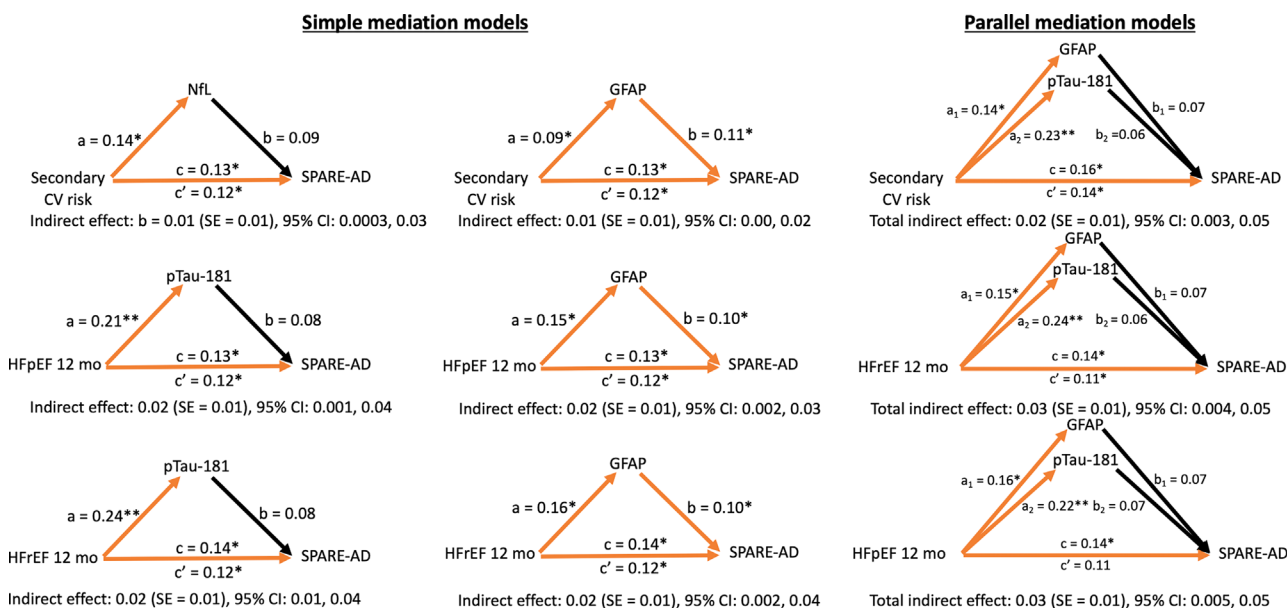


FIGURE 4: Mediation analyses. Orange paths represent significant associations, and black paths represent nonsignificant associations. a , a_1 , a_2 = paths a ; b , b_1 , b_2 = paths b ; c = total effect; c' = direct effect. $^*p < 0.05$, $^{**}p < 0.001$. Neurofilament light chain (NFL) and glial fibrillary acidic protein (GFAP) analyses: $n = 600$; tau phosphorylated at threonine-181 (pTau-181) analyses: $n = 520$. Confidence intervals (CIs) are based on 10,000 bootstrapped samples. Covariates for all analyses include age, sex, race, education, and *APOE* ϵ 4. For parallel mediation models, the total indirect effect reflects the mediation effects of both GFAP and pTau-181 simultaneously. CV = cardiovascular; HFpEF = heart failure with preserved ejection fraction; HFrEF = heart failure with reduced ejection fraction; SE = standard error; SPARE-AD = Spatial Pattern of Abnormality for Recognition of Early Alzheimer's Disease. [Color figure can be viewed at www.annalsofneurology.org]

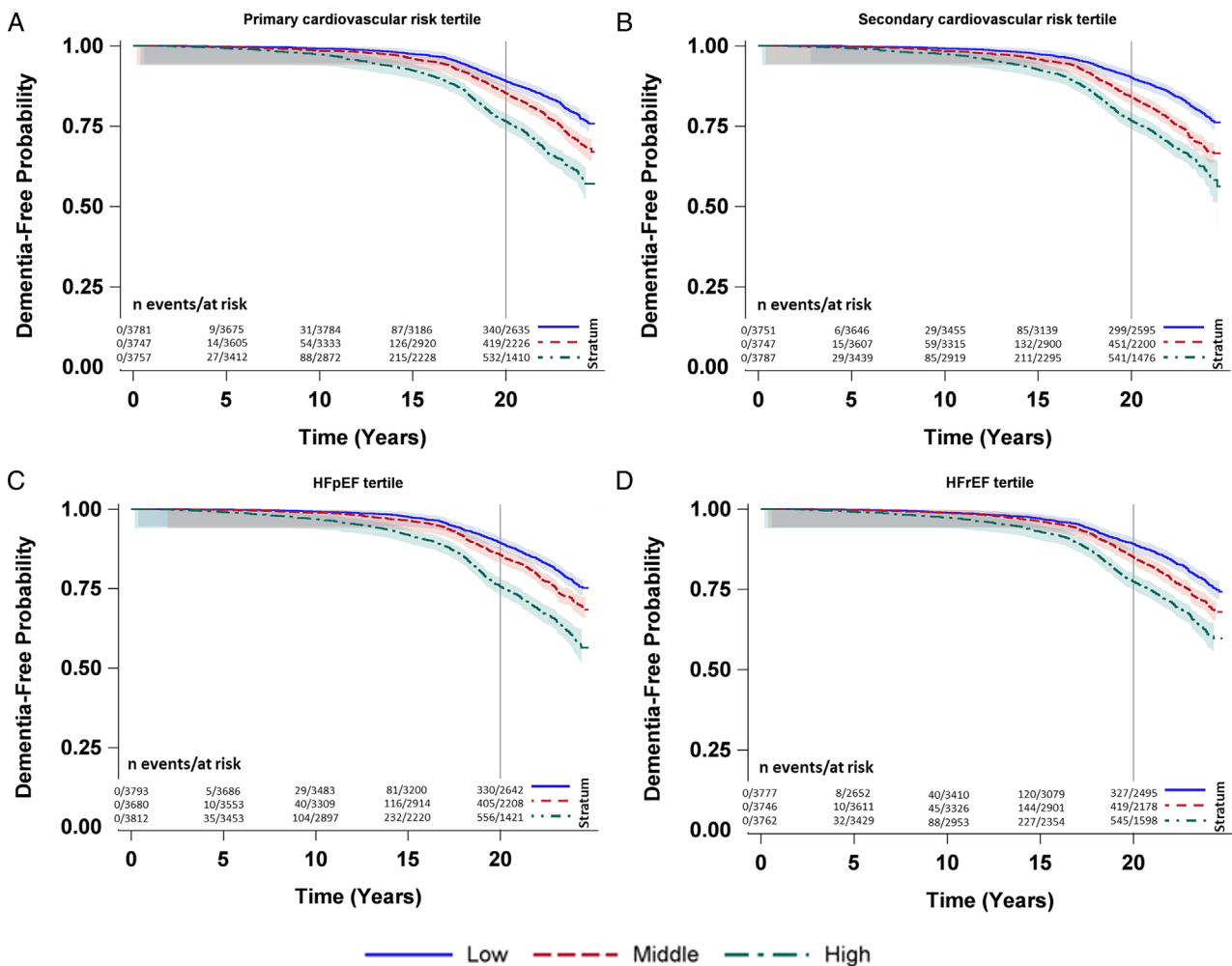


FIGURE 5: Kaplan–Meier curves for time to dementia onset stratified based on proteomic indicators of cardiovascular health. Kaplan–Meier curves are shown for time to dementia onset by (A) primary cardiovascular risk, (B) secondary cardiovascular risk, (C) heart failure with preserved ejection fraction (HFpEF), and (D) heart failure with reduced ejection fraction (HFrEF) tertiles in the Atherosclerosis Risk in Communities study. N = 11,285 for all analyses. [Color figure can be viewed at www.annalsofneurology.org]

increase in dementia risk, respectively. Importantly, these associations remained similar after excluding participants with coronary heart disease, stroke, and suspected heart failure (based on the use of heart failure medications) at the time of protein measurement (Table S16).

Discussion

The present study examined whether validated proteomic indicators of peripheral health were associated with plasma biomarkers of AD and neurodegenerative disease, PET measures of AD neuropathological changes, MRI-defined brain atrophy, and dementia risk. We found that participants with a proteomic signature indicating higher risk for future kidney disease, cardiovascular events, and poor prognosis in the context of heart failure tended to have concentrations of A β and greater concentrations of pTau-181, NfL, and GFAP in plasma, even among participants without clinically defined kidney or

cardiovascular disease. Among participants with neuropathologically defined preclinical AD (as defined by PET), greater primary cardiovascular risk and heart failure prognosis were associated with higher levels of cortical A β . Although several proteomic indicators of health, primarily cardiometabolic health, were associated with cortical tau accumulation in the ITG and entorhinal cortex, these associations did not survive multiple comparison correction. We further demonstrated that proteomic indicators of cardiovascular risk and poor heart failure prognosis were associated with greater AD-like patterns of brain atrophy, and in a separate cohort, increased dementia risk (when measured during midlife) over a 25-year follow-up period. Overall, results suggest that proteomic indicators of peripheral health, especially cardiovascular health, are associated with plasma and cortical biomarkers of AD and neurodegenerative disease, even in a group of largely healthy older adults.

As blood-based biomarkers for ADRD become more widely used, it is important to understand how health factors (eg, comorbid disease) may influence blood biomarker levels, either directly, or indirectly through their effects on underlying neuropathology.⁴⁶ To this end, we found peripheral health, at a proteomic level, accounted for a large amount of variation in plasma NfL (50%), but only a small amount of variation in $A\beta_{42/40}$ (12%). These findings suggest that plasma NfL levels—or the neuronal injury that results in greater NfL levels—can be to a large extent influenced by peripheral health factors, more so than $A\beta_{42/40}$ (Fig 6). By comparison, peripheral health indicators together accounted for approximately 30% of variation in plasma pTau-181 and GFAP levels. There was considerable variation in the relative importance of each health measure for each of the 4 ADRD biomarkers. This exploratory study identified several associations not well documented in the literature, including the strong associations between body composition measures and pTau-181, NfL, and GFAP,⁴⁶ and the inverse association of resting energy rate with NfL, and GFAP. Although these results provide insight into the peripheral health factors that may influence ADRD biomarkers in older adults, they will need to be replicated in targeted analyses.

In the present study, proteomic indicators of greater primary cardiovascular risk and poor HFpEF prognosis were associated with plasma $A\beta_{42/40}$ and, to a lesser degree, cortical $A\beta$. Although the general patterns of protein-based risk factor associations with plasma and cortical $A\beta$ were similar, the sample size for cortical $A\beta$ was smaller, with considerably less statistical power to detect effects. Although prior studies suggest that plasma $A\beta_{42/40}$ is predictive of cortical $A\beta$ PET positivity,⁴⁷ several studies—including one from our group⁴⁸—show that plasma $A\beta_{42/40}$ declines prior to and is predictive of elevations in cortical $A\beta$ (measured using PET).⁴⁹ Thus, given the cognitively normal sample, $A\beta_{42/40}$ may provide a more meaningful indicator of $A\beta$ burden, particularly among this sample, where 71% of participants do not have elevations in cortical amyloid. Although heart failure was essentially absent in our primary cohort, the present study suggests that the same proteins associated with poor heart failure prognosis may also be dysregulated in cognitively normal participants with elevated $A\beta$ pathology. In contrast to heart failure prognosis risk, kidney prognosis was associated with greater plasma $A\beta_{42/40}$,⁵⁰ but showed no evidence of a relationship to cortical $A\beta$.

Several proteomic indicators of cardiometabolic, cardiovascular, and kidney function/prognosis were associated

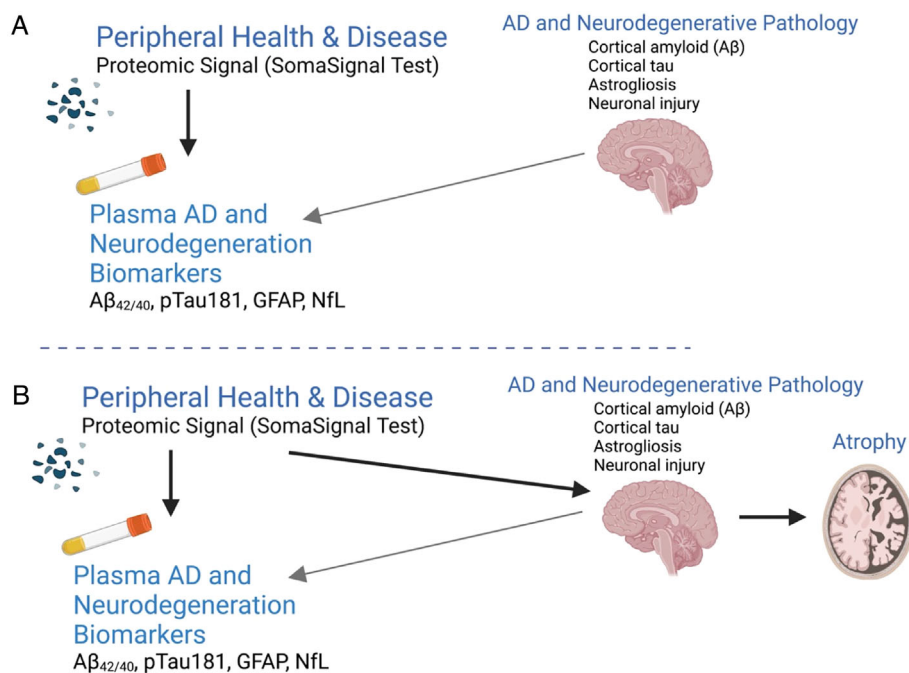


FIGURE 6: Associations between peripheral health and disease, plasma biomarkers, and brain health. (A) Alzheimer disease (AD)/neurodegenerative pathology can independently influence levels of plasma Alzheimer's Disease and Related Dementias (ADRD) biomarkers. Concurrently, peripheral health can directly influence plasma ADRD biomarkers in a central nervous system (CNS)-independent manner. (B) Peripheral health can directly affect AD/neurodegenerative pathology. AD/neurodegenerative pathology can, in turn, influence levels of plasma ADRD biomarkers. Concurrently, peripheral health can directly influence plasma ADRD biomarkers in a CNS-independent manner. $A\beta$ = amyloid- β ; GFAP = glial fibrillary acidic protein; NfL = neurofilament light chain; pTau181 = tau phosphorylated at threonine-181. Figure created with BioRender.com. [Color figure can be viewed at www.annalsofneurology.org]

with pTau-181, a measure of phosphorylated soluble tau.^{46,51} Although prior studies have demonstrated that pTau-181 and cortical tau are correlated, these two biomarkers may reflect different stages of tau progression,⁵² as the plasma pTau-181 onset is believed to occur prior to that of cortical tau in AD pathogenesis.⁵³ Our results suggest that cardiovascular and kidney health may be more strongly associated with soluble phosphotau (early stage) than cortical fibrillary (later stage) tau captured using PET imaging. However, the sample size for cortical tau was small, thus decreasing the chance of detecting significant effects. It is also possible that the lack of association between protein-based health estimates and cortical tau may be a function of the health of the current sample, in which severe cardiovascular and kidney disease were uncommon.

In addition to demonstrating an association between poorer cardiovascular prognosis and an AD-like pattern of brain atrophy, our mediation analyses suggest that reactive astrogliosis and soluble pTau partially mediate this relationship. These results are consistent with prior studies, which found that heart failure may lead to increased phosphorylation of tau,⁵⁴ a known risk factor for brain atrophy.⁵⁵ Notably, GFAP and pTau-181 only partially explained the association between cardiovascular risk, heart failure, and increased brain atrophy. Therefore, other neuropathological processes not measured in the present study may also mediate the link between cardiovascular health and neurodegeneration. For example, prior work suggests that heart failure has reciprocal associations with dementia through several mechanisms including, but not limited to, reduced cerebral blood flow, hypoxia, microvascular dysfunction, systemic inflammation, and oxidative stress.^{54,56} Although the presence of a significant indirect effect supports the hypothesized connection between cardiovascular risk factors, tau phosphorylation/astrogliosis, and brain atrophy, confounding assumptions cannot be ruled out,⁵⁷ and as such these results should be considered nondirectional associations rather than causal relationships.

In clinical and research settings, cardiovascular disease is typically defined based on symptoms, a clinical diagnosis, or a collection of diagnoses.^{6,8,58} These methods likely do not capture the full spectrum of disease severity, particularly at the lowest and highest ends of the spectrum. The continuous nature of the protein-based peripheral health indices used in the present study allows for the assessment of subthreshold to severe disease risk, increasing the ability to detect associations that may otherwise be obscured. These findings are strengthened by results from the analyses demonstrating robust associations in participants without clinically defined cardiovascular or kidney

disease. By demonstrating the link between plasma proteomic health indicators, ADRD biomarkers, and future dementia risk, the present study demonstrates that proteomic health indices of non-central nervous system (CNS) disease may complement efforts for early identification of dementia risk and even aid in the identification of the most relevant early stage interventions in a personalized medicine approach.

The present study has several strengths, including the use of multiple large community-based samples and comprehensive proteomic measures of peripheral health, as well as the incorporation of plasma, MRI, and PET biomarkers of ADRD. However, several limitations should be considered. First, proteomic indicators of health were constructed and validated using cohorts that consisted primarily of White participants; thus, it is unclear whether the proteomic health indicators derived in BLSA and ARIC are optimal for the sizable subset of non-White participants included in the current analyses. The use of varying sample sizes for the examination of plasma- versus PET-based outcome measures is another factor that limits the interpretation of the current results. We suspect, for example, that some null results found in the A β and tau PET analyses are due to the smaller sample sizes, resulting in limited statistical power. Furthermore, although each of the SSTs has been previously validated, a proportion of these indices could not be directly validated in the BLSA because gold standard clinical measures were not available. Finally, the present study was primarily cross-sectional and therefore does not provide information on the effect of changing peripheral health on longitudinal ADRD biomarkers or on ADRD biomarker progression. Despite these limitations, the present study suggests that peripheral health factors likely influence levels of ADRD biomarkers and the underlying neuropathological processes associated with neurodegeneration and greater dementia risk. These findings demonstrate the potential utility of a scalable protein-based examination of peripheral health to advance the understanding of CNS disease.

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Author Contributions

H.E.D., C.P., A.M., S.M.R., M.S., J.Co., P.P., L.F., A.S., S.A.W., and K.A.W. contributed to the conception and design of the study. H.E.D., C.P., G.N.D., Z.P., M.R.D., M.B., Y.A., A.M., C.D., S.M.R., K.L., M.S., J.Co., T.M., J.Co., L.F., S.A.W., and K.A.W. contributed to acquisition and analysis of data. H.E.D. and K.A.W. contributed to drafting a significant portion of the manuscript and figures.

Potential Conflicts of Interest

The SomaLogic coauthors (C.P., K.L., M.S., and S.A.W.) were/are all employees of SomaLogic, which has a commercial interest in the results. The remaining authors have no competing interests.

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