BMI and Allostatic Load Are Directly Associated with Longitudinal Increase in Plasma Neurofilament Light among Urban Middle-Aged Adults

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ABSTRACT

Background: Plasma neurofilament light chain (NfL) is a novel biomarker for age-related neurodegenerative disease. We tested whether NfL may be linked to cardiometabolic risk factors, including BMI, the allostatic load (AL) total score (AL_{total}), and related AL continuous components (AL_{comp}). We also tested whether these relations may differ by sex or by race.

Methods: We used data from the HANDLS (Healthy Aging in Neighborhoods of Diversity across the Life Span) study [$n = 608$, age at visit 1 (v_1: 2004–2009): 30–66 y, 42% male, 58% African American] to investigate associations of initial cardiometabolic risk factors and time-dependent plasma NfL concentrations over 3 visits (2004–2017; mean $\pm$ SD follow-up time: 7.72 $\pm$ 1.28 y), with outcomes being NfL_{v1} and annualized change in NfL (δNfL). We used mixed-effects linear regression and structural equations modeling (SM).

Results: BMI was associated with lower initial ($\gamma_{01} = -0.014 \pm 0.002$, $P < 0.001$) but faster increase in plasma NfL over time ($\gamma_{11} = +0.0012 \pm 0.0003$, $P < 0.001$), a pattern replicated for AL_{total}. High-sensitivity C-reactive protein (hsCRP), serum total cholesterol, and resting heart rate at v_1 were linked with faster plasma NfL increase over time, overall, while being uncorrelated with NfL_{v1} (e.g., hsCRP $\times$ Time, full model: $\gamma_{11} = +0.004 \pm 0.002$, $P = 0.015$). In SM analyses, BMI's association with δNfL was significantly mediated through AL_{total} among women [total effect (TE) = $+0.0014 \pm 0.00038$, $P < 0.001$; indirect effect = $+0.00042 \pm 0.00019$, $P = 0.025$; mediation proportion = 30%], with only a direct effect (DE) detected among African American adults (TE = $+0.0011 \pm 0.0004$, $P = 0.015$; DE = $+0.0010 \pm 0.00048$, $P = 0.034$). The positive associations between AL_{total}/BMI and δNfL were mediated through increased glycated hemoglobin (HbA1c) concentrations, overall.

Conclusions: Cardiometabolic risk factors, particularly elevated HbA1c, should be screened and targeted for neurodegenerative disease, pending comparable longitudinal studies. Other studies examining the clinical utility of plasma NfL as a neurodegeneration marker should account for confounding effects of BMI and AL. *J Nutr* 2022;152:535–549.

Keywords: neurofilament light, allostatic load, body mass index, urban adults, race, cognition

Introduction

When axons are damaged with age and in many neurodegenerative diseases, certain cytoskeletal proteins referred to as neurofilaments are often released into the extracellular space, then the cerebrospinal fluid (CSF), and finally may transmigrate into blood at a lower concentration (1). Neurofilament light chain (NfL) is a novel biomarker for neurodegenerative diseases detectable in blood, reflecting axonal degeneration. Accumulating data indicate that concentrations of plasma NfL are associated with Alzheimer disease (AD) and other neurodegenerative diseases (2–8). In fact, plasma NfL concentrations are associated with cognitive decline in nondemented adults (9, 10) and are able to predict the onset of AD (11, 12). Plasma NfL is attractive as a biomarker, because it uses less invasive procedures than do CSF assessments. NfL measured in CSF is positively correlated with plasma NfL (13, 14) and plasma NfL concentrations are associated with neuroimaging measures of cognition (1, 15, 16). Compared with neuroimaging measures or tests of cognitive performance, plasma NfL as a biomarker reduces both time and expense in assessing risk of dementia with high-risk

groups in randomized controlled trials. As for CSF NfL, plasma NfL concentration exhibits an upward-trending trajectory with age. This positive correlation may be in part explained by increased BMI and other related metabolic disorders such as renal dysfunction as was shown in 2 recent studies (17, 18), which in turn are largely determined by poor dietary quality and other nutritional factors (19–24). Obesity, directly measured with BMI, along with its associated cardiometabolic disorders and markers of inflammation [e.g., abdominal obesity, hypertension, dyslipidemia, hyperglycemia, elevated blood C-reactive protein, reduced serum albumin (ALB)], become more prevalent with age, particularly between early and mid-life (25, 26); they are also associated with later-life cognitive decline and impairment (27–31) and adverse neuroimaging outcomes (32–38). Moreover, neurocognitive outcomes were associated with CSF biomarkers of neurodegeneration (e.g., Aβ42:40 ratio, tau, and NfL) (39–41), as well as plasma NfL in more recent studies (2–4, 15, 40, 42–46). However, it is still unknown whether age-related cardiometabolic disorders are independent risk factors for the rate of increase in plasma NfL over time.

If these associations exist then they are likely to differ markedly across sociodemographic factors, particularly across sex and race groups, given the sex- and race-specific associations between cardiometabolic risk and neurocognitive aging outcomes (47–50). Moreover, previous reports have shown a direct association between cardiometabolic risk and adverse neurocognitive outcomes, and of increased plasma NfL with those same outcomes. Therefore, a positive association between cardiometabolic risk and plasma NfL would suggest that cardiometabolic risk needs to be accounted for as a potential confounder in studies of the clinical utility of plasma NfL as an early marker of neurodegeneration. Plasma NfL may also be a pathway through which cardiometabolic risk is linked to neurocognitive outcomes.

The present study investigated the longitudinal associations of BMI (continuous and categorical) and the allostatic load (AL) [total score (AL$_{total}$) and components] with plasma NfL (baseline BMI/AL compared with baseline plasma NfL; baseline BMI/AL compared with annual rate of change in plasma NfL), independently of key exogenous confounders, and across sex

and race. The study also assessed whether AL$_{total}$ mediated the association between BMI and the annual rate of change in plasma NfL, across sex and race groups. Finally, the study tested which individual components of the AL mediated the total effects (TEs) of AL and BMI on annual rate of change in plasma NfL, overall and across sex and race groups.

Methods

Database
The sample was selected from the HANDLS (Healthy Aging in Neighborhoods of Diversity across the Life Span) study (51). Initiated in 2004, HANDLS is a longitudinal study involving socioeconomically diverse White and African-American adult women and men who resided in Baltimore, MD. Baseline data (visit 1, v$_1$) were collected in 2 phases during 2004–2009. Phase I consisted of a home visit, whereby recruitment, consent, and screening procedures as well as a household in-person interview were performed, including the first 24-h dietary recall. During Phase II (v$_1$), an in-person complete physical health examination was performed within Medical Research Vehicles (MRVs) including a second 24-h dietary recall. Participants were invited to participate in follow-up in-person visits [visit 2 (v$_2$): 2009–2013 and visit 3 (v$_3$): 2013–2017] whereby similar protocols as for v$_1$ (Phase II) were applied. Fasting blood samples were drawn from participants who provided written informed consent during in-person examinations. The study protocol of HANDLS was approved by the Institutional Review Board of the National Institute of Environmental Health Sciences, NIH.

Study sample
In our present study, ≤3 repeats on plasma NfL concentrations were available from v$_1$, v$_2$, and/or v$_3$. AL components and therefore AL$_{total}$ were measured also at ≤3 visits. However, in this study, we only examine v$_1$ exposures. As shown in the study design flowchart (**Figure 1**), among 3720 initially recruited HANDLS participants, $n = 694$ had complete v$_1$, v$_2$, and/or v$_3$ data on plasma NfL. Of those participants, $n = 608$ had data on v$_1$ AL. Mean ± SD follow-up time (between v$_1$ and v$_3$) for the final analytic sample with complete v$_3$ data ($n = 596$ participants) was 7.72 ± 1.28 y. **Supplemental Method 1** shows a detailed description of sample selection with respect to the plasma NfL outcome. Compared with the initial sample with incomplete data for our analysis, the final sample had a lower proportion of individuals living below poverty (27% compared with 44%, $P < 0.001$, χ^2 test). Of $n = 608$, 606 had complete data to compute the observed annualized NfL change (δNfL$_{obs}$), and thus an additional $n = 2$ were excluded for the structural equations modeling (SM) analysis.

Plasma NfL
Fasting blood samples were collected between 09:30 and 11:30 into EDTA blood collection tubes. The tubes were centrifuged at 4°C and at $600 \times g$ for 15 min followed by the removal of the buffy coat. These steps were repeated twice and samples were visually examined for hemolysis. Plasma samples were stored in aliquots at −80°C after their collection. Plasma NfL concentrations were quantified using the Simoa® NF-light Advantage Kit (Quanterix) following kit instructions. Samples from the different visits were run on the same plate for each individual and plates were balanced for individuals within each demographic group (race/sex/poverty). Plasma samples were diluted 4-fold, and concentrations were adjusted for this dilution correction. Pooled plasma samples from 2 individuals were run in duplicate on all plates; intra-assay and interassay CVs were 4.5% and 7%, respectively. The limit of detection was 0.152 pg/mL and the lower limit of quantification was 0.696 pg/mL. The upper limit of detection was 1872 pg/mL. Plasma NfL was the main outcome of interest, measured for ≤3 repeats per participant, at v$_1$, v$_2$, and/or v$_3$.
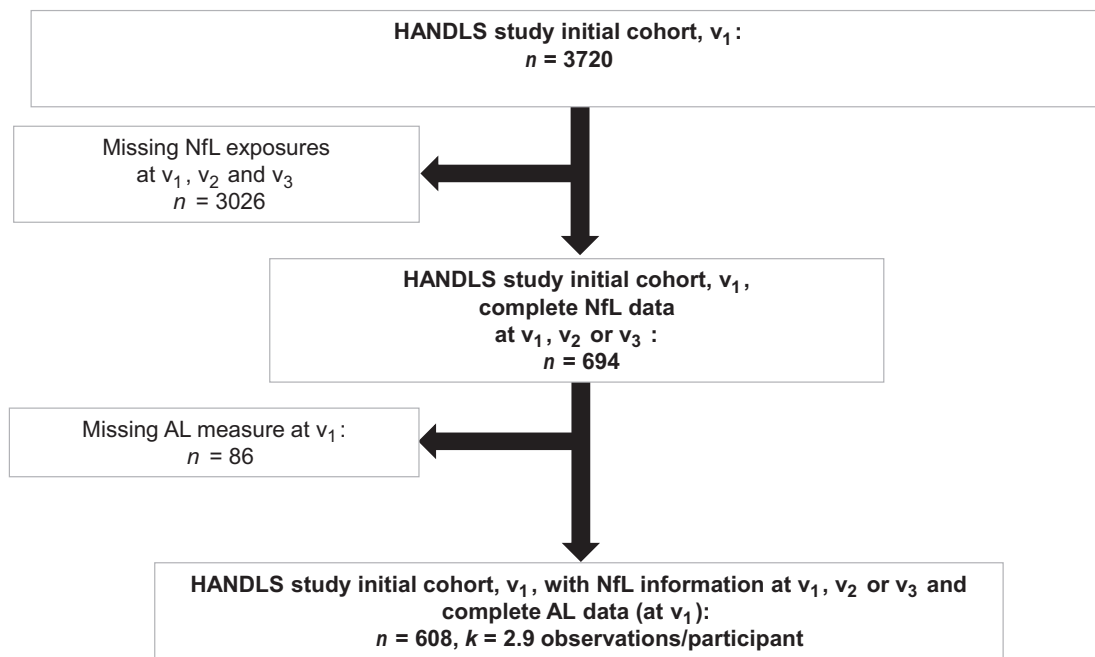
FIGURE 1 Participant flowchart. AL, allostatic load; HANDLS, Healthy Aging in Neighborhoods of Diversity Across the Life Span; NfL, plasma neurofilament light chain; v_1, visit 1; v_2, visit 2; v_3, visit 3.

AL

We relied on a previously reported method to compute AL_{total} (52). This method sums cardiovascular [systolic blood pressure (SBP) and diastolic blood pressure (DBP), pulse rate], metabolic [total cholesterol, HDL cholesterol, glycated hemoglobin (HbA1c), sex-specific waist-to-hip ratio (WHR)], and inflammatory [serum ALB and high-sensitivity C-reactive protein (hsCRP)] risk indicators. As summarized in **Supplemental Table 1**, multiple clinical criteria were used to obtain risk indicators which were subsequently summed with equal weighting to compute an AL_{total} that ranges between 0 and 9. The higher the AL_{total}, the more the overall cardiometabolic risk. Total cholesterol (mg/dL), HDL cholesterol (mg/dL), hsCRP (mg/dL), ALB (g/dL), and HbA1c (%) were determined by contract laboratories (Quest Diagnostics), using reference analytical methods. Using standard protocols, trained examiners measured WHR, radial pulse (beats/min), and SBP and DBP (mm Hg). In particular, blood pressure was measured using a mercury sphygmomanometer and the arithmetic means of left and right SBP and DBP were used in this analysis.

BMI

BMI was calculated as weight divided by height (kg/m^2). In part of the analysis, the TE of BMI on plasma NfL change was tested as potentially being mediated through AL_{total}. In addition, both continuous BMI and weight status (categorical BMI) were included among potential exposures, alternative to AL_{total}, in mixed-effects linear regression models. Weight status was defined as follows: BMI_{v1} <18.5—underweight; BMI_{v1} ≥18.5 and <25—normal weight; BMI_{v1} ≥25 and <30—overweight; and BMI_{v1} ≥30—obese.

Covariates

We assessed multiple covariates as potential confounders, given previous significant associations with plasma NfL, and these are considered antecedent risk factors to the AL. These included v_1 age (continuous; y), sex (male, female), race (white, African American), poverty status (below compared with above 125% of the federal poverty line), and educational attainment (less than high school, high school, more than high school). We operationalized poverty status using the 2004 US Census Bureau poverty thresholds (53) based on household income and total family size (including children <18 y old). Some of the lifestyle and health-related factors were considered

as potential confounders, given their potential impact on both AL and plasma NfL, although they were not necessarily on the causal pathway between AL and plasma NfL. Those factors were current smoking status (0 = no compared with 1 = yes), illicit drug use (0 = no compared with 1 = yes, using any of marijuana, opiates, and cocaine), the Healthy Eating Index 2010 (HEI-2010) (54) whereby overall diet quality was measured based on food- and macronutrient-related US dietary guidelines for Americans, total energy intake (kcal/d), and the 20-item Center for Epidemiologic Studies-Depression (CES-D) total score for depressive symptoms (55). Sex and race were the main effect modifiers in our analyses. Basic sociodemographic covariates were complete by design, whereas other measures assessed during the MRV phase of v_1 had some missing data. However, after accounting for missingness in all key variables (v_1 AL and δNfL), covariates had <5% missingness individually out of the final eligible sample (n = 608). Thus, multiple imputation was conducted as described in the next section.

Statistical analysis

All analyses were conducted using Stata release 16 (56). First, study sample characteristics were described in terms of fixed, baseline, and longitudinal changes in key variables across race and sex, using means and proportions, as well as bivariate linear, logistic, and multinomial logit models to examine racial and sex differences in continuous, binary, and categorical multilevel covariates, respectively. We then further adjusted those models for the remaining sociodemographic factors among age, sex, race, and poverty status to determine whether racial and sex differences remained statistically significant. Second, for testing our main hypotheses, a series of mixed-effects linear models were conducted (**Supplemental Method 2**). The outcome in these models was plasma NfL measured longitudinally with ≤3 repeats, whereas the main exposure was AL_{total} measured at v_1 (2004–2009). The modeling process consisted of 2 model sets, with an increasing level of adjustment for potentially confounding covariates. These covariates were assumed to confound the relation between v_1 AL_{total} and v_1 plasma NfL as well as v_1 AL_{total} and annualized change in plasma NfL, and thus were included among the main effects and interacted with Time. Model 1 adjusted for only sociodemographic variables: age at v_1, sex, race, poverty status, and educational attainment. Model 2 adjusted for all other lifestyle and health-related covariates listed in the Covariates section, excluding BMI at v_1. We ensured sample size consistency across

models by conducting multiple imputation for covariates (aside from sociodemographics). This was accomplished with chained equations (5 imputations, 10 iterations), with all covariates used simultaneously in the estimation process, similarly to previous studies (57, 58). Nine AL continuous components (AL_{comp}) measured at v_1 were considered as secondary predictors, substituting AL_{total} in these models, separately. Thus, in these multiple mixed-effects linear regression models, we applied Models 1 and 2 to 1 key exposure (AL_{total}) and 9 secondary predictors (AL_{comp}), 1 key outcome (plasma NfL) with ≤ 3 repeats [effect of AL_{total} on v_1 plasma NfL (NfL_{v1}) and annualized change in NfL (δNfL) over time between v_1 and v_3], and 2 main stratifying variables (race and sex). In all these models, plasma NfL was $\log_e$ transformed, as done in other studies [e.g., (45)]. Using a simplified model with Time as the only predictor, annualized change in plasma NfL was also estimated for each participant in the final analytic sample, by predicting random effects from the model and estimating the empirical Bayes estimator for annualized change in plasma NfL. This estimation process used the largest available sample with 1, 2, or 3 measures on plasma NfL and assumed missingness of outcome at random. Given that the variance of this estimator is significantly different from the observed annualized change, it was only used to validate δNfL_{obs} (**Supplemental Figure 1**). The latter was estimated by taking the arithmetic mean for annualized changes between v_1 and v_2, between v_2 and v_3, and between v_1 and v_3. Racial and sex differences in the association between v_1 AL_{total} and plasma NfL at v_1 were tested using $AL_{total} \times$ Race and $AL_{total} \times$ Sex interaction terms in separate models, respectively. In each of these models, heterogeneity by race and sex in the association between AL_{total} and δNfL were tested by also adding $AL_{total} \times$ Time $\times$ Race and $AL_{total} \times$ Time $\times$ Sex, respectively. This modeling process was repeated for BMI and weight status, substituting AL_{total}.

Two sets of structural equations models were constructed to test pathways explaining annual rate of change in plasma NfL, predicted from a simple mixed-effects regression model with random effects added to intercept and slope (δNfL_{obs}), through mediating pathways involving several cardiometabolic risk factors. The first SM set examined whether BMI at v_1 was associated with δNfL_{obs} through AL_{total} at v_1, overall and stratifying separately by sex and race. Thus, this SM set attempted to test whether AL_{total} mediated the association between BMI and δNfL. In contrast, a second SM set examined individual AL_{comp} (e.g., total cholesterol) as alternative mediators between AL_{total} at v_1 and δNfL_{obs}. In all these models, exogenous covariates included v_1 age, sex, race, poverty status, education, current smoking, current illicit drug use, CES-D total score, HEI-2010, and mean energy intake (kcal/d) at v_1. These exogenous covariates were allowed to predict all 3 endogenous variables in the system, including (δNfL_{obs}), (v_1 BMI), (v_1 AL_{total}), and (v_1 AL_{comp}). AL_{comp} included v_1 WHR, v_1 serum ALB, v_1 hsCRP, v_1 HbA1c, v_1 total cholesterol, v_1 HDL cholesterol, v_1 resting heart rate (RHR), v_1 SBP, and v_1 DBP. **Supplemental Method 3** provides a detailed description of the SM methods and the estimated parameters and statistics. In a sensitivity analysis with δNfL_{obs} as the final outcome, we examined the mediating effect of AL_{comp} in the BMI–δNfL_{obs} relation, overall and by sex and race following a similar analytic strategy.

In all models (mixed-effects and SM), sample selectivity potentially caused by missingness on exposure and outcome data, relative to the initially recruited sample, was corrected by utilizing a 2-stage Heckman selection process. As a first stage, using a probit model, we predicted an indicator of selection with sociodemographic factors. Those were, in this case, v_1 age, race, sex, and poverty status. This model yielded an inverse Mills ratio (IMR), a function of the probability of being selected conditional on those sociodemographic factors. At the second stage, the main models testing the key hypotheses were estimated using multiple mixed-effects linear and SM models, adding among adjusted factors the IMR in addition to the aforementioned covariates (59).

We set the type I error rate a priori for main effects and interactions to 0.05 and 0.10, respectively (60). We illustrated some of the main findings from specific mixed-effects linear regression models using predictive margins (with estimated 95% CIs) of plasma NfL outcome across time, and by AL_{total} exposure, overall or stratified

by race and/or sex. Pictural representations of SM models were also utilized, where appropriate, to illustrate the potential mediating effects of cardiometabolic risk factors, while stratifying by sex and race.

Results

Study sample characteristics by sex and race

Table 1 describes the characteristics of the study sample, while examining differences by sex and by race. The most notable differences were in BMI, which was higher among women than among men, whereas the reverse was true for total caloric intake and current illicit drug use ($P < 0.05$). AL_{total} on average reflected greater cardiometabolic risk among white than among African American adults (1.98 compared with 1.78, $P = 0.044$). Sex and racial differences were also detected in AL_{comp}. Specifically, men were at higher cardiometabolic risk than women on HDL cholesterol and DBP, whereas the reverse was true for serum ALB, hsCRP, and total cholesterol. Moreover, African American adults were at higher risk of lower ALB concentrations, and at lower risk of higher HDL cholesterol, than their White counterparts. The $\log_e$-transformed plasma NfL concentrations at v_1 and v_3 were all on average higher among men than among women, with no difference detected by sex for δNfL_{obs}. At each of v_1 and v_3, $\log_e$-transformed plasma NfL concentration was also higher among White adults, even though δNfL_{obs} did not differ across racial groups.

BMI, weight status, and their longitudinal association with plasma NfL

Our main hypotheses of associations of BMI (and weight status) with time-dependent plasma NfL concentrations were examined by a series of mixed-effects linear regression models, with key findings presented in **Table 2**. Overall, baseline concentrations of plasma NfL were inversely associated with BMI ($\gamma_{01} = -0.014 \pm 0.002$, $P < 0.001$) and higher weight status in both the reduced and full models. In contrast, BMI ($\gamma_{11} = +0.0012 \pm 0.0003$, $P < 0.001$) and higher weight status at baseline were linked to faster increase in plasma NfL over time. Both associations were driven by the contrast between obesity and normal weight ($\gamma_{01} = -0.234 \pm 0.045$, $P < 0.001$; $\gamma_{11} = +0.017 \pm 0.006$, $P < 0.010$). The results were largely homogeneous across sex and race with few exceptions, particularly for the reduced model. When examining standardized regression coefficients (b), 1 SD increase in BMI was linked to a -0.19 SD lower baseline plasma NfL and with a 0.015-fold annual increase in SD of plasma NfL, yielding an increase of 0.15 SD over a period of 10 y. Both of these cross-sectional and longitudinal standardized effect sizes are considered weak to modest.

AL_{total} and AL_{comp} and their longitudinal association with plasma NfL

Following a similar modeling approach (**Table 3**), we examined the associations of AL_{total} and AL_{comp} in relation to time-dependent change in plasma NfL. Overall, there was a clear association between AL_{total} at v_1 and faster increase in plasma NfL ($P < 0.001$) and the same exposure was associated with lower baseline plasma NfL ($P < 0.05$). Although largely homogeneous by sex and by race, this association differed markedly by these 2 sociodemographic groups when each component of AL was considered as the main exposure. One notable finding is that v_1 HbA1c was consistently associated

TABLE 1 Study sample characteristics by sex and by race: HANDLS, 2004–2017[1]

	Overall (n = 608)	Women (n = 352)	Men (n = 256)	P_{sex}	White adults (n = 257)	African American adults (n = 351)	P_{race}
Sociodemographic, lifestyle, and health-related factors at v_1							
Men, %	42.1	0.0	100.0	—	39.7	43.9	0.30
African American, %	57.7	56.0	60.2	0.30	0.0	100.0	—
Age, y	47.7 ± 0.4	47.7 ± 0.5	47.6 ± 0.5	0.90	48.4 ± 0.5	47.2 ± 0.5	0.11
Below poverty, %	27.0	28.1	25.4	0.45	26.1	27.6	0.67
Education, %							
<High school	4.9	5.0	4.7	0.78	6.8	3.4	0.093
High school	58.2	57.1	59.8	Ref.	59.5	57.3	Ref.
>High school	36.9	37.9	35.5	0.53	33.7	39.3	0.28
Current illicit drug use, % yes	15.6	9.7	23.7	<0.001*	12.3	18.0	0.058
Current tobacco use, % yes	40.0	38.9	46.1	0.084	40.5	43.0	0.54
Healthy Eating Index-2010 total score	42.3 ± 0.5	42.9 ± 0.7	41.3 ± 0.7	0.12	41.4 ± 0.8	42.9 ± 0.7	0.18
Energy intake, kcal/d	1993 ± 40	1697 ± 47	2399 ± 71	<0.001*	1996 ± 73	1991 ± 63	0.97
CES-D total score	14.3 ± 0.4	14.6 ± 0.6	13.9 ± 0.7	0.41	15.2 ± 0.7	13.6 ± 0.6	0.070*
BMI$_{v_1}$, kg/m²	30.3 ± 0.3	31.8 ± 0.4	28.3 ± 0.4	<0.001*	30.4 ± 0.5	30.2 ± 0.4	0.84
Weight status at v_1, %							
Underweight: BMI$_{v_1}$ <18.5	3.0	2.8	3.1	0.23	3.1	2.8	0.84
Normal: BMI$_{v_1}$ ≥18.5, <25	20.9	16.2	27.3	<0.001*	19.8	21.7	0.72
Overweight: BMI$_{v_1}$ ≥25, <30	30.4	26.1	36.3	<0.001*	31.5	29.6	0.72
Obese: BMI$_{v_1}$ ≥30	45.7	54.8	33.2	Ref.	45.5	45.9	Ref.
AL$_{total}$ at v_1	1.87 ± 0.05	1.91 ± 0.06	1.80 ± 0.08	0.28	1.98 ± 0.07	1.78 ± 0.06	0.044
AL$_{comp}$ at v_1							
WHR	0.95 ± 0.02	0.94 ± 0.04	0.96 ± 0.00	0.75	0.99 ± 0.05	0.92 ± 0.00	0.081
Serum albumin, g/dL	4.33 ± 0.01	4.28 ± 0.01	4.40 ± 0.02	<0.001*	4.36 ± 0.02	4.30 ± 0.01	0.006*
hsCRP[2] mg/L	0.74 ± 0.05	0.99 ± 0.07	0.40 ± 0.08	<0.001*	0.81 ± 0.07	0.69 ± 0.08	0.26
HbA1c, %	5.85 ± 0.04	5.85 ± 0.05	5.86 ± 0.06	0.93	5.83 ± 0.06	5.87 ± 0.04	0.55
Total cholesterol, mg/dL	186.1 ± 1.7	190.2 ± 2.2	180.5 ± 2.6	0.004*	189.6 ± 2.7	183.6 ± 2.1	0.075
HDL cholesterol, mg/dL	53.5 ± 0.7	55.9 ± 0.9	50.3 ± 1.1	<0.001*	50.2 ± 0.9	56.0 ± 1.0	<0.001*
Resting heart rate, beat/min	66.9 ± 0.5	67.3 ± 0.6	66.4 ± 0.7	0.32	67.7 ± 0.7	66.4 ± 0.6	0.14
Systolic blood pressure, mm Hg	119.5 ± 0.7	119.1 ± 0.9	120.0 ± 1.0	0.48	119.3 ± 1.1	119.7 ± 0.8	0.76
Diastolic blood pressure, mm Hg	72.9 ± 0.4	71.7 ± 0.5	74.5 ± 0.7	0.001*	72.4 ± 0.6	73.2 ± 0.6	0.32
Plasma NfL, log$_e$ transformed							
NfL$_{v_1}$	1.98 ± 0.02	1.95 ± 0.03	2.03 ± 0.03	0.039*	2.11 ± 0.03	1.89 ± 0.03	<0.001*
NfL$_{v_3}$	2.35 ± 0.02	2.28 ± 0.03	2.44 ± 0.04	0.002*	2.41 ± 0.04	2.30 ± 0.03	0.038
δNfL$_{obs}$[3]	0.0492 ± 0.0027	0.0462 ± 0.0043	0.0532 ± 0.0047	0.20	0.0468 ± 0.0044	0.0508 ± 0.0033	0.47

[1] Values are means ± SEs for continuous variables or percentages for categorical variables. BMI in kg/m². * $P < 0.05$ upon further adjustment for age, sex, race, and poverty status in multiple linear and multinomial logit models. AL$_{comp}$, allostatic load continuous components; AL$_{total}$, allostatic load total score; BMI$_{v_1}$, BMI at v_1; CES-D, Center for Epidemiologic Studies-Depression; HANDLS, Healthy Aging in Neighborhoods of Diversity Across the Life Span; HbA1c, glycated hemoglobin; hsCRP, high-sensitivity C-reactive protein; NfL, neurofilament light; δNfL$_{obs}$, observed annualized rate of change in plasma neurofilament light between visit 1 and visit 3.

[2] Log$_e$ transformed.

[3] Validated against the empirical Bayes estimator predicted from a mixed-effects linear regression model with NfL as the outcome and Time as the only predictor (Pearson's $r > 0.80$). $n = 606$. See **Table 4** for sample sizes within sex and race strata. 1 SD of δNfL$_{obs}$ is 0.0665. All other SDs can be obtained from this table as sqrt(N) × SE to assess clinically meaningful effects.

Adiposity, allostatic load, neurofilament light 539

TABLE 2 BMI and weight status (at v_1) and their association with baseline plasma NfL and annualized change in plasma NfL between v_1 and v_3, overall and by sex and race: mixed-effects linear regression models; HANDLS, 2004–2017[1]

	Overall (n = 608)	Women (n = 352)	Men (n = 256)	P_{sex}[2]	White adults (n = 257)	African American adults (n = 351)	P_{race}[3]
Model 1A							
BMI_{v1}, γ_{0a}	−0.015 ± 0.002***	−0.014 ± 0.003***	−0.016 ± 0.005***	0.56	−0.014 ± 0.004***	−0.014 ± 0.003***	0.72
BMI_{v1} × Time, γ_{1a}	+0.0012 ± 0.0003***	+0.0011 ± 0.0003***	+0.0014 ± 0.0006**	0.65	+0.0007 ± 0.0005	+0.0014 ± 0.0004***	0.21
Model 2A							
BMI_{v1}, γ_{0a}	−0.014 ± 0.002***	−0.013 ± 0.003***	−0.016 ± 0.005**	0.58	−0.014 ± 0.004***	−0.013 ± 0.003***	0.81
BMI_{v1} × Time, γ_{1a}	+0.0012 ± 0.0003***	+0.0012 ± 0.0003***	+0.0014 ± 0.0006*	0.60	+0.0008 ± 0.005	+0.0013 ± 0.0003***	0.15
Model 1B							
[Underweight vs. Normal], γ_{0a}	+0.170 ± 0.103	+0.176 ± 0.129	+0.160 ± 0.165	1.00	+0.343 ± 0.155*	+0.028 ± 0.137	0.10
[Overweight vs. Normal], γ_{0a}	−0.085 ± 0.047	−0.025 ± 0.064	−0.122 ± 0.071	0.27	+0.034 ± 0.073	−0.163 ± 0.052**	0.054
[Obese vs. Normal], γ_{0a}	−0.245 ± 0.045***	−0.223 ± 0.057***	−0.245 ± 0.072**	0.75	−0.177 ± 0.069*	−0.281 ± 0.059***	0.16
[Underweight vs. Normal] × Time, γ_{1a}	−0.016 ± 0.013	−0.003 ± 0.016	−0.034 ± 0.022	0.23	−0.015 ± 0.019	−0.014 ± 0.018	0.91
[Overweight vs. Normal] × Time, γ_{1a}	+0.001 ± 0.006	+0.009 ± 0.008	+0.007 ± 0.010	0.89	−0.002 ± 0.009	+0.016 ± 0.008*	0.15
[Obese vs. Normal] × Time, γ_{1a}	+0.017 ± 0.006**	+0.023 ± 0.007**	+0.010 ± 0.010	0.22	+0.011 ± 0.009	+0.022 ± 0.008**	0.39
Model 2B							
[Underweight vs. Normal], γ_{0a}	+0.138 ± 0.104	+0.109 ± 0.127	+0.163 ± 0.167	0.95	+0.344 ± 0.155*	−0.020 ± 0.137	0.093
[Overweight vs. Normal], γ_{0a}	−0.077 ± 0.047	−0.015 ± 0.062	−0.115 ± 0.072	0.27	+0.041 ± 0.073	−0.156 ± 0.062*	0.061
[Obese vs. Normal], γ_{0a}	−0.234 ± 0.045***	−0.206 ± 0.056***	−0.239 ± 0.074**	0.70	−0.171 ± 0.070*	−0.274 ± 0.060***	0.17
[Underweight vs. Normal] × Time, γ_{1a}	−0.018 ± 0.013	−0.005 ± 0.016	−0.036 ± 0.022	0.20	−0.023 ± 0.019	−0.011 ± 0.018	0.91
[Overweight vs. Normal] × Time, γ_{1a}	+0.009 ± 0.006	+0.009 ± 0.008	+0.006 ± 0.010	0.94	−0.002 ± 0.009	+0.015 ± 0.008	0.14
[Obese vs. Normal] × Time, γ_{1a}	+0.017 ± 0.006**	+0.0237 ± 0.007**	+0.008 ± 0.010	0.23	+0.014 ± 0.009	+0.020 ± 0.008*	0.34

[1] Values are fixed-effects γ ± SE. Models 1A and 1B included each of v_1 BMI and weight status (z scored), separately as the main predictor for v_1 NfL and NfL annualized rate of change over time, using a series of mixed-effects linear regression models, carried out in the overall population, and stratified by sex and by race, separately. These models adjusted only for age, sex, race, poverty status, educational attainment, and the inverse Mills ratio. Models 2A and 2B followed a similar approach but adjusted further for selected lifestyle and health-related factors, namely current drug use, current tobacco use, Healthy Eating Index-2010, total energy intake, and the Center for Epidemiologic Studies-Depression total score. * $P < 0.05$; ** $P < 0.010$; *** $P < 0.001$ for null hypothesis that fixed effect $\gamma = 0$. HANDLS, Healthy Aging in Neighborhoods of Diversity Across the Life Span; NfL, neurofilament light chain; v_1, visit 1; v_3, visit 3.
[2] Based on separate models testing the statistical significance for Sex × BMI/[weight status] and Sex × BMI/[weight status] × Time in models unstratified by sex or race in which these 2-way and 3-way interaction terms were included for each sociodemographic factor, separately.
[3] Based on separate models testing the statistical significance for Race × BMI/[weight status] and Race × BMI/[weight status] × Time in models unstratified by sex or race in which these 2-way and 3-way interaction terms were included for each sociodemographic factor, separately.

TABLE 3 AL_{total} and AL_{comp} (at v_1) and their association with baseline plasma NfL and annualized change in plasma NfL between v_1 and v_3, overall and by sex and race: mixed-effects linear regression models; HANDLS, 2004–2017[1]

	Overall (n = 608)	Women (n = 352)	Men (n = 256)	P_{sex}[2]	White adults (n = 257)	African American adults (n = 351)	P_{race}[3]
Model 1A							
AL_{total}, γ_{0a}	−0.038 ± 0.015*	−0.029 ± 0.019	−0.039 ± 0.023	0.52	−0.005 ± 0.023	−0.057 ± 0.020**	0.15
AL_{total} × Time, γ_{1a}	+0.0066 ± 0.0018***	+0.006 ± 0.002**	+0.008 ± 0.003*	0.75	+0.004 ± 0.003	+0.008 ± 0.002**	0.25
Model 2A							
AL_{total}, γ_{0a}	−0.033 ± 0.015*	−0.027 ± 0.018	−0.037 ± 0.024	0.68	−0.003 ± 0.023	−0.050 ± 0.019*	0.65
AL_{total} × Time, γ_{1a}	+0.0067 ± 0.0018***	+0.006 ± 0.002**	+0.008 ± 0.003*	0.75	+0.004 ± 0.003	+0.008 ± 0.002**	0.19
Model 1B							
WHR, γ_{0a}	+0.053 ± 0.033	+0.063 ± 0.030*	−1.247 ± 0.465**	<0.001	−0.065 ± 0.032	−1.047 ± 0.349**	0.008
WHR × Time, γ_{1a}	+0.004 ± 0.004	+0.003 ± 0.004	+0.119 ± 0.062	0.066	+0.0035 ± 0.0040	+0.074 ± 0.045	0.084
Model 2B							
WHR, γ_{0a}	+0.053 ± 0.032	+0.064 ± 0.029*	−1.179 ± 0.474**	0.001	−0.064 ± 0.032	−0.991 ± 0.345**	0.008
WHR × Time, γ_{1a}	+0.005 ± 0.004	+0.003 ± 0.004	+0.112 ± 0.063	0.077	+0.0034 ± 0.0039	+0.0711 ± 0.044	0.064
Model 1C							
ALB, γ_{0a}	−0.092 ± 0.064	+0.045 ± 0.078	−0.301 ± 0.106**	0.004	−0.064 ± 0.101	−0.127 ± 0.083	0.90
ALB × Time, γ_{1a}	+0.014 ± 0.008	+0.003 ± 0.010	+0.029 ± 0.014*	0.14	+0.010 ± 0.012	+0.017 ± 0.011	0.56
Model 2C							
ALB, γ_{0a}	−0.107 ± 0.064	+0.007 ± 0.077	−0.303 ± 0.107**	0.006	−0.062 ± 0.102	−0.146 ± 0.082	0.81
ALB × Time, γ_{1a}	+0.013 ± 0.008	+0.003 ± 0.010	+0.027 ± 0.014	0.17	+0.007 ± 0.012	+0.017 ± 0.011	0.46
Model 1D[4]							
hsCRP, γ_{0a}	−0.008 ± 0.008	−0.015 ± 0.010	+0.004 ± 0.014	0.19	−0.010 ± 0.012	−0.008 ± 0.011	0.90
hsCRP × Time, γ_{1a}	+0.004 ± 0.002*	+0.006 ± 0.002**	+0.001 ± 0.003	0.059	+0.005 ± 0.003*	+0.003 ± 0.002	0.53
Model 2D[4]							
hsCRP, γ_{0a}	−0.008 ± 0.008	−0.015 ± 0.010	+0.003 ± 0.015	0.24	−0.010 ± 0.013	−0.018 ± 0.011	0.88
hsCRP × Time, γ_{1a}	+0.004 ± 0.002*	+0.006 ± 0.002**	+0.001 ± 0.003	0.074	+0.005 ± 0.002*	+0.004 ± 0.002	0.54
Model 1E							
HbA1c, γ_{0a}	+0.026 ± 0.019	+0.032 ± 0.025	+0.015 ± 0.028	0.78	+0.080 ± 0.025**	−0.035 ± 0.027	0.007
HbA1c × Time, γ_{1a}	+0.0078 ± 0.0023**	+0.0098 ± 0.0052*	+0.0061 ± 0.0036	0.35	+0.0084 ± 0.0030**	+0.0068 ± 0.0034*	0.70
Model 2E							
HbA1c, γ_{0a}	+0.030 ± 0.019	+0.030 ± 0.024	+0.021 ± 0.029	0.98	+0.076 ± 0.025**	−0.024 ± 0.028	0.021
HbA1c × Time, γ_{1a}	+0.0076 ± 0.0023**	+0.0099 ± 0.0030**	+0.0055 ± 0.0037	0.30	+0.0088 ± 0.0029**	+0.0051 ± 0.0035	0.60
Model 1F							
CHOL, γ_{0a}	−0.0004 ± 0.0004	+0.0001 ± 0.0005	−0.0011 ± 0.0007	0.13	−0.0004 ± 0.0006	−0.0004 ± 0.0006	0.89
CHOL × Time, γ_{1a}	+0.00014 ± 0.00005**	+0.00006 ± 0.00006	+0.00025 ± 0.00009**	0.069	+0.00016 ± 0.00007*	+0.00012 ± 0.00007	0.66
Model 2F							
CHOL, γ_{0a}	−0.0004 ± 0.0004	+0.0001 ± 0.0005	−0.0011 ± 0.0007	0.17	−0.0004 ± 0.0006	−0.0004 ± 0.0006	0.87
CHOL × Time, γ_{1a}	+0.00014 ± 0.00005**	+0.00006 ± 0.00006	+0.00026 ± 0.00009**	0.060	+0.00017 ± 0.00007*	+0.00014 ± 0.00007	0.69
Model 1G							
HDL-C, γ_{0a}	+0.0034 ± 0.0010**	+0.0029 ± 0.0013*	+0.0037 ± 0.0017*	0.35	+0.0031 ± 0.0019	+0.0034 ± 0.0012**	0.88
HDL-C × Time, γ_{1a}	−0.00017 ± 0.00014	−0.00030 ± 0.00016	−0.00002 ± 0.00023	0.33	−0.00010 ± 0.00024	−0.00018 ± 0.00017	0.80

(Continued)

Adiposity, allostatic load, neurofilament light   541

TABLE 3 (*Continued*)

	Overall (n = 608)	Women (n = 352)	Men (n = 256)	P_{sex}[2]	White adults (n = 257)	African American adults (n = 351)	P_{race}[3]
Model 2G							
HDL-C, γ_{0a}	+0.0030 ± 0.0010**	+0.0025 ± 0.0013	+0.0036 ± 0.0017*	0.54	+0.0030 ± 0.0019	+0.0028 ± 0.0013*	0.89
HDL-C × Time, γ_{1a}	−0.0002 ± 0.0001	−0.00032 ± 0.00007	−0.00001 ± 0.00024	0.31	−0.00019 ± 0.00024	−0.00013 ± 0.00017	0.81
Model 1H							
RHR, γ_{0a}	−0.0017 ± 0.0015	−0.0031 ± 0.0020	−0.00043 ± 0.0024	0.43	−0.00205 ± 0.00231	−0.0018 ± 0.0020	0.99
RHR × Time, γ_{1a}	+0.00087 ± 0.00019***	+0.00077 ± 0.00024**	+0.00096 ± 0.00031**	0.60	+0.00069 ± 0.00028*	+0.00100 ± 0.00026***	0.47
Model 2H							
RHR, γ_{0a}	−0.0019 ± 0.0015	−0.0029 ± 0.0019	−0.0005 ± 0.0024	0.51	−0.00213 ± 0.00230	−0.0017 ± 0.0020	0.89
RHR × Time, γ_{1a}	+0.00087 ± 0.00019***	+0.00081 ± 0.00024**	+0.00096 ± 0.00031**	0.68	+0.00062 ± 0.00028*	+0.0010 ± 0.0002***	0.46
Model 1I							
SBP, γ_{0a}	−0.0033 ± 0.0011**	−0.0037 ± 0.0013**	−0.0023 ± 0.0019	0.67	−0.0052 ± 0.0016**	−0.0013 ± 0.0016	0.023
SBP × Time, γ_{1a}	+0.00040 ± 0.00014**	+0.00019 ± 0.00016	+0.00074 ± 0.00025**	0.062	+0.00024 ± 0.00019	+0.00056 ± 0.00020**	0.36
Model 2I							
SBP, γ_{0a}	−0.0030 ± 0.0011**	−0.0035 ± 0.0013**	−0.0020 ± 0.0010	0.64	−0.0049 ± 0.0016**	−0.0007 ± 0.0016	0.019
SBP × Time, γ_{1a}	+0.00039 ± 0.00014**	+0.0002 ± 0.0002	+0.00070 ± 0.00026**	0.063	+0.00021 ± 0.00019	+0.00053 ± 0.00020**	0.27
Model 1J							
DBP, γ_{0a}	−0.0052 ± 0.0017**	−0.0082 ± 0.0021***	−0.0012 ± 0.0027	0.047	−0.0104 ± 0.0026***	−0.0015 ± 0.002	0.002
DBP × Time, γ_{1a}	+0.0005 ± 0.0002*	+0.0003 ± 0.0003	+0.0007 ± 0.0003*	0.32	+0.0003 ± 0.0003	+0.00063 ± 0.00027*	0.50
Model 2J							
DBP, γ_{0a}	−0.0045 ± 0.0017**	−0.0071 ± 0.0021**	−0.0010 ± 0.0027	0.069	−0.0098 ± 0.0027***	−0.0005 ± 0.0021	0.002
DBP × Time, γ_{1a}	+0.00048 ± 0.00021*	+0.00034 ± 0.00027	+0.00068 ± 0.00034	0.32	+0.0002 ± 0.0003	+0.00056 ± 0.00028*	0.38

[1]Values are fixed effects γ ± SE. Models 1A–1J included each of v_1 AL$_{total}$ and AL$_{comp}$, separately as the main predictor for v_1 NfL and NfL annualized rate of change over time, using a series of mixed-effects linear regression models, carried out in the overall population, and stratified by sex and by race, separately. These models adjusted only for age, sex, race, poverty status, educational attainment, and the inverse Mills ratio. Models 2A–2J followed a similar approach but adjusted further for selected lifestyle and health-related factors, namely current drug use, current tobacco use, Healthy Eating Index-2010, total energy intake, and the Center for Epidemiologic Studies-Depression total score. *P < 0.05; **P < 0.010; ***P < 0.001 for null hypothesis that fixed effect γ = 0. ALB, albumin; AL$_{comp}$, allostatic load continuous components; AL$_{total}$, allostatic load total score; CHOL, total cholesterol; DBP, diastolic blood pressure; HANDLS, Healthy Aging in Neighborhoods of Diversity Across the Life Span; HbA1c, glycated hemoglobin; HDL-C, HDL cholesterol; hsCRP, high-sensitivity C-reactive protein; NfL, neurofilament light chain; RHR, resting heart rate; SBP, systolic blood pressure; v_1, visit 1; v_3, visit 3; WHR, waist:hip ratio.

[2]Based on separate models testing the statistical significance for Sex × AL and Sex × AL × Time in models unstratified by sex or race in which these 2-way and 3-way interaction terms were included for each sociodemographic factor, separately.

[3]Based on separate models testing the statistical significance for Race × AL and Race × AL × Time in models unstratified by sex or race in which these 2-way and 3-way interaction terms were included for each sociodemographic factor, separately.

[4]Log$_e$ transformed. All the continuous predictors, including main exposure variables, were centered at their respective means.
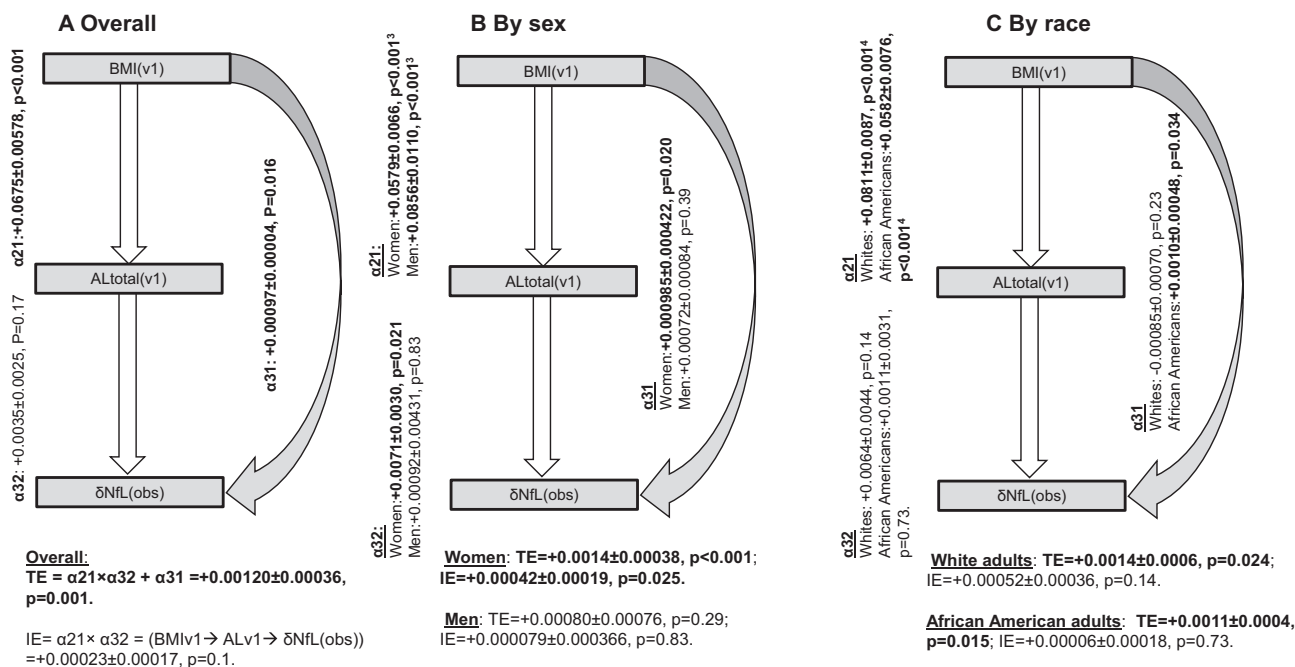
FIGURE 2 BMI at $v_1 \rightarrow$ AL at $v_1 \rightarrow \delta NfL_{obs}$, overall and by sex and race: structural equations model; HANDLS (Healthy Aging in Neighborhoods of Diversity Across the Life Span), 2004–2017. Values are unstandardized path coefficients $\alpha \pm$ SE, TEs, direct effects, and IEs with associated P values. See Table 4 for sample sizes, overall and by strata. Exogenous variables in the models were age, sex, race, poverty status, educational attainment, current drug use, current tobacco use, Healthy Eating Index-2010, total energy intake, the Center for Epidemiologic Studies-Depression total score, and the inverse Mills ratio. [1] P value associated with null hypothesis of no difference in path coefficient α, by sex, using the Wald test (χ^2 test, 1 df) for group invariance. [2] P value associated with null hypothesis of no difference in path coefficient α, by race, using the Wald test (χ^2 test, 1 df) for group invariance. *AL, allostatic load; IE, indirect effect; TE, total effect; v_1, visit 1; δNfL_{obs}, observed annualized rate of change in plasma neurofilament light chain between visit 1 and visit 3.

with higher plasma NfL at baseline and faster increase in plasma NfL among White adults in both the reduced and full models ($P < 0.010$ for HbA1c main effect and HbA1c × Time), with $P = 0.007$ for the HbA1c × Race parameter. Other notable findings included an inverse relation between v_1 WHR and v_1 plasma NfL among men, with the reverse found in women and among African American adults and no association detected among White adults. In contrast, inverse associations of v_1 SBP and/or DBP with v_1 plasma NfL were mostly detected among White adults. Moreover, v_1 serum ALB was inversely linked with plasma NfL at v_1 among men, an association not detected in women ($P < 0.01$ for ALB × sex).

hsCRP, cholesterol, and RHR at v_1 were among the AL_{comp} associated with faster increase in plasma NfL over time in the total sample, whereas they were uncorrelated with baseline plasma NfL. In contrast, SBP and DBP had different associations with baseline NfL compared with δNfL, suggesting that both measures were inversely related to first-visit NfL as well as being associated with faster increase in NfL over time. Finally, higher HDL cholesterol at v_1 was associated with higher v_1 plasma NfL, in the total sample.

AL_{total} and AL_{comp} as a mediator between BMI and annualized change in plasma NfL

Figure 2 shows the results from a structural equations model in which AL_{total} at v_1 was tested as a potential mediator between BMI at v_1 and annualized change in plasma NfL. Our results suggested that the TE of BMI at v_1 on δNfL_{obs} indicated a positive association between the 2 factors in the total sample (TE >0, $P < 0.05$). This was also the case among women. However, only among women the indirect

effect (IE) indicated a large portion of this TE was mediated through AL_{total}, with an estimated mediation proportion of 30% (TE $= +0.0014 \pm 0.00038$, $P < 0.001$; IE $= +0.00042 \pm 0.00019$, $P = 0.025$). In other groups and overall, TE was statistically significant mostly as a direct effect (DE) and there was no significant IE through AL_{total}. Specifically, among African American adults, most of the TE was a DE (TE $= +0.0011 \pm 0.0004$, $P = 0.015$; DE $= +0.0010 \pm 0.00048$, $P = 0.034$).

AL_{comp} as mediators between AL_{total} and annualized change in plasma NfL

Table 4 presents results from the structural equation model testing DEs and IEs of AL_{total} on δNfL_{obs} through alternative AL_{comp}, while stratifying by sex and by race groups. Focusing on models with significant TEs, the IE was statistically significant only for HbA1c as the primary mediating factor in the total sample (IE $= +0.0031 \pm 0.0012$, $P < 0.05$; TE $= +0.0062 \pm 0.0023$, $P < 0.05$; mediation proportion: 50%). Most other models with significant TEs (e.g., WHR, hsCRP, total cholesterol, SBP, DBP) of AL_{total} on δNfL_{obs} indicated that another pathway was at play not including each of those AL_{comp}.

Supplemental Table 2 tests similar mediating effects but replacing AL_{total} with AL_{comp} while examining them overall and by race and sex. The results indicate that HbA1c, overall and among women, is also among the main mediators in the relation between BMI and δNfL_{obs}, as was the case for the AL_{total}–δNfL_{obs} association. Specifically, a TE of $+0.00140 \pm 0.0004$ ($P < 0.001$) was detected among women, of which $+0.00023 \pm 0.00011$ ($P = 0.028$) was explained by the IE of HbA1c at baseline (or a mediation proportion

TABLE 4 AL_{total} (at v_1) → AL_{comp} (at v_1) → δNfL_{obs}, overall and by sex and race: structural equations model; HANDLS, 2004–2017[1]

	Overall (n = 606)	Women (n = 351)	P_{sex}[2]	Men (n = 255)	White adults (n = 255)	African American adults (n = 351)	P_{race}[3]
WHR							
$AL_{total}→WHR$	+0.0411 ± 0.0185*	+0.0560 ± 0.0333	0.39	+0.0273 ± 0.0027***	+0.0600 ± 0.0439	+0.0277 ± 0.0026***	0.46
$WHR→\delta NfL_{obs}$	+0.0054 ± 0.0051	+0.0038 ± 0.0045	0.032	+0.1989 ± 0.0908*	+0.0045 ± 0.0054	+0.0848 ± 0.0592	0.18
$AL_{total}→\delta NfL_{obs}$	+0.0059 ± 0.0023*	+0.0098 ± 0.0028***	0.012	−0.0037 ± 0.0046	+0.0089 ± 0.0038*	+0.0013 ± 0.0033	0.13
TE (AL_{total})	+0.0062 ± 0.0023**	+0.0101 ± 0.0028***	—	+0.0018 ± 0.0040	+0.0092 ± 0.0038*	+0.0036 ± 0.0029	—
IE (AL_{total})	+0.0002 ± 0.0002	−0.0002 ± 0.0003	—	+0.0054 ± 0.0025*	+0.0003 ± 0.0004	+0.0023 ± 0.0017	—
ALB							
$AL_{total}→ALB$	−0.0368 ± 0.0093***	−0.0488 ± 0.0126***	0.099	−0.0179 ± 0.0138	−0.0391 ± 0.0140**	−0.0394 ± 0.0126**	0.99
$ALB→\delta NfL_{obs}$	−0.0034 ± 0.0101	−0.0057 ± 0.0118	0.47	+0.0098 ± 0.0178	−0.0311 ± 0.0169	+0.0140 ± 0.0124	0.031
$AL_{total}→\delta NfL_{obs}$	+0.0060 ± 0.0023*	+0.0098 ± 0.0029**	0.11	+0.0019 ± 0.0040	+0.0079 ± 0.0038*	+0.0041 ± 0.0030	0.43
TE (AL_{total})	+0.0062 ± 0.0023	+0.0101 ± 0.0028***	—	+0.0018 ± 0.0040	+0.0092 ± 0.0038*	+0.0036 ± 0.0029	—
IE (AL_{total})	+0.0001 ± 0.0004	+0.0003 ± 0.0006	—	−0.0002 ± 0.0003	+0.0012 ± 0.0008	−0.0006 ± 0.0005	—
hsCRP[4]							
$AL_{total}→hsCRP$	+0.5602 ± 0.0400***	+0.6194 ± 0.0534***	0.12	+0.4928 ± 0.0611***	+0.5687 ± 0.0524***	+0.5377 ± 0.0580***	0.69
$hsCRP→\delta NfL_{obs}$	−0.0009 ± 0.0024	+0.0018 ± 0.0028	0.13	−0.0055 ± 0.0040	−0.0011 ± 0.0045	−0.001 ± 0.0027	0.98
$AL_{total}→\delta NfL_{obs}$	+0.0067 ± 0.0027*	+0.0090 ± 0.0033**	0.41	+0.0045 ± 0.0044	+0.0098 ± 0.0046*	+0.0041 ± 0.0033	0.31
TE (AL_{total})	+0.0062 ± 0.0023**	+0.0101 ± 0.0028***	—	+0.0017 ± 0.0040	+0.0092 ± 0.0038	+0.0036 ± 0.0029	—
IE (AL_{total})	−0.0005 ± 0.0013	+0.0011 ± 0.0017	—	−0.0027 ± 0.0020	−0.0006 ± 0.0026	−0.0005 ± 0.0014	—
HbA1c							
$AL_{total}→HbA1c$	+0.3657 ± 0.0285***	+0.3922 ± 0.0347***	0.52	+0.3544 ± 0.0479***	+0.4169 ± 0.0490***	+0.3200 ± 0.0338***	0.10
$HbA1c→\delta NfL_{obs}$	+0.0085 ± 0.0033**	+0.0072 ± 0.0043	0.71	+0.0098 ± 0.0051	+0.0074 ± 0.0048	+0.0095 ± 0.0046*	0.76
$AL_{total}→\delta NfL_{obs}$	+0.0030 ± 0.0026	−0.0073 ± 0.0032*	0.10	−0.0017 ± 0.0043	+0.0061 ± 0.0043	+0.0006 ± 0.0033	0.31
TE (AL_{total})	+0.0062 ± 0.0023*	+0.0101 ± 0.0028***	—	+0.0018 ± 0.0040	+0.0092 ± 0.0038	+0.0036 ± 0.0029	—
IE (AL_{total})	+0.0031 ± 0.0012*	+0.0028 ± 0.0017	—	+0.0035 ± 0.0019	+0.0031 ± 0.0020	+0.0030 ± 0.0015*	—
CHOL							
$AL_{total}→CHOL$	+4.4555 ± 1.4354**	+2.4277 ± 1.9497	0.079	+7.4950 ± 2.1194***	+5.8848 ± 2.3200*	+3.8843 ± 1.8562*	0.50
$CHOL→\delta NfL_{obs}$	+0.0000 ± 0.0001	+0.0000 ± 0.0001	0.86	+0.0000 ± 0.0001	−0.0001 ± 0.0001	+0.0001 ± 0.0001	0.23
$AL_{total}→\delta NfL_{obs}$	+0.0061 ± 0.0023**	+0.0100 ± 0.0028***	0.088	+0.0016 ± 0.0040	+0.0095 ± 0.0038*	+0.0032 ± 0.0029	0.19
TE (AL_{total})	+0.0062 ± 0.0023**	+0.0101 ± 0.0028	—	+0.0017 ± 0.0040	+0.0092 ± 0.0038	+0.0036 ± 0.0029	—
IE (AL_{total})	+0.0001 ± 0.0003	+0.0001 ± 0.0002	—	+0.0002 ± 0.0009	−0.0003 ± 0.0006	+0.0004 ± 0.0004	—
HDL-C							
$AL_{total}→HDL-C$	−5.7701 ± 0.5346***	−5.3073 ± 0.7133***	0.51	−6.0073 ± 0.8018***	−4.9331 ± 0.6840	−6.2943 ± 0.7649	0.18
$HDL-C→\delta NfL_{obs}$	−0.0001 ± 0.0002	−0.0001 ± 0.0002	0.96	−0.0001 ± 0.0003	−0.0002 ± 0.0003	+0.0000 ± 0.0002	0.35
$AL_{total}→\delta NfL_{obs}$	+0.0058 ± 0.0025*	+0.0097 ± 0.0030**	0.12	+0.0014 ± 0.0044	+0.0084 ± 0.0042*	+0.0035 ± 0.0032	0.72
TE (AL_{total})	+0.0062 ± 0.0023	+0.0101 ± 0.0028***	—	+0.0017 ± 0.0040	+0.0092 ± 0.0038	+0.0036 ± 0.0029	—
IE (AL_{total})	+0.0004 ± 0.0010	+0.0004 ± 0.0011	—	+0.0004 ± 0.0019	+0.0008 ± 0.0017	+0.0001 ± 0.0013	—

(Continued)

TABLE 4 (Continued)

	Overall (n = 606)	Women (n = 351)	Men (n = 255)	P_{sex}[2]	White adults (n = 255)	African American adults (n = 351)	P_{race}[3]
RHR							
AL$_{total}$→RHR	+2.7833 ± 0.3786***	+2.7503 ± 0.4903***	+3.1124 ± 0.5822***	0.63	+3.0838 ± 0.5950***	+2.7980 ± 0.4917***	0.71
RHR→δNfL$_{obs}$	+0.0003 ± 0.0002	+0.0003 ± 0.0003	+0.0004 ± 0.0004	0.85	+0.0001 ± 0.0004	+0.0005 ± 0.0003	0.48
AL$_{total}$→δNfL$_{obs}$	+0.0052 ± 0.0024*	+0.0093 ± 0.0029**	+0.0005 ± 0.0042	0.085	+0.0088 ± 0.0040*	+0.0022 ± 0.0030	0.19
TE (AL$_{total}$)	+0.0062 ± 0.0023**	+0.0101 ± 0.0028**	+0.0018 ± 0.0040	—	+0.0092 ± 0.0038*	+0.0036 ± 0.0029	—
IE (AL$_{total}$)	+0.0010 ± 0.0007	+0.0008 ± 0.0008	+0.0012 ± 0.0013	—	+0.0004 ± 0.0012	+0.0014 ± 0.0009	—
SBP							
AL$_{total}$→SBP	+5.5879 ± 0.4957***	+6.0208 ± 0.6872***	+4.8958 ± 0.7195***	0.26	+6.0278 ± 0.8259***	+5.2905 ± 0.6207***	0.48
SBP→δNfL$_{obs}$	+0.0045 ± 0.0025	+0.0002 ± 0.0002	+0.0004 ± 0.0003	0.54	+0.0003 ± 0.0003	+0.0003 ± 0.0003	0.91
AL$_{total}$→δNfL$_{obss}$	+0.0003 ± 0.0002	+0.0091 ± 0.0031**	−0.0003 ± 0.0043	0.076	+0.0076 ± 0.0042	+0.0020 ± 0.0032	0.28
TE (AL$_{total}$)	+0.0062 ± 0.0023*	+0.0101 ± 0.0028***	+0.0017 ± 0.0040	—	+0.0092 ± 0.0038*	+0.0036 ± 0.0029	—
IE (AL$_{total}$)	+0.0016 ± 0.0011	+0.0010 ± 0.0013	+0.0020 ± 0.0017	—	+0.0016 ± 0.0017	+0.0016 ± 0.0013	—
DBP							
AL$_{total}$→DBP	+3.1337 ± 0.3384***	+3.2288 ± 0.4412***	+2.9112 ± 0.5342***	0.65	+2.7516 ± 0.5047***	+3.4213 ± 0.4603***	0.33
DBP→δNfL$_{obs}$	+0.0001 ± 0.0003	−0.0001 ± 0.0003	+0.0003 ± 0.0005	0.41	+0.0000 ± 0.0005	+0.0001 ± 0.0003	0.90
AL$_{total}$→δNfL$_{obs}$	+0.0059 ± 0.0025*	+0.0105 ± 0.0030***	+0.0008 ± 0.0042	0.057	+0.0091 ± 0.0040*	+0.0032 ± 0.0031	0.25
TE (AL$_{total}$)	+0.0062 ± 0.0023**	+0.0101 ± 0.0028***	+0.0017 ± 0.0040	—	+0.0092 ± 0.0038*	+0.0036 ± 0.0029	—
IE (AL$_{total}$)	+0.0003 ± 0.0009	−0.0004 ± 0.0011	+0.0010 ± 0.0014	—	+0.0001 ± 0.0013	+0.0004 ± 0.0012	—

[1] Exogenous variables in the models were age, sex, race, poverty status, educational attainment, current drug use, current tobacco use, Healthy Eating Index-2010, total energy intake, the Center for Epidemiologic Studies-Depression total score, and the inverse Mills ratio. * $P < 0.05$; ** $P < 0.010$; *** $P < 0.001$ for null hypothesis that path coefficient $\alpha = 0$. ALB, albumin; AL$_{comp}$, allostatic load continuous components; AL$_{total}$, allostatic load total score; CHOL, total cholesterol; DBP, diastolic blood pressure; HANDLS, Healthy Aging in Neighborhoods of Diversity Across the Life Span; HbA1c, glycated hemoglobin; HDL-C, HDL cholesterol; hsCRP, high-sensitivity C-reactive protein; IE, indirect effect; RHR, resting heart rate; SBP, systolic blood pressure; TE, total effect; v$_1$, visit 1; WHR, waist:hip ratio; δNfL$_{obs}$, annualized rate of change in plasma neurofilament light chain between visit 1 and visit 3.

[2] P value associated with null hypothesis of no difference in path coefficient α, by sex, using the Wald test (χ^2 test, 1 df) for group invariance.

[3] P value associated with null hypothesis of no difference in path coefficient α, by race, using the Wald test (χ^2 test, 1 df) for group invariance.

[4] Log$_e$ transformed.

Adiposity, allostatic load, neurofilament light   545

of 16.4%). A similar pattern was observed overall with a mediation proportion ~19%. There were no significant IEs of HbA1c detected among men, white, or African American adults in the association between BMI and δNfL_{obs}.

Discussion

This study is the first that we know of to examine associations comprehensively and longitudinally between cardiometabolic risk factors and plasma NfL, particularly in a racially diverse community-based sample of middle-aged urban adults. Among our key findings, BMI and AL_{total} were associated with lower initial but faster increase in plasma NfL over time. hsCRP, serum total cholesterol, and RHR at v_1 were linked with faster increase in plasma NfL over time overall. In SM analyses, the association of BMI with δNfL was significantly mediated through AL_{total} among women and overall was mediated through HbA1c concentrations.

Previous studies
Plasma NfL and its association with neurocognitive outcomes.
NfL has been posited as a biomarker of neuronal injury and recently the development of sensitive and accurate methods to measure plasma NfL has led to the examination of whether this noninvasive biomarker may be an indicator of neurodegeneration. Cross-sectional studies have reported that plasma NfL is elevated in patients with mild cognitive impairment (MCI) and AD and that these concentrations correlate with other neurocognitive measures (15). Individuals with mild cognitive impairment or with AD dementia had higher baseline plasma NfL and longitudinal analyses showed faster rates of NfL were correlated with rates of cognitive and imaging measures as well as CSF biomarker concentrations (2). In fact, serum/plasma NfL predicts future development of sporadic and familial AD and is associated with faster cognitive decline and also with brain structure alterations (3, 4, 40, 42). However, plasma NfL was associated with changes in brain white matter and AD but not with preclinical phases of AD in another study (43). In nondemented adults, plasma NfL also was associated with cognitive decline (45, 61). These data are indicative that plasma NfL may have value in monitoring neurodegenerative disease progression. Furthermore, these studies point to plasma NfL as an easily accessible biomarker that shows promise for delineating early neurodegeneration in the presymptomatic stages of AD.

Cardiometabolic risk and its association with neurocognitive outcomes.
The association of obesity and other cardiometabolic factors with dementia is complex. These complexities lie in when during the life span these factors are considered and the fact that they are often accompanied by a myriad of risk factors. For example, midlife obesity is associated with a significant risk of dementia and structural brain changes (27, 32). However, closer in time to the onset of disease (~5–10 y) low BMI is associated with increased dementia risk, possibly owing to behavioral changes that accompany dementia including reduced physical activity and caloric intake (62, 63). Our data agree with the association of midlife obesity with dementia risk because BMI was associated with a faster increase of plasma NfL over time. AL_{total} was also associated with a faster increase in plasma NfL over time, indicating that other cardiometabolic risk factors

may also influence dementia risk. Cardiovascular disease risk factors including hsCRP, serum total cholesterol, and RHR at v_1 were components of AL_{total} that were linked to higher NfL longitudinally. Vascular disorders, such as hypertension, also have complex risk associations with dementia. Evidence indicates that hypertension at midlife increases risk of dementia but is protective or not a significant risk factor for the elderly (>80 y old) (31, 33, 64). Taken together, modifiable cardiometabolic risk factors at midlife may have long-term consequences that affect dementia risk and may be amenable to interventions for at-risk populations.

Cardiometabolic risk, adiposity, and their association with plasma NfL.
Plasma NfL and CSF NfL concentrations increase with age. Aging is associated with chronic health conditions, in particular cardiometabolic risk. Therefore, cardiometabolic risk and other age-associated disease processes may be potential explanations for the observed increases in NfL over the lifetime. Despite this, few studies have examined cardiometabolic risk factors and plasma NfL and findings are contrary to expectations, which demonstrates the need for further research. For instance, recent studies suggest that there is an inverse relation between BMI and plasma NfL in healthy samples (17) and several clinical samples, including individuals with type 2 diabetes (18), multiple sclerosis (17), and women with anorexia (65, 66). However, a few studies found no relation between BMI and plasma NfL in healthy controls (18, 66). Furthermore, 1 study examining renal function, another aspect of cardiometabolic risk, found a significant, positive correlation between serum creatinine concentrations and plasma NfL in nondemented samples aged 60 y or older in both healthy controls and patients with diabetes (18). Finally, another study examining glucose metabolism, yet another aspect of cardiometabolic risk, found that, in patients with type 1 diabetes, those with more frequent and severe hypoglycemic episodes had significantly higher plasma NfL than did those with less frequent and less severe hypoglycemic episodes (67). Notably, the plasma NfL concentrations did not differ between healthy controls and patients with type 1 diabetes who had fewer and less severe hypoglycemic episodes. This, in tandem with the fact that both type 1 diabetes groups showed no differences in their cardiometabolic profiles, suggests that hypoglycemia, in particular, is associated with plasma NfL, which may indicate neuronal damage.

Given the paucity of research in cardiometabolic risk and NfL, several of our study findings are novel. Our findings indicate that AL mediates the association between BMI and the rate of change in plasma NfL only among women. Thus, plasma NfL increase over time is determined by BMI in women, a relation largely explained by the multimorbidity index of AL_{total}, reflecting cardiometabolic risk. In contrast, among African American adults, the putative effect of BMI on rate of change in plasma NfL is largely a DE perhaps explained by other factors associated with global adiposity that are not part of the multimorbidity index of AL_{total}. Sex and race differences in mediating effects of AL_{total} in the BMI–δNfL_{obs} relation may be explained by the possible inadequacy of the summary score of AL in some subgroups as opposed to the AL_{comp}. Our additional analyses indicated that, overall, HbA1c is the most likely mediator in the relation between BMI and change in plasma NfL over time, given the observed significant TEs and IEs, suggesting that the adverse potential effect of BMI on NfL

over time is at least in part explained by co-occurrence of an elevated HbA1c with elevated BMI.

Moreover, HbA1c was the component of AL that explained its TE on rate of change in NfL reflecting glucose metabolism disorders. This AL_{comp} consistently explained the association overall between BMI and rate of change in NfL, with the mediation mostly detected among women. Finally, measures of inflammation, lipid metabolism, and hemodynamics were all related to increased plasma NfL over time in the total sample, without affecting baseline values of plasma NfL. This was not the case for SBP and DBP, which were associated with lower NfL at first visit, as well as having a direct relation with increase in NfL over time. This suggests that in this population of urban middle-aged adults, these 3 components of AL may have utility in predicting the pace of increase in plasma NfL over time, independently of its initial value.

Strengths and limitations

Our study has several notable strengths. First, this study has included adequate numbers of African American adults to power subset analysis, which is critical for the field to move forward and examine the differential risk of dementia among African American adults. As far as we know, it is the first study to examine these important research questions on the association between cardiometabolic risk and plasma NfL. This suggests cardiometabolic risk may be important to consider when examining the clinical utility of plasma NfL for predicting neurocognitive outcomes. In addition, plasma NfL may be on a pathway through which cardiometabolic risk can influence neurocognitive outcomes. Second, we ascertained temporality of association with a longitudinal study design examining baseline exposures against change in outcomes over time. Third, the sample size is large and adequately powered to test those associations across sex and race groups. Fourth, advanced statistical techniques were used, including multiple linear mixed-effects regression and structural equation models, to test those associations and their heterogeneity across sex and race, while adjusting for key potential confounders and for sample selectivity using 2-stage Heckman selection.

Nevertheless, our study has ≥1 notable limitation, which is the relatively young age of our sample, leading to a low baseline plasma NfL compared with previous studies with older participants. Thus, the rate of increase in plasma NfL may have occurred at a slower pace than in older adults. Many studies have indicated that increased risk of adverse cognitive outcomes later in life is a function of cardiometabolic and related lifestyle risk factors at midlife (27, 28). Therefore, our study shows that a midlife putative marker of neurodegeneration may in fact be longitudinally associated with cardiometabolic risk among middle-aged adults.

Conclusions

In conclusion, we report an association between cardiometabolic risk and an increase over time in plasma NfL. The association of BMI with δNfL was mediated through AL_{total} in women and was mainly explained by elevations in HbA1c. Given that NfL may be a pathway through which cardiometabolic risk can lead to neurodegeneration, prevention efforts aimed at reducing plasma NfL should target cardiometabolic risk factors, particularly reduction of HbA1c concentrations.

Data Availability

Upon request, data can be made available to researchers with approved proposals, after they have agreed to confidentiality as required by our Institutional Review Board. Policies are publicized on https://handls.nih.gov. Data access requests can be sent to principal investigators (PIs) 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 2 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." Code book and statistical analysis script can be readily obtained from the corresponding author, upon request, by e-mail contact at baydounm@mail.nih.gov.

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Body mass index and allostatic load are directly associated with longitudinal increase in plasma neurofilament light among urban middle-aged adults

By Beydoun, M. A. et al.

Supplementary Method 1: NfL sample selection

Plasma NfL was quantified in a sub-cohort of participants from HANDLS from visits v_1 (2004-2009), v_2 (2009-2013) and v_3 (2013-2018), from which we extracted data from only v_1 and v_2 for our present study. This sub-sample included participants from the HANDLS SCAN, an ancillary neuroimaging sub-study, (n=238)(1) This sub-study of the HANDLS cohort excluded participants with a history of dementia, stroke, transient ischemic attack, and carotid endarterectomy, MRI contraindications, terminal illness, HIV positivity or other neurological disorders (1). All HANDLS SCAN participants included in this sub-study had donated plasma samples at three different visits except for one participant that had samples from only 2 of 3 visits. In addition, we also included participants (n=463; 1389 samples) that donated plasma samples at v_1, v_2 and v_3, who were HIV negative, had complete cognitive tests [Trailmaking test, part A (TRAILS A) and Digits Span-Forward (DS-F)[at v_1 and v_2, Centers of Epidemiologic Studies-Depression (CES-D) scores at all 3 visits and with no history of HIV, stroke, transient ischemic attack, dementia, epilepsy, Parkinson's disease or brain cancer. Participants (n=3) were also included who had plasma samples available from v_1, v_2 and v_3, who also had genome wide DNA methylation data at v_1(2-4). These participants had the exclusions listed above. Thus, overall, N=694 HANDLS participants had plasma NfL data at v_1 ; N=709 at v_2 and N=707.

Supplementary Table 1. Allostatic load indicator criteria(5).

	High-risk clinical
Waist:Hip	>0.9 for men; > 0.85 for women (6)
Albumin (g/dL)	< 3.8 (7)
C-reactive protein (mg/dL)	≥ 0.3 (8)
Total cholesterol (mg/dL)	≥240(9)
HDL (mg/dL)	<40(9)
Glycated hemoglobin (%)	≥6.4(10, 11)
Resting heart rate (beat/min)	≥90(12)
Systolic BP	≥140(13)
Diastolic BP	≥90(13)

Supplementary method 2: Mixed-effects regression models

The main multiple mixed-effects regression models can be summarized as follows:

Multi-level models   vs. **Composite models**

Eq. 1.1-1.4

$$Y_{ij} = \pi_{0i} + \pi_{1i}Time_{ij} + \varepsilon_{ij}$$

$$\pi_{0i} = \gamma_{00} + \gamma_{0a}X_{aij} + \sum_{k=1}^{l}\gamma_{0k}Z_{ik} + \zeta_{0i}$$

$$\pi_{1i} = \gamma_{10} + \gamma_{1a}X_{aij} + \sum_{m=1}^{n}\gamma_{1m}Z_{im} + \zeta_{1i}$$

$$Y_{ij} = \gamma_{00} + \gamma_{0a}X_{aij} + \sum_{k=1}^{l}\gamma_{0k}Z_{ik}$$
$$+ \gamma_{10}Time_{ij} + \gamma_{1a}X_{aij}Time_{ij}$$
$$+ \sum_{m=1}^{n}\gamma_{1m}Z_{im}Time_{ij}$$
$$+ (\zeta_{0i} + \zeta_{1i}Time_{ij} + \varepsilon_{ij})$$

Where Y_{ij} is the outcome (plasma NfL measured at v_1, v_2 and/or v_3) for each individual "i" and visit "j"; π_{0i} is the level-1 intercept for individual i; π_{1i} is the level-1 slope for individual i; γ_{00} is the level-2 intercept of the random intercept π_{0i}; γ_{10} is the level-2 intercept of the slope π_{1i}; Z_{ik} is a vector of fixed covariates for each individual *i* that are

used to predict level-1 intercepts and slopes, which can include socio-demographic variables among others. In this analysis, mixed-effects regression models included AL total score exposure measured at v_1 (X_{ij}) along with covariates (Z_{ij}). ζ_{0i} and ζ_{1i} are level-2 disturbances; ε_{ij} is the within-person level-1 disturbance (14).

It is worth noting that the models were fit using the entire HANDLS cohort with complete data on either v_1, v_2 or v_3 on NfL was used to improve reliability of predicted estimates. Empirical bayes estimators for annual rate of change in NfL (δNfL) were also predicted from time-interval mixed-effects models, with up to 3 repeats on plasma NfL as the outcome and covariates between v_1 Age, sex, race and poverty status. This estimate was used as a validation tool, against observed annualized change in NfL between those 3 visits. The latter was computed as the arithmetic mean in the annualized changes of Log_e transformed NfL between v1 and v2; v2 and v3; and v1 and v3. The individual-level observed annualized rate of change (δNfL$_{obs}$) depended on 1, 2 or 3 values of annualized changes and thus there was no additional missing data for this estimate. δNfL$_{obs}$ was used in structural equations models described in **Supplementary Method 3**. The scatter plot of δNfLobs and δNfLbayes is shown in **Supplementary Figure 1**.

Supplementary Figure 1. Observed vs. empirical bayes estimator for annualized rate of change in Log_e transformed NfL, Pearson's r=0.83, p<0.001
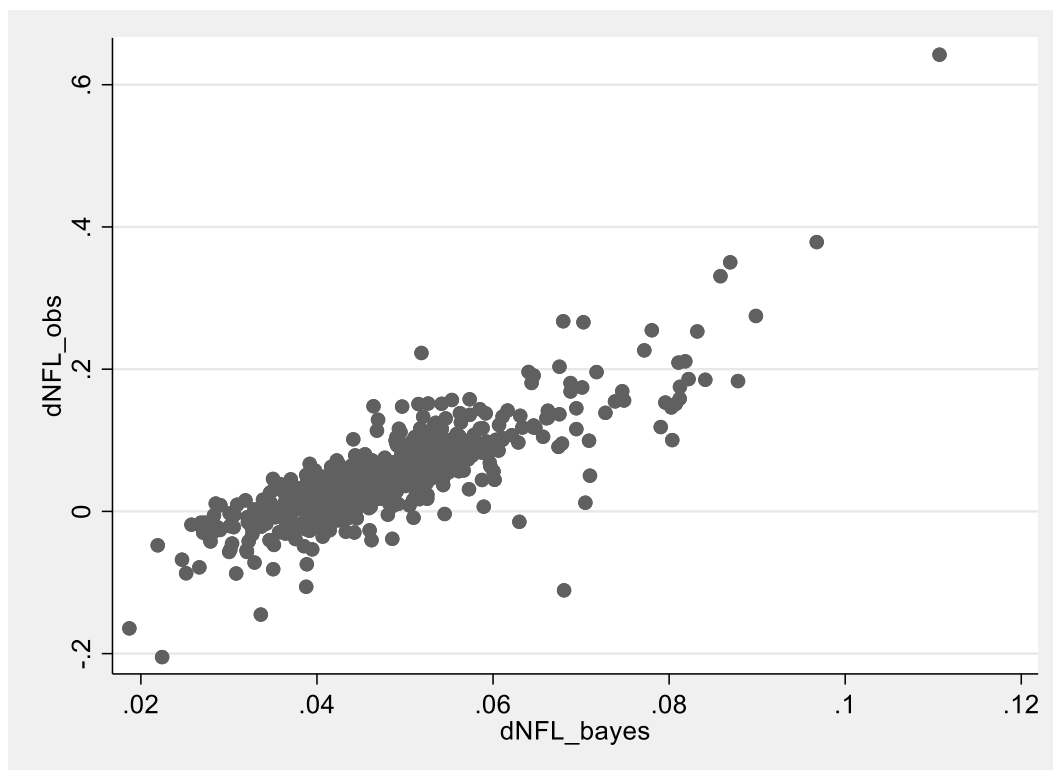
Supplementary method 3: Structural Equations Models

Total, direct and indirect effects with 95%CI and p-values were estimated in the overall population, as well as the individual sex and race strata. Invariance in the path coefficient parameters was also tested across sex and across race groups, using a Wald (χ^2) test with 1 d.f.. Stratified structural equations models (SM) by sex or race, allowed to test those pathways for each group separately. Model fit indices were also examined for the overall sample models. The general structure of the models were as follows:

$$X(v1) = \sum_{i=1}^{k} \alpha_{Z_{i_{1i}}} Z_i + e_1 \qquad (1)$$

$$M(v1) = \alpha_{21} \ X(v1) + \sum_{i=1}^{k} \alpha_{Z_{i_2}} Z_i + e_2 \qquad (2)$$

$$Y = \alpha_{31} \ X(v1) \ + \alpha_{32} M(v1) + \sum_{i=1}^{k} \alpha_{Z_{i_3}} Z_i + e_3 \qquad (3)$$

Where $X(v_1)$ is visit 1 exposure, $M(v_1)$ is visit 1 mediator and Y is the main longitudinal outcomes, namely annualized rate of change in NfL (δNfL_{obs}). In the first set of SM models, $X(v_1)$ was BMI at v_1, while $M(v_1)$ was AL_{total} at v_1. In the second set of SM models, $X(v_1)$ was AL_{total} at v_1, while $M(v_1)$ were the 9 alternative continuous components of AL at v_1, Z_i is a vector exogenous socio-demographic, lifestyle or health-related covariates (either fixed or measured at v_1; See Covariates section) that are allowed to predict X, M and Y, i is the number of covariate terms. Based on Equations (1)–(3): α_{31} = direct effect; $\alpha_{21} \times \alpha_{32}$ = indirect effect; total effect = $\alpha_{31} + \alpha_{21} \times \alpha_{32}$. Mediation was determined by the statistical significance and direction of the indirect effect at a type I error of 0.05 and was interpreted based on the direction of that indirect effect (+ or −). It is expected that BMI would have a direct relationship with AL_{total}, which would have a direct association with rate of change in NfL. In contrast, the relationship between AL_{total} and AL_{comp} depends on the criteria used to define AL_{total}. For instance, WHR, SBP, DBP, HbA1c, CRP, CHOL and HR are all expected to be directly associated with AL_{total}, whereas ALB and HDL are expected to be inversely related to AL_{total}. Nevertheless, a high risk AL_{comp} is hypothesized to be associated with faster increase in plasma NfL over time, and to be mediating the association between AL_{total}

and δNfL_{obs}. Thus, in both sets of SM models, the indirect effect is hypothesized to be positive and statistically significant, either in the total population or within strata.

Supplementary Table 2. BMI (at v_1) → AL_{comp} (at v_1) → annualized change in NfL between v_1 and v_3 (δNfL_{obs}), overall and by sex and race: Structural equations model; HANDLS, 2004-2017[4]

	Overall (n =606)	Women (n =351)	Men (n =255)	P_{sex} [1]	Whites (n =255)	African Americans (n =351)	P_{race} [2]
WHR							
BMI→WHR	+0.0012±0.0029	-0.0004±0.0050	**+0.0059±0.0005*****	0.17	-0.0013±0.007	**+0.0027±0.0004*****	0.57
WHR→δNfL$_{obs}$	+0.0062±0.0050	+0.0054±0.0044	+0.1708±0.094	0.078	+0.0057±0.0053	+0.064±0.054	0.54
BMI → δNfL$_{obs}$	**+0.0012±0.0004****	**+0.0014±0.0003*****	-0.0002±0.0009	0.11	**+0.0013±0.0006***	+0.0009±0.0005	0.54
Total effect (BMI)	**+0.0012±0.004****	**+0.0014±0.0004*****	+0.0008±0.0008	---	**+0.0014±0.0006***	**+0.0011±0.0004***	---
Indirect effect (BMI)	+0.0000±0.0000	-0.0000±0.0000	+0.0010±0.0006	---	-0.0000±0.0000	+0.00017±0.00015	---
ALB							
BMI →ALB	**-0.0104±0.0014*****	-0.0122±0.0016	-0.0048±0.0027	0.50	**-0.0113±0.0021*****	**-0.0102±0.0019*****	0.70
ALB→δNfL$_{obs}$	+0.0020±0.010	+0.0001±0.012	+0.0064±0.0176	0.80	-0.0281±0.0175	+0.0209±0.0126	0.023
BMI → δNfL$_{obs}$	**+0.0012±0.0004****	**+0.0014±0.0004****	+0.0008±0.0008	0.019	+0.0011±0.0006	**+0.0013±0.0005****	0.76
Total effect (BMI)	**+0.0012±0.0004****	**+0.0014±0.0003*****	+0.0008±0.0008	---	**+0.0013±0.0006***	**+0.0011±0.0005***	---
Indirect effect (BMI)	-0.0000±0.0001	-0.0000±0.0002	-0.0000±0.0001	---	+0.0003±0.0002	-0.0002±0.0001	---
hsCRP [3]							
BMI →hsCRP	**+0.0651±0.0068*****	**+0.0796±0.008*****	**+0.0288±0.0133***	0.001	**+0.078±0.009*****	**+0.0530±0.0096*****	0.052
hsCRP→δNfL$_{obs}$	-0.0005±0.0022	+0.0023±0.0027	-0.0045±0.0036	0.13	+0.0003±0.0042	-0.0012±0.0025	0.76
BMI → δNfL$_{obs}$	**+0.0012±0.0003****	**+0.0012±0.0004****	+0.0009±0.0008	0.74	+0.0014±0.0007	**+0.0012±0.0005***	0.82
Total effect (BMI)	**+0.0012±0.0004****	**+0.0014±0.0004*****	+0.0008±0.0008	---	**+0.0014±0.0006***	**+0.0010±0.0004***	---
Indirect effect (BMI)	-0.0000±0.0001	+0.0002±0.0002	-0.0001±0.0001	---	+0.0000±0.0003	-0.0001±0.0001	---
HbA1C							
BMI →HbA1C	**+0.0312±0.005*****	**+0.0248±0.005*****	**+0.0486±0.009*****	0.035	**+0.0389±0.0086*****	**+0.0256±0.0057*****	0.20
HbA1C→δNfL$_{obs}$	**+0.0084±0.0030****	**+0.0094±0.0038***	+0.0083±0.0047	0.86	+0.0087±0.0044	+0.0079±0.0042	0.85
BMI → δNfL$_{obs}$	**+0.0010±0.0004***	**+0.00012±0.0004****	+0.00040±0.0007	0.38	+0.0010±0.0006	+0.0009±0.0005	0.91
Total effect (BMI)	**+0.0012±0.0004****	**+0.0014±0.0004*****	+0.00080±0.00080	---	**+0.0014±0.0006***	**+0.0011±0.0004***	---
Indirect effect (BMI)	**+0.00026±0.00010***	**+0.00023±0.00011***	+0.00040±0.00025	---	+0.00034±0.00019	+0.00020±0.00012	---
CHOL							
BMI →CHOL	-0.225±0.228	-0.301±0.269	+0.069±0.426	0.46	+0.106±0.378	-0.475±0.287	0.22
CHOL→δNfL$_{obs}$	+0.00005±0.00006	+0.00008±0.00008	+0.00003±0.00011	0.72	-0.00002±0.00010	+0.00013±0.00008	0.25
BMI → δNfL$_{obs}$	**+0.0012±0.0004****	**+0.0014±0.0004*****	+0.00080±0.00076	0.46	**+0.0014±0.006***	**+0.0011±0.0004***	0.76
Total effect (BMI)	**+0.0012±0.0004****	**+0.0014±0.0004*****	+0.00080±0.00076	---	**+0.0014±0.0006***	**+0.0011±0.0004***	---
Indirect effect (BMI)	-0.00001±0.00002	-0.00002±0.0003	+0.00000±0.00002	---	-0.00000±0.00001	-0.00006±0.00005	---

HDL-C

BMI →HDL-C	**-0.826±0.086*** **	**-0.770±0.097*** **	**-0.0898±0.164*** **	0.50	**-0.718±0.112*** **	**-0.8833±0.1200*** **	0.31
HDL-C→δNfL$_{obs}$	-0.00004±0.00017	-0.00006±0.00021	+0.00006±0.00029	0.99	-0.00021±0.00034	+0.00006±0.00020	0.48
BMI → δNfL$_{obs}$	**+0.0012±0.0004** **	**+0.0014±0.0004** **	+0.0007±0.0008	0.50	+0.0012±0.0007	**+0.0011±0.0004* **	0.92
Total effect (BMI)	**+0.0012±0.0004** **	**+0.0014±0.0004*** **	+0.0008±0.0008	---	**+0.0014±0.0006* **	**+0.0011±0.0004* **	---
Indirect effect (BMI)	+0.00003±0.00014	+0.0000±0.0002	+0.0001±0.0003	---	+0.0002±0.0002	-0.00006±0.0002	---

RHR

BMI →RHR	**+0.266±0.061*** **	**+0.177±0.069** **	**+0.5000±0.116*** **	0.017	**+0.2008±0.1000* **	**+0.3258±0.0774*** **	0.32
RHR→δNfL$_{obs}$	+0.00037±0.00024	+0.0005±0.0003	+0.0003±0.0008	0.69	+0.0003±0.0004	+0.0004±0.0003	0.80
BMI → δNfL$_{obs}$	**+0.0011±0.0004** **	**+0.0013±0.004** **	+0.0007±0.0008	0.46	**+0.0013±0.0006* **	**+0.0009±0.0005* **	0.63
Total effect (BMI)	**+0.0012±0.0004** **	**+0.0014±0.0004*** **	+0.0008±0.0008	---	**+0.0014±0.0006* **	**+0.0011±0.0004* **	---
Indirect effect (BMI)	+0.0001±0.0001	+0.00008±0.00006	+0.00013±0.00021	---	+0.00006±0.00008	+0.00014±0.00011	---

SBP

BMI →SBP	**+0.698±0.081*** **	**+0.669±0.098*** **	**+0.754±0.146*** **	0.63	**+0.962±0.133*** **	**+0.513±0.102*** **	0.007
SBP→δNfL$_{obs}$	+0.0003±0.0002	+0.0002±0.0002	+0.0004±0.0003	0.63	+0.0003±0.0003	+0.0002±0.0002	0.91
BMI → δNfL$_{obss}$	**+0.0010±0.0003** **	**+0.0013±0.0004** **	+0.0005±0.0008	0.39	+0.0011±0.0007	**+0.0010±0.0005* **	0.86
Total effect (BMI)	**+0.0012±0.0004** **	**+0.0014±0.0004*** **	+0.0008±0.0008	---	**+0.0014±0.0006* **	**+0.0011±0.0004* **	---
Indirect effect (BMI)	+0.00018±0.00013	+0.00015±0.00014	+0.00031±0.00025	---	0.0003±0.0003	+0.0001±0.0001	---

DBP

BMI →DBP	**+0.318±0.056*** **	**+0.3460±0.0625*** **	**+0.236±0.110* **	0.39	**+0.312±0.083*** **	**+0.310±0.075*** **	0.99
DBP→δNfL$_{obs}$	+0.0001±0.0003	-0.00001±0.00033	+0.0003±0.0004	0.54	+0.0002±0.0005	+0.0001±0.0003	0.88
BMI → δNfL$_{obs}$	**+0.0012±0.0004** **	**+0.0014±0.0004*** **	+0.0007±0.0008	0.43	**+0.0013±0.0006* **	**+0.0011±0.005* **	0.73
Total effect (BMI)	**+0.0012±0.0004** **	**+0.0014±0.0004*** **	+0.0008±0.0008	---	**+0.0014±0.0006* **	**+0.0011±0.0004* **	---
Indirect effect (BMI)	+0.00004±0.00009	-0.0000±0.0001	+0.0001±0.0001	---	0.00005±0.00014	+0.0000±0.0001	---

Abbreviations: ALB=Albumin; AL$_{comp}$=Continuous components of the allostatic load; BMI=Body Mass Index; CES-D=Center for Epidemiologic Studies-Depression; CHOL=Total cholesterol; δ=Annualized rate of change; DBP=Diastolic Blood Pressure; HANDLS=Healthy Aging in Neighborhoods of Diversity Across the Life Span; HbA1C=Glycated Hemoglobin; HDL-C=High Density Lipoprotein-cholesterol; HEI=Healthy Eating Index; RHR=Resting Heart Rate; hsCRP=High sensitivity C-reactive Protein; NfL=plasma Neurofilament Light; SBP=Systolic Blood Pressure; v$_1$=Visit 1; v$_2$=Visit 2; v$_3$=Visit 3.

[1] *P*-value associated with null hypothesis of no difference in path coefficient α, by sex, using Wald test (χ^2 test, 1 d.f.) for group invariance.

[2] *P*-value associated with null hypothesis of no difference in path coefficient α, by race, using Wald test (χ^2 test, 1 d.f.) for group invariance.

[3] hsCRP was Log$_e$ transformed.

[4] Exogenous variables in the models were: age, sex, race, poverty status, educational attainment, current drug use, current tobacco use, HEI-2010, total energy intake, the CES-D total score and the inverse mills ratio.

*P<0.05; **P<0.010; ***P<0.001 for null hypothesis that path coefficient α=0.

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