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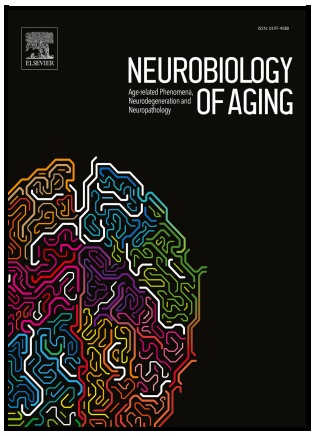

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Plasma neurofilament light and brain volumetric outcomes among middle-aged urban adults

Running head: Plasma neurofilament light and brain volumes

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ABBREVIATIONS

Core for Translational Research in Imaging @ Maryland (C-TRIM)

δ (annualized change)

False Discovery Rate (FDR)

Fluid-Attenuated Inversion Recovery (FLAIR)

Field of View (FOV)

Gray Matter (GM)

Healthy Aging in Neighborhoods of Diversity across the Life Span (HANDLS) study

HANDLS SCAN visit (v_{scan})

High School (HS)

Institutional Review Board (IRB)

Intracranial Volume (ICV)

Magnetization prepared rapid gradient echo (MP-RAGE)

Magnetic Resonance Imaging (MRI)

Medical Research Vehicle (MRV)

Multiplicative intrinsic component optimization (MICO)

Multi-atlas region Segmentation utilizing Ensembles (MUSE)

Neurofilament Light (NfL)

Regions of Interest (ROI)

Structural MRI (sMRI)

Total Brain Volume (TBV)

United States (US)

Visit 1 (v_1)

Visit 2 (v_2)

White Matter (WM)

White Matter Lesion (WML)

White Matter Lesion Volume (WMLV)

World Health Organization (WHO)

ABSTRACT

Elevated plasma neurofilament light chain (NfL) is associated with dementia, though underlying mechanisms remain unknown. We examined cross-sectional relationships of time-dependent plasma NfL with selected brain structural magnetic resonance imaging (sMRI) prognostic markers of dementia. The sample was drawn from the Healthy Aging in Neighborhoods of Diversity Across the Life Span (HANDLS) study, selecting participants with complete v_1 (2004-2009) and v_2 (2009-2013) plasma NfL exposure and ancillary sMRI data at v_{scan} (2011-2015, n=179, mean v_1 to v_{scan} time: 5.4y). Multivariable-adjusted linear regression models were conducted, overall, by sex, and by race, correcting for multiple testing with q-values. $NfL_{(v1)}$ was associated with larger WMLV (both Log_e transformed), after 5-6 years' follow-up, overall (β=+2.131±0.660, b=+0.29, p=0.001, q=0.0029) and among females. $NfL_{(v2)}$ was linked to a 125 mm^3 lower left hippocampal volume (p=0.004, q=0.015) in reduced models, mainly among males, as was observed for annualized longitudinal change in NfL (δNfL_{bayes}). Among African American adults, $NfL_{(v1)}$ was inversely related to total, gray and white matter volumes. Plasma NfL may reflect future brain pathologies in middle-aged adults.

Keywords:

Neurofilament Light Chain, hippocampus, brain volumes, white matter lesion, aging.

INTRODUCTION

Axonal damage in neurons can lead to the release of cytoskeletal proteins such as neurofilaments into the extracellular space and subsequently into the cerebrospinal fluid (CSF), and then into the blood at a lower concentration(Zhao et al., 2019). Therefore, exploring the use of neurofilaments, in particular neurofilament light (NfL), as an indicator of neuroaxonal damage has become a recent focus. Importantly, blood-based markers have many advantages over using cerebrospinal fluid (CSF) and neuroimaging measures to monitor neurodegeneration. Blood sample acquisition is noninvasive, more cost effective, and accessible to diverse clinical settings and more feasible for time-dependent assessment. Methods measuring blood-levels of NfL have recently become more sensitive (Kuhle et al., 2016), a development with a potential for large-scale applications in clinical practice and in randomized clinical trials to identify high-risk groups for all-cause and Alzheimer's Disease (AD) dementia(Raket et al., 2020). The focus of much prior research has been on the association of plasma NfL with late AD (de Wolf et al., 2020), despite plasma NfL's link to frontotemporal degeneration(Scherling et al., 2014), multiple sclerosis (Teunissen et al., 2005), traumatic brain injury(Shahim et al., 2016) and other neurodegenerative diseases, including vascular dementia(Khalil et al., 2018), which rendered NfL indicative of general neuroaxonal damage and a marker for non-specific neurodegeneration. Recent data also indicates that NfL may be a useful biomarker in the early preclinical stages of AD (Giacomucci et al., 2022).

Blood NfL is associated with neurodegenerative diseases as well as cerebrovascular events and diseases, being elevated in patients with ischemic stroke (Uphaus et al., 2019) and small vessel disease (Duering et al., 2018) and associated with stroke severity and white matter lesion volume (WMLV) among ischemic stroke patients, predicting adverse clinical outcomes(Egle et al., 2021; Uphaus et al., 2019). Consequently, blood NfL levels may also

reflect neuroaxonal damage caused by both acute and progressive cerebrovascular events. Generally, reduced cortical and hippocampal brain volumes, as well as increased WMLV were linked to poor cognitive performance associated with AD (Beydoun et al., 2021a; Hsu et al., 2018; Walter et al., 2019). Recently, CSF NfL levels was positively correlated with WMLV(Jonsson et al., 2010). With NfL (in blood or CSF) capturing sub-cortical large-caliber axonal degeneration (Norgren et al., 2003), plasma NfL concentrations strongly correlate with corresponding CSF NfL concentrations (Raket et al., 2020), adding to NfL's clinical utility for monitoring both neurocognitive and cerebrovascular diseases.

Plasma NfL's association with brain aging neuroimaging measures (He et al., 2020; Khalil et al., 2020; Merluzzi et al., 2019; Mielke et al., 2019; Nyberg et al., 2020; Peters et al., 2020; Rajan et al., 2020; Rubsamen et al., 2021) has been tested without evaluating racial and gender group differentials. Associations of plasma NfL measured at different time points with follow-up brain volumetric markers of subclinical brain pathology prognostic of future stroke and dementia among healthy middle-aged adults is largely unknown. Thus, our study (i) Examined baseline plasma NfL's association with follow-up brain volumetric outcomes, including global, cortical gray and white matter, hippocampal, and white matter lesion volumes; (ii) Examined NfL at follow-up in relation to these brain volumetric outcomes at follow-up; (iii) Examined annualized change in NfL and "tracking high" and "tracking low" NfL longitudinal exposures in relation to these brain volumetric outcomes at follow-up (iv) Tested sex and race as putative effect modifiers in these associations.

METHODS

Study Design

The Healthy Aging in Neighborhoods of Diversity across the Life Span (HANDLS) study includes a socio-demographically diverse sample of middle-aged White and African American urban adults selected using an area probability strategy ($Agev_1$: 30-64y, Baltimore, MD) (Evans et al., 2010). HANDLS, an ongoing prospective cohort study initiated by the National Institute on Aging in 2004(Evans et al., 2010), included interviews among identified participants through random sampling of addresses within each census tract, with eligibility criteria listed elsewhere (Evans et al., 2010).

The initial recruitment and examination were composed of two phases, with Phase 1 including a dietary interview and several demographic and psychosocial scales elicited from participants within their homes. Phase 2 examined participants on Medical Research Vehicles (MRV) parked in close proximity to their neighborhoods(Evans et al., 2010). MRV exams elicited a second dietary interview from participants and included other physical, psychosocial and medical assessments, personal and family health history, neuropsychological tests, physical performance by a brief screening battery, physical examination by a physician, and inventories to assess depressive symptoms (Evans et al., 2010). Eight hours or longer of fasting was required prior to MRV visits, where plasma specimens were collected, aliquoted and frozen at −80°C. Phases 1 and 2 data are labelled as visit 1 (v_1, 2004-2009). Comparable follow-up MRV visits were conducted, including at visit 2 (v_2, 2009-2013). For our present study, only visit 1 covariates, including biochemical and hematological indices, were selected for our analyses.

In our present study, we included participants with complete and valid sMRI data at the HANDLS SCAN visit and complete NfL data at v_1 and v_2 (**Figure 1**). HANDLS SCAN recruited participants from consecutive waves of first and second follow-up examinations (2011-2015).

Participants excluded from the HANDLS SCAN examination had self-reported histories of HIV, neurological and/or terminal diseases, stroke, transient ischemic attack, carotid endarterectomy or had specific MRI contraindications (e.g., indwelling ferromagnetics). The sample recruited into HANDLS SCAN represented the overall HANDLS study sample in educational attainment, poverty status, and sex ($p>0.05$), but had more White and younger participants ($p<0.05$).

Here we analyzed plasma NfL data from visits 1 (v_1: 2004-2009) and 2 (v_2:2009-2013) in relation to follow-up data measured in a sub-sample of N_{max}=238 participants within the HANDLS SCAN sub-study (v_{scan}: 2011-2015) (Waldstein et al., 2017). Thus, this was a cross-sectional analysis with outcomes measured at one time point and exposures measured as part of the MRV visits (v_1 or v_2); outcomes were MRI assessments obtained from v_{scan} reflecting brain volume and WMLV (Waldstein et al., 2017). Mean±SD follow-up time between v_1 and v_{scan} was 5.61y±1.90.

Of the initial 3,720 participants, two sub-samples were identified from v_1 (n=694) and v_2 (n=709) (**Figure 1**). These sub-samples were used for the LASSO covariate selection process (See **Supplemental Method** 4), further restricting to participants with complete data on NfL at both visits 1 and 2, and more importantly to the HANDLS SCAN sub-study participants with complete sMRI data (n=238), yielding a final sample of 179 participants with complete brain sMRI and NfL (v_1 and v_2) data. This final sample (N=179), when compared with the remaining excluded participants from the initial n=3,720, had higher proportions of White adults (59% vs. 40%, $P<0.05$) and individuals living above poverty (69% vs. 58%, $P<0.05$). Moreover, we conducted a sensitivity analysis whereby we excluded individuals with a history of head injuries and with a total Mini-Mental State Exam (Folstein et al., 1975) v_1 score <23, yielding n=147 with normal cognition and free from head injuries.

Written informed consent was provided by all participants. HANDLS and HANDLS-SCAN study protocols were approved by the National Institute on Environmental Health Sciences Institutional Review Board (IRB) of the National Institutes of Health. Furthermore, HANDLS- SCAN was approved by the IRBs of the University of Maryland School of Medicine and the University of Maryland, Baltimore County.

Brain sMRI: Volumetric outcomes

Cranial MRI assessments were conducted on a Siemens Tim-Trio 3.0 Tesla unit scanner. We used magnetization prepared rapid gradient echo (MP-RAGE) to perform volumetric measurements for anatomical regions and volumetric measures were estimated per region of interest (ROI). **Supplemental method 1** details methods used to estimate ROI-specific volumes and the quality assurance. A multimodal lesion segmentation technique was used based on supervised learning, which utilizes a model trained on manually segmented lesions and then applies them to segment ischemic lesions(Lao et al., 2008). The method relies on co-registering T1, T2, FLAIR and PD scans, histogram normalization to a template image, extraction of features, voxel wise label assignment and elimination of false-positives. We applied a novel multi-atlas label fusion methodology to segment the brain into anatomical ROIs(Doshi J., 2013). We computed volumetric measurements for normal and abnormal (with lesion) tissue within each ROI, and then grouped those into larger anatomical regions using a hierarchical representation. Specifically, WM lesions were identified by segmenting hyperintensities on FLAIR images. We used an automated multi-modal segmentation method that uses supervised learning. The method was trained on an external training set with FLAIR and T1-weighted MRI images and voxelwise ground-truth WM lesion labels. We segmented T1-weighted image of each subject into 145 regions of interest (ROIs) using a multi-atlas label fusion method(Doshi et

al., 2016). These ROIs were also used for calculating larger derived ROIs in multiple resolution levels, including total GM and total WM, using a pre-defined ROI hierarchy. ROI labels for each subject were used to calculate regional volumes of different tissue types, as well as to calculate regional volumes of abnormal tissues, specifically regional WM lesions in this present study.

The current study focused on hippocampal volumes [Left(L) and Right(R)] and overall WMLV (Log_e transformed) as primary outcomes, while also examining total brain volume (TBV), GM and WM volume, as well as ROI-specific WMLV (cubic root transformed), as secondary outcomes of interest. In addition, regional volumes within GM and WM, taking laterality into account, was also examined as a post hoc analysis [i.e., L/R, regional WM and GM for "frontal", "temporal", "parietal" and "occipital" regions]. However, this analysis was only presented if GM and/or WM showed a significant association with each of 2 main exposures, namely v_1 or v_2 NfL. All analyses with lesion volume and small ROIs (e.g., hippocampal volumes or ROI-specific WMLV) were adjusted for intracranial volume (ICV).

NfL at v_1 and v_2

After an overnight fast, MRV visit blood samples were collected (9:30 am through 11:30 am) into EDTA blood collection tubes, which were centrifuged at 600*g* for 15 min, with the buffy coat removed. These latter steps were repeated twice, while visually inspecting for hemolysis. Upon aliquoting, plasma samples were stored at -80°C. Plasma NfL concentrations were quantified using the Simoa® NF-light Advantage Kit by Quanterix (Billerica, MA, USA), after following kit instructions. Samples from distinct visits were run on one plate for each individual and plates were balanced for all individuals within each demographic stratum (race/sex/poverty). After four-fold dilution of plasma samples, concentrations were adjusted for dilution correction. Pooled plasma samples from two individuals were run in duplicate on all plates and average

intra-assay and inter-assay coefficient of variations were 4.5% and 7%, respectively. Information on limits of detection and quantification for the runs of this specific assay have been described previously (Beydoun et al., 2021b). Plasma NfL was assessed with ≤ 2 repeats/participant at v_1 and/or v_2. The NfL_{v1} and NfL_{v2} exposures are detailed in **supplemental Method 2**. NfL_{v1} is the baseline NfL measured at v_1 (2004-2009), while NfL_{v2} was measured at first follow-up visit v_2 (2009-2013). Both of these observed measurements are exposures of interest in the present study. A secondary exposure of interest is "tracking high" or "tracking low" of NfL over time, defined by a common median level of untransformed plasma NfL. These two exposures are binary with 1=tracking high/low between the two visits (i.e., >median at both visits for "tracking high" and ≤median value for "tracking low"); 0=otherwise. This analysis is only presented for selected outcomes whereby at least one of two previous exposures is found to be significantly associated with those outcomes of interest, thus enhancing interpretation of visit-specific findings. We also report the δNfL as the annualized rate of change between $NfLv_1$ and $NfLv_2$ measurements (Beydoun et al., 2021b) for descriptive purposes (see **supplemental method 3).**

Covariates

In all models, exposure-outcome associations were adjusted for v_1 age (years), sex (male=1, female=0: primary stratifying variable), self-identified race (African American=1, White=0), self-reported household income either <125%=1 or ≥125% =0 of the 2004 Health and Human Services poverty guidelines (termed poverty status) (Nilsson et al., 2019), and time (days) between v_1 MRV visit and v_{scan}. Models also included ICV for select volumetric outcomes, namely hippocampal volume (L/R), total and small ROI-specific lesion volumes, and small ROI-specific brain volumes. All models were further stratified by sex (main potential effect modifier), and by race (secondary potential effect modifier). Additional covariates were added to models

after screening for their associations with NfL exposures using machine learning techniques **(online supplemental method 4)**. Those are considered as explanatory pathways by which main exposures may be linked with outcomes of interest.

Statistical analyses

We used Stata version 16.0 (STATA, 2019) for all analyses. We computed means and proportions of sample characteristics and tested for sex differences using Student's *t* and chi-square tests as appropriate. Regression modeling (linear and multinomial logit) was used in order to assess sex differences in various measures, including exposures, outcomes and covariates, after adjustment for race, poverty status and age in one model, and additional adjustment for ICV in a second model. We further described the sample characteristics by tertiles of NfL_{v1}, and NfL_{v2} (**Supplementary Table 1**).

To test the main hypotheses, a series of multivariable regression models was then estimated with sequential covariate adjustment, using the complete sample with further sex stratification, wherein each of two exposures were added as potential predictors for each sMRI outcome measured at v_{scan}. For every model, we obtained estimates of standardized *b* which we interpreted as a 1 SD change in sMRI outcome per 1 SD change in the specified continuous exposure (i.e., NfL at v_1 and v_2). We decided a priori to classify standardized *b* estimates >0.20 as moderate-to-strong and estimates between 0.10 and 0.20 as weak-to-moderate. In another set of secondary models, we considered annualized rate of change in NfL (Log_e transformed) as a main exposure, while adjusting for NfL at v_1, Log_e transformed along with other potentially confounding covariates, using a similar modeling strategy.

We conducted our analysis in four stages. Our first analysis (*Analysis A*) included measures of total brain, total WM, and total GM volumes. Our second analysis (*Analysis A'*) was a post-hoc regional analysis for *Analysis A* that detailed cortical volumes within GM and WM (i.e., as L/R; GM/WM; frontal, temporal, parietal and occipital); this yielded 16 post-hoc outcomes. Results from *Analysis A'* were presented only if, for a given model, at least one *Analysis A* exposure-outcome association was statistically significant ($P_{uncorr}<0.05$) in a given sample. In *Analysis B*, we examined L/R hippocampal volumes as our primary outcomes. In *Analysis C*, total WM lesion volume (Log_e transformed) was our outcome of interest. If at least one model showed significant findings for exposure-outcome association at type I error of 0.05, another analysis was conducted examining small ROI-specific associations (*Analysis C'*). For each stage, we considered models with minimal covariate adjustment as our primary analyses to test the hypotheses of interest. The post hoc analysis (*Analysis A'* and *C'*) and subsequent models with sequential covariate adjustment were considered secondary analyses, as were race-specific analyses conducted for *Analyses A* and *C*.

Secondary outcomes were WMLV at each independent ROI (**Supplemental Table 1,** 61 WM-related ROIs with a non-zero WMLV). These selected regional WMLV were cubic-root transformed, and the resulting values were then transformed into standardized z-scores. Additionally, of the 61 WM-related ROIs, only 24 were selected having >5% with non-zero WMLV on the untransformed scale. A sensitivity analysis was conducted on the untransformed regional WMLVs for comparative purposes. We utilized volcano plots as a visualization tool for these select findings from ROI-specific models, focusing on **Model 1** minimally adjusted model, corrected for ICV (R Foundation for Statistical Computing, 2013). These plots display Log_{10} (p-values) for each set of models, with exposures being alternatively NfL at v_1 or NfL at v_2, against

standardized beta coefficients (*b*) on the X-axis, highlighting findings with larger *b*. The volcano plots visualize ROIs in terms of uncorrected p-value <0.05 showing different colors depending on whether effect size *b* >0.20. Visualization of ROI-specific *b* with standard brain images was carried out using FSLeyes software (Jenkinson et al., 2002; Jenkinson and Smith, 2001) applied to these sMRI results for ROI-specific lesion volumes (URL: https://fsl.fmrib.ox.ac.uk/fsl/-fslwiki/FSLeyes).

Type I error was set at 0.05 for uncorrected p-values. We corrected for multiple testing using the false discovery rate (FDR, q-value). Each stage of analysis conducted for the overall and stratified samples were treated as separate hypotheses (i.e., *Analyses A-C*: overall vs. stratified by sex). In doing so, we adjusted for multiplicity within analysis and across strata. We used this correction specifically for the model with minimal covariate adjustment (i.e., Model 1) for each of *Analyses A-C.* We reported FDR q-values when P_{uncorr}<0.05 for exposure-outcome associations. Statistical significance in Model 1 was determined when FDR q-value<0.05, while a q-value<0.10 but ≥0.05 suggested a trend. Our models with sequential covariate adjustment (Models 2-6) were presented as secondary analyses designed to test mediating pathways between exposures and outcomes of interest (**online supplemental method 4**). Another sensitivity analysis was conducted in the sub-sample that was free from head injuries and was considered as having normal cognitive performance at v1 based on the MMSE total score (n=163).

RESULTS

The main characteristics of the study sample are presented in **Table 1**, overall and stratified by sex. The total selected sample consisted of 80 males and 99 females (N=179), with mean±SD age of 48.3±9.4y, of whom 41.3% were African American adults and 68.7% living above poverty. Lengths of follow-up were comparable across sex. No sex differences were detected for poverty status, age and race, nor in the NfL exposure distributions or the annualized change in NfL between v_1 and v_2. In contrast, males (vs. females) had higher odds of pre-diabetes, significantly higher urinary specific gravity, serum uric acid and serum creatinine levels, and serum albumin. Larger brain volumes were found among males, with the exception of hippocampal volume as % of ICV which was higher among females. No sex differences were detected in WMLV. Upon adjustment for age, race and poverty status, none of the statistically significant sex differences were markedly attenuated. Upon further adjustment ICV, however, sex differences were reversed for WM volumes whereby accounting for ICV women had larger total WM volume, and results became largely null for total GM volume. **Table S2** findings are summarized in **Online supplemental Result 1**.

Tables 2-3 and **S3-S4** tested whether NfL measured at v_1 (Table 1) or v_2 (Table 3) was associated with neuroimaging markers of brain aging. The data was examined in both the overall sample and stratified by sex. Upon correction for multiple testing ($q<0.05$) in minimally adjusted models which were corrected for ICV in analyses B and C, $NfL_{(v1)}$ had a moderately strong association with larger WMLV at follow-up v_{scan} visit, after a period of 5-6 years of follow-up, overall ($\beta=+2.131\pm0.660$, $b=+0.29$, $p=0.001$, $q=0.0029$). This association was largely unaltered with addition of other covariates, including v_1 BMI (**Table 2**, Model 2), or other upstream potentially confounding variables including measures of kidney and liver disease (**Table S3**, Model 3-6). This association was also significantly stronger among females, particularly in

models adjusting for visit 1 BMI ($P_{sex \times NfL(v1)}$=0.007). In fact, in Model 2 among females, $NfL_{(v1)}$ exposure 1 unit increase was linked to 4-point increase in Log_e transformed WMLV, p=0.001. This association remained largely unaltered in models further adjusted for upstream covariates (**Table S3**, Models 3-6).

When examining plasma NfL exposure at visit 2, with an average of ~1.1 year follow-up to sMRI outcome, $NfL_{(v2)}$ was linked to smaller hippocampal volumes in general, with the left hippocampus showing a slightly stronger effect size compared with the right hippocampus, overall (b=-0.19 vs. b=-0.14, respectively, q<0.05). More specifically, 1 unit increase in Log_e transformed NfL at v_2 was linked to a left hippocampal volume that was lower by 125 mm^3 in the overall sample (p=0.004, q=0.015), an association that was not altered by addition of other baseline covariates including v_1 BMI (**Table 3**, Model 2 and Models 3-6 in **Table S4**). This association was also mainly detected in males, even though heterogeneity of this effect by sex was not detected (p>0.05 for interaction term sex×$NfL_{(v2)}$ in a separate unstratified model).

Our exploratory analyses stratified by race as shown in **Tables S5-S6**, indicated that those main associations of NfL with WMLV and hippocampal volumes were largely homogeneous across racial groups. In contrast, there was significant heterogeneity detected in the relationship between $NfL_{(v1)}$ and TBV, GM and WM volume; an inverse association was found among African American but not among White participants in most models, particularly those adjusted for diabetes and serum glucose (Model 3). Sensitivity analyses using the largest available samples for each analysis did not alter our main findings (data not shown). Most notably, a 1 unit increase in NfL exposure at v_1 (Log_e transformed) was associated with a 1.93-unit increase in the Log_e transformed WMLV outcome, overall (β=+1.93±0.60, p=0.001, b=0.27, n=197), an association that was significantly stronger in females (β=+2.37, p=1.00, b=0.020, n=107) and

remained significant with adjustment for BMI and other potential confounders. Moreover, a more proximal NfL exposure at v_2 1 unit increase was linked to an average of 115 mm^3 smaller left hippocampal volume outcome (β=-115.2±39.1, p=0.004, b=-0.17, n=213, for Model 1). These results were comparable for the Right hippocampus, were stronger in men and slightly altered with addition of other covariates to Model 1. Both Left and Right hippocampal volumes were smaller when NfL exposure was "tracking high" over time, by retaining a value greater than 8 pg/mL, overall and among males (**Table S7**), a relationship not detected among females. For instance, left hippocampal volume was ~138 mm^3 smaller in the chronically elevated NfL group compared to the rest of the sample (β=-138±51, p=0.007, Model 1), overall. Conversely, tracking low for NfL exposure (i.e. ≤8 pg/mL) trended towards an inverse relationship with WMLV outcome among females in most models, particularly models adjusted for BMI (Model 2: β=-2.20±1.02, p=0.035) (**Table S7**).

In another set of secondary models (**Table 4**), annualized change in NfL (δNfL_{bayes}) was associated with smaller hippocampal volumes in the total population, in both the reduced and the BMI-adjusted models, independently of NfLv1. In contrast, NfLv1 was the main exposure associated with greater WMLV at follow-up, independently of δNfL_{bayes}.

Key findings were largely unaltered in the sub-sample that was free from head injuries and was deemed as having normal cognition at v_1. Most notably, overall (n=163), Nfl at v1 exposure was positively associated with WMLV in both the reduced (β±SE: 2.57±0.76, p=0.001) and BMI-adjusted models (β±SE: 2.87±0.79, p<0.001), with significant interaction by sex and stronger effects among females. However, association between NfL at v2 and left hippocampal volumes were attenuated in this selected sample (n=163), with this exclusion with most results indicating a marginally significant association across models, reflecting a smaller hippocampal

volume with higher NfL at v2 (P<0.10). Findings stratified by race were also comparable between the two samples (N=163 vs. N=179) for both NfL at v1 and v2. All main and sensitivity analysis Output will be made available on github link: https://github.com/baydounm/HANDLS_NFL_SMRI_PAPER_SUPPDATA.

Finally, when regional small ROI-specific WMLV were left untransformed, results remained similar with respect to main regions affected by elevated NfL levels at baseline. The statistically significant ROI-specific WMLV results (cubic root transformed) at type I error of 0.05 are presented in **Table S8** and **Figure 2**. Most notably, the top two hits with effect sizes >0.25 were right posterior limb of the internal capsule WMLV (b=0.38, p<0.001) and right frontal lobe WMLV (b=0.26, p=0.002).

DISCUSSION

Summary of findings

This study is among a few to examine the relationships of plasma NfL concentrations at two consecutive visits (v_1 and v_2) with key structural brain MRI markers, including hippocampal, global and cortical regional brain volumes, and WMLV, in a racially and socio-economically diverse sample of urban adults. Among key findings, $NfL_{(v1)}$ was significantly associated with larger WMLV at a 5-6 year follow-up period. This association was stronger in females. For the short time ~1 year follow-up, elevated NfL levels were significantly associated with a smaller hippocampal volume. Specifically, and in the overall sample, a 1 unit increase in Log_e transformed NfL at v_2 was linked to a left hippocampal volume that was lower by 125 mm^3, a pattern observed for annualized longitudinal change in NfL (δNfL_{bayes}). Interestingly, this association was mainly detected in males. Most of these associations were not altered by

adjusting for biomedical and lifestyle factors. Among African American adults only, $NfL_{(v1)}$ was inversely related to total, gray and white matter volumes.

Previous human studies

Currently, methods for diagnosing and monitoring neuropathology rely on expensive imaging modalities that have limited availability. CSF biomarkers, which include NfL, have also been used but require invasive procedures. Accordingly, less invasive biomarkers of neuroaxonal damage and neurocognitive decline are needed to identify individuals at risk for AD and other neurodegenerative diseases. Data indicate that plasma NfL may be a suitable option for a biomarker. Recent technological advances indicate that NfL levels measured in blood, i.e., plasma NfL, are associated with AD diagnosis and various cognitive, imaging and biochemical disease measures(Jin et al., 2019; Mattsson et al., 2017; Zhao et al., 2019). CSF NfL inversely associates with Clinical Dementia Rating Scale scores, the Recognition Memory Test(Weston et al., 2019) and the cognitive sub-scale of an AD assessment battery(Mattsson et al., 2019), is elevated in early stages of dementia, and is a strong predictor for cognitive decline in Aβ positive individuals(Bos et al., 2019) and in the general non-demented older adult population(Merluzzi et al., 2019). Given that Aβ positivity alone was not sufficient for predicting symptoms of cognitive decline in AD, identifying additional markers of neurodegeneration downstream from Aβ accumulation is highly useful for screening individuals in pre-symptomatic trials(Weston et al., 2019).

Recent reports suggest differences in levels of plasma NfL even in early stages of AD. For example, plasma NfL levels are elevated based on underlying pathology (Aβ+vs Aβ-) in adults with subjective cognitive decline (SCI), MCI and AD (Giacomucci et al., 2022). Furthermore, in a cross-sectional study elevated plasma NfL levels were reported in SCI-Aβ+

patients compared to normal controls with Aβ- and correlated with brain amyloid and cognitive decline(Huang et al., 2022). Plasma NfL was predictive of longitudinal cognitive decline in SCI and MCI patients (Bangen et al., 2021) and in acute post stroke patients with subjective cognitive decline(Wang et al., 2021). Therefore, plasma NfL levels may be a useful marker not only of stage of cognitive decline, but also by the underlying pathology; thus, being suitable as a biomarker in early stages of AD.

Because of the strong association between plasma and CSF NfL levels, and the invasiveness of acquiring CSF, plasma NfL may be a better screening tool. This is supported by evidence that plasma NfL may accurately predict estimated year of onset for dementia in familial and autosomal dominant AD (Sanchez-Valle et al., 2018; Weston et al., 2017). Also, recent studies have shown that serum or plasma NfL are direct indicators of axonal degeneration based on neuroimaging markers, including gray and white matter pathology(Jakimovski et al., 2019; Mielke et al., 2019; Sun et al., 2020), acting as proxies for hypometabolism in AD-vulnerable brain regions, particularly in Aβ-positive individuals(Benedet et al., 2019). Generally, axonal demyelination triggers inefficient energy utilization, mitochondrial dysfunction and oxidative stress accumulation, alterations that increase axonal fragmentation resulting in neurodegeneration(Fischer et al., 2013). Such pathology can spread at independent tract locations and their associated gray matter structures(Bergsland et al., 2017). Since such axonal retraction does not often occur instantaneously, it is more likely that baseline plasma NfL rather than follow-up or change in NfL, is associated with change or follow-up outcomes of neurodegeneration or adverse cognitive performance(Jakimovski et al., 2019; Trapp et al., 1998). This is in line with our current findings.

As plasma NfL is a marker of neuroaxonal damage, recent data suggests that in addition to having potential clinical utility in neurodegenerative diseases, blood NfL may also be a useful indicator of neuronal injury due to cerebrovascular disease. Here, we found that elevated NfL_{v1} was associated with greater WMLV. WM lesions reflect small vessel disease, which is a highly prevalent brain pathology causing vascular cognitive impairment, stroke and mortality(Pantoni, 2010). Moreover, serum NfL was higher in patients with genetically defined and sporadic small vessel disease and associated with greater MRI mean diffusivity, WM hyperintensity volume, lacune volume, and microbleed count(Duering et al., 2018). Patients with vascular dementia had higher levels of serum NfL compared to controls(Yuan et al., 2020) and after ischemic stroke, serum NfL levels were associated with secondary degeneration of white matter tracts(Tiedt et al., 2018) and WMLV (Uphaus et al., 2019). Therefore, plasma NfL may be linked to future subclinical brain pathologies that increase risk for disability and mortality. This is important given that imaging modalities are costly, require specialized machinery and personnel, and detailed post-imaging analysis. Our cohort notably consists of middle-aged African American and White adults without a history of cerebrovascular or neurodegenerative diseases, suggesting that plasma NfL shows promise as a biomarker for future brain pathologies in the absence of previous diagnoses. In previous secondary analyses of a comparable sample of HANDLS SCAN participants, we show that adverse volumetric outcomes considered in this study are associated with decline in cognitive performance over time in several domains of cognition, including v1 and v2 cognitive measurements which preceded the HANDLS SCAN visit (see supplemental methods 4 in (Beydoun et al., 2021a). More specifically, that study indicated a slower decline over time for certain cognitive domains was associated with larger MRI scan volumes in the region of the hippocampus, along with smaller WMLV and larger cortical brain volumes

(Beydoun et al., 2021a). In particular, larger hippocampal volumes were associated with slower decline based on the test of visual memory and attention, whereas faster decline based on an executive function test was related to larger WMLV, especially among African American adults (Beydoun et al., 2021a). WM volume at follow-up, particularly among men, was related to slower decline on the Digits Span-Forward test, reflecting attention domain of cognitive function(Beydoun et al., 2021a). Conversely, faster decline in executive function was linked to smaller temporal GM cortical volumes (Beydoun et al., 2021a).

In our short-term follow-up analyses of NfL_{v2} with MRI-based assessed subclinical brain pathology, we found that NfL was strongly associated with smaller hippocampal volumes. As hippocampal atrophy is associated with cognitive decline and dementia, particularly AD(Hsu et al., 2018; Walter et al., 2019), these findings lay important groundwork for identifying factors that may be linked to subclinical brain volume changes. In agreement with this idea, in analyses of an aging cohort from the Austrian Stroke Prevention Family Study, serum NfL was associated with smaller brain volume and future brain atrophy (Khalil et al., 2020). In a cohort of Hispanic and non-Hispanic adults with cognitive impairment, the etiology of which is due to a many diverse neurodegenerative diseases, serum NfL was associated with hippocampal atrophy(Barker et al., 2021). In longitudinal analyses, higher baseline plasma NfL was associated with a longitudinal decline in hippocampal volume(Mielke et al., 2019; Rajan et al., 2020). All together, these studies suggest associations between blood NfL levels and changes in brain volume which could, in turn, influence neuropsychological functioning. It is possible that the main reason we did not detect an association between medium term NfL and hippocampal volume is that there is a threshold effect beyond which NfL can affect this volume and earlier NfL tended to be lower than later NfL in this cohort.

Strengths and limitations

This study has several strengths, including its novel examination of relationships between markers of neuroaxonal damage and brain structural sMRI measures, which reflect global and regional volumes and WMLV, potentially underlying various neuropathologies. Although cross-sectional in design, this study provided 5-6 years of latency between NfL exposure and outcome (brain MRI measures), while considering shorter term NfL measured 1-2 years prior to follow-up as an additional exposure of interest, in addition to "tracking high", "tracking low" and annualized change in NfL as secondary longitudinal exposures. Furthermore, given the importance of sex in both NfL and cognitive impairment, we examined these hypotheses separately among males and females and adjusted basic models for multiple testing and potential confounders for socio-demographic, lifestyle, and health-related factors, including hematological and other nutritional biomarkers. These analyses also considered heterogeneity of associations by race, which is important given the disparities in risk of vascular dementia, cognitive decline, and Alzheimer's disease between African American and White adults(Matthews et al., 2019). Importantly, our cohort is relatively young free from neurological and cerebrovascular disease, indicating that plasma NfL can be equally useful for monitoring brain health in middle-aged adults. Our study findings did indicate that there was some heterogeneity across sex and race, in the association between plasma NfL and adverse brain volumetric outcomes, though this heterogeneity was outcome-specific.

Nevertheless, our study has several limitations. First, latency between exposure and outcome may render findings speculative as opposed to a cohort study with repeated outcomes, allowing testing of baseline exposure against annualized change in outcome. This latency period between exposure and outcome differed among participants, though it had a central tendency of 5-6 years.

Thus, we controlled for the follow-up time in our models. Moreover, the lack of baseline sMRI measure should be addressed in future studies of similar populations, and future longitudinal studies should examine change in volumetric outcomes in relation to short term and medium term NfL or in relation to tracking high vs. tracking low NfL as well as estimated annualized change in NfL. Second, although we did not directly assess cognitive decline along with volumetric outcomes, a previous study of ours conducted this secondary analysis confirming that the selected volumetric outcomes are reflective of cognitive decline over time and its results were summarized earlier (Beydoun et al., 2021a). Third, residual confounding is a potential problem given the observational nature of this study. Fourth, our study did not have a large enough sample on APOE genotype or other plasma neurodegeneration markers to conduct analyses with those markes included as either potential confounders or effect modifiers. Finally, our findings are in middle-aged urban White and African American adults, which may limit the generalizability to older elderly populations.

Conclusions

In summary, baseline plasma NfL was consistently associated with larger WMLV, particularly among females. On the other hand, follow-up and annualized longitudinal change in plasma NfL were both linked to smaller bilateral hippocampal volumes, overall, with a stronger effect found in the left hippocampal volume and among males. Among African American adults, $NfL_{(v1)}$ was inversely related to total, gray and white matter volumes. These associations were robust to further adjustment with key potential confounders. Thus, plasma NfL may be an independent marker for medium-term increase in lesion volumes, while its increase over time and its elevation in the shorter term may potentially be a marker for smaller hippocampal volumes normalized to the ICV independently of other confounding factors. It may also reflect future

smaller cortical volumes among African American adults. Therefore, plasma NfL may be a promising biomarker to reflect future subclinical brain pathologies in middle-aged adults.

DECLARATION

1- Ethical Approval and Consent to participate

All participants provided written informed consent at each wave of the Healthy Aging in Neighborhoods of Diversity across the Life Span (HANDLS) study. The study protocol was reviewed and approved the National Institutes of Health Institutional Review Board, protocol
number 09-AG- N248.

2- Consent for publication

Not applicable

3 - Availability of data and materials

The study protocol (09-AG-N248) received approval from the National Institute on Environmental Health Sciences' Institutional Review Board (IRB) of the National Institutes of Health (NIH). Upon request, data can be made available to researchers with approved proposals, after they have agreed to confidentiality as required by our IRB. Policies are publicized on: https://handls.nih.gov. Data access request can be sent to principal investigators (PI) or the study manager, Jennifer Norbeck at norbeckje@mail.nih.gov. These data are owned by the National Institute on Aging at the NIH. The PIs have made those data restricted to the public for two main reasons: "(1) The study collects medical, psychological, cognitive, and psychosocial information on racial and poverty differences that could be misconstrued or willfully manipulated to promote racial discrimination; and (2) Although the sample is fairly large, there are sufficient identifiers that the PIs cannot guarantee absolute confidentiality for every participant as we have stated in acquiring our confidentiality certificate."(Beydoun et al., 2019)

4- Competing interests and disclaimers

All authors declare no conflict of interest. The views expressed in this article are those of the authors and do not necessarily reflect the official policy or position of Fort Belvoir Community Hospital, the Defense Health Agency, Department of Defense, or U.S. Government. Reference to any commercial products within this publication does not create or imply any endorsement by Fort Belvoir Community Hospital, the Defense Health Agency, Department of Defense, or U.S. Government.

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6- Authors' contributions

All authors reviewed the manuscript. MAB: Conceptualization, plan of analysis, statistical analysis, literature review, write-up of parts of the manuscript, revision of the manuscript. NNH: Conceptualization, data acquisition, plan of analysis, literature search and review, write-up of parts of the manuscript, revision of the manuscript. HAB: Plan of analysis, assistance with statistical analysis, literature search and review, write-up of parts of the manuscript, revision of the manuscript. JW: Plan of analysis, assistance with statistical analysis, literature search and review, assistance with statistical analysis, write-up of parts of the manuscript, revision of the manuscript. AIM: Plan of analysis, literature search and review, write-up of parts of the manuscript, revision of the manuscript. LIK: Data acquisition, literature search and review, write-up of parts of the manuscript, revision of the manuscript. CD: Data acquisition, plan of analysis, write-up of parts of the manuscript, revision of the manuscript. RPG: Data acquisition, plan of analysis, write-up of parts of the manuscript, revision of the manuscript. SLS: Data acquisition, plan of analysis, write-up of parts of the manuscript, revision of the manuscript. GE: Data acquisition, plan of analysis, assistance with data management and statistical analysis, prepared parts of Figure 2, write-up of parts of the manuscript, revision of the manuscript. MKE: Data acquisition, plan of analysis, write-up of parts of the manuscript, revision of the manuscript. ABZ: Data acquisition, data management, plan of analysis, write-up of parts of the manuscript, revision of the manuscript. SRW: Conceptualization, data acquisition, data management, plan of analysis, write-up of parts of the manuscript, revision of the manuscript.

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Table 1. Study sample characteristics of eligible study sample by sex; HANDLS (v_1: 2004-2009; v_2: 2009-2013) and HANDLS-SCAN 2011-2015[a]

	Total *(N=179)*	*Females* *(N=99)*	*Males* *(N=80)*	P_{sex}
Socio-demographic, lifestyle and health-related factors at v_1				
	%, Mean±SD	%, Mean±SE	%, Mean±SE	
Sex, % males	44.7	—	—	
Age_{v1}	48.3±9.4	47.4±0.95	48.2±0.98	0.59
Race, % African American	41.3	40.4	42.5	0.77[b]
% above poverty	68.7	63.6	75.0	0.10
Time between v_1 and v_{scan} (years)	5.42±1.73	5.46±0.18	5.36±0.19	0.70

Time between v_2 and v_{scan}(years)	1.13±1.21	1.17±0.12	1.07±0.14	0.59
Imputed covariates, % or Mean±SE				
Body mass index, $kg.m^{-2}$	29.3±0.5	30.2±0.7	28.3±0.6	0.047
Diabetes				
No	71.6	78.8	62.8	__
Pre-diabetes	17.7	11.1	25.8	0.011
Diabetes	10.7	10.1	11.4	0.47
Plasma glucose, mg/dL	99.9±2.1	96.5±2.2	104.1±3.8	0.073
Creatinine, mg/dL	0.89±0.02	0.79±0.02	1.01±0.03	<0.001
Urine Specific Gravity	1.0193±0.0004	1.0183±0.0006	1.02047±0.0007	0.025
Blood urea nitrogen, mg/dL	13.75±0.31	13.33±0.40	14.27±0.50	0.14
Alkaline Phosphatase, U/L	75.2±1.6	77.9±2.2	71.9±2.2	0.059
Uric acid, mg/dL	5.50±0.11	4.98±0.14	6.13±0.15	<0.001
Albumin, g/dL	4.34±0.02	4.28±0.03	4.42±0.03	0.001
Eosinophils, %	2.75±0.15	2.54±0.21	3.00±0.20	0.12
25-hydroxyvitamin D, ng/mL	22.3±0.8	21.9±1.25	23.0±1.2	0.58
Current drug use, % yes	20.1	21.8	18.0	0.55
Self-rated health, %				
Poor/fair	21.8	25.3	17.5	__
Good	36.9	37.4	36.3	0.42
Very good/Excellent	41.3	37.4	46.3	0.15
	%, Mean±SD	%, Mean±SE	%, Mean±SE	
NfL, Log_e transformed (v_1)				
Mean±SD	2.01±0.53	1.97±0.05	2.06±0.06	0.28
Median	1.97	1.97	1.98	
IQR	1.68;2.26	1.62;2.27	1.74;2.25	
NfL, Log_e transformed (v_2)				
Mean±SD	+2.22±0.58	+2.15±0.054	+2.30±0.07	0.091

Median	+2.18	+2.12	+2.19	
IQR	+1.84;2.56	1.78;2.55	+1.94;+2.56	
δNfL, observed, annualized (Empirical bayes)				
Mean±SD	+0.038±0.074	+0.033±0.057	+0.044±0.92	0.32
"Tracking high" v_1 through v_2: NfL>8 pg/mL	36.3	35.4	37.5	0.77
"Tracking low", v_1 through v_2: NfL≤8 pg/mL	36.3	37.4	35.0	0.74
sMRI measures, mm^3	*(N=179)*	*(N=99)*	*(N=80)*	
Global brain volumes	***mean±SD***	***mean±SE***	***mean±SE***	
Total brain volume	1,142,888±118,106	1,082,462±8,173	1,217,666±12,741	<0.001[d]
Gray Matter	642,412±65,224	611,599±4,794	680,543±7,160	<0.001
White Matter	457,267±52,875	432,087±3,779	488,427±5,882	<0.001[d]
Regional cortical brain volumes	***mean±SD***	***mean±SE***	***mean±SE***	
Left Brain				
Frontal GM	93,214±10,104	89,163±802	98,228±1,145	<0.001
Frontal WM	85,326±10,333	80,698±760	91,053±1,171	<0.001[d]
Temporal GM	50,289±6,140	47,233±427	54,072±674	<0.001[c]
Temporal WM	49,913±6,054	46,476±431	52,942±689	<0.001
Parietal GM	46,149±5,674	44,208±466	48,552±664	<0.001[d]
Parietal WM	43,897±5,610	41,365±419	47,030±626	<0.001[d]
Occipital GM	38,075±5,221	36.164±437	40,440±588	<0.001
Occipital WM	21,028±2,957	19,757±232	22,600±327	<0.001
Right Brain				
Frontal GM	93,300±10,316	88,900±790	98,744±1,166	<0.001
Frontal WM	87,552±10,687	82,820±783	93,407±1,221	<0.001[d]
Temporal GM	50,289±6,140	48,266±438	54,878±644	<0.001

Temporal WM	49,913±6,054	47,041±429	53,465±679	<0.001
Parietal GM	46,149±5,674	44,654±470	49,395±660	<0.001
Parietal WM	41,683±5,452	39,211±407	44,743±607	<0.001
Occipital GM	39,335±5,402	36,957±409	42,277±605	<0.001
Occipital WM	20,816±2,931	19,512±236	22,429±311	<0.001
Hippocampal volume	***mean±SD***	***mean±SE***	***mean±SE***	
Hippocampus, Left	3,537±386	3,414±29	3,688±48	<0.001
Hippocampus, Right	3,827±413	3,706±34	3,978±50	<0.001
White matter lesion volume, Log_e transformed	***mean±SD***	***mean±SE***	***mean±SE***	
	5.65±3.84	5.30±0.44	6.08±0.34	0.18
Hippocampal volumes, % of intracranial volume				
Hippocampus, Left	0.265±0.024	0.272±0.002	0.257±0.003	<0.001
Hippocampus, Right	0.286±0.025	0.295±0.002	0.277±0.003	<0.001
Intracranial volume, mm^3	***mean±SD***	***mean±SE***	***mean±SE***	
	1,339,313±142,093	1,259,338±9661	1,438,281±14184	<0.001

Abbreviations: Age_{v1}=age measured at HANDLS visit 1 (2004-2009); IQR=Interquartile Range; GM=Gray Matter; HANDLS=Healthy Aging in Neighborhoods of Diversity Across the Life Span; HANDLS-SCAN=Brain magnetic resonance imaging scan ancillary study of HANDLS; IQR=Interquartile range (25th-75th percentile); NfL=Neurofilament Light; sMRI=Structural Magnetic Resonance Imaging; v_1=visit 1 of HANDLS (2004-2009); v2=visit 2 of HANDLS (2009-2013); v_{scan}=HANDLS-SCAN visit (2011-2015); WM=White Matter; WRAT-3=Wide Range Achievement Test, 3rd version.

[a] Values are Mean±SD for totals and Mean±SE for stratum-specific, or % (except for imputed data where it was Mean±SE for totals). Volumes are expressed in mm^3. P_{sex} was obtained from nd *t*-tests for the unimputed covariates and from multinomial logit and linear regression models for the imputed data. Additional models with sex, race, age and poverty status were conducted to test whether the sex differences were independent other socio-demographic factors. All statistically significant sex differences at type I error of 0.05 retained their statistical significance after further adjustment for age, race and poverty status.

[b] P<0.05 for null hypothesis that mean or proportion are equal between males and females after further adjustment for ICV.

[c] Direction of difference between males and females reverse upon further adjustment for ICV.

Table 2. Minimally and BMI-adjusted associations from analyses A (global, GM and WM volumes), A' (regional cortical GM/WM), B (hippocampal volume) and C (White matter lesion volume) vs. visit 1 NfL, Log_e transformed or NfL_{v1} (overall and stratified by sex): ordinary least square analyses; HANDLS 2004-2009 and HANDLS-SCAN 2011-2015[a]

	***Model 1**: Minimally adjusted*					***Model 2**: BMI-adjusted, sensitivity analysis (SA)*[b]			
Total sample (N=179)	*β1*	*(SE1)*	*b1*	*P1*	*q-value 1*	*β2*	*(SE2)*	*P2*	*Interaction by sex*
sMRI, Analysis A									
Total brain	-1,042	(15,863)	-0.005	0.95	__	+1,841	(16,514)	0.91	0.59
GM	-5,763	(8,503)	-0.046	0.50	__	-3,707	(8,844)	0.68	0.23
WM	+2,207	(7,725)	+0.022	0.76	__	+2,987	(8,048)	0.71	0.76
sMRI, Analysis B									
Hippocampus, Left	-49.3	(52.1)	-0.067	0.35	__	-39.9	(54.2)	0.46	0.24
Hippocampus, Right	-39.4	(53.8)	-0.050	0.47	__	-28.4	(56.0)	0.61	0.31
Analysis C									
White matter lesion volume, ***Log$_e$ transformed***	**+2.131**	**(0.660)**	**+0.29**	**0.001**	**0.0029**	**+2.376**	**(0.685)**	**0.001**	**0.007**
Males (N=80)									
sMRI, Analysis A									
Total brain	-7,470	(24,369)	-0.036	0.76	__	-6,446	(24,763)	0.80	__
GM	-11,061	(12,684)	-0.096	0.39	__	-10,057	(12,870)	0.44	__
WM	-1,46	(11,879)	-0.01	0.90	__	-1,653	(12,078)	0.89	__

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sMRI, Analysis B									
Hippocampus, Left	-92.6	(76.1)	-0.12	0.23	__	-85.3	(77.1)	0.27	__
Hippocampus, Right	-63.4	(78.3)	-0.08	0.42	__	-48.6	(78.7)	0.54	__
Analysis C									
White matter lesion volume, ***Log$_e$ transformed***	**+1.48**	**(0.71)**	**+0.27**	**0.040**	**0.080**	**+1.44**	**(0.72)**	**0.049**	__
Females (N=99)									
sMRI, Analysis A									
Total brain	+7,503	(20,835)	+0.046	0.72	__	+17,727	(22,535)	0.43	__
GM	+938	(11,624)	+0.010	0.94	__	+7,992	(12,522)	0.53	__
WM	+8,132	(10,113)	+0.109	0.42	__	+12,452	(10,958)	0.26	__
sMRI, Analysis B									
Hippocampus, Left	+56.8	(70.0)	+0.097	0.42	__	+82.6	(76.2)	0.28	__
Hippocampus, Right	+28.7	(75.5)	+0.043	0.70	__	+36.8	(82.5)	0.66	__
sMRI, Analysis C									
White matter lesion volume, ***Log$_e$ transformed***	**+2.896**	**(1.146)**	**+0.332**	**0.013**	**0.053**	**+4.005**	**(1.217)**	**0.001**	__

Abbreviations: Age$_{v1}$=age measured at HANDLS visit 1 (2004-2009); FDR=False Discovery Rate; GM=Gray Matter; HANDLS=Healthy Aging in Neighborhoods of Diversity Across the Life Span; HANDLS-SCAN=Brain magnetic resonance imaging scan ancillary study of HANDLS; NfL=Neurofilament Light; SE=Standard Error; sMRI=Structural Magnetic Resonance Imaging; v_1=visit 1 of HANDLS (2004-2009); v2=visit 2 of HANDLS (2009-2013); v_{scan}=HANDLS-SCAN visit (2011-2015); WM=White Matter.

[a] Values are adjusted linear regression coefficients β with associated SE, standardized beta, uncorrected p-values, corrected q-values (false discovery rate) and results of sensitivity analysis. (N) is the sample size in each analysis. Standardized betas for NfL are computed as SD in outcome per SD in visit 1 NfL, Log$_e$ transformed. Q-values presented only for uncorrected P-values<0.05 for model 1. Model 1 was adjusted for Age$_{v1}$, sex, race, poverty status, intracranial volume (Analyses B and C) and time of follow-up between visit 1 and v_{scan}. Volumes are expressed in mm^3.

[b] Model 2 is a sensitivity analysis further adjusting Model 1 for BMI after screening using machine learning techniques (See Supplemental methods 2).

Table 3 Minimally-adjusted and BMI-adjusted associations from analyses A (global GM and WM volumes), A' (regional cortical GM/WM), B (hippocampal volume) and C (White matter lesion volume) vs. visit 2 NfL, Log_e transformed or NfL_{v2} (overall and stratified by sex): ordinary least square analyses; HANDLS 2009-2013 and HANDLS-SCAN 2011-2015[a]

	***Model 1**: Minimally adjusted*					***Model 2**: BMI-adjusted, sensitivity analysis (SA)*[b]			
Total sample (N=179)	*β1*	*(SE1)*	*b1*	*P1*	*q-value1*	*β2*	*(SE2)*	*P2*	*Interaction by sex*
sMRI, Analysis A									
Total brain	-3,967	(13, 209)	-0.02	0.76	__	-3,050	(13, 320)	0.82	0.38
GM	-5,622	(7,078)	-0.05	0.43	__	-4,874	(7,128)	0.50	0.30
WM	-1,217	(6,435)	-0.01	0.85	__	-1,036	(6,495)	0.87	0.37
sMRI, Analysis B									
Hippocampus, Left	**-125.0**	**(42.4)**	**-0.19**	**0.004**	**0.015**	**-122.2**	**(42.8)**	**0.005**	0.14
Hippocampus, Right	**-100.4**	**(44.2)**	**-0.14**	**0.024**	**0.049**	**-97.1**	**(44.6)**	**0.031**	0.14
Analysis C									
White matter lesion volume, ***Log_e transformed***	+0.466	(0.565)	+0.07	0.41	__	+0.496	(0.570)	0.39	0.015
Males (N=80)									
sMRI, Analysis A									
Total brain	-15,556	(20, 664)	-0.08	0.45	__	-16,032	(20, 821)	0.44	__
GM	-10,368	(10, 778)	-0.10	0.34	__	-10,844	(10, 831)	0.32	__
WM	-7,68	(10, 067)	-0.0	0.45	__	-7,65	(10, 154)	0.45	__

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sMRI, Analysis B									
Hippocampus, Left	**-145.4**	**(63.1)**	**-0.21**	**0.024**	0.19	**-149.4**	**(63.2)**	**0.021**	__
Hippocampus, Right	-120.9	(65.5)	-0.17	0.069	__	-127.4	(64.9)	0.054	__
Analysis C									
White matter lesion volume, ***Log$_e$ transformed***	-0.276	(0.619)	-0.06	0.66	__	-0.250	(0.622)	0.69	__
Females (N=99)									
sMRI, Analysis A									
Total brain	+7,559	(18,576)	0.050	0.69	__	+11,430	(18,933)	0.55	__
GM	-2,310	(10,364)	-0.026	0.82	__	+358	(10,530)	0.97	__
WM	+7,876	(9,012)	+0.11	0.38	__	+9,352	(9,207)	0.31	__
sMRI, Analysis B									
Hippocampus, Left	-24.1	(62.9)	-0.04	0.70	__	-18.8	(64.5)	0.77	__
Hippocampus, Right	-15.5	(67.6)	-0.02	0.82	__	-15.0	(69.4)	0.83	__
sMRI, Analysis C									
White matter lesion volume, ***Log$_e$ transformed***	+1.29	(1.05)	0.16	0.22	__	+1.59	(1.07)	0.14	__

Abbreviations: Age$_{v1}$=age measured at HANDLS visit 1 (2004-2009); FDR=False Discovery Rate; GM=Gray Matter; HANDLS=Healthy Aging in Neighborhoods of Diversity Across the Life Span; HANDLS-SCAN=Brain magnetic resonance imaging scan ancillary study of HANDLS; NfL=Neurofilament Light; SE=Standard Error; sMRI=Structural Magnetic Resonance Imaging; v_1=visit 1 of HANDLS (2004-2009); v2=visit 2 of HANDLS (2009-2013); v_{scan}=HANDLS-SCAN visit (2011-2015); WM=White Matter.

[a] Values are adjusted linear regression coefficients β with associated SE, standardized beta, uncorrected p-values, corrected q-values (false discovery rate) and results of sensitivity analysis. (N) is the sample size in each analysis. Standardized betas are computed as SD in outcome per SD in Nfl at v2. Q-values presented only for uncorrected P-

values<0.05 for model 1. Model 1 was adjusted for Age_{v1}, sex, race, poverty status, intracranial volume (Analyses B and C) and time of follow-up between visit 1 and v_{scan}. Volumes are expressed in mm^3.

[b] Model 2 is a sensitivity analysis further adjusting Model 1 for BMI at visit 1 after screening using machine learning techniques (See Supplemental methods 2).

[c] P<0.10 for null hypothesis that exposure×sex 2-way interaction term is =0 in the unstratified model with exposure and sex included as main effects.

Table 4 Minimally-adjusted and BMI-adjusted associations from analyses A (global GM and WM volumes), A' (regional cortical GM/WM), B (hippocampal volume) and C (White matter lesion volume) vs. annualized change in Log_e transformed NfL (empirical bayes estimator; δNfL_{bayes}) and visit 1 NfL, Log_e transformed or NfL_{v1}: ordinary least square analyses; HANDLS 2004-2013 and HANDLS-SCAN 2011-2015[a]

	***Model 1**: Minimally adjusted*				***Model 2**: BMI-adjusted, sensitivity analysis (SA)*[b]		
Total sample (N=179)	*β1*	*(SE1)*	*b1*	*P1*	*β2*	*(SE2)*	*P2*
sMRI, Analysis A							
Total brain							
δNfL_{bayes}	-33,686	(96825)	-0.021	0.73	-46,622	(31,050)	0.14
NfL_{v1}	-3,137	(17005)	-0.015	0.85	-2,145	(5628)	0.70
GM							
δNfL_{bayes}	-31,771	(51,863)	-0.036	0.54	-38,370	(26,728)	0.15
NfL_{v1}	-7,738	(9,109)	-0.063	0.40	-6,603	(4,845)	0.18
WM							
δNfL_{bayes}	-16,424	(47,153)	-0.023	0.73	-22,022	(22,517)	0.33
NfL_{v1}	1,186	(8,281)	+0.012	0.89	1,126	(4,082)	0.78
sMRI, Analysis B							
Hippocampus, Left							
δNfL_{bayes}	**-894**	**(310)**	**-0.172**	**0.004**	**-899**	**(310)**	**0.004**
NfL_{v1}	***-104***	***(55)***	***-***	***0.0***	***-95***	***(56)***	***0.0***

			0.144	***56***			***94***
Hippocampus, Right							
δNfL_{bayes}	**-725**	**(323)**	**-0.130**	**0.026**	**-731**	**(324)**	**0.024**
NfL_{v1}	-84	(57)	-0.111	0.14	-73	(59)	0.22
Analysis C							
White matter lesion volume, , ***Log_e transformed***							
δNfL_{bayes}	-2.55	(4.02)	-0.050	0.53	-2.67	(4.02)	0.51
NfL_{v1}	**+1.97**	**(0.71)**	**0.271**	**0.006**	**+2.21**	**(0.73)**	**0.003**

Abbreviations: Age_{v1}=age measured at HANDLS visit 1 (2004-2009); δNfL_{bayes}=annualized rate of change in NfL, empirical bayes estimator; FDR=False Discovery Rate; GM=Gray Matter; HANDLS=Healthy Aging in Neighborhoods of Diversity Across the Life Span; HANDLS-SCAN=Brain magnetic resonance imaging scan ancillary study of HANDLS; NfL=Neurofilament Light; SE=Standard Error; sMRI=Structural Magnetic Resonance Imaging; v_1=visit 1 of HANDLS (2004-2009); v2=visit 2 of HANDLS (2009-2013); v_{scan}=HANDLS-SCAN visit (2011-2015); WM=White Matter.

[a] Values are adjusted linear regression coefficients β with associated SE, standardized beta, uncorrected p-values and results of sensitivity analysis. (N) is the sample size in each analysis. Standardized betas are computed as SD in outcome per SD in Nfl exposures. Model 1 was adjusted for Age_{v1}, sex, race, poverty status, intracranial volume (Analyses B and C) and time of follow-up between visit 1 and v_{scan}. Volumes are expressed in mm^3.

[b] Model 2 is a sensitivity analysis further adjusting Model 1 for BMI at visit 1 after screening using machine learning techniques (See Supplemental methods 2).

FIGURE LEGENDS

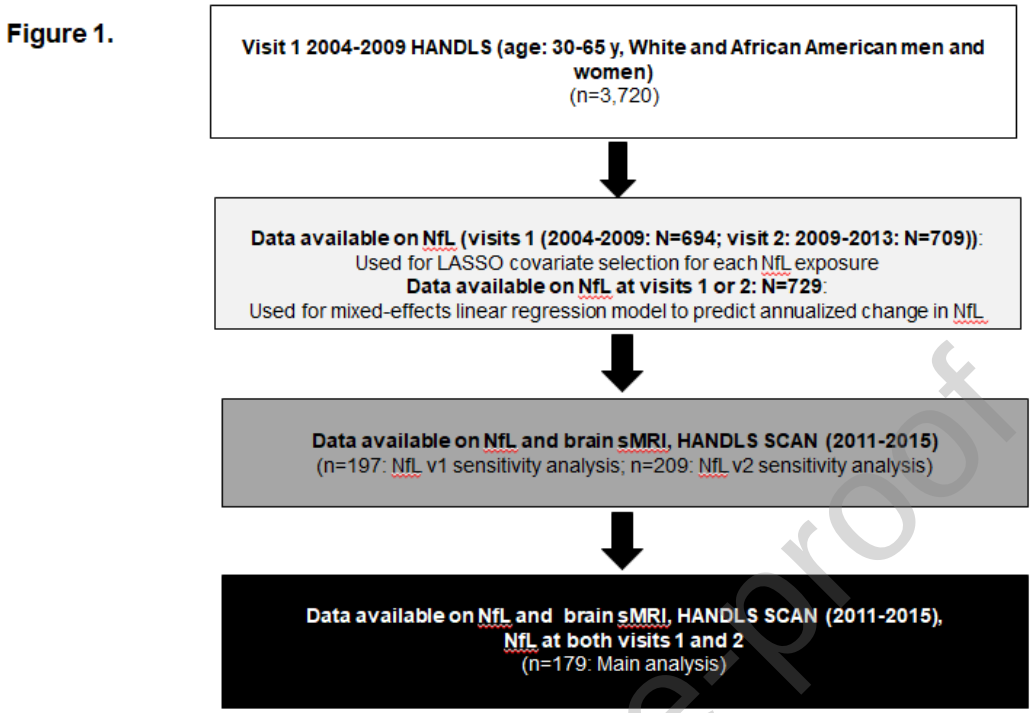
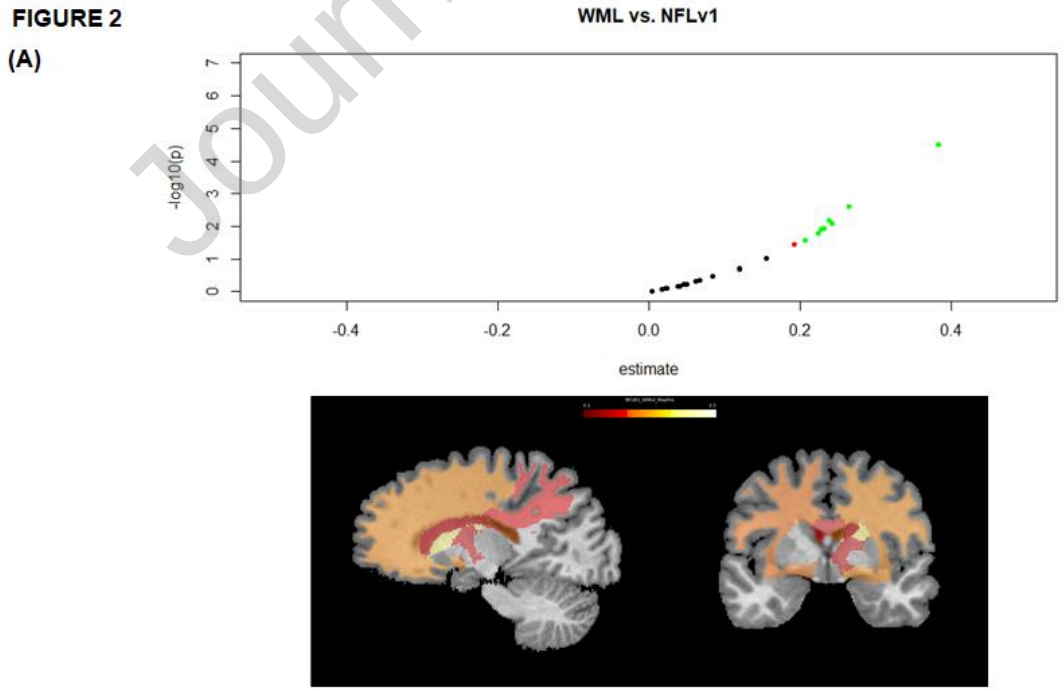


Figure 1. **Study participant schematic: HANDLS 2004-2013 and HANDLS-SCAN 2011-2015[a]**

Abbreviations: HANDLS=Healthy Aging in Neighborhoods of Diversity Across the Life Span.

[a]Visit 1 refers to HANDLS 2004-2009; Visit 2 refers to HANDLS 2009-2013; and HANDLS-SCAN visit (v_{scan}) was carried out between 2011 and 2015.



(B)

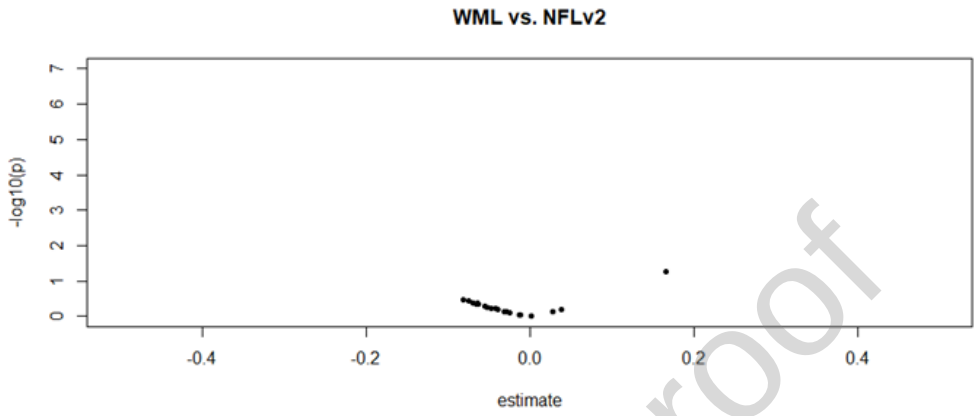


Figure 2. Volcano plots and brain images of WMLV vs. (A) NfL(v_1) and (B) NfL(v_2) : HANDLS 2004-2009/2009-2013 and HANDLS-SCAN 2011-2015[a,b,c]

Abbreviations: HANDLS=Healthy Aging in Neighborhoods of Diversity Across the Life Span; HANDLS-SCAN=HANDLS brain MRI ancillary study; MRI=Magnetic Resonance Imaging; WMLV=White Matter Lesion Volumes.

[a] In both the volcano plot and the brain images: Values are effect sizes from adjusted linear regression models with $NfL_{(v1)}$ and $NfL_{(v2)}$ (Log_e transformed and z-scored) as alternative exposures and outcomes being regional small ROI WMLV, cubic root transformed. The Log_e transformed value was then z-scored. The multiple linear models were adjusted for age, race, poverty status, time of follow-up between visit 1 and v_{scan}, and the intracranial volume.

[b] The volcano plot represents the results of the two models for each exposure, with outcomes being regional small ROI WMLVs. "Estimate" refers to the standardized beta coefficient or effect size; -Log10(p) is the associated negative Log base 10 of the p-value for each regional association. Red dots represent $p<0.05$ with $b<+0.20$ for positive associations or $b>-0.20$ for negative associations; Green dots represent $p<0.05$ with $b>+0.20$ for positive associations or $b<-0.20$ for negative associations.

[c] The brain images represent the same results using FSLEYES software for visualizing effect sizes. Those effect sizes were selected for regions with $p<0.05$, using a color gradient. Colder (blue) colors are for negative associations (smaller WMLV with higher NfL exposure) and warmer colors (red through yellow) are for positive associations (larger WMLV with higher NfL exposure). Lighter colors indicate stronger effect sizes.

CRediT authorship contribution statement

MAB: Study concept, plan of analysis, data management, statistical analysis, literature search and review, write-up of parts of the manuscript, revision of the manuscript.

NNH: Study concept, plan of analysis, data acquisition, literature search and review, write-up of parts of the manuscript, revision of the manuscript.

HAB: Plan of analysis, assistance with statistical analysis, literature review, write-up of parts of the manuscript, revision of the manuscript.

JW: Plan of analysis, assistance with statistical analysis, literature search and review, write-up of parts of the manuscript, revision of the manuscript.

AIM: Plan of analysis, literature search and review, write-up of parts of the manuscript, revision of the manuscript.

LIK: Data acquisition, write-up of parts of the manuscript, revision of the manuscript.

CD: Data acquisition, write-up of parts of the manuscript, revision of the manuscript.

RPG: Data acquisition, write-up of parts of the manuscript, revision of the manuscript.

SLS: Data acquisition, write-up of parts of the manuscript, revision of the manuscript.

GE: Data acquisition, image analysis, assistance with statistical analysis, write-up of parts of the manuscript, revision of the manuscript.

MKE: Data acquisition, write-up of parts of the manuscript, revision of the manuscript.

ABZ: Data acquisition, plan of analysis, data management, write-up of parts of the manuscript, revision of the manuscript.

SRW: Data acquisition, plan of analysis, data management, literature search and review, write-up of parts of the manuscript, revision of the manuscript.

VERIFICATION

1. All authors must disclose:

(a) Any actual or potential conflicts of interest including any financial, personal or other relationships with other people or organizations within three years of beginning the work submitted that could inappropriately influence (bias) their work. Examples of potential conflicts of interest which should be disclosed include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding. If there are no actual or potential conflicts of interest, please state this. Should a significant conflict of interest be present, the Editors reserve the right to reject the article on that basis.

(b)Whether any author's institution has contracts relating to this research through which it or any other organization may stand to gain financially now or in the future.

(c) Any other agreements of authors or their institutions that could be seen as involving a financial interest in this work.

The author declare no conflict of interest for (a), (b) or (c).

2. Please disclose sources of financial support related to the manuscript being submitted.

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3. Please verify that the data contained in the manuscript being submitted have not been previously published, have not been submitted elsewhere and will not be submitted elsewhere while under consideration at Neurobiology of Aging.

This manuscript was not submitted or published elsewhere.

4. When applicable, provide statements verifying that appropriate approval and procedures were used concerning human subjects and animals.

This manuscript was exempted from a full protocol by the National Institute on Aging's IRB and has received approval.

5. Please verify that all authors have reviewed the contents of the manuscript being submitted, approve of its contents and validate the accuracy of the data.

All authors have indeed reviewed the contents of the manuscript being submitted and approve its contents and have validated the accuracy of the data.

Highlights

- Plasma NfL is associated with increased brain white matter lesion volume.
- Plasma NfL is associated with reduced hippocampal volumes.
- Plasma NfL is a promising blood-based biomarker for poor brain volumetric outcomes.