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Red Cell Distribution Width, Anemia, and Brain Volumetric Outcomes Among Middle-Aged Adults

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Abstract.

Background: Anemia and red cell distribution width (RDW) have been linked to poor cognitive performance, pending studies of underlying mechanisms.

Objective: We examined cross-sectional relationships of initial RDW status (v_1), RDW change (δ), and anemia with brain structural magnetic resonance imaging (sMRI) markers, including global and cortical brain and hippocampal and white matter lesion (WML) volumes, 5–6 years later.

Methods: Data were used from three prospective visits within the Healthy Aging in Neighborhoods of Diversity Across the Life Span (HANDLS) study with complete v_1 (2004–2009) and v_2 (2009–2013) exposures and ancillary sMRI data at v_{scan} (2011–2015, $n = 213$, mean v_1 to v_{scan} time: 5.7 years). Multivariable-adjusted linear regression models were conducted, overall, by sex, by race, and within non-anemics, correcting for multiple testing with q-values.

Results: In minimally adjusted models (socio-demographics and follow-up time), $anemia_{v1}$ and RDW_{v1} were consistently associated with smaller bilateral hippocampal volumes overall, and among females ($q < 0.05$), without significant sex differences. RDW_{v1} was related to smaller select regional cortical brain gray and white matter volumes in hematological measure-adjusted models; $anemia_{v1}$ was associated with larger WML volumes only among Whites.

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Conclusion: In summary, baseline anemia and RDW were consistently associated with smaller bilateral hippocampal volumes, particularly among females, while anemia was linked to larger WML volume among Whites. In hematological measure-adjusted models, baseline RDW was linked to smaller regional gray and white matter volumes. Pending studies with sMRI repeats, randomized controlled trials are needed, demonstrating associations of anemia and elevated RDW with reduced brain volumes and cognitive dysfunction.

Keywords: Aging, anemia, brain volumes, hippocampus, red cell distribution width, white matter lesion

INTRODUCTION

Aging has been linked to chronic conditions such as diabetes, hypertension, and cognitive impairment, including Alzheimer's disease (AD) and other dementias [1] which are recognized as among the largest unmet medical needs [2]. Anemia, half of which is caused by iron deficiency, affects 33% of the world's population [3]. It is defined by the World Health Organization (WHO) as blood hemoglobin (Hb) <13 g/dL among males and <12 g/dL among females [4]. Its prevalence increases with age, and it is independently associated with poor quality of life, and poor health and physical function [1], while constituting an important risk factor for cognitive impairment and early markers of AD [5–12]. The anemia-cognitive impairment relationship was attributed to reduced oxygen access by obligate aerobic cortical brain tissue [13]. This relationship is also attributed to lower blood oxygen-carrying capacity triggering brain hypoperfusion, leading to oxidative stress, inflammation, and neurodegeneration [14]. Furthermore, both anemia and elevated Hb have been implicated in cerebral hypoxia [8, 15] and are patterned by age, with older individuals facing greater risks [16]. Generally, reduced cortical and hippocampal brain volumes, as well as increased white matter lesion volumes (WMLV) were linked to dysfunction in key domains of cognition associated with AD [17–20]. Aside from iron deficiency as the main cause of anemia, reduced Hb can be driven by other micronutrient deficiencies such as folate and B-12 deficiencies and may be triggered by untreated chronic infections, such as *Helicobacter pylori* infection [21, 22]. Many of these infections have been recently linked with AD [23–26].

Importantly, red cell distribution width (RDW) is a useful marker for variations in red blood cell sizes (i.e., anisocytosis) that predicts chronic disease morbidity and mortality [27–31], particularly among non-anemic individuals [31]. Moreover, among the non-anemic, elevated RDW was linked to worse cognitive performance on a verbal memory test and to higher dementia prevalence in two recent studies [32, 33], with similar associations reported elsewhere [5, 34, 35]. Furthermore, elevated RDW was closely linked to anemia and to worse cognitive outcomes including reaction time and reasoning [5]. This implies that anemia is correlated with poorer cognitive performance and suggests a possible deficit in heme synthesis or iron metabolism as an underlying trait of cognitive aging [5]. In mouse models, the amyloid-β protein precursor exhibited ferroxidase activity [36] and iron biochemistry was correlated with amyloid-β (Aβ) deposition in animal models [37]. RDW was among independent correlates of elevated blood homocysteine (Hcy) in a recent study [38] and elevated Hcy is among established risk factors for incident AD based on a recent meta-analysis [39].

Despite evidence from epidemiological and basic animal studies of an association between anemia (and RDW) with cognitive performance and select biomarkers of AD (e.g., Aβ), few mechanistic studies have examined the association of anemia (or RDW) with brain imaging markers related to cognitive performance and contributing to the AD brain phenome, including hippocampal, cortical brain, and WMLV [40]. These studies indicated that anemia was associated with smaller whole brain gray matter (GM), while RDW was linked to more severe or larger WMLV [41–43], while none thus far have examined associations with hippocampal volumes. Moreover, anemia is more prevalent among women compared with men [33]. RDW is directly correlated with anemia [33], and cortical brain volumes are larger in men versus women, independently of age, race, and poverty status. These observations suggest that the relationship between RDW/anemia versus brain volumetric markers may be patterned by sex as well.

In a socio-economically and racially diverse sample of urban adults accounting for heterogeneity by sex, we examined relationships of anemia and status and change in RDW with key brain volume markers linked to episodic memory and other domains of cognition including hippocampal and cortical brain volumes and WMLV. We hypothesized that first-visit and change over time in RDW as well as first-visit anemia were related to smaller hippocampal and

cortical brain volumes, while being linked to greater WMLV. We explored sex and race differences in the associations between those exposures and volumetric outcomes. These relationships with RDW exposures were also tested among the non-anemic sub-group [32, 33].

MATERIALS AND METHODS

Database: HANDLS and HANDLS SCAN

An area probability strategy was used to select a socio-demographically diverse sample of middle-aged White and African American urban adults ($Agev_1$: 30–64 years, Baltimore city, MD) into the Healthy Aging of Neighborhoods of Diversity across the Life Span (HANDLS) study [44]. HANDLS is an ongoing prospective cohort study initiated by the National Institute on Aging in 2004 [44]. Interviews were conducted among participants identified by random sampling of addresses within each census tract. Participants were invited to join the study when meeting the following eligibility criteria: 1) ages 30–64; 2) not currently pregnant; 3) not within 6 months of active cancer treatment; 4) not diagnosed with AIDS; 5) capable of providing written informed consent; 6) able to produce valid government-issued identification and verifiable address [44].

The initial recruitment and examination consisted of two phases: Phase 1 whereby a dietary interview and various demographic and psychosocial scales were completed in participants' homes and Phase 2 whereby participants were examined on Medical Research Vehicles (MRV) parked in their neighborhoods [44]. Examinations included the second dietary interview and other physical, medical, and psychosocial measures such as Dual X-ray absorptiometry for bone mineral density and body composition, an electrocardiogram, intima-media thickness by ultrasound, personal and family health history, physical examination by a physician, physical performance by a brief screening battery, neuropsychological tests, and inventories to assess depressive symptoms [44]. Participants were asked to fast for $\geq$8 h before their MRV visits, and 2 mL serum specimens were collected and frozen at –80°C. Data collected at Phases 1 and 2 are labelled as visit 1 (v_1, 2004–2009). Follow-up visits included comparable MRV visits. At visit 2 (v_2, 2009–2013), blood draws were analyzed at one of two laboratory facilities compared with visit 1, namely Quest Diagnostics, both of which yielding standardized biochemical and hematological indices for longitudinal analysis.

All participants provided written informed consent. Study protocols for HANDLS and HANDLS SCAN were approved by the National Institute on Environmental Health Sciences Institutional Review Board (IRB) of the National Institutes of Health. Moreover, HANDLS SCAN was approved by the IRBs of the University of Maryland School of Medicine and the University of Maryland, Baltimore County.

This study analyzed hematological data (anemia and RDW) from visit 1 (v_1: 2004–2009) and change between v_1 and v_2 (2009–2013) for RDW in relation to follow-up data measured in a sub-sample of $N_{max} = 240$ participants within the HANDLS SCAN sub-study (v_{scan}: 2011–2015) [45]. Thus, in this cross-sectional analysis with outcomes measured one time point, exposure variables were 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 [45]. The mean follow-up time between v_1 and v_{scan} was 5.61 ± 1.90 years.

Study sample

The initial HANDLS cohort included 3,720 participants (30–65 years, African Americans and Whites, Phase 1, v_1). We included participants with complete and valid MRI data at follow-up and complete RDW data at v_1 and/or v_2 and complete anemia data based on sex-specific Hb cut-points (Fig. 1). HANDLS SCAN recruited participants from consecutive waves of first and second follow-up examinations. Exclusions were based on self-reported histories of HIV, cerebrovascular, neurological, vascular, and terminal diseases, or MRI contraindications (e.g., indwelling ferromagnetics). The sample recruited represented the overall study sample in educational attainment, poverty status, and sex ($p > 0.05$), but had more white and younger participants ($p < 0.05$).

Thus, of the initial 3,720 participants, 2,744 had data on v_1 RDW, 2,267 at v_2, and 3,017 at either visit. From this group, v_1 Hb was complete among 2,744 participants. This sub-group was further restricted to HANDLS SCAN sub-study participants, yielding a final sample of 213 participants with complete data on brain MRI parameters of interest, RDW at either visit and v_1 Hb. Moreover, 191 of those 213 participants were non-anemic at v_1 and 183 were non-anemic at v_1 and/or v_2. Comparing the final sample ($N = 213$) with the remaining excluded participants

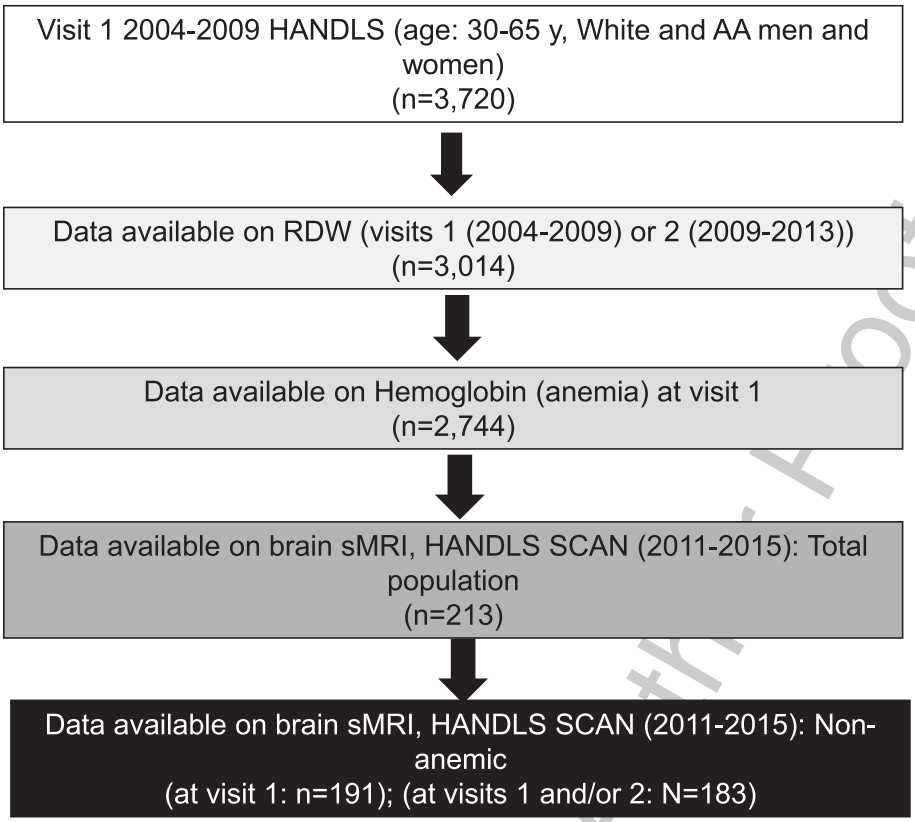

Fig. 1. Study participant schematic: HANDLS 2004–2013 and HANDLS-SCAN 2011–2015[a]. HANDLS, Healthy Aging in Neighborhoods of Diversity Across the Life Span. [a]Visit 1 refers to HANDLS 2004–2009; Visit 2 refers to HANDLS 2009–2013; and HANDLS-SCAN visit (v_{scan}) was carried out between 2011 and 2015.

from the initial $n = 3{,}720$, the final sample had higher proportions of Whites (59% versus 40%, $p < 0.05$) and individuals living above poverty (68% versus 58%, $p < 0.05$). Sample selectivity for the non-anemic group at v_1 (i.e., $n = 191$) was similar with respect to race, while no differences were detected for the non-anemic group at both or at least one visit (i.e., $n = 183$) versus those excluded.

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). Supplementary Method 1 details methods used to estimate ROI-specific volumes and the quality assurance as well as voxel-based morphometry (VBM) methods. A multi-modal 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 [46]. 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 [47]. 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.

The current study focused on hippocampal volumes [Left (L) and Right (R)] as primary outcomes, while also examining total brain volume (TBV), GM and white matter (WM) volume, as well as WMLV 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 with regions being

"frontal", "temporal", "parietal" and "occipital"]. However, this analysis was only presented if GM and/or WM showed a significant association with each of three main exposures, namely v_1 RDW, δ RDW between v_1 and v_2 (annualized) and v_1 anemia. Sensitivity analyses were also carried out on continuous v_1 Hb levels, and selected small volumetric outcomes (i.e., hippocampal and WML volumes) expressed as % TBV or adjusted in the model for TBV.

RDW at v_1 and δRDW

RDW was calculated by automated Coulter DXH 800 hematology analyzer as part of peripheral complete blood count (Beckman Coulter, Brea, CA). The analyzer underwent regular calibration every three months and quality control procedures [48]. Clinical analysis typically includes two RDW measurements, i.e., the RDW-CV (unit: %), which we adopted in this study, and the RDW-Standard Deviation (SD, unit: fL) from which RDW-CV is obtained. RDW-CV = RDW-SD $\times$ 100/MCV, MCV being the mean cell volume. The normal range for RDW-CV is 11.0–15.0%, and it depends on width of the distribution (normal range: 40–55 fL) curve and MCV [49]. In addition to RDW(v_1), annual rate of change in RDW between v1 and v2 (aka δRDW) was also of interest (see Supplementary Method 2). RDW(v_1) was considered among potential confounders in models where anemia was the main exposure of interest.

Anemia

Using electronic cell sizing/cytometry/microscopy, Hb was determined from a sample of 1 ml of blood drawn from subjects after overnight fast and refrigerated for ≤ 6 days (Quest Diagnostics). We defined anemia based on the World Health Organization as low blood Hb levels (< 13 g/dL in males and < 12 g/dL in females [4] for v_1. A similar criterion was applied to v_2 Hb to define anemia at v_2. Non-anemic participants at one or both visits were selected out for a secondary analysis for RDW exposures. Specifically, for RDW(v_1), absence of anemia was defined only for v_1, while in the case of δRDW, non-anemic reflected v_1, v_2, or both. Continuous v_1 Hb was mainly considered as a potential confounder in models with RDW and δRDW as main exposures of interest. However, v_1 Hb was also a secondary exposure of interest.

Covariates

All models were adjusted for v_1 age (year), sex (male = 1, female = 0: primary stratifying variable), self-identified race (African American = 1, White = 0), self-reported household income either <125% or $\geq$ 125% of the 2004 Health and Human Services poverty guidelines (termed poverty status) [50], and time (days) between v_1 MRV visit and v_{scan}. Models were stratified by sex. Additional covariates were added to models after we found them linked with anemia and/or RDW exposures and are considered as explanatory pathways by which main exposures may be linked to each of the key outcomes of interest. Description and modeling approaches are summarized in Supplementary Method 3 and the next section. In addition, race was considered a secondary stratifying variable.

Statistical analysis

Analyses were conducted using Stata version 16.0 [51]. First, means and proportions of sample characteristics were compared by sex using Student's *t* and chi-square tests for continuous and categorical variables, respectively. Multivariable adjusted models (linear for continuous measures; multinomial logit or logistic for categorical variables) also tested sex differences in sample characteristics, while adjusting for age, race, and poverty status. This was done for unimputed exposures, outcomes and covariates as well as additional imputed covariates. As a supplementary analysis, sample characteristics were also described across anemia and RDW tertiles at v_1. Second, for the main hypotheses, we ran on the overall sample and by sex, a series of multiple ordinary least square linear regression models. These primary models (minimally adjusted Model 1) included each of three exposures predicting each sMRI outcome measured at v_{scan}, while adjusting for key confounders (i.e., age at v_1, sex, race, poverty status, and time (days) elapsed between v_1 and v_{scan}). Parameters estimated included unstandardized $\beta \pm$ SE, uncorrected *p*-value and the standardized *b*. The latter was interpreted as the fraction of 1 SD change in sMRI outcome per 1 SD change in a continuous exposure (i.e., RDW and δRDW) and was considered moderate-to-strong if > 0.20, and weak-to-moderate if between 0.10 and 0.20.

Analyses were sub-divided into four sets, depending on the sMRI outcome type. The first analysis included three measures (*Analysis A*): Total brain,

total WM, and total GM volumes. The second was a *post-hoc* regional analysis for analysis A (termed *Analysis A'*), detailing cortical volumes within GM and WM (i.e., as L/R; GM/WM; frontal, temporal, parietal, and occipital), thereby yielding 16 *post-hoc* outcomes. This analysis was only presented if, per model, exposure and for each stratification group, at least one *Analysis A* exposure-outcome association was statistically significant ($p_{uncorr} < 0.05$). Thus, it was not included among models that were adjusted for multiple testing, given that it was a secondary analysis. *Analysis B* focused on L/R hippocampal volumes as two main outcomes, while total WM lesion volume was a singular outcome for *Analysis C*. The minimally adjusted models (Model 1, *Analyses A*, *B,* and *C*) were conducted to test the primary hypotheses of interest. The *post hoc* analysis (*Analysis A'*) and subsequently further covariate-adjusted models, as well as models among non-anemic participants were considered secondary analyses.

Type I error was set at 0.05 for uncorrected *p*-values. Multiple testing was adjusted for using false discovery rate (FDR, q-value), while considering the three analyses/stratification status as separate hypotheses (i.e., *Analyses A-C*: overall versus stratified by sex), thus adjusting for multiplicity in exposures, outcomes within analysis, and strata for the sex-stratified models. This multiple testing correction was only applied to the minimally adjusted models (i.e., Model 1) for each of Analyses A, B, and C, using the original (i.e., unimputed) data, being the main model of interest. FDR q-values were only reported for this model 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. Five additional models (Models 2–6) conducted on a multiple imputed data, whereby only covariates were imputed, were presented as secondary analyses aimed at testing mediating pathways between exposures and outcomes of interest (Supplementary Method 3).

All analyses were also applied to the non-anemic sub-sample at v_1 for the $RDW_{(v1)}$ and the non-anemic at v_1 and/or v_2 for δRDW, without correction for multiple testing (see Fig. 1). Additionally, TBV was entered into selected models (Models 1–6, Analyses B and C) with outcomes being hippocampal and WMLV. This secondary analysis was conducted to examine associations net of TBV, as a proxy to intracranial volume (ICV), overall, by sex and in the non-anemic for RDW exposures, and by race as a secondary stratifying variable. *Analysis A* was also conducted separately among Whites and African Americans as a secondary analysis. To examine the association of Hb in its entire spectrum with volumetric outcomes (as opposed to anemic versus non-anemic), Model 2 was conducted and predictive margins estimate by sex and by race, with TBV adjusted for, in the case of hippocampal and WML volume outcomes. Findings were plotted with 95%CI and overlayed with crude data points. Exploration of an association between hippocampal volumes and cognitive performance over time (adjusted for TBV and socio-demographics) was also presented as supplementary analysis.

Finally, to visualize key findings, anemic individuals were propensity-score matched with the non-anemic group, on age, sex, race, poverty status, and length of follow-up (days), using Mahalanobis distance within the *psmatch2* command in Stata [51]. Volumetric differences were then examined by presenting super-imposed images of the anemic group and those of the non-anemic matched controls, and presenting a voxel wise map of differences in volumes, using VBM methods [52] (Supplementary Method 1). We hypothesize that the anemic group will have more voxel-specific associations showing smaller volumes than the non-anemic, at a type I error of 0.10. This error rate was adjusted for multiple testing using FDR. Nevertheless, this analysis was only conducted as an illustration with more emphasis placed on the hippocampal volume differences by anemia status in the total sample.

Data availability statement

Data are available upon request to researchers with valid proposals who agree to the confidentiality agreement as required by our Institutional Review Board. We publicize our policies on our website https://handls.nih.gov, which contains the code book for the parent study, HANDLS. Requests for data access may be sent to the PIs or the study manager, Jennifer Norbeck at E-mail: norbeckje@mail.nih.gov. These data are owned by the National Institute on Aging at the National Institutes of Health. The Principal Investigators, have restricted public access to these data because 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. Analytic scripts and code book specific to HANDLS SCAN can be obtained from the corresponding author upon request.

RESULTS

Study sample characteristics are presented in Table 1, across sex. Overall, the selected sample consisted of 99 males and 114 females, with mean ± SD age of 47.5 ± 9.0 years, 41.3% of whom were African American and 67.6% living above poverty. No sex difference was detected in terms of length of follow-up between v1 and v_{scan}. On the other hand, males were more likely than females to live above poverty, while having lower mean $RDW_{(v1)}$ (13.7 versus 14.3, $p = 0.005$) and were trending toward a lower anemia(v_1) prevalence (6.1% versus 14.0%, $p = 0.06$), while having significantly higher Hb levels ($p < 0.001$). Males also generally had larger brain volumes compared with females, differences remaining significant after adjustment for age, race, and poverty status. This also applied to hippocampal volumes, with mean bilateral differentials of 283–310 mm^3 ($p < 0.05$). These associations were reversed ($M < F$), when hippocampal volumes were expressed as % of TBV, particularly for right hippocampal volume (0.394% in males versus 0.408% in females, $p = 0.003$). In contrast, no sex differences in WMLV were detected, expressed both as mm^3 and as % of TBV. Other imputed covariates that exhibited sex differences that survived adjustment for age, race and poverty status included C-reactive protein (Males(M)<Females(F)), albumin ($M > F$), cholesterol: HDL-C ratio ($M > F$), triglycerides ($M > F$), Creatinine ($M > F$), mean cell hemoglobin ($M > F$), serum iron ($M > F$), and ESR ($M < F$). Moreover, in the total sample ($N = 213$), $RDW_{(v1)}$ was moderately and inversely correlated with Hgb_{v1} ($r = –0.54$, $p < 0.001$), (data not shown). Supplementary Table 1 examines study characteristics distributions across anemia and $RDW_{(v1)}$ tertile groups, overall and by sex and indicated that brain volumes were generally smaller with anemia and elevated $RDW_{(v1)}$ (See Supplementary Table 1 results). Moreover, MCH and serum iron were consistently lower among the anemic and among participants with elevated $RDW_{(v1)}$ exposures, overall and by sex. CRP was among factors that were directly associated with the RDW_{v1} exposure; while lower albumin was observed among the anemic compared with the non-anemic, particularly among females.

Tables 2–4 and Supplementary Tables 2–4 test the main hypotheses of interest. All findings are presented overall, stratifying by sex, and for non-anemic individuals (RDW exposures). After correction for multiple testing ($q < 0.05$), $anemia_{(v1)}$ and $RDW_{(v1)}$ (but not δRDW) were associated with smaller hippocampal volumes at v_{scan}, overall and among females, though without significant effect modification by sex (exposure × sex $p > 0.05$).

More specifically, $anemia_{(v1)}$ was associated with a smaller left hippocampal volume, even after further adjustment for other hematological measures (Table 2, Model 2), both overall and among females. This association was somewhat attenuated ($p < 0.10$) in further adjusted models, particularly among females (e.g., Supplementary Table 2, Models 5–6). The independence of this relationship with right hippocampus was less evident, suggesting potential mediating effects of other hematological measures, as well as lifestyle and health-related factors. Additional control for TBV, however, did not alter these associations in all models (Supplementary Table 5, Models 1–6). Moreover, in most models, anemia at v_1 was consistently associated with reduced Left and Right hippocampal volumes among African American participants, with weaker associations found among Whites ($p > 0.05$ for $Anemia_{v1}$ × Race interaction in separate model with all main effects included). More importantly, anemia at v_1 was associated with larger WMLV among Whites ($p < 0.001$), with a significant interaction by race ($p < 0.05$). This finding was robust to additional adjustment for various groups of covariates (Supplementary Table 6).

Similarly, $RDW_{(v1)}$ was linked to smaller left and right hippocampal volumes, overall and among females in the minimally adjusted model (Table 3, Model 1). Upon adjustment for other hematological measures, including hemoglobin, most of these associations became non-significant (Table 3, Model 2 versus Model 1: $p > 0.05$), with the exception of $RDW_{(v1)}$ versus right hippocampus in the total sample ($p = 0.039$). This association (overall, $RDW_{(v1)}$ versus right Hippocampus) was slightly attenuated by adding inflammatory markers among covariates to Model 2 (i.e., Model 4, Supplementary Table 3). Nevertheless, when TBV was added to Models 2–6, all these associations were largely non-significant (Supplementary Tables 5 and 6). Thus, the net effect of $RDW_{(v1)}$ on hippocampal volumes was only significant in minimally adjusted models when adding TBV, and only among females. Moreover, there was an inverse association, overall, between $RDW_{(v1)}$

Table 1
Study sample characteristics of eligible study sample by sex; HANDLS 2004–2009 and HANDLS-SCAN 2011–2015[a]

	Total (*N* = 213)	Females (*N* = 114)	Males (*N* = 99)	p_{sex}
Socio-demographic, lifestyle and health-related factors at v_1				
	%, Mean ± SD	%, Mean ± SE	%, Mean ± SE	
Sex, % males	46.5	–	–	
Age_{v1}	47.5 ± 9.0	47.4 ± 0.85	47.7 ± 0.90	0.82
Race, % African American	41.3	42.1	40.4	0.80
% above poverty	67.6	62.3	73.7	0.075
Time between v_1 and v_{scan} (y)	5.63 ± 1.87	5.71 ± 0.17	5.53 ± 0.20	0.49
Imputed covariates, % or Mean ± SE				
Education, y				
<High School	7.1	7.5	6.7	0.86
High School	54.3	54.7	53.7	–
>High School	38.6	37.7	39.6	0.83
WRAT-3 score	43.6 ± 0.50	43.5 ± 0.6	43.7 ± 0.8	0.89
Current smoker, % yes	45.5	48.6	42.0	0.34
HEI-2010 total score	42.3 ± 0.8	43.5 ± 1.2	40.8 ± 1.1	0.13
Serum vitamin B-12, pg/mL	520.6 ± 17.0	536.3 ± 26.6	502.4 ± 20.0	0.32
Serum folate, ng/mL	15.1 ± 0.4	15.0 ± 0.6	15.2 ± 0.6	0.84
C-reactive protein, mg/L	4.3 ± 0.6	5.7 ± 1.0	2.7 ± 0.5	0.011
Albumin, g/dL	4.34 ± 0.02	4.28 ± 0.03	4.41 ± 0.03	0.001
White blood cell, count × 10^9/L	6.6 ± 0.2	6.9 ± 0.2	6.4 ± 0.2	0.073
Waist size, cm	98.9 ± 1.1	98.9 ± 1.6	99.0 ± 1.5	0.98
Total cholesterol, mg/dL	190.6 ± 3.1	192.9 ± 4.4	187.9 ± 4.4	0.43
Cholestrol:HDL-Cholesterol ratio	3.9 ± 0.1	3.7 ± 0.1	4.1 ± 0.2	0.031
Triglycerides, mg/dL	123.8 ± 5.0	112.9 ± 5.1	136.4 ± 8.9	0.018
Creatinine, mg/dL	0.90 ± 0.03	0.79 ± 0.03	1.02 ± 0.03	<0.001
Other hematological measures at v_1				
Imputed covariates, % or Mean ± SE				
Mean Cell Hemoglobin, pg	30.3 ± 0.18	29.9 ± 0.3	30.8 ± 0.2	0.013
Serum iron, μg/dL	88.0 ± 2.7	78.4 ± 3.4	98.9 ± 3.9	<0.001
Erythrocyte Sedimentation Rate, mm/h	13.2 ± 0.7	16.5 ± 1.0	9.4 ± 0.9	<0.001
	%, Mean ± SD	%, Mean ± SE	%, Mean ± SE	
RDW (v_1)				
CV (%)	14.0 ± 1.5	14.3 ± 0.17	13.7 ± 0.09	0.005
Median	13.6	13.9	13.5	
IQR	13.1;14.3	13.1;14.6	13,1;14.1	
RDW (v_2-v_1, annual), δ RDW				
CV (%)	+0.050 ± 0.070	+0.056 ± 0.008	–0.053 ± 0.005	0.72
Median	+0.05	+0.052	+0.049	
IQR	–0.41;+0.36	–0.41;0.31	–0.09;+0.36	
Hemoglobin, g/dL (v1)	13.98 ± 4.96	13.24 ± 0.13	14.84 ± 0.10	<0.001
Anemia (v_1)				
Yes, %	10.3	14.0	6.1	0.056
Anemia (v_1 and v_2)	*(N = 195)*	*(N = 105)*	*(N = 90)*	
Yes, %	6.2	8.6	3.3	<0.001
sMRI measures, mm^3	*(N = 213)*	*(N = 114)*	*(N = 99)*	
Global brain volumes	***mean±SD***	***mean±SE***	***mean±SE***	
Total brain volume	973,661 ± 102,546	924,506 ± 6,596	1,030,264 ± 10,532	<0.001
Gray Matter	515,836 ± 55,311	491,389 ± 3,712	543,987 ± 5,784	<0.001
White Matter	457,925 ± 50,467	433,117 ± 3,215	486,278 ± 5,147	<0.001
Regional cortical brain volumes	***mean±SD***	***mean±SE***	*mean±SE*	
Left Brain				
Frontal GM	90,081 ± 10,329	85,976 ± 732	94,807 ± 1,093	<0.001
Frontal WM	92,157 ± 10,515	87,520 ± 721	97,497 ± 1,087	<0.001
Temporal GM	49,114 ± 5,712	46,497 ± 387	52,127 ± 582	<0.001
Temporal WM	52,175 ± 5,967	49,136 ± 355	55,675 ± 614	<0.001
Parietal GM	43,764 ± 5,660	41,920 ± 404	45,886 ± 630	<0.001
Parietal WM	46,758 ± 5,636	44,310 ± 384	49,577 ± 589	<0.001

(*Continued*)

Table 1
(*Continued*)

	Total (*N* = 213)	Females (*N* = 114)	Males (*N* = 99)	p_{sex}
Occipital GM	34,458 ± 4,553	32,769 ± 343	36,403 ± 474	<0.001
Occipital WM	22,479 ± 3,050	21,124 ± 228	24,039 ± 296	<0.001
Right Brain				
Frontal GM	89,733 ± 10,409	85,495 ± 728	94,614 ± 1,100	<0.001
Frontal WM	94,385 ± 11,003	89,439 ± 728	100,081 ± 1,150	<0.001
Temporal GM	50,367 ± 5,688	47,676 ± 397	53,465 ± 562	<0.001
Temporal WM	52,364 ± 5,815	49,440 ± 366	55,731 ± 588	<0.001
Parietal GM	44,294 ± 5,631	42,510 ± 426	46348 ± 610	<0.001
Parietal WM	44,274 ± 5,442	41,822 ± 374	47,099 ± 556	<0.001
Occipital GM	34,373 ± 4,612	32,473 ± 336	36,562 ± 473	<0.001
Occipital WM	23,314 ± 3,071	21,818 ± 210	25,037 ± 301	<0.001
Hippocampal volume	***mean*±*SD***	***mean*±*SE***	***mean*±*SE***	
Hippocampus, Left	3,597 ± 427	3,452 ± 32	3,762 ± 32	<0.001
Hippocampus, Right	3,893 ± 428	3,762 ± 33	4,045 ± 46	<0.001
White matter lesion volume	***mean*±*SD***	***mean*±*SE***	***mean*±*SE***	
	1,299 ± 2,227	1,401 ± 234	1,181 ± 190	0.47
Hippocampal volumes, % of total brain volume				
Hippocampus, Left	0.370 ± 0.034	0.374 ± 0.003	0.366 ± 0.003	0.074
Hippocampus, Right	0.401 ± 0.035	0.408 ± 0.003	0.394 ± 0.003	0.003
White matter lesion volume, % of total brain volume	0.135 ± 0.234	0.150 ± 0.025	0.118 ± 0.020	0.32

Age_{v1}, age measured at HANDLS visit 1 (2004–2009); HDL, high density lipoprotein; HEI-2020, Healthy Eating Index, 2010 release; CV, coefficient of variation; IQR, interquartile range; δRDW, red cell distribution width annualized change between visits 1 and 2; 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; HDL, high density lipoprotein; HEI-2010, Healthy Eating Index, 2010 version; IQR, interquartile range (25^{th}-75^{th} percentile); RDW, red cell distribution width; sMRI, structural magnetic resonance imaging; v_1, visit 1 of HANDLS (2004–2009); v_2, visit 2 of HANDLS (2009–2013); v_{scan}, HANDLS-SCAN visit (2011–2015); WM, white matter; WRAT-3, Wide Range Achievement Test, 3^{rd} 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). For RDW, medians and inter-quartile ranges (IQR) were also provided. Volumes are expressed in mm^3. p_{sex} was obtained from χ^2 and *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.

and several global and cortical regional brain volumes in models 2–6 (Table 3 and Supplementary Table 3). These volumes included total GM, total WM, right and left frontal GM, and left parietal and occipital GM. The relationship between total GM and $RDW_{(v1)}$ was notably attenuated with further adjustment for education, WRAT-3 score and smoking (Model 6 versus Model 2, Table 3 and Supplementary Table 3). Nevertheless, those associations were not detected in the minimally adjusted Model 1, which was not adjusted for hemoglobin and other hematological measures. Moreover, there was some evidence of an association between $RDW_{(v1)}$ and WMLV in some but not all models among Whites, even upon correction for hemoglobin level and other hematological measures (Supplementary Table 6). Furthermore, no significant relationships were detected between anemia or $RDW_{(v1)}$ exposures and TBV, GM, or WM within each racial group (Models 1–6, Supplementary Table 7). As stated earlier and shown in Supplementary Table 4, our analyses showed that longitudinal change in RDW (δRDW) was not associated with any of the main volumetric outcomes. Exploratory analyses of an association between hippocampal, WML, and global/cortical volumes and cognitive performance over time, and between hemoglobin levels and key volumetric outcomes are shown in Supplementary Methods 4 and 5. This exploratory analysis showed, that in fact, slower declines over time on specific domains of cognition are related to larger v_{scan} hippocampal volumes, smaller WMLV and larger cortical brain volumes. More specifically, larger hippocampal volumes (L/R, as %TBV) were linked to slower decline on test of visual memory and attention, while faster decline on a test of executive function was linked to larger WMLV, particularly among African Americans. WM volumes at follow-up, especially among men, were linked to slower decline on the Digits Span-Forward test, which reflects the domain of attention. In contrast, faster decline on the domain of executive function was associated with smaller

Table 2
Minimally and hematological measure adjusted associations from analyses A (global, GM, and WM volumes), B (hippocampal volume), and C (White matter lesion volume) versus visit 1 Anemia (overall and stratified by sex): ordinary least square analyses; HANDLS 2004–2009 and HANDLS-SCAN 2011–2015[a]

	Model 1: *Minimally adjusted*				***Model 2:*** *Hematological measures adjusted, sensitivity analysis (SA)*[b]			
Total sample (*N* = **213**)	*β1*	*(SE1)*	*P1*	*q-value1*	*β2*	*(SE2)*	*P2*	*Interaction by sex*
sMRI, Analysis A								
Total brain	+788	(19,331)	0.97	–	+11,155	(23,411)	0.63	0.17
GM	–503	(10,307)	0.96	–	+4,716	(12,453)	0.71	0.19
WM	+1,291	(9,844)	0.90	–	+6,439	(11,974)	0.59	0.19
sMRI, Analysis B								
Hippocampus, Left	–280	(88)	0.002	0.010	–244	(108)	0.025	0.67
Hippocampus, Right	–215	(91)	0.019	0.046	–167	(111)	0.13	0.58
Analysis C								
White matter lesion volume	+499	(502)	0.32	–	+741	(612)	0.23	0.18
Males (N = 99)								
sMRI, Analysis A								
Total brain	–20,924	(43,230)	0.63	–	–23,319	(49,149)	0.63	–
GM	–9,959	(22,644)	0.66	–	–10,570	(25,903)	0.68	–
WM	–10,966	(22,186)	0.62	–	–12,749	(25,158)	0.61	–
sMRI, Analysis B								
Hippocampus, Left	–198	(197)	0.32	–	–219	(230)	0.34	–
Hippocampus, Right	–108	(200)	0.59	–	–114	(233)	0.63	–
Analysis C								
White matter lesion volume	–545	(786)	0.59	–	–699	(897)	0.44	–
Females **(N = 114)**								
sMRI, Analysis A								
Total brain	+12,751	(18,614)	0.50	–	21,472	(23,534)	0.36	–
GM	+6,007	(10,195)	0.56	–	9,752	(12,823)	0.45	–
WM	+6,743	(9,403)	0.4q8	–	11,719	(11,917)	0.33	–
sMRI, Analysis B								
Hippocampus, Left	–326	(86)	<0.001	0.003	–276	(109)	0.013	–
Hippocampus, Right	–265	(91)	0.005	0.018	–207	(115)	0.075	–
Analysis C								
White matter lesion volume	+929	678	0.17	–	+1,402	(857)	0.11	–

Age_{v1}, age measured at HANDLS visit 1 (2004–2009); CV, coefficient of variation; ESR, erythrocyte sedimentation rate; 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; MCH, mean cell hemoglobin; RDW, red cell distribution width; SA, sensitivity analysis; SE, standard error; sMRI, structural magnetic resonance imaging; v_1, visit 1 of HANDLS (2004–2009); v_2, 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. Q-values presented only for uncorrected *p*-values <0.05 for model 1. Model 1 was adjusted for Age_{v1}, sex, race, poverty status 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 selected hematological measures [i.e., RDW+other hematological measures (MCH, Serum iron, ESR)] after screening using machine learning techniques (See Supplementary Methods 2).

temporal GM cortical volumes (Supplementary Method 4). This exploratory analysis also showed that, in Model 1, but not in Model 2, hemoglobin level was associated with larger hippocampal volumes among females. Among African Americans, and for Left hippocampal volume, the positive association between Hb and this regional volume was significant for both Models 1 and 2. There was also an inverse relationship between Hb and WML volumes among Whites, which was slightly attenuated between Models 1 and 2. There was no association detected between Hb and global brain volumes (Supplementary Method 5).

Figure 2 illustrates the contrast in left hippocampal volume between cases of anemia ($n = 22$) and their propensity score matched controls ($n = 22$), accounting for age, sex, race, poverty status, and length of follow-up. On average, a 7.6% smaller hippocampal volume in the anemia group compared to matched controls ($p < 0.05$, *t*-test) was detected within this case-control study, based on manual volumetry. The directionality of the differences between

Table 3
Minimally and hematological measure adjusted associations from analyses A (global, GM and WM volumes), A' (regional cortical GM/WM), B (hippocampal volume), and C (White matter lesion volume) versus visit 1 RDW (overall and stratified by sex; and among non-anemic participants): ordinary least square analyses; HANDLS 2004–2009 and HANDLS-SCAN 2011–2015[a]

	***Model 1:** Minimally adjusted*					***Model 2:** Hematological measures-adjusted, sensitivity analysis (SA)[b]*			
Total sample (N = **213**)	*β1*	*(SE1)*	*b1*	*P1*	*q-value1*	*β2*	*(SE2)*	*P2*	*Interaction by sex*
sMRI, Analysis A									
Total brain	–4,208	(3,899)	–0.06	0.28	–	–11,808	(5413)	0.030	0.38
GM	–2,343	(2,078)	–0.07	0.26	–	–6,471	(2,880)	0.026	0.39
WM	–1,865	(1,987)	–0.06	0.35	–	–5,337	(2,768)	0.055	0.41
sMRI, Analysis A'									
Left Brain									
Frontal GM	–	–	–	–	–	–1,473	(551)	0.008	0.047 (*M* > F)
Frontal WM	–	–	–	–	–	–1,451	(598)	0.016	0.21
Temporal GM	–	–	–	–	–	–192	(309)	0.54	0.89
Temporal WM	–	–	–	–	–	–618	(323)	0.057	0.94
Parietal GM	–	–	–	–	–	–832	(309)	0.008	0.43
Parietal WM	–	–	–	–	–	–458	(322)	0.16	0.23
Occipital GM	–	–	–	–	–	–593	(248)	0.018	0.72
Occipital WM	–	–	–	–	–	–260	(170)	0.13	0.92
Right Brain									
Frontal GM	–	–	–	–	–	–1,364	(564)	0.017	0.063 (*M* > F)
Frontal WM	–	–	–	–	–	–1,545	(625)	0.014	0.20
Temporal GM	–	–	–	–	–	–374	(308)	0.23	0.74
Temporal WM	–	–	–	–	–	–616	(313)	0.050	0.99
Parietal GM	–	–	–	–	–	–571	(312)	0.069	0.64
Parietal WM	–	–	–	–	–	–202	(309)	0.51	0.34
Occipital GM	–	–	–	–	–	–468	(245)	0.057	0.68
Occipital WM	–	–	–	–	–	–130	(167)	0.44	0.21
sMRI, Analysis B									
Hippocampus, Left	–40	(18)	–0.15	0.028	0.046	–44	(25)	0.083	0.31
Hippocampus, Right	–40	(18)	–0.14	0.031	0.046	–54	(26)	0.039	0.56
Analysis C									
White matter lesion volume	16	(102)	+0.01	0.88	–	+108	(142)	0.45	0.70
Males (**N = 99**)									
sMRI, Analysis A									
Total brain	–9,939	(11,543)	–0.09	0.39	–	–17,935	(13,478)	0.19	–
GM	–4,914	(6,047)	–0.08	0.42	–	–8,514	(7,093)	0.23	–
WM	–5,025	(5,925)	–0.09	0.40	–	–9,420	(6,908)	0.18	–
sMRI, Analysis B									
Hippocampus, Left	–2	(53)	–0.00	0.97	–	+1	(63)	0.99	–
Hippocampus, Right	–18	(54)	–0.04	0.73	–	–48	(63)	0.45	–
Analysis C					–				
White matter lesion volume	+138	(210)	+0.07	0.51		302	(246)	0.22	–
Females (**N = 114**)									
sMRI, Analysis A									
Total brain	–2,027	(3,554)	–0.05	0.57	–	–6,265	(5,448)	0.25	–
GM	–1,079	(1,946)	–0.05	0.58	–	–4,207	(2,971)	0.16	–
WM	–948	(1,796)	–0.05	0.60	–	–2,058	(2,751)	0.46	–
sMRI, Analysis B									
Hippocampus, Left	–50	(17)	–0.28	0.004	0.018	–51	(26)	0.051	–
Hippocampus, Right	–45	(18)	–0.24	0.013	0.038	–41	(26)	0.14	–
sMRI, Analysis C									
White matter lesion volume	–20	(131)	–0.02	0.88	–	+24	(200)	0.90	–
Non-Anemic (**N = 191**)									
sMRI, Analysis A									
Total brain	–5,017	(6,046)	–0.05	0.41	–	–8,790	(6,726)	0.19	–
GM	–2,704	(3,229)	–0.05	0.40	–	–4,931	(3,580)	0.17	–
WM	–2,312	(3,086)	–0.05	0.45	–	–3,859	(3,448)	0.27	–

(*Continued*)

Table 3
(*Continued*)

	Model 1: *Minimally adjusted*					***Model 2:*** *Hematological measures-adjusted, sensitivity analysis (SA)*[b]			
Total sample (N = **213**)	*β1*	*(SE1)*	*b1*	*P1*	*q-value1*	*β2*	*(SE2)*	*P2*	*Interaction by sex*
sMRI, Analysis B									
Hippocampus, Left	–27	(28)	–0.07	0.34	–	–33	(32)	0.29	–
Hippocampus, Right	–42	(29)	–0.10	0.16	–	–50	(33)	0.12	–
Analysis C									
White matter lesion volume	+11	147	0.00	0.94	–	95	(164)	0.56	–

Age_{v1}, age measured at HANDLS visit 1 (2004–2009); CV, coefficient of variation; ESR, erythrocyte sedimentation rate; 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; Hb, hemoglobin; MCH, mean cell hemoglobin; RDW, red cell distribution width; SE, standard error; sMRI, structural magnetic resonance imaging; v_1, visit 1 of HANDLS (2004–2009); v_2, 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 RDW are computed as SD in outcome per SD in visit 1 RDW. Q-values presented only for uncorrected *p*-values <0.05 for model 1. Model 1 was adjusted for Age_{v1}, sex, race, poverty status 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 selected hematological measures [i.e., Hb+other hematological measures (MCH, Serum iron, ESR)] after screening using machine learning techniques (See Supplementary Methods 2).

anemia cases and controls using VBM is summarized in Fig. 2A, while Fig. 2B shows the voxels that were statistically significant between anemia and control groups at a type I error of 0.10. This figure suggests that most voxel differences between the two groups indicate larger volumes among controls at $p < 0.10$, given the predominance of warmer colors (yellow-orange: T-score for control-case>0) versus cooler colors (T-score for control-case <0). Nevertheless, using FDR to adjust for multiple testing, none of those voxels remained statistically significant at $q < 0.05$. The same methodology and findings applied to the right hippocampus (Fig. 2C, D). For both L and R hippocampus, the total number of voxels with $p < 0.10$ was 1,202. Despite loss of significance after controlling for multiple testing in the case of VBM, results were comparable with manual volumetry of the hippocampal region in terms of the general directionality of significant associations.

DISCUSSION

This study is among few to examine the relationships of anemia status (v_1), RDW status (v_1), and change (δ) with key structural brain MRI markers, including hippocampal, global, and cortical regional brain volumes, as well as WMLV, measured 5.7 years after v_1, on average, in a racially and socioeconomically diverse sample of urban adults. Among key findings, in minimally adjusted models (sociodemographics and follow-up time), $anemia_{v1}$ and $RDW_{(v1)}$ (but not δRDW) were consistently associated with smaller bilateral hippocampal volumes overall, and among females ($q < 0.05$), without significant sex differences. $RDW_{(v1)}$ was related to smaller select regional cortical brain GM and WM volumes in hematological measure-adjusted models; $anemia_{v1}$ was associated with larger WMLV only among Whites.

Previous human studies

No epidemiologic study thus far has demonstrated a clear relationship between anemia (or RDW) and hippocampal volume. Among notable studies, in a sample of mostly black, urban-dwelling older adults, Hb levels were investigated against cognitive performance and brain volume measures. In regression models adjusted for co-morbidities, lower Hb associated with smaller GM and ICV, with a trend observed for WM [43]. In parallel to these findings, lower Hb was associated with poorer performance on a task reflecting processing speed, though no relationship was found with memory or executive function [43]. More recently, among 5,267 older adults without dementia participating in the Rotterdam study and who had brain MRI, Hb was assessed in relation to vascular brain disease, global cerebral perfusion, and structural connectivity [11]. The study found that cerebral microbleeds were more common with anemia and that hemoglobin levels inversely correlated to cerebral perfusion ($p < 0.0001$) [11]. Similar to our study, there was no indication of a linear relationship between anemia (or RDW) and WMLV,

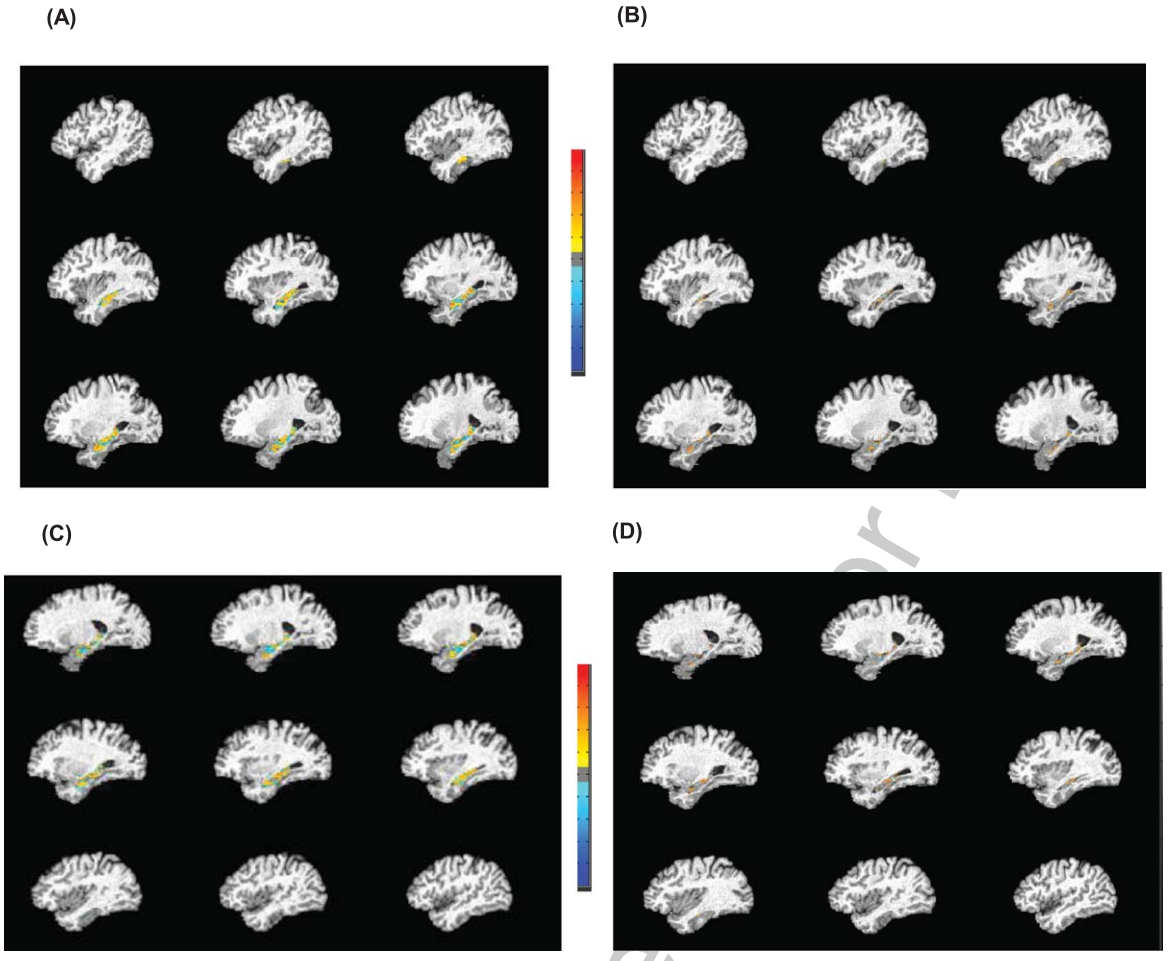

Fig. 2. Mean Left (A and B) and Right (C and D) hippocampal volumes in anemic cases at v_1 versus selected controls with propensity score matching in total sample: voxel-based morphometry. A) Initial images without showing statistically significant voxels: yellow/red means controls volumes >case volumes at each voxel, based on T-scores (see color bar for gradient). Light blue/dark blue means the opposite direction of association. B) Image with statistically significant voxels at $p < 0.10$, T-scores. T-scores ranged between –3.105053 and +4.4977. C) Initial images without showing statistically significant voxels: yellow/red means controls volumes >case volumes at each voxel, based on T-scores (see color bar for gradient). Light blue/dark blue means the opposite direction of association. D) Image with statistically significant voxels at $p < 0.10$, T-scores. T-scores ranged between –3.38557 to +4.038. Propensity score matching accounting for age, sex, race, poverty status and length of follow-up between v_1 and v_{scan}.

overall, though our study found a significant association between anemia and WMLV among Whites [11]. Nevertheless, a recent human study on RDW and cranial imaging revealed that higher RDW might be associated with poorer periventricular and subcortical WM scores, reflecting greater burden of WM lesions, among subjects with dementia [41]. Similarly, another study found that in fact RDW was linked with severity of WML, in a large sample of older adults ($n = 1{,}006$ non-stroke individuals), independently of other hematological markers, including Hb [42]. It is plausible that anemia or RDW's associations with WMLVs can more readily be detected in older adults and less so among middle-aged adults as is the case in our sample. Our current study findings suggest that RDW and anemia are consistently associated with lower hippocampal volumes among middle-aged urban adults, with most of these results being more robust among females, in minimally adjusted models, and even after correction for TBV. This finding coupled with earlier studies that connect hippocampal atrophy with cognitive decline and occurrence of AD [17–20, 53], strengthen our previous observation of an association between elevated RDW and poorer performance in the domain of verbal memory [33]. It also suggests that anemia

and elevated RDW may be mediating the association between chronic infections and AD occurrence (e.g., *Helicobacter pylori* infection [23–26]), possibly through iron, folate, and cobalamin deficiencies that lead to hippocampal atrophy. However, further studies are needed to uncover this pathway.

Biological mechanisms

Iron deficiency negatively impacts various neuronal processes, including myelination producing lasting changes in the hippocampus, amygdala, and prefrontal cortex [54]. Most of these negative impacts might, in fact, be irreversible [54]. In addition, in both animal and human models, iron deficiency has been linked to cognitive deficits correlated with changes in neural plasticity affecting memory and learning. A loss of postsynaptic transmission required for synaptic plasticity and activity-dependent neuronal gene expression has been attributed to the learning and memory deficits exhibited by humans and animals exposed to fetal or early postnatal iron deficiency [55].

Studies on iron deficiency in animals provide evidence of neuronal malfunction and structural abnormalities. For example, an early morphometric study of iron deficient Sprague-Dawley rat brains revealed deficient white matter formation compared to controls, and the deficit was only partially recouped upon iron supplementation [56]. The structural damages extend to the hippocampal region in a task-related experiment on Sic11a2 (hipp/hipp) mice model, where iron deficiency appeared to correlate with longer mean escape times on a cues task, compared to their wild type littermates [57]. The loss of spatial and procedural memory has been attributed to reduced iron availably in the formation of mice fetal hippocampus [57]. In an attempt to recover some of the damages triggered by early life iron deficiency in rats, a high-dose iron supplementation (10X than normal) was differentially associated with improved neurochemical profiles of the prefrontal cortex and hippocampus. The hippocampal expression of myelination markers and dopamine 1 receptors were downregulated in C57BL mice as a result of iron deficiency from another study [58].

Strengths and limitations

This study has several strengths, most notably its novel examination of associations between anemia-related biomarkers with brain structural sMRI measures reflecting global and regional volumes and WMLV, potentially underlying various neuropathologies. Although cross-sectional in design, this study provided 5–6 years of latency between exposure ($RDW_{(v1)}$ and anemia) and outcome (brain MRI measures), while considering longitudinal change in RDW as an additional exposure of interest. Moreover, given the importance of sex in both anemia and cognitive impairment, we examined our hypotheses separately among males and females and adjust our basic models for multiple testing and potential confounding for socio-demographic, lifestyle, and health-related factors, including hematological and other nutritional biomarkers. Our analyses also considered heterogeneity of associations by race.

Nevertheless, our study has several limitations. First, the 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 across participants, though it had a central tendency of 5–6 years. Thus, we adjusted for the follow-up time in our models. Moreover, the lack of a baseline sMRI measure should be remedied in future studies of comparable populations. Second, residual confounding is a possibility given the observational nature of the study. Third, in the main models, no ICV corrections were performed in the context of ROIs because: a) differences in ICV are mostly influenced by sex and age [59], which were controlled for in all of our multivariable analyses, b) we were concerned with ROI actual volumes, rather than volumes relative to the entire brain, c) ICV is highly correlated with the majority of ROIs, and therefore, distinguishing ICV would explain most ROI variability, and d) bias in ICV estimation is well-established [60]. Nevertheless, when we adjusted for TBV, as a proxy for ICV, findings from our analyses with hippocampal volume outcomes remained largely unaltered, particularly in minimally adjusted models and among females. Finally, our findings can only be generalized to US middle-aged urban White and African American adults, and thus can be extrapolated to at least 14 US urban settings with comparable racial composition to Baltimore city.

CONCLUSIONS

In summary, baseline anemia and RDW were consistently associated with smaller bilateral hippocam-

pal volumes, particularly among females, while anemia was linked to larger WMLV among Whites. In hematological measure-adjusted models, baseline RDW was linked to smaller regional GM and WM volumes. Pending further studies with sMRI repeats, randomized controlled trials are needed, demonstrating direct associations of anemia and elevated RDW with reduced brain volumes and cognitive dysfunction.

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SUPPLEMENTARY MATERIAL

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Supplementary Material

Red Cell Distribution Width, Anemia, and Brain Volumetric Outcomes Among Middle-Aged Adults

Supplementary Method 1. Brain structural/volumetric (s) magnetic resonance imaging (MRI) detailed description:

HANDLS description

sMRI

In addition to standard axial T1, T2, FLAIR images, high-resolution axial T1-weighted MPRAGE (TE = 2.32 ms, TR = 1900 ms, TI = 900 ms, flip angle = 9º, resolution = 256 × 256 × 96, FOV = 230 mm, sl. Thick. = 0.9 mm) of the brain was obtained for structural imaging. We used images as anatomic references and for the extraction of parameters of regional and whole brain volumes. T1-weighted MP-RAGE images covered the whole brain at a thickness of 1.2 mm for 160 sagittal slices (TR/TE/TI=2300/2.9/900 ms; FOV 25.6 cm). These images were then converted to axial sections for comparative purposes.

The Section for Biomedical Image Analysis at the University of Pennsylvania developed techniques in-house to preprocess structural MRI scans. First, extra-cranial material on the T1-weighted images was removed using a multi-atlas registration method [1]. Bias was corrected using multiplicative intrinsic component optimization (MICO) method [2]. MUlti-atlas region Segmentation utilizing Ensembles (MUSE), segmented the pre-processed images into a set of anatomical regions of interest (ROIs) [3]. MUSE integrates a broad ensemble of labeled templates by using a number of warping algorithms, regularization atlases and parameters [3].

Quality assurance

The Core for Translational Research in Imaging @ Maryland (C-TRIM), managed by the Department of Diagnostic Radiology at UMB's School of Medicine, has instituted several quality control measures to ensure highest level of quality and safety. The research-dedicated scanner undergoes routine quality data assurance as mandated by the American College of Radiology [4]. In addition, the AD Neuroimaging Initiative phantom is used to assess weekly signal-to-noise ratio and monthly structural distortions [5]. We periodically check the reliability of diffusion data by utilizing the National Institutes of Standards and Technology diffusion phantom in order to ensure that the measurements from diffusion MRI are stable [6].

Voxel-based morphometry methods

These methods are automated fairly user friendly, time-efficient and can detect focal microstructural differentials in brain anatomy (in vivo) across groups of people, while reducing decision-making as to which structures to evaluate [7]. Moreover, VBM has a similar accuracy to manual volumetry, based on several validation studies [7]. The processing of images followed several steps: T1 weighted scan of subjects were preprocessed using an automated pipeline which included magnetic field inhomogeneity correction [8], extraction of brain using multi-atlas skull-stripping [1]. Anatomical ROIs were segmented for each subject using multi-atlas segmentation method [3]. Right and Left hippocampus tissue density maps were computed using RAVENS algorithm [9] after segmentation. RAVENS method involved tissue segmentation followed by

nonlinear registration to atlas space (Jakob Atlas) whose intensity encodes volume deformation from source to target at a voxel. Volumetric differences were then examined by computing group differences between anemic and non-anemic matched control group (using 3dttest++ AFNI) and presented voxel-wise map of differences in volume, significant differences at type I error of 0.10 and correcting for multiple voxels comparison within a region using FDR $q < 0.05$. Visualization of findings were also used to corroborate results from raw regional volumetry of the hippocampus (L/R), specifically with respect to group comparisons showing predominance of controls (or cases) having greater volumes than cases (or controls) among all significant voxels.

Supplementary Method 2. Mixed-effects linear regression models and empirical Bayes estimation

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

Multi-level models versus **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 (RDW) 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 and included baseline age (Age_{base}) among other covariates. $X_{ija,}$ represents the main predictor variables. In this case, all predictor variables were socio-demographic and used for prediction. ζ_{0i} and ζ_{1i} are level-2 disturbances; ε_{ij} is the within-person level-1 disturbance. Main effect of TIME (γ_{1a}) and interactions with socio-demographic factors (γ_{1a}) along with random effects ζ_{1i} were used to estimate each individual slope π_{1i}, also known as the empirical bayes estimator. The time interval model is described in details in this methodological paper [10]. Since time is measured as year elapsed since visit 1 up till visit 2, the interpretation of π_{1i} is the predicted individual-level annual rate of change in the outcome Y_{ij}, between visits 1 and 2. This empirical bayes estimator of slope was used to examine association between annual rates of change in each of RDW versus brain MRI markers. Below are the results of the mixed effects regression models for each of the RDW exposure:

Table II.1. Mixed-effects linear regression model for RDW over time, with random intercept and slope and fixed effects for v1 age, sex, race, and poverty status interacted with TIME.

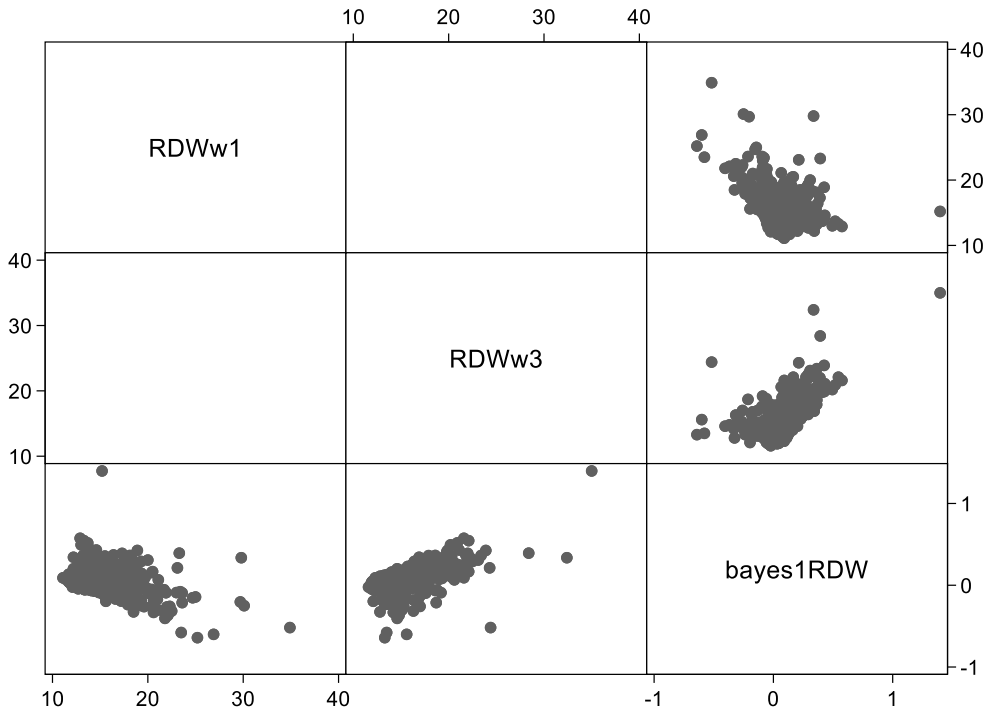
	RDW (n=3,017, k=1.7)
Intercept (γ_{00}±SE)	14.09±0.18***
Time (γ_{10}±SE)	+0.02±0.04
Age(v_1) γ_{01}±SE	-0.000±0.003
Age(v_1)×Time, γ_{11}±SE	0.001±0.001
Sex (0=Female, 1=Male), γ_{02}±SE	-0.48±0.06***
Sex×Time, γ_{12}±SE	+0.013±0.014
Race (0=Whites, 1=AA), γ_{03}±SE	+0.658±0.064***
Race×Time, γ_{13}±SE	+0.004±0.014
Poverty (0=Below, 1=Above), γ_{04}±SE	-0.13±0.06*
Poverty×Time, γ_{14}±SE	-0.025±0.014
Var (ζ_{0i})	1.97±0.11
Var (ζ_{1i})	0.03±0.01
Var (ε_{ij})	0.80±0.09

***p<0.001; **p<0.010; *p<0.05

The empirical bayes estimator for annual rate of change in RDW can be summarized as follows:

$\gamma_{10} + \gamma_{11}\times$ Age + $\gamma_{12}\times$ Sex + $\gamma_{13}\times$ Race + $\gamma_{14}\times$ Poverty + ζ_{1i}

Figure II.1 Baseline (v1), follow-up(v2) and annual rates of change in RDW scatter plot



RDWw1=RDW at visit 1 (HANDLS wave 1); RDWw3= RDW at visit 2 (HANDLS wave 3); bayes1RDW=Empirical bayes estimator of annual rate of change in RDW or δRDW.

Supplementary Method 3. Additional covariates, LASSO regression, and multiple imputations

A. Additional covariates:

A.1. Socio-demographic

Additional socio-demographic confounders included educational attainment (0 ≤ High School (HS); 1 = HS and 2 ≥ HS), the Wide Range Achievement Test (WRAT) letter and word reading subtotal scores to measure literacy, and marital status (1=married, 0=not married) [11].

A.2. Lifestyle

Smoking and drug use

Current use of opiates, marijuana or cocaine ("current" versus "never or former") and smoking status ("current" versus "never or former") were considered.

Adiposity measures

Measured body mass index (BMI, kg/m^2), waist circumference, and waist-hip-ratio were considered among potential confounders.

Healthy Eating Index 2010-

The Healthy Eating Index (HEI-2010) total score, based on two 24-h recalls administered at baseline, was used as a measure of overall dietary quality. See steps for calculating HEI-2010 at http://appliedresearch.cancer.gov/tools/hei/tools.html and http://handls.nih.gov/06Coll-dataDoc.html.

Dietary Approaches to Stop Hypertension (DASH)

DASH diet adherence score, based on eight nutrients, was determined for each participant using the formula reported by Mellen et al. [12]. The nine target nutrients were: total fat, saturated fat, protein, fiber, cholesterol, sodium, calcium, magnesium, and potassium. Micronutrient goals were expressed per 1000 kcal. The total DASH score was generated by the sum of all nutrient targets met. If the participant achieved the DASH target for a nutrient, a value of one was assigned, and if an intermediate target for a nutrient was achieved, a value of 0.5 was assigned. A value of zero was assigned if neither target was met. The maximum DASH score was nine; individuals meeting approximately half of the DASH targets (DASH score = 4.5) were considered DASH adherent [12].

Mean Adequacy Ratio (MAR)

Diet quality was also assessed using Nutrient Adequacy Ratio (NAR) and Mean Adequacy Ratio (MAR) scores [13, 14]. NAR score was determined by taking each participant's daily intake of a nutrient divided by the Recommended Dietary Allowance (RDA) for that nutrient. NAR scores were determined for 17 micronutrients: vitamins A, C, D, E, B_6, B_{12}, folate, iron, thiamin, riboflavin, niacin, copper, zinc, calcium, magnesium, phosphorus, and selenium. The RDA was adjusted for participants' ages and sexes and vitamin C was adjusted for smokers [15]. The NAR score was converted into a percent with values exceeding 100 truncated to 100. MAR scores were calculated by averaging the NAR scores: MAR= (∑NAR scores)/17 [16]. NAR and MAR were calculated separately for each daily-intake and then averaged. MAR scores, based on food intakes only, were used as the nutrient-based diet quality variable.

Supplemental use

The HANDLS dietary supplement questionnaire was adapted from the 2007 NHANES instrument [17]. Information on Over-The-Counter (OTC) vitamin and mineral supplements, antacids, prescription supplements, and botanicals were reported, and supplement users were asked about dose strength, dose amount consumed, length of supplement use (converted to days), frequency of use (daily, monthly, seasonally, annually), and if each supplement was taken the day prior to interview[11]. Participants had to provide supplement bottles during their dietary interview at the follow-up visit (i.e., visit 2).

A HANDLS dietary supplement database was developed by trained nutritionists and registered dietitians. This database consisted of four files integrated to generate daily intake of each nutrient consumed by a dietary supplement user. [See detailed description at the HANDLS study website: https://handls.nih.gov/].

Depressive symptoms

Depressive symptoms were operationalized using the CES-D at baseline and follow-up. The 20-item CES-D is a self-reported symptom rating scale assessing affective and depressed mood.[18] A score of ≥16 on the CES-D is reflective of elevated depressive symptoms (EDS), [19] and predicts clinical depression based on the Diagnostic and Statistical Manual, fourth edition (DSM-IV) criteria.[20] Four CES-D sub-domains exhibiting an invariant factor structure between The National Health and Nutrition Examination Survey I and pilot HANDLS data [21] were computed. We tested our hypotheses using total and domain-specific CES-D scores: **(1)** Somatic complaints; **(2)** Depressive affect; **(3)** Positive affect and **(4)** Interpersonal problems.[21]

A.3. Health-related

Baseline chronic conditions included self-reported history measurement, biomarker-based measurement, and medication-based measurement, of type 2 diabetes, hypertension, dyslipidemia, cardiovascular disease, and inflammatory disease. Dyslipidemia was based on a combination of self-report, HDL, total cholesterol, triglyceride criteria, and statin use. Similarly, type 2 diabetes was determined using a combination of self-report, serum glucose criteria and medication. The same was conducted for hypertension. Additionally, a composite of cardiovascular disease history was added in which self-reported stroke, congestive heart failure, non-fatal myocardial infarction or atrial fibrillation combined into a yes/no variable. Similarly, inflammatory disease was a binary composite of multiple sclerosis, systemic lupus, gout, rheumatoid arthritis, psoriasis, Thyroid disorder and Crohn's disease. The use of NSAIDs (prescription and over the counter) and statins over the past two weeks were considered separately as potential covariates.

A.4. Other biomarkers

All laboratory tests selected for this study were done at Quest Diagnostics, Chantilly, VA.

Serum cholesterol and atherogenic indices

Total cholesterol (TC), High density lipoprotein-cholesterol (HDL-C) and Triacylglycerols (TA) were assessed using a spectrophotometer (Olympus 5400). Low density lipoprotein-cholesterol (LDL-C) was calculated as TC-(HDL-C+TA/5) and directly measured in a sub-sample (N=236) using a spectrophotometer (Olympus 5400). The correlation between those with baseline

calculated LDL-C and those with measured LDL-C was r~0.95. From these calculations, two relative measures were obtained, namely TC: HDL-C and LDL-C: HDL-C ratios. These were termed "atherogenic indices" and have been previously studied in relation to various cardiovascular outcomes that found them to be positively associated with measures of atherosclerosis and coronary heart disease [22-24].

Serum uric acid (SUA)

SUA measurements are useful in the diagnosis and treatment of renal and metabolic disorders, including renal failure, gout, leukemia, psoriasis, starvation or other wasting conditions, as well as in patients receiving cytotoxic drugs. Using 1 ml of fasting blood serum, uric acid was measured using a standard spectrophotometry method. The reference range for adult men is 4.0-8.0 mg/dL, whereas for women the range is 2.5-7.0 mg/dL (http://www.questdiagnostics.com/testcenter/TestDetail.action?ntc=905). Other reference ranges were also recently suggested and depend on the menopausal status of women. Those reference ranges are based on predictive value for gout outcomes among healthy individuals and do not necessarily predict other pathologies. Thus, based on recent research evidence, a "normal" SUA value is suggested to be <6.0 mg/dL for all healthy adult individuals.

Serum albumin

Using 0.5-1 mL samples of plasma prepared with heparin and refrigerated for up to 30 days, albumin was measured with spectrophotometry, with an expected reference range of 3.6-5.1 g/dL [25, 26].

High sensitivity C-reactive protein (CRP)

High sensitivity CRP (hs-CRP) was analyzed with an immunoturbidimeter (Siemens/Behring Nephelometer II), using 0.5-1 mL of plasma. A range of 1-10 mg/dL indicates average to high cardiovascular risk and >10 mg/dL suggests an infection or a chronic inflammation.

Serum creatinine

Using participant fasting blood specimens, baseline serum creatinine was measured at the National Institute on Aging, Clinical Research Branch Core Laboratory, using a modified kinetic Jaffe method (CREA method, Dade Dimension X-Pand Clinical Chemistry System, Siemens Healthcare Diagnostics Inc., Newark, DE) for a small group of participants (n=88). However, a majority of participants (n=1,528) had baseline serum creatinine analyzed at Quest Diagnostics, Inc. by isotope dilution mass spectrometry (IDMS) (Olympus America Inc., Melville, NY) and standardized to the reference laboratory, Cleveland Clinic. While inter-assay coefficients of variation (CV) for this sample could not be calculated due to the use of only one or the other measurement of creatinine at baseline, only intra-assay CVs (mean/SD) could be estimated. These were 0.192 and 0.187 for the CREA and the IDMS methods, respectively.

HbA1c

Glycated hemoglobin is derived from the nonenzymatic addition of glucose to amino groups of hemoglobin. HbA1c is a specific glycated hemoglobin that results from the attachment of glucose to the N-terminal valine of the hemoglobin b-chain. Numerous assays were subsequently developed to measure glycated hemoglobins. The principle of all methods is to separate the glycated and nonglycated forms of hemoglobin [27]. This can be accomplished based on

differences in charge (usually by HPLC) or structure (usually immunoassays or boronate affinity chromatography). In this study, HPLC was used (Quest diagnostics).

White blood cell inflammatory markers

Fasting blood samples were collected from participants at baseline and follow-up to determine total white blood cell count (K/mm^3), using electronic Cell Sizing, counting, cytometry, and microscopy (http://www.questdiagnostics.com/testcenter/TestDetail.action?ntc=7064).

Serum 25-hydroxyvitamin D, folate and cobalamin

Participants were asked to fast for ≥8 h prior to the MRV visits, and serum specimens in volumes of 2 mL were collected and frozen at −80°C. Similar procedures were adopted for serum folate and cobalamin, both measured using chemiluminescence immunoassay (Siemens Centaur) by Quest Diagnostics, Chantilly, VA [28, 29], and previously validated against other automated methods with coefficient of variation (CV) <10% [30, 31].

25(OH)D were measured using slightly revised methodologies between v_1 and v_2. In this study, only the v_1 measure was used. At v_1, total levels of serum 25(OH)D (in ng/ml; D_2 and D_3) were measured using tandem mass spectrometry (interassay CV, 8.6%) at Massachusetts General Hospital for less than 60 days later, as recommended for frozen samples [32]. Blood samples drawn at examination were stored at −80°C.

Dietary and supplemental intakes of vitamin D, folate and cobalamin were shown to moderately correlate with their corresponding serum biomarkers in HANDLS and national surveys [11, 33, 34].

Hemoglobin and other hematological measures

Hemoglobin (Hb)

Similarly, using electronic cell sizing/cytometry/microscopy, Hb was assayed from a sample of 1 ml of blood drawn from participants after an overnight fast, and refrigerated up to 6 days (Quest diagnostics).

Other hematological markers

Ferritin: Ferritin is decreased in iron deficiency anemia and increases with iron overload. It is measured with immunoassay with reference ranges of 20-380 ng/mL among men and 10-232 ng/mL among women [35].

Erythrocyte Sedimentation Rate (ESR): Using 5 mL of refrigerated whole blood stored in lavender top EDTA tubes, the ESR was tested within 24 h of blood draw. This test used automated modified Westergren photochemical capillary-stopped flow kinetic analysis.[36, 37] The Mayo Clinic reports a reference of 0-22 mm/h for men and 0-29 mm/h for women [38] and is considered a proxy measure for serum fibrinogen [39].

Serum iron: 0.5-1 mL of fasting serum was collected, transported at room temperature (with heparin added) and refrigerated or frozen subsequently. Serum iron was measured with spectrophotometry, [40, 41] with reference ranges for men aged ≥30 y set at 50-180 μg/dL, and for women: 20-49 y (40-190 μg /dL) and 50+y (45-160 μg /dL) [41].

MCV: Also known as erythrocyte mean corpuscular volume, MCV is measured using standard electronic cell sizing/counting/cytometry/microscopy. Similar to other hemogram measures (e.g. ESR), a microtainer 1 mL whole blood in an EDTA (lavender-top) tube was transported at room temperature to the laboratory facility [36].

MCH: The hematologic index MCH was calculated as follows: MCH = Hb/RBC.

B. Least absolute shrinkage and selection operator (LASSO) regression procedure

In order to select the appropriate set of predictive models for RDW, we used a statistical learning method for variable selection known as adaptive LASSO and compared it to cross-validation LASSO (cvLASSO) and lowest BIC LASSO. Socio-demographic variables (age, sex, race/ethnicity, poverty status) were force entered as fixed terms into all models. The LASSO then selected among the other covariates listed above as variables that should be retained. Covariates were imputed using chained equations (5 imputations, 10 iterations), accounting for their level of measurement. Socio-demographic factors were entered into all the chained equations. Continuous covariates were entered as outcomes in a series of linear regression models, while binary and categorical variables were entered into a series of multinomial logit regression models.

LASSO is a covariate selection methodology that is superior to both generalized linear models without covariate selection as well as the usually applied stepwise or backward elimination process.[42] In fact, stepwise selection is often trapped into a local optimal solution rather than the global optimal solution and backward elimination can be time-consuming given the large number of variables in the full model [42]. These methods often ignore stochastic errors or uncertainty incurred during variable selection, with the LASSO estimate being defined as:

$\beta(\text{lasso}) = \arg min_{\beta} \| y - \sum_{j=1}^{p} x_j \beta_j \|^2 + \lambda \sum_{j=1}^{p} |\beta_j|$

with λ being a nonnegative regularization parameter.[42] The second term of the equation termed the "l1 penalty" is a key portion of this equation that ensures the success of the lasso method of covariate selection. This method was shown to discover the right sparse representation of the model, given certain conditions. Nevertheless, this method can produce biased estimates for larger coefficients. Thus, there a number of scenarios whereby the LASSO can yield inconsistent results. Recent methods have shown that an adaptive version of the LASSO gave more consistent findings, particularly when compared with the nonnegative garotte, another popular variable selection technique.

In our modeling approach, we used this convex optimization technique with l_1 constraint known as adaptive LASSO as one of three methods to select the final linear regression models. The model is trained on a random half sample of the total population (first imputation out of 5) and validated against the other half sample to check robustness of findings, by comparing R^2 between samples. One model was selected among the cvLASSO, adaptive LASSO or minBIC LASSO, depending on how close the R^2 are between half-samples. This parsimonious model selected for RDW (measured at v_1 and empirical Bayes slope estimator measured between v_1 and v_2) as 2 potential outcomes is then run on the entire population and a backward elimination process is carried out to keep only significant covariates at type I error = 0.10. Thus, the selected model through LASSO was used as a starting point for further backward elimination. Backward elimination was conducted on the imputed data for the entire sample, rather than the half sample for the first imputation.

In our analysis, the following LASSO models were selected, and the final model included is shown also in this Table.

Table III.1. Results of LASSO selection models and backward elimination

	Selected covariates[1]			
	cvLASSO	**Min BIC LASSO**	**Adaptive LASSO**	**Reduced model**
RDW (v1)	MCH, Hb, Creatinine, smoking, CES-D, age, Cholesterol:HDL ratio, HEI-2010 total score, CVD, sex, WHR, CRP, B-12, WBC, Triglycerides, Poverty status, race, WRAT total score, albumin, cholesterol, Hypertension medication, Iron, education, current drugs, HbA1C	MCH, Hb, Creatinine, smoking, CES-D, age, Cholesterol:HDL ratio, HEI-2010 total score, CVD, sex, WHR, CRP, B-12, WBC, poverty status, race, albumin cholesterol	**MCH, Hb, Creatinine, smoking, CES-D, age, Cholesterol:HDL ratio, HEI-2010 total score, CVD, sex, WHR, CRP, B-12, WBC, Triglycerides, poverty status, race, WRAT total score, NSAIDS, albumin.**	**MCH, Hb, Creatinine, smoking, age, Cholesterol:HDL ratio, HEI-2010 total score, sex, CRP, B-12, WBC, Triglycerides, poverty status, race, WRAT total score.**
RDW (v2-v1, annual)	**Poverty status, Hb, race, age, WBC, MCV, WHR, CVD and sex.**	**Poverty status, Hb, race, age, WBC, MCV, WHR, CVD and sex.**	**Poverty status, Hb, race, age, WBC, MCV, WHR, CVD and sex.**	**Poverty status, Hb, race, age, WBC, MCV**
Anemia (v1)	ESR, RDW, MCH, Albumin, Serum iron, race, WBC, age, WRAT total score, Cholesterol, Folate, B12, Inflammatory conditions, education, WC, married, diagnosed hypertension, vitamin supplements, current drugs, WHR, Triglycerides, 25(OH)D, poverty status, sex.	ESR, RDW, MCH, Albumin, Serum iron, race, WBC, age, poverty status.	**ESR, RDW, MCH, Albumin, Serum iron, race, WBC, age, WRAT total score, Cholesterol, Folate, B12, Inflammatory conditions, education, WC, poverty status, sex.**	**ESR, RDW, MCH, Albumin, Serum iron, race, WBC, age, Cholesterol, Folate, B12, education, WC, poverty status, sex.**

B-12, vitamin B-12 (cobalamin); BIC, Bayesian information criterion; BMI, body mass index; CES-D, Center for Epidemiologic Studies-Depression; CRP, C-reactive Protein; cv, cross-validation; CVD, Self-reported cardiovascular disease; DASH, Dietary Approaches to Stop Hypertension; ESR, erythrocyte sedimentation rate; HbA1c, glycated hemoglobin; HDL, high density lipoprotein cholesterol; LASSO, Least absolute shrinkage and selection operator; HEI-2010, Healthy Eating Index, 2010 revision; MAR, mean adequacy ratio; MCH, mean cell hemoglobin; MCV, mean cell volume; NSAIDS, non-steroidal anti-inflammatory drugs; RDW, red cell distribution width; WBC, white blood cells; WC, waist circumference; WHR, waist-hip-ratio

[1]Bolded sets of covariates are the ones that are selected at each step of the model selection process. A full row of bolded sets of covariates indicates that the selection process is equivalent, and that backward elimination did not reduce the model further.

The final common set of covariates that were chosen using the reduced model for each exposure was:

Anemia(v1): RDW(v1), age, sex, race, poverty status, ESR, MCH, Serum iron, Creatinine, albumin, cholesterol, Cholesterol:HDL ratio, HEI-2010 total score, CRP, B-12, folate, WBC, Triglycerides, smoking, WC, WRAT total score, education.

RDW(v1) and RDW (v2-v1, annual): Hb(v1), age, sex, race, poverty status, ESR, MCH, MCV, Serum iron, Creatinine, albumin, cholesterol, Cholesterol:HDL ratio, HEI-2010 total score, CRP, B-12, folate, WBC, Triglycerides, smoking, WC, WRAT total score, education.

From these, six models were constructed:

Model 1: Only socio-demographic

Model 2: Socio-demographic + hematological measures [i.e., Hb for RDW (or δRDW) and RDW for anemia + other iron status measures (MCH, Serum iron, ESR).

Model 3: Socio-demographic +hematological measures + other nutritional/dietary (HEI-2010 total score, B-12, folate).

Model 4: Socio-demographic +hematological measures +inflammatory (CRP, albumin, WBC).

Model 5: Socio-demographic +hematological measures+ adiposity and metabolic factors (WC, cholesterol, Cholesterol:HDL ratio, Triglycerides, Creatinine)

Model 6: Socio-demographic + hematological measures + other (education, WRAT, smoking).

C. Full description of the modeling approach:

Using multiple imputed data (k=5 imputations), a sensitivity analysis (SA) adjusted for additional covariates, selected with a multi-step process detailed in Supplementary Method 3, that included machine learning, followed by backward elimination and finally selection of a common pool of covariates that were independent predictors of at least one of 3 exposures. The pool of covariates initially selected had exhibited associations with either hematological measures and/or cognitive outcomes in previous studies. Thus, the final modeling approach consisted of a minimally adjusted basic model, i.e., Model 1 conducted on the unimputed data. Subsequently, the SA was carried out on multiple imputed data, with the following modeling approach:
Model 2: Model 1 +hematological measures [i.e., Hb for RDW (or δRDW) and RDW for anemia + other hematological measures (MCH, Serum iron, ESR).
Model 3: Model 2 + other nutritional/dietary (Healthy Eating Index-2010 total score, B-12, folate); Model 4: Model 2+inflammatory (high sensitivity C-reactive protein, albumin, White blood cells); Model 5: Model 2+ adiposity and metabolic factors (WC, cholesterol, Cholesterol:HDL ratio, Triglycerides, Creatinine); Model 6: Model 2 + other covariates (education, WRAT, smoking). For this SA, formal effect modification testing was conducted by including 2-way interaction terms between exposure and sex in the non-stratified model, with a type I error of 0.10 used for 2-way interaction terms due to reduced statistical power [43].

Supplementary Method 4. Hippocampal, WML, and global/cortical volumes versus cognitive performance change over time

A large battery of cognitive tests was available in HANDLS at v1 and v2, from which annualized rates of change were directly computed using complete case analysis for each test score. Detailed descriptions of those cognitive tests and their scoring are available in previous studies[26, 44, 45]. Participants with non-valid test scores were excluded from this analysis as were the participants who were not eligible for the current study. Thus, out of the 213 participants who were eligible for this study, our current analysis sample size ranged between n=147 (Brief Test of Attention, BTA) and n=190 (Clock Drawing Test, CDT). The analysis consisted of a series of multiple linear regression models, with outcome Y being each of the volumetric outcomes and main predictor X being one of 11 cognitive performance change measures (annualized). All these change measures were in the direction of higher change in score → slower decline or faster improvement, except for Trails A and B and for BVRT (See abbreviations under Table IV.1). The baseline (v1) score was also included in these models as a potential confounder. MMSE scores were normalized as was done in previous studies [46], while TRAILS A and TRAILS B were Log_e transformed. Volumes were expressed in mm3, while cognitive test score change were retained in their original units (e.g., seconds to completion for TRAILS A and B; errors for BVRT).

Both hippocampal and WML volumes were standardized by total brain volume (TBV), dividing each by TBV and multiplying by 100 in the final analysis. This analysis was compared with another one whereby TBV was entered into the model. For cortical and global volumes, TBV was not entered into the models. All models were adjusted for Age_{v1}, sex, race, poverty status and length of follow-up between v_1 and v_{scan}. Heterogeneity in the main association by sex or race was tested using 2-way interaction between cognitive change predictors and those socio-demographic factors in the unstratified model, at a type I error of 0.10. Main findings are summarized in Table IV.1, listing findings with $p<0.10$.

Table IV.1. Annual rate of change in cognitive performance (X) versus volumetric outcomes (Y)[a]

	Overall β±SE	p	Heterogeneity by sex[b]	Heterogeneity by race[b]
Hippocampal and WML volumes, as % TBV				
Right Hippocampal volume (Y) versus δBVRT (X)	-0.007±0.003	0.026	No	No
Left Hippocampal volume (Y) versus δBTA (X)	+0.015±0.006	0.018	No	No
WMLV (Y) versus δTRAILS B (X)	+0.31±0.15	0.034	No	Yes
Among Whites	-0.04±0.14	0.78	—	—
Among AA	+0.76±0.28	0.009	—	—
Global and cortical brain volumes, mm^3				
TBV (Y) versus δDS-F (X)	+32302±17515	0.062	Yes	No
Among women	+12465±18541	0.50	—	—
Among men	+54902±31214	0.083	—	—
WM (Y) versus δDS-F (X)	+18941±8875	0.034	Yes	No
Among women	+5,557±9,498	0.56	—	—
Among men	+35,204±15,726	0.029	—	—
FRONTAL GM, LEFT (Y) versus δTRAILS B (X)	-8830±5066	0.083	No	No
TEMPORAL GM, LEFT (Y) versus δDS-F (X)	+2113±1009	0.038	No	No
TEMPORAL GM, LEFT (Y) versus δTRAILS B (X)	-6535±2800	0.021	No	No
TEMPORAL GM, RIGHT (Y) versus δTRAILS B (X)	-4677±2804	0.097	No	No
TEMPORAL WM, LEFT (Y) versus δDS-F (X)	+2657±1045	0.012	Yes	No
Among women	+1040±1045	0.32	—	—
Among men	+4582±1919	0.020	—	—
TEMPORAL WM, RIGHT (Y) versus δDS-F (X)	+2763±1022	0.0076	Yes	No
Among women	+1156±1088	0.29	—	—
Among men	+4811±1813	0.010	—	—
PARIETAL GM, RIGHT (Y) versus δCDT (X)	-1955±1104	0.078	Yes	Yes
Among women	-3989±1400	0.005	—	—
Among men	-244±1758	0.89	—	—
Among Whites	-5210±1460	0.001	—	—
Among AA	+1878±1619	0.25	—	—
PARIETAL WM LEFT (Y), versus δDS-F (X)	+2129±1045	0.043	Yes	No
Among women	+932 ±1137	0.42	—	—
Among men	+3533±1857	0.061	—	—
PARIETAL WM, RIGHT (Y) versus δDS-F (X)	+2002±1001	0.047	Yes	No
Among women	+689±1,132	0.54	—	—
Among men	+3422±1731	0.052	—	—
OCCIPITAL GM, RIGHT (Y) versus δDS-F (X)	+1350±795	0.092	No	No
OCCIPITAL WM, LEFT (Y) versus δDS-F (X)	+1219±521	0.021	No	No

OCCIPITAL WM, RIGHT (Y) versus δDS-F (X)	+1240±520	0.018	No	No

Age_{v1}, Age at visit 1; δ, annualized rate of change; BTA, Brief Test of Attention; BVRT, Benton Visual Retention Test; CDT, Clock Drawing Test; DS-F, Digits Span-Forward; GM, gray matter; TBV, total brain volume; TRAILS B, Trailmaking Test, part B; WM, white matter.

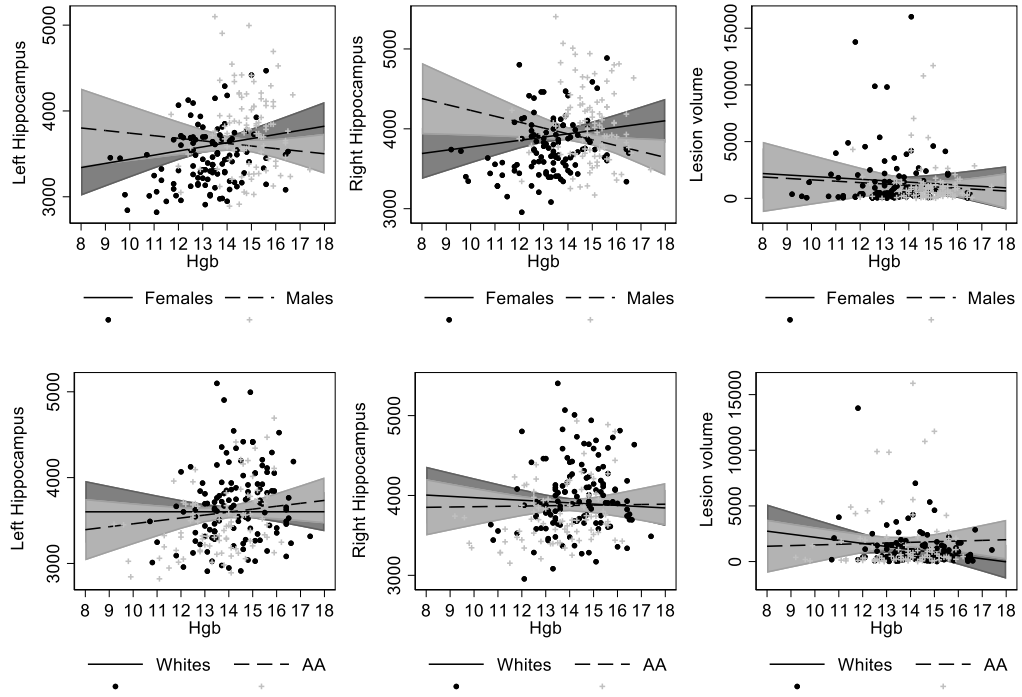
[a]Values are regression coefficients (β±SE) from multiple linear regression models with Y=brain volumetric outcomes at v_{scan} and X=annualized rates of change in cognitive performance test scores between v1 and v2. All models were adjusted for Age_{v1}, sex, race, poverty status and length of follow-up between v_1 and v_{scan}.

[b] Heterogeneity by sex or race was determined by adding a 2-way interaction term to the unstratified models, between X and race or sex. Yes=2-way interaction term is statistically significant at type I error of 0.10: No=otherwise, for the specific potential effect modifier (i.e., sex or race).

Supplementary Method 5. Hemoglobin levels and key volumetric outcomes

Additional analyses were conducted to explore the association between hemoglobin levels and the key volumetric outcomes by sex and race, using Models 1 and 2 and examining crude correlation with scatterplot matrices, across socio-demographic factors. Models 1 and 2 were additionally adjusted for total brain volume for the following outcomes: L/R hippocampal volumes and white matter lesion volumes (*note*: WML lesion volume was abbreviated as "Lesion volume"). The 3 remaining outcomes were total brain volume, gray matter and white matter global volumes. Predictive margins (with 95% CI) were obtained from multiple linear models with interaction by sex or race with Hb in Model 2. Scatterplots were crude representation of the correlation between Hb and each of the outcomes across sex or race. Tables show the results from Model 2, stratified by sex or race for each of the outcomes. In addition, heterogeneity by sex or race is also tested, by adding a 2-way interaction term in the unstratified model, at a type I error rate of 0.05.

Figure V.1 Hb (X) versus Hippocampal and lesion volumes (Y), Model 2 adjusted for total brain volume: stratified by sex and by race



AA, African American; ESR, erythrocyte sedimentation rate; Hb/Hgb, hemoglobin level; MCH, mean cell hemoglobin; RDW, red cell distribution width.
Model 2 was adjusted for visit 1 age, sex, race, poverty status, length of follow-up, total brain volume and RDW at visit 1 + other visit 1 hematological measures (MCH, Serum iron, ESR).

Table V.1 Hb versus Hippocampal and lesion volumes, Models 1 and 2 adjusted for total brain volume: multiple linear regression models stratified by sex and by race[a]

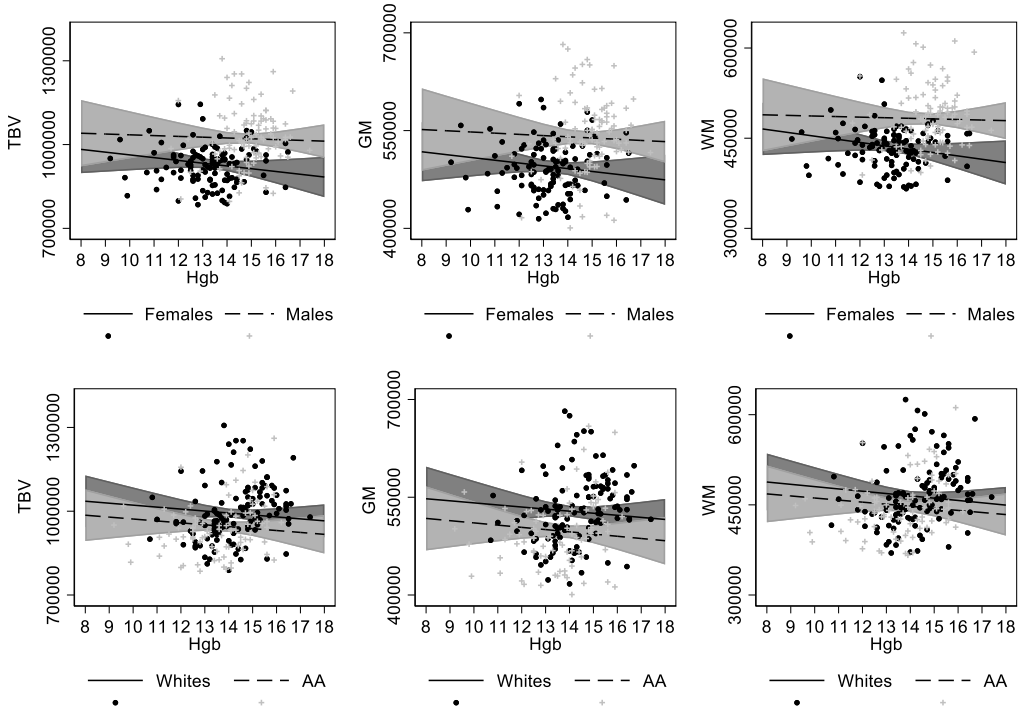
	Females N=114	Males N=99	Whites N=125	African Americans N=88
Model 1: X=Hb				
Y=Left Hippocampus	**+59.6±21.3**	-14.8±34.8	+2.43±29.4	**+59.7±22.8**
	p=0.006	p=0.66	p=0.93	**p=0.011**
Y=Right Hippocampus	**+51.2±21.0**	-66.0±34.2	-12.9±29.0	+32.6±22.5
	p=0.017	p=0.056	p=0.66	p=0.15
Y=Lesion volume	-48.9±174.2	-108.4±183.3	**-272.4±135.4**	+98.8±222.1
	p=0.78	p=0.56	**p=0.046**	p=0.66
Model 2: X=Hb				
Y=Left Hippocampus	+40.0±31.2	-22.5±40.5	-22.8±36.9	**+71.4±34.4**
	p=0.20	p=0.58	p=0.54	**p=0.041**
Y=Right Hippocampus	+37.2±30.7	-78.3±40.6 [a]	-38.1±35.7	+42.3±33.7
	p=0.23	p=0.057	p=0.29	p=0.21
Y=Lesion volume	-89.6±257.6	-214.3±214.9	-317.0±169.4	-4.2±333.0
	p=0.73	p=0.32	p=0.064	p=0.99

ESR, erythrocyte sedimentation rate; Hb, hemoglobin; MCH, mean cell hemoglobin.

[a]Values are regression coefficients (β±SE) from multiple linear regression models with Y=brain volumetric outcomes at v_{scan} and X=Hb measured at v_1. Model 1 was adjusted for visit 1 age, sex, race, poverty status, length of follow-up and total brain volume. Model 2 was additionally adjusted for RDW at visit 1 + other visit 1 hematological measures (MCH, Serum iron, ESR).

[b] $p<0.05$ for interaction by sex or race in separate model with 2-way interaction between Hb and each of those two socio-demographic factors, Model 2.

Figure V.2. Hb (X) versus Total brain volume, gray and white matter volumes (Y), Model 2: stratified by sex and by race



AA, African American; ESR, erythrocyte sedimentation rate; GM, gray matter volume; Hb/Hgb, hemoglobin level; MCH, mean cell hemoglobin; RDW, red cell distribution width; TBV, total brain volume; WM, wite matter volume
Model 2 was adjusted for visit 1 age, sex, race, poverty status, length of follow-up, and RDW at visit 1 + other visit 1 hematological measures (MCH, Serum iron, ESR).

Table V.2 Hb (X) versus global brain volume (Y), Models 1 and 2 adjusted for total brain volume: multiple linear regression models, stratified by sex and by race[a]

	Females N=114	Males N=99	Whites N=125	African Americans N=88
Model 1				
Total brain volume	-2,532±4,806 p=0.60	-757±10,062 p=0.94	-7,098±7,052 p=0.32	+3,148±6,626 p=0.64
Gray Matter volume	-650±2,633 p=0.81	-1005±52,608 p=0.85	-3,148±3,651 p=0.39	+1,821±3.636 p=0.62
Whit Matter volume	-1,882±2,424 p=0.44	+248.4±5,164 p=0.96	-3,950±3,681 p=0.29	+1,327±3,296 p=0.69
Model 2				
Total brain volume	-5,011±7,080 p=0.48	-7,134±11,767 p=0.55	-8,913±8,965 p=0.32	-2,450±9,756 p=0.80
Gray Matter volume	-1,077±3,861 p=0.78	-4,660±6,193 p=0.45	-4,849±4,618 p=0.30	+365±5,364 p=0.95
Whit Matter volume	-3,935±3,576 p=0.27	-2,477±6,030 p=0.68	-4,064±4,702 p=0.39	-2,815±4,850 p=0.56

ESR, erythrocyte sedimentation rate; Hb, hemoglobin; MCH, mean cell hemoglobin.
[a]Values are regression coefficients (β±SE) from multiple linear regression models with Y=brain volumetric outcomes at v_{scan} and X=Hb measured at v_1. Model 1 was adjusted for visit 1 age, sex, race, poverty status and length of follow-up. Model 2 was additionally adjusted for RDW at visit 1 + other visit 1 hematological measures (MCH, Serum iron, ESR).

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Supplementary Table 1. Study sample characteristics of eligible study sample by anemia (v1 and v1/v2) status and by RDW(v1) tertiles, overall, among males and among females; HANDLS 2004-2009 and HANDLS-SCAN 2011-2015[a]

	Anemia status at v1		*Anemia status at v1/v2*		*RDW at v1, tertiles*		
	Non-anemic	*Anemic*	*Non-anemic (v1 and/or v2)*	*Anemic (v1 & v2)*	*T1*	*T2*	*T3*
Total sample	*(N=191)*	*(N=22)*	*(N=183)*	*(N=12)*	*(N=72)*	*(N=70)*	*(N=71)*
Demographic factors							
Sex, % males	48.7	27.3	47.5	25.0	47.2	54.3	38.0
Age_{v1}	47.9±8.7	44.7±10.8	48.2±8.6	43.6±12.6	47.4±9.1	48±8.4	47.3±9.5
Race, % AA	37.2[b]	77.3[b]	39.3	66.7	29.2[c]	38.6[c]	56.3[c]
% above poverty	68.6	59.1	70.5	58.3	69.4	71.4	62.0
Time between v_1 and v_{scan} (y)	5.60±1.91	5.93±1.58	5.54±1.91	5.09±1.18	5.93±1.81	5.39±1.79	5.56±2.00
Imputed covariates, % or Mean±SE							
Education, y							
<High School	7.4	4.6	7.8	0.0	9.4	8.9	3.1
High School	53.2	63.6	52.7	75.0	46.7	58.0	58.3
>High School	39.4	31.8	39.6	25.0	43.9	33.1	38.6
WRAT-3 score	43.9±0.5	41.0±1.4	43.8±0.5	42.1±2.2	44.0±0.8	43.2±1.0	43.6±0.8
Current smoker, % yes	47.1	31.8	44.2	25.0	40.0	45.7	51.0
HEI-2010 total score	42.4±0.8	41.6±2.9	42.6±0.9	39.2±4.0	42.4±1.6	41.9±1.4	42.5±1.3
Serum vitamin B-12, pg/mL	526±18	474±43	531±19	490±66	563±32	499±29	499±28
Serum folate, ng/mL	15.1±0.5	14.4±1.2	15.2±0.5	15.0±1.1	15.6±0.8	15.6±0.7	14.0±0.7
C-reactive protein, mg/L	3.98±0.63	7.15±1.73	4.3±0.7	6.0±2.1	3.0±0.6[c]	3.14±0.6[c]	6.8±1.5[c]
Albumin, g/dL	4.36±0.02[b]	4.17±0.07[b]	4.34±0.02	4.19±0.08	4.38±0.03	4.36±0.03	4.29±0.04
White blood cell, count*10^9/L	6.72±0.16	6.00±0.43	6.65±0.16	5.56±0.37	6.60±0.23	6.16±0.22	7.18±0.32
Waist size, cm	98.7±1.1	100.6±4.1	99.0±1.2	103.8±6.1	95.7±1.6[c]	99.0±1.8[c]	102.2±2.2[c]
Total cholesterol, mg/dL	193.4±3.3[b]	166.0±7.1[b]	192.0±3.4	165.9±7.9	192.2±6.3	195.4±5.1	184.2±4.7
Cholesterol: HDL-Cholesterol ratio	3.95±0.11[b]	3.10±0.19[b]	3.95±0.11	3.41±0.25	3.89±0.19	4.10±0.16	3.61±0.17
Triglycerides, mg/dL	128.1±5.4[b]	86.8±7.6[b]	127±6	101.3±10.6	130.5±9.4[c]	137.7±10.1[c]	103.4±5.0[c]
Creatinine, mg/dL	0.90±0.03	0.84±0.08	0.90±0.02	0.86±0.13	0.90±0.04	0.89±0.03	0.89±0.05
Other hematological measures at v_1							
Imputed covariates, % or Mean±SE							
Mean Cell Hemoglobin, pg	30.8±0.1[b]	26.2±0.8[b]	30.7±0.2[b]	26.7±1.2	31.5±0.2[c]	30.9±0.2[c]	28.7±0.4[c]
Serum iron, µg/dL	93.2±2.6[b]	42.4±5.1[b]	90.2±2.7[b]	48.2±6.8	100.3±3.6[c]	96.3±4.4[c]	67.2±4.7[c]
Erythrocyte Sedimentation Rate, mm/h	12.9±0.8	15.7±2.7	13.4±0.8	17.3±4.6	12.1±1.2	14.6±1.5	12.9±1.2
RDW (v_1)							
CV (%)	13.7±1.1[b]	16.5±2.6[b]	13.8±1.2[b]	16.2±2.8[b]	12.8±0.4[c]	13.7±0.2[c]	15.5±1.7[c]
Median	13.5	16.7	13.5	14.6	12.9	13.6	14.9
IQR	13.0;14.1	14;17.8	13.0;14.2	13.8;19.0	12.6;13.1	13.5;13.9	14.3;15.8
sMRI measures	*(N=191)*	*(N=22)*	*(N=183)*	*(N=12)*	*(N=72)*	*(N=70)*	*(N=71)*
Global cortical brain volumes,* cm^3 *(mean±SD)							
Total brain volume	977.6±104.1	939.5±81.3	976.5±103.1	946.2±78.7	981.4±89	985.9±109.4	953.8±106.7
Gray Matter	518.1±56.2	496.5±43.5	517.5±55.8	503.8±40.4	522.2±46.1[c]	521.3±59.8[c]	504±58.1[c]
White Matter	459.5±51.3	443±40.6	459.1±50.6	442.5±42.2	459.2±46.9	464.5±52.5	449.8±51.5
***Regional cortical brain volumes*, cm^3 *(mean±SD*)**							
Left Brain							
Frontal GM	90.3±10.4	87.9±9.6	90.3±10.4	89.7±7.7	91.7±9[c]	90.7±11.1[c]	87.9±10.5[c]
Frontal WM	92.4±10.7	90.3±9.2	92.3±10.6	90.7±8.5	92.9±10	93.3±10.6	90.3±10.8
Temporal GM	49.4±5.9[b]	46.7±3.4[b]	49.3±5.8	46.8±3.7	49.8±5.2[c]	49.7±6.1[c]	47.9±5.7[c]

Temporal WM	52.5±6.1[b]	49.7±4.3[b]	52.4±6	49.4±4.8	52.3±5.4	53±6.3	51.2±6.1
Parietal GM	43.9±5.7	42.5±4.8	44±5.7	43.3±4.5	44.2±4.9	44.4±6	42.6±6
Parietal WM	46.9±5.7	45.2±4.8	46.9±5.7	45.5±5.4	46.9±5.5	47.6±5.9	45.8±5.4
Occipital GM	34.7±4.6[b]	32.3±3.8[b]	34.7±4.6	33.1±3	34.9±3.9	35.1±4.7	33.4±4.9
Occipital WM	22.6±3.1[b]	21.3±2.8[b]	22.6±3.1	20.9±2.1	22.4±2.8	22.9±3.1	22.1±3.2
Right Brain							
Frontal GM	90.0±10.5	87.6±9.5	89.8±10.5	89.8±8.1	91.2±9.1[c]	90.3±10.8[c]	87.7±11[c]
Frontal WM	94.6±11.2	92.2±8.9	94.6±11.1	92.7±8.8	94.8±10.4	95.7±11.2	92.7±11.3
Temporal GM	50.7±5.7[b]	47.7±4.1[b]	50.6±5.8	47.4±4.6	51±4.9	50.8±6.3	49.3±5.8
Temporal WM	52.6±5.9[b]	49.9±4.8[b]	52.6±5.8	49.6±5.5	52.4±5.1	53.3±6.3	51.4±5.9
Parietal GM	44.4±5.7	43.2±4.8	44.4±5.7	44.2±4.5	44.4±5	45.1±5.9	43.4±5.9
Parietal WM	44.4±5.5	42.9±4.9	44.3±5.4	42.6±5.3	44.2±5.2	45±5.8	43.6±5.3
Occipital GM	34.6±4.7[b]	32.2±3.1[b]	34.6±4.7	32.5±2.8	34.9±3.9	34.7±4.9	33.5±4.9
Occipital WM	23.4±3.1	22.5±2.5	23.4±3.1	22.4±2.1	23.5±3	23.5±3.1	23±3.1
Hippocampal volume, mm^3							
Hippocampus, Left	3,636±423[b]	3,252±286[b]	3,622±426[b]	3,276±259[b]	3,627±359	3,662±492	3,501±410
Hippocampus, Right	3,928±430[b]	3,595±272[b]	3,915±435[b]	3,629±272[b]	3,944±380[c]	3,942±476[c]	3,794±414[c]
White matter lesion volume, mm^3	1,242±2,113	1,794±3,069	1,258±2,143	2,136±4,033	975±1,586	1,604±2,510	1,327±2,463
Males	*(N=93)*	*(N=6)*	*(N=87)*	*(N=3)*	*(N=34)*	*(N=38)*	*(N=27)*
Demographic factors							
Age$_{v1}$	47.6±8.8	49.3±8.8	48.3±8.6	47.6±12.9	47.2±9.6	47.1±8.1	49.2±8.8
Race, % AA	36.6[b]	100.0[b]	36.7[b]	100.0[b]	26.5	42.1	55.6
% above poverty	74.2	66.7	77.0	66.7	76.5	76.3	66.7
Time between v_1 and v_{scan} (y)	5.54±1.87	6.28±1.56	5.43±1.85	5.34±0.17	5.74±1.71	5.33±1.90	5.76±1.99
Imputed covariates, % or Mean±SE							
Education, y							
<High School	7.1	0.0	7.6	0.0	12.9	5.3	0.0
High School	52.9	66.7	52.0	66.7	42.4	57.4	64.0
>High School	40.0	33.3	40.5	33.3	44.7	37.4	36.0
WRAT-3 score	43.8±0.9	42.0±2.3	44.0±0.9	39.3±2.7	44.0±1.4	42.5±1.6	44.9±1.1
Current smoker, % yes	41.5	50.0	38.6	33.3	37.6	41.6	48.1
HEI-2010 total score	40.9±1.1	40.3±5.9	40.9±1.2	38.8±12.5	40.3±2.2	41.1±2.0	41.4±1.8
Serum vitamin B-12, pg/mL	512±21[b]	347±37[b]	521±22	356±81	523±29	475±28	514±50
Serum folate, ng/mL	15.2±0.6	14.2±2.1	15.4±0.7	17.1±2.1	15.9±1.2	14.9±0.9	14.5±1.2
C-reactive protein, mg/L	2.8±0.6	1.3±0.2	2.8±0.6	1.2±0.5	2.5±0.9[c]	1.8±0.8[c]	4.3±1.2[c]
Albumin, g/dL	4.41±0.03	4.52±0.07	4.39±0.03	4.50±0.12	4.45±0.04	4.38±0.04	4.41±0.06
White blood cell, count*10^9/L	6.42±0.22	5.22±0.54	6.32±0.21	5.33±0.85	6.10±0.26	6.04±0.29	7.10±0.57
Waist size, cm	99.2±1.5	94.7±6.7	99.9±1.60	100.7±10.5	97.6±2.55[c]	100.0±2.02[c]	99.2±3.4[c]
Total cholesterol, mg/dL	188.7±4.6	175.5±12.2	188.4±4.8	157.3±13.6	182.7±8.2	193.0±7.4	187.4±6.5
Cholesterol: HDL-Cholesterol ratio	4.19±0.16[b]	2.70±0.23[b]	4.24±0.16	3.00±0.26	3.88±0.23	4.53±0.26	3.77±0.31
Triglycerides, mg/dL	140±9	80.0±7.0	142.0±9.7	84.0±3.2	141.1±15.5[c]	154.7±17.0[c]	104.8±7.8[c]
Creatinine, mg/dL	1.02±0.03	1.00±0.05	1.02±0.03	1.00±0.08	1.02±0.05	1.00±0.04	1.06±0.07
Other hematological measures at v_1							
Imputed covariates, % or Mean±SE							
Mean Cell Hemoglobin, pg	31.0±0.2[b]	27.6±0.7[b]	31.0±0.2[b]	27.9±1.42[b]	31.65±0.23[c]	30.91±0.22[c]	29.65±0.51[c]
Serum iron, µg/dL	102.2±3.9[b]	48.7±6.9[b]	100.9±4.0[b]	52.3±11.7[b]	108.3±5.9[c]	103.9±6.3[c]	80.1±7.4[c]
Erythrocyte Sedimentation Rate, mm/h	9.3±0.9	10.0±2.9	9.8±1.0	10.7±4.4	7.8±1.3	10.2±1.6	10.2±1.78
RDW (v_1)							
CV (%)	13.6±0.8[b]	15.3±1.3[b]	13.5±0.8	14.3±0.7	12.8±0.3[c]	13.6±0.2[c]	14.8±0.7[c]

Median	13.5	15.4	13.5	14.1	12.9	13.6	14.7
IQR	13.0;14.0	14.1;16.6	13.0;14.0	13.7;15.1	12.6;13.1	13.5;13.9	14.2;15.2
sMRI measures	*(N=93)*	*(N=6)*	*(N=87)*	*(N=3)*	*(N=34)*	*(N=38)*	*(N=27)*
Global cortical brain volumes,* cm^3 *(mean±SD)							
Total brain volume	1,034.7±102.8	962.3±121.3	1033.4±102.3	987.8±146.9	1039.9±72.9	1032.2±115.7	1015.4±123.4
Gray Matter	546.6±56.6	503.8±62.5	545.8±56.8	521.9±71.3	550.9±35.1	545.3±65.1	533.6±68.7
White Matter	488.1±50.5	458.5±59.8	487.5±49.7	465.9±76.4	489±43.8	487±53.5	481.8±57.8
***Regional cortical brain volumes,* cm^3 *(mean±SD*)**							
Left Brain							
Frontal GM	95.3±10.7	87.0±12.2	95.4±10.8	92.1±12.1	96.9±7.7	95±12.2	91.8±12
Frontal WM	97.8±10.7	92.9±13.3	97.8±10.5	96.4±15	98.6±9.4	97.4±11.1	96.3±12.3
Temporal GM	52.4±5.8	48.4±4.8	52.2±5.7	49.6±6.1	52.5±4.6	52.1±6.5	51.7±6.2
Temporal WM	55.9±6	52.1±6.7	55.8±6	51.4±9.3	55.6±5.4	55.8±6.4	55.6±6.7
Parietal GM	46.1±6.2	43.2±7.6	46±6.2	45.3±9	46.6±4.1	46.1±6.9	44.6±7.5
Parietal WM	49.8±5.8	46.7±6.9	49.7±5.7	47.5±9.5	50.1±5.3	49.7±6.1	48.7±6.2
Occipital GM	36.6±4.6[b]	32.6±4.8[b]	36.6±4.7	33.9±4.8	36.8±3.5	36.6±5	35.6±5.6
Occipital WM	24.1±2.9	22.6±3.2	24.1±2.9	22.4±2.5	24±2.7	24.3±3	23.7±3.2
Right Brain							
Frontal GM	95.1±10.7	87.4±13.3	95±10.8	92.5±13.7	96.7±7.4	94.5±11.7	92.1±13.2
Frontal WM	100.4±11.3	94.5±13.8	100.4±11.1	98.2±16.6	100.8±10.2	100.3±11.5	99±13
Temporal GM	53.6±5.6	50.6±5.4	53.6±5.6	51±7.6	53.5±3.9	53.6±6.6	53.2±6.1
Temporal WM	55.9±5.7	52.7±7.3	55.8±5.7	52.2±10.2	55.6±4.9	56±6.4	55.5±6.3
Parietal GM	46.5±6.1	44.3±6.3	46.4±6.1	46.5±7.3	46.8±4.1	46.7±6.7	45.2±7.3
Parietal WM	47.3±5.5	44.4±6.3	47.2±5.5	45±8.7	47.5±4.8	47.2±6	46.5±5.9
Occipital GM	36.8±4.6[b]	32.3±4.1[b]	36.7±4.7	33.4±4.9	37.2±3.3	36.5±5.1	35.8±5.6
Occipital WM	25.2±3[b]	22.2±2.3[b]	25.1±2.9	22.5±2.7	25.5±2.8	24.9±3	24.6±3.3
Hippocampal volume, mm^3							
Hippocampus, Left	3,782±459	3,459±303	3771.8±457.9	3415.6±134.9	3734.6±326.5	3806.2±558.3	3736.2±449.7
Hippocampus, Right	4,059±465	3,814±293	4049±471.6	3868.4±71.7	4032.3±383	4069.1±533	4025.3±449.2
White matter lesion volume, mm^3	1,173±1,914	1,306±1,664	1208.4±1970	93.2±89.1	941.2±1957.6	1118.8±1400.6	1571.4±2367.4
Females	*(N=98)*	*(N=16)*	*(N=96)*	*(N=9)*	*(N=38)*	*(N=32)*	*(N=44)*
Demographic factors							
Age$_{v1}$	48.1±8.6[b]	43.1±11.2[b]	48.1±8.7	42.3±13	47.5±8.6	49.2±8.7	46.1±9.8
Race, % AA	37.8[b]	68.8[b]	41.7	55.6	31.6[c]	34.4[c]	56.8[c]
% above poverty	63.3	56.3	64.6	55.6	63.2	65.6	59.1
Time between v_1 and v_{scan} (y)	5.65±1.95	5.81±1.64	5.64±1.96	5.00±1.37	6.11±1.90	5.48±1.69	5.44±2.02
Imputed covariates, % or Mean±SE							
Education, y							
<High School	7.7	6.3	7.9	0.0	6.3	13.1	4.5
High School	53.5	62.5	53.3	77.7	50.5	58.8	55.5
>High School	38.8	31.3	38.8	22.2	43.2	28.1	40.0
WRAT-3 score	44.0±0.6[b]	40.7±1.7[b]	43.6±0.6	43.0±2.8	43.9±0.8	44.0±1.1	42.9±1.0
Current smoker, % yes	52.4	25.0	49.4	22.0	42.1	50.6	52.7
HEI-2010 total score	43.7±1.2	42.1±3.5	44.2±1.3	39.2±4.0	44.3±2.4	43.0±2.0	43.1±1.9
Serum vitamin B-12, pg/mL	539±30	522±52	541±30	534±81	598±54	528±53	490±33
Serum folate, ng/mL	15.1±0.7	14.5±1.4	15.1±0.7	14.4±1.3	15.3±1.2	16.4±1.0	13.7±0.9
C-reactive protein, mg/L	5.1±1.1	9.3±2.1	5.7±1.1	7.6±2.6	3.47±0.9[c]	4.73±0.94[c]	8.31±2.26[c]
Albumin, g/dL	4.33±0.03 [b]	4.04±0.06 [b]	4.30±0.03 [b]	4.09±0.08 [b]	4.31±0.04	4.34±0.04	4.22±0.04
White blood cell, count*10^9/L	7.00±0.24	6.30±0.55	6.94±0.24	5.64±0.43	7.03±0.38	6.31±0.33	7.23±0.39

Waist size, cm	98.3±1.7	102.9±5.1	98.2±1.69	104.8±7.6	94.0±2.1 [c]	97.7±3.0 [c]	104.1±2.9 [c]
Total cholesterol, mg/dL	197.9±4.8 [b]	162.4±8.7 [b]	195.3±4.9	168.8±9.7	200.6±9.2	198.2±6.9	182.3±6.5
Cholesterol: HDL-Cholesterol ratio	3.73±0.15	3.26±0.24	3.70±0.14	3.55±0.31	3.90±0.29	3.59±0.16	3.51±0.20
Triglycerides, mg/dL	116.7±5.6	89.4±10.1	114.0±5.4	107.1±13.7	121.0±11.2 [c]	117.5±7.9 [c]	102.6±6.6 [c]
Creatinine, mg/dL	0.79±0.03	0.78±0.10	0.78±0.03	0.81±0.17	0.79±0.04	0.77±0.03	0.79±0.07
Other hematological measures at v_1							
Imputed covariates, % or Mean±SE							
Mean Cell Hemoglobin, pg	30.6±0.20 [b]	25.7±1.1 [b]	30.3±0.2 [b]	26.3±1.6 [b]	31.4±0.2 [c]	30.8±0.3 [c]	28.0±0.5 [c]
Serum iron, µg/dL	84.7±3.4 [b]	40.0±6.6 [b]	80.5±3.4 [b]	46.8±8.5 [b]	93.2±4.2 [c]	87.1±5.9 [c]	59.3±5.8 [c]
Erythrocyte Sedimentation Rate, mm/h	16.3±1.1	17.9±3.5	16.8±1.1	19.6±5.8	16.0±1.6	19.8±2.3	14.6±1.5
RDW (v_1)							
CV (%)	13.8±1.3[b]	16.9±2.8[b]	14.0±1.5[b]	16.8±3.0[b]	12.8±0.4[c]	13.7±0.2[c]	16.0±2.0[c]
Median	13.7	17.5	13.7	17.8	12.9	13.8	15.2
IQR	13.0;14.3	13.9;19.0	13.0;14.5	13.8;19.7	12.5;13.1	13.5;13.9	14.4;17.5
sMRI measures	*(N=98)*	*(N=16)*	*(N=96)*	*(N=9)*	*(N=38)*	*(N=32)*	*(N=44)*
Global cortical brain volumes,* cm^3 *(mean±SD)							
Total brain volume	923.5±71.7	931.0±63.7	925±72.5	932.3±47.4	929.1±67.2	930.8±70	915.9±74.1
Gray Matter	491±40.3	493.7±36.3	491.8±40.4	497.7±28.5	496.6±39.4	492.9±36.9	485.8±41.9
White Matter	432.4±34.9	437.2±31.3	433.2±35.4	434.6±26.9	432.6±31.1	437.9±36.9	430.2±35.5
***Regional cortical brain volumes,* cm^3 *(mean±SD*)**							
Left Brain							
Frontal GM	85.6±7.6	88.3±8.8	85.7±7.6	89±6.5	86.9±7.4	85.6±6.8	85.4±8.9
Frontal WM	87.2±7.7	89.3±7.4	87.3±7.9	88.8±5.2	87.7±7.5	88.4±7.6	86.7±8
Temporal GM	46.6±4.4	46.1±2.6	46.6±4.3	45.9±2.3	47.3±4.4[c]	47±4.2[c]	45.5±3.8[c]
Temporal WM	49.2±3.9	48.8±2.7	49.3±3.9	48.8±2.7	49.3±3.3	49.8±4.4	48.6±3.7
Parietal GM	41.9±4.5	42.2±3.5	42.1±4.4	42.6±2.4	42.1±4.6	42.4±3.8	41.4±4.5
Parietal WM	44.2±4.2	44.7±3.8	44.3±4.2	44.8±3.8	44±3.9	45.1±4.6	44±3.9
Occipital GM	32.9±3.7	32.2±3.5	33±3.7	32.8±2.6	33.1±3.3	33.2±3.6	32.1±3.9
Occipital WM	21.2±2.4	20.8±2.6	21.3±2.5	20.3±1.8	21±2.1	21.3±2.5	21.1±2.7
Right Brain							
Frontal GM	85.1±7.7	87.7±8.2	85.2±7.6	88.9±6.2	86.2±7.5	85.3±7.2	85±8.6
Frontal WM	89.1±7.9	91.3±6.7	89.3±8	90.9±4.8	89.4±7.2	90.4±8.1	88.8±8.1
Temporal GM	47.8±4.4	46.6±3	47.8±4.4	46.2±2.8	48.7±4.6	47.5±3.9	46.9±4.1
Temporal WM	49.5±4	48.9±3.2	49.6±4	48.8±3.5	49.5±3.4	50±4.5	48.9±3.9
Parietal GM	42.5±4.6	42.8±4.3	42.6±4.7	43.5±3.4	42.3±4.8	43.2±4.2	42.2±4.6
Parietal WM	41.7±3.9	42.3±4.4	41.8±4	41.8±4	41.2±3.4	42.5±4.5	41.8±4.1
Occipital GM	32.5±3.7	32.2±2.8	32.7±3.7	32.2±2	32.8±3.2	32.6±3.8	32±3.8
Occipital WM	21.7±2.2	22.7±2.7	21.8±2.2	22.4±2.1	21.7±1.8	21.8±2.4	21.9±2.5
Hippocampal volume, mm^3							
Hippocampus, Left	3497.8±333[b]	3174.9±245.7[b]	3485.6±343.3[b]	3229.6±279.3[b]	3530.5±363.3[c]	3490.6±331.5[c]	3357.4±310.1[c]
Hippocampus, Right	3802.6±354.1[b]	3512.9±221[b]	3794.3±360.9[b]	3549.1±267.6[b]	3864.7±363.4[c]	3791.5±350.6[c]	3651.7±319.6[c]
White matter lesion volume, mm^3	1,307±2,293	1,977±3,484	1,302±2,298	2,817±4,502	1,005±1,187	2,180±3,323	1,176±2,535

Age$_{v1}$, age measured at HANDLS visit 1 (2004-2009); CV, coefficient of variation; 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); RDW, red cell distribution width; sMRI, structural magnetic resonance imaging; T1-T3, tertiles; v_1, visit 1 of HANDLS (2004-2009); v_2, visit 2 of HANDLS (2009-2013); v_{scan}, HANDLS-SCAN visit (2011-2015); WM, white matter.

[a] Values are Mean±SD, or %. For RDW, medians and inter-quartile ranges (IQR) were also provided. Volumes are expressed in mm^3 for hippocampal volumes and white matter lesion volume and cm^3 otherwise.

[b] $p<0.05$ for null hypothesis of no difference between anemic or non-anemic, t-test; [c] $p<0.05$ for null hypothesis of no trend across tertiles of RDW and δRDW.

Supplementary Table 1 Results

Overall, African Americans were consistently more represented in the anemic group (versus non-anemic group); and in the uppermost tertiles of RDW, compared with the lowest tertiles, with a dose-response relationship. RDW_{v1} means were also higher in the anemic groups versus non-anemic. In the left brain, there were consistent associations of $anemia_{(v1)}$ and $RDW_{(v1)}$ tertiles with reduced temporal GM. Higher $RDW_{(v1)}$ tertile was also linked to smaller frontal GM volumes, with a dose-response relationship. This association was also found for the right frontal GM, while $anemia_{(v1)}$ was linked to smaller temporal GM and WM in the right brain. Mean left and right hippocampal volumes were generally smaller in the anemic group and with higher RDW tertiles, associations and trends found only among females. WMLV was not related to anemia, RDW tertiles. Anemic participants had lower serum albumin and lipids compared to the non-anemic, while elevated RDW was associated with elevated CRP and reduced serum vitamin B-12 levels among others.

Supplementary Table 2. Hematological measure and other covariate-adjusted associations from analyses A (global GM and WM volume), B (hippocampal volume), and C (White matter lesion volume) versus visit 1 anemia (overall and stratified by sex): ordinary least square analyses; HANDLS 2004-2009 and HANDLS-SCAN 2011-2015: Sensitivity analyses[a]

	Model 3		*Model 4*		*Model 5*		*Model 6*	
Total sample (N=213)	*β3*	*(SE3)*	*β4*	*(SE4)*	*β5*	*(SE5)*	*β6*	*(SE6)*
sMRI, Analysis A								
Total brain	+13,512	(23,707)	+8,707	(23,623)	+17,450	(23,717)	11,647	23,631
GM	+6,819	(12,595)	+3,519	(12,565)	+8,723	(12,568)	4,813	12,552
WM	+6,694	(12,131)	+5190	(12,090)	+8,727	(12,188)	6,834	12,109
sMRI, Analysis B								
Hippocampus, Left	-241	(110)[d]	-248	(109)[d]	-208	(109)[c]	-219	(108)[d]
Hippocampus, Right	-150	(113)	-165	(112)	-131	(112)	-145	(111)
Analysis C								
White matter lesion volume	+691	(623)	+782	(617)	+844	(620)	+786	(621)
Males (N=99)								
sMRI, Analysis A								
Total brain	+214	(51,225)	-33,768	(52,453)	-10,982	(50,744)	-11,406	(49,120)
GM	+2,606	(26,882)	-15,260	(27,730)	-1884	(26,480)	-4,148	(25,728)
WM	+2392	(26,382)	-18,508	(26,824)	-9098	(26,119)	-7,258	(25,325)
sMRI, Analysis B								
Hippocampus, Left	-205	(243)	-285	(246)	-134	(234)	-164	(230)
Hippocampus, Right	-49	(246)	-131	(251)	-26	(238)	-85	(236)
Analysis C								
White matter lesion volume	-865	(948)	-538	(950)	-902	(923)	-727	(917)
Females (N=114)								
sMRI, Analysis A								
Total brain	+19,789	(24,138)	21,663	(24,107)	+25,490	(23,978)	+26,744	(24,599)
GM	+10,704	(13,148)	9,819	(13,128)	+12,272	(13,070)	+12,579	(13,431)
WM	+9,084	(12,138)	11,844	(12,216)	+13,218	(12,167)	+14,165	(12,440)
sMRI, Analysis B								
Hippocampus, Left	-283	(111)[d]	-276	(110)[d]	-255	(111)[d]	-207	(112)[c]
Hippocampus, Right	-210	(117)[c]	-196	(116)[c]	-192	(119)	-143	(117)
Analysis C								
White matter lesion volume	+1,410	(880)	+1,439	(877)	+1,604	(870)[c]	1598	(887)[c]

Age_{v1}, age measured at HANDLS visit 1 (2004-2009); B-12, serum cobalamin; CV, coefficient of variation; ESR, erythrocyte sedimentation rate; 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; HDL, high density lipoprotein; MCH, mean cell hemoglobin; RDW, red cell distribution width; SE, standard error; sMRI, structural magnetic resonance imaging; v_1, visit 1 of HANDLS (2004-2009); v_2, visit 2 of HANDLS (2009-2013); v_{scan}, HANDLS-SCAN visit (2011-2015); WM, white matter; WRAT, Wide Range Achievement Test.

[a] Values are adjusted linear regression coefficients β with associated SE. (N) is the sample size in each analysis. Model 2 in Table 2 was adjusted for Age_{v1}, sex, race, poverty status and time of follow-up between visit 1 and v_{scan} and selected hematological status measures [i.e., RDW + other hematological measures (MCH, Serum iron, ESR)]. Volumes are expressed in mm^3.

[b] Model 3 is a sensitivity analysis further adjusting Model 2 (Table 2) for selected nutritional/dietary factors (Healthy Eating Index-2010 total score, B-12, folate); Model 4 is a sensitivity analysis further adjusting Model 2 (Table 2) for selected inflammatory markers (high sensitivity C-reactive protein, albumin, White blood cells); Model 5 is a sensitivity analysis further adjusting Model 2 (Table 2) for selected adiposity and metabolic factors (Waist

circumference, cholesterol, Cholesterol:HDL ratio, Triglycerides, Creatinine); Model 5 is a sensitivity analysis further adjusting Model 2 (Table 2) for other selected covariates (education, WRAT, smoking). Selection of covariates beyond socio-demographics was done using machine learning techniques followed by backward elimination for each exposure. Common covariates to all exposures were then selected. (See Supplementary Methods 2).

[c] $p<0.10$; [d] $p<0.05$; [e] $p<0.010$ for null hypothesis that exposure main effect is =0 in each model, stratified or unstratified.

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

Supplementary Table 3. Hematological measure and other covariate-adjusted associations from analyses A (global GM and WM volumes), A' (regional cortical GM/WM), B (hippocampal volume) and C (White matter lesion volume) versus visit 1 RDW (overall and stratified by sex): ordinary least square analyses; HANDLS 2004-2009 and HANDLS-SCAN 2011-2015: Sensitivity analyses[a]

	Model 3		*Model 4*		*Model 5*		*Model 6*	
Total sample (N=213)	*β3*	*(SE3)*	*β4*	*(SE4)*	*β5*	*(SE5)*	*β6*	*(SE6)*
sMRI, Analysis A								
Total brain	-10,826	(5,506)[c]	-11,269	(5,508)[d]	-12,499	(5,466)[d]	-10,078	(5,556)[c]
GM	-5,894	(2,925)[d]	-6,125	(2,930)[d]	-6,912	(2,898)[d]	-5,561	(2,952)[c]
WM	-4,932	(2,818)[c]	-5,144	(2,820)[c]	-5,587	(2,809)[d]	-4,516	(2,847)
sMRI, Analysis A'								
Left Brain								
Frontal GM	-1,294	(557)[d, f]	-1,438	(559)[d, f]	-1,577	(546)[e, f]	__	
Frontal WM	-1,331	(608)[d]	-1,410	(609)[d]	-1,516	(603)[d]	__	
Temporal GM	-170	(316)	-165	(315)	-229	(315)	__	
Temporal WM	-560	(328)	-588	(328)[c]	-644	(328)[c]	__	
Parietal GM	-816	(316)[d]	-776	(313)[d]	-817	(310)[e]	__	
Parietal WM	-447	(328)	-440	(327)	-482	(327)	__	
Occipital GM	-539	(250)[d]	-556	(252)[d]	-620	(251)[d]	__	
Occipital WM	-246	(172)	-255	(173)	-268	(172)	__	
Right Brain								
Frontal GM	-1,156	(569)[d, f]	-1,310	(575)[d, f]	-1,494	(562)[e, f]	__	
Frontal WM	-1,421	(636)[d]	-1,486	(637)[d]	-1,614	(632)[d]	__	
Temporal GM	-370	(313)	-375	(314)	-415	(314)	__	
Temporal WM	-586	(319)[c]	-602	(319)[c]	-628	(317)[d]	__	
Parietal GM	-550	(319)[c]	-534	(318)[c]	-586	(314)[c]	__	
Parietal WM	-196	(316)	-194	(315)	-231	(314)	__	
Occipital GM	-425	(247)[c]	-416	(247)[c]	-492	(248)[d]	__	
Occipital WM	-112	(170)	-125	(170)	-140	(170)	__	
sMRI, Analysis B								
Hippocampus, Left	-45	(26)[c]	-39	(26)	-51	(26)[d]	-42	(26)
Hippocampus, Right	-56	(26)[d]	-47	(26)[c]	-59	(26)[d]	-52	(26)[d]
Analysis C								
White matter lesion volume	+114	(145)	127	(144)	76	(144)	92	(146)
Males (N=99)								
sMRI, Analysis A								
Total brain	-16,703	(13,432)	-15,549	(13,749)	-16,944	(14,019)	-12,823	(13,931)
GM	-7,870	(7,037)	-7,514	(7,258)	-7,121	(7,296)	-5,266	(7,294)
WM	-8,833	(6,932)	-8,035	(7,033)	-9,822	(7,237)	-7,557	(7,183)
sMRI, Analysis B								
Hippocampus, Left	+1	(64)	+0.80	(65)	-2	(64)	+25	(65)
Hippocampus, Right	-49	(64)	-50	(65)	-46	(64)	-30	(66)
Analysis C								
White matter lesion volume	+318	(249)	+304	(247)	+229	(255)	+246	(258)
Females (N=114)								
sMRI, Analysis A								
Total brain	-6,198	(5,736)	-5,891	(5,601)	-7,616	(5,582)	-6,742	(5,631)
GM	-4,348	(3,126)	-3,974	(3,051)	-4,962	(3,044)	-4,511	(3,077)
WM	-1,850	(2,875)	-1,917	(2830)	-2,654	(2,830)	-2,231	(2,839)
sMRI, Analysis B								
Hippocampus, Left	-55	(27)[d]	-45	(26)[c]	-59	(26)[d]	-58	(26)[d]

Hippocampus, Right	-44	(28)	-32	(27)	-45	(28)	-44	(27)
Analysis C								
White matter lesion volume	+35	(211)	+48	(206)	-19	(206)	+29	(206)
Non-Anemic (N=191)								
sMRI, Analysis A								
Total brain	-7,970	(6,824)	-7205	(6,832)	-9,567	(6,953)	-8,245	(6,938)
GM	-4,493	(3,627)	-4,051	(3,636)	-5,659	(3,683)	-4658.026	(3,691)
WM	-3,477	(3,500)	-3,154	(3,509)	-3,908	(3,582)	-3,587	(3,560)
sMRI, Analysis B								
Hippocampus, Left	-35	(32)	-28	(33)	-51	(33)	-32	(33)
Hippocampus, Right	-52	(33)	-46	(33)	-63	(33)[c]	-53	(34)
Analysis C								
White matter lesion volume	+109	(167)	+99	(167)	+93	(171)	+52	(170)

Age_{v1}, age measured at HANDLS visit 1 (2004-2009); B-12, serum cobalamin; CV, coefficient of variation; ESR, erythrocyte sedimentation rate; 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; Hb, hemoglobin; HDL, high density lipoprotein; MCH, mean cell hemoglobin; RDW, red cell distribution width; SE, standard error; sMRI, structural magnetic resonance imaging; v_1, visit 1 of HANDLS (2004-2009); v_2, visit 2 of HANDLS (2009-2013); v_{scan}, HANDLS-SCAN visit (2011-2015); WM, white matter; WRAT, Wide Range Achievement Test.

[a] Values are adjusted linear regression coefficients β with associated SE. (N) is the sample size in each analysis. Model 2 in Table 3 was adjusted for Age_{v1}, sex, race, poverty status and time of follow-up between visit 1 and v_{scan} and selected hematological status measures [i.e., Hb + other hematological measures (MCH, Serum iron, ESR)]. Volumes are expressed in mm^3.

[b] Model 3 is a sensitivity analysis further adjusting Model 2 (Table 3) for selected nutritional/dietary factors (Healthy Eating Index-2010 total score, B-12, folate); Model 4 is a sensitivity analysis further adjusting Model 2 (Table 3) for selected inflammatory markers (high sensitivity C-reactive protein, albumin, White blood cells); Model 5 is a sensitivity analysis further adjusting Model 2 (Table 3) for selected adiposity and metabolic factors (Waist circumference, cholesterol, Cholesterol:HDL ratio, Triglycerides, Creatinine); Model 5 is a sensitivity analysis further adjusting Model 2 (Table 3) for other selected covariates (education, WRAT, smoking).

[c]$p<0.10$; [d]$p<0.05$; [e]$p<0.010$ for null hypothesis that exposure main effect is =0 in each model, stratified or unstratified.

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

Supplementary Table 4. Minimally and hematological measure-adjusted associations from analyses A (global GM and WM volumes), A' (regional cortical GM/WM), B (hippocampal volume), and C (White matter lesion volume) versus δRDW (overall and stratified by sex; and among non-anemic participants): ordinary least square analyses; HANDLS 2004-2009 and HANDLS-SCAN 2011-2015[a]

	***Model 1**: Minimally adjusted*					***Model 2**: Hematological measure-adjusted, sensitivity analysis (SA)[b]*			
Total sample (N=213)	*β1*	*(SE1)*	*b1*	*P1*	*q-value1*	*β2*	*(SE2)*	*P2*	*Interaction by sex*
sMRI, Analysis A									
Total brain	+32,329	(83,473)	0.02	0.70	__	36,283	(84,498)	0.67	0.30
GM	+24,518	(44,490)	+0.03	0.58	__	27,089	(44,976)	0.55	0.36
WM	+7,810	(42,518)	+0.011	0.85	__	9,193	(43,119)	0.83	0.28
sMRI, Analysis B									
Hippocampus, Left	+25.8	(391)	+0.004	0.95	__	-69.9	(394.6)	0.86	0.082
Hippocampus, Right	-79.6	(398)	-0.012	0.84	__	-159.8	(402.3)	0.69	0.43
Analysis C									
White matter lesion volume	+2,572	(2,164)	+0.08	0.24	__	2,552	(2,188)	0.25	0.43
Males (N=99)									
sMRI, Analysis A									
Total brain	-125,737	(204,149)	-0.06	0.54	__	-112,996	(213,885)	0.60	__
GM	-45,725	(107,021)	-0.04	0.67	__	-36,469	(112,469)	0.75	__
WM	-80,012	(104,662)	-0.08	0.45	__	-76,527	(109,549)	0.49	__
sMRI, Analysis B									
Hippocampus, Left	-893	(930)	-0.10	0.34	__	-1,064	(986)	0.28	__
Hippocampus, Right	-441	(947)	-0.05	0.64	__	-365	(992)	0.71	__
Analysis C									
White matter lesion volume	-2,415	(3,716)	-0.06	0.52	__	-2,656	(3,888)	0.50	__
Females (N=114)									
sMRI, Analysis A									
Total brain	+86,006	(77,450)	+0.10	0.27	__	+86,747	(79,107)	0.27	__
GM	+51,893	(42,345)	+0.11	0.22	__	+53,777	(43,209)	0.22	__
WM	34,113	(39,218)	+0.08	0.31	__	+32,973	(39,902)	0.41	__
sMRI, Analysis B									
Hippocampus, Left	+300	(383)	+0.07	0.43	__	+244	(382)	0.52	__
Hippocampus, Right	+12	(396)	+0.00	0.98	__	-40	(396)	0.92	__
sMRI, Analysis C									
White matter lesion volume	+4,390	(2,826)	+0.14	0.12	__	+4,306	(2,876)	0.14	__
Non-Anemic, v_1 or v_2 or both (N=183)									
sMRI, Analysis A									
Total brain	+10,878	(90,261)	+0.01	0.90	__	+53,463	(94,241)	0.57	__
GM	+6,477	(48,046)	+0.01	0.89	__	+30,604	(50,002)	0.54	__

WM	4,401	(46,163)	+0.01	0.92	__	+22,858	(48,447)	0.64	__
sMRI, Analysis B									
Hippocampus, Left	+110	(430)	+0.02	0.80	__	+116	(456)	0.80	__
Hippocampus, Right	+4	(446)	+0.00	0.99	__	+28	(471)	0.95	__
sMRI, Analysis C									
White matter lesion volume	1,435	(2,269)	+0.04	0.53		+830	(2,401)	0.73	

Age_{v1}, age measured at HANDLS visit 1 (2004-2009); CV, coefficient of variation; δRDW, red cell distribution width annualized change between visits 1 and 2; ESR, erythrocyte sedimentation rate; 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; Hb, hemoglobin; MCH, mean cell hemoglobin; SE, standard error; sMRI, structural magnetic resonance imaging; v_1, visit 1 of HANDLS (2004-2009); v_2, 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 δRDW are computed as SD in outcome per SD in δRDW. Q-values presented only for uncorrected p-values<0.05 for model 1. Model 1 was adjusted for age, sex, race, poverty status 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 selected hematological measures [i.e., Hb + other hematological measures (MCH, Serum iron, ESR)] after screening using machine learning techniques (See Supplementary 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.

Supplementary Table 5. Summary of findings from sensitivity analyses (for hippocampal and lesion volume outcomes), adjusted for total brain volume as proxy to intracranial volume, overall, by sex and among the non-anemic, HANDLS-SCAN 2011-2015

	Model 1	Model 2	Model 3	Model 4	Model 5	Model 6
	Anemia at v_1					
Brain volumes, mm^3	***Overall (n=213)***					
Hippocampus, Left	-282.1±72.4, p<0.001	-273.7±89.0 p=0.002	-276.8±90.5 p=0.003	-271.2±89.9 p=0.003	-253.4±90.7 p=0.006	-249.4±89.7 p=0.006
Hippocampus, Right	-216.9±72.5 p=0.003	-198.9± 88.6 p=0.026	-189.4±89.8 p=0.036	-190.0±89.2 p=0.034	-181.2±90.1 p=0.046	-178.1±89.4 p=0.048
Lesion volume	+496.9±499.5 [c] p=0.32	+702.0 ±608.8 [c] p=0.25	642.9±619.9 [c] p=0.30	+752.3±613.2 [c] p=0.22	788.5 ±618.7 [c] p=0.20	+743.7 ±617.5 [c] p=0.23
	Males (n=99)					
Hippocampus, Left	-134.2±146.0 p=0.36	-144.3±168.4 p=0.39	-206.3±176.3 p=0.25	-173.8±178.7 p=0.33	-100.2±173.6 p=0.57	-128.1 ±172.7 p=0.46
Hippocampus, Right	-43.3±150.0 p=0.77	-39.1±172.9 p=0.82	-49.8±181.7 p=0.79	-20.3±186.4 p=0.91	8.4±178.4 p=0.96	-47.9 ±176.7 p=0.79
Lesion volume	-516.9±789.1 p=0.51	-655.0±898.8 p=0.47	-865.6±947.4 p=0.36	-487.1±954.3 p=0.61	-880.0±922.8 p=0.34	-699.9±914.7 p=0.45
	Females (n=114)					
Hippocampus, Left	-351.6±78.7 p<0.001	-317.5±100.0 p=0.002	-321.0±101.8 p=0.002	-317.6±101.4 p=0.002	-302.2±102.6 p=0.004	-257.3±102.7 p=0.014
Hippocampus, Right	-296.9±78.8 p<0.001	-260.9±99.8 p=0.010	-259.0±101.8 p=0.013	-250.2±100.4 p=0.014	-257.5±103.1 p=0.014	-206.9±102.2 p=0.046
Lesion volume	+842.8±671.1 p=0.21	+1,259.9±849.8 p=0.14	+1,267.6±872.3 p=0.15	+1,298.1±871.0 p=0.14	+1,459.7±869.8 p=0.097	1440.2±884.8 p=0.11
	RDW at v_1					
	Overall (n=213)					
	Model 1	Model 2	Model 3	Model 4	Model 5	Model 6
Hippocampus, Left	-29.1±15.1 [c] p=0.055	-13.2±21.3 [c] p=0.54	-16.5±21.7 [c] p=0.45	-9.04 ±21.7 [c] p=0.68	-18.9 ±21.7 [c] p=0.39	-16.3±21.7 p=0.45
Hippocampus, Right	-28.2±14.9 p=0.059	-20.1±21.0 p=0.34	-24.7±21.3 p=0.25	-15.2±21.2 p=0.48	-23.6±21.3 p=0.27	-24.1±21.4 p=0.26
Lesion volume	28.8±101.5 p=0.78	148.1±142.8 p=0.30	151.7 ±145.6 p=0.30	+ 164.5±144.8 p=0.26	114.7±145.3 p=0.43	129.2± 146.6 p=0.38
	Males (n=99)					
Hippocampus, Left	+29.2±39.3 p=0.46	+58.2±46.7 p=0.22	55.6±47.2 p=0.24	52.0±47.1 p=0.27	50.1±48.4 p=0.30	64.6±49.4 p=0.19
Hippocampus, Right	12.5±40.3 p=0.76	9.03±46.9 p=0.85	4.8±47.2 p=0.92	0.4±47.6 p=0.99	+5.4± 48.4 p=0.91	11.0±49.6 p=0.83

Lesion volume	153.4±211.7 p=0.47	334.6±248.1 p=0.18	351.8±251.4 p=0.17	327.8±249.3 p=0.19	260.5±256.9 p=0.31	275.0±259.2 p=0.29
	Females (n=114)					
Hippocampus, Left	-45.9±15.7 p=0.004	-39.4±24.1 p=0.11	-44.2±25.1 p=0.081	-34.4±24.4 p=0.16	-45.8±24.9 p=0.069	-45.9±24.1 p=0.059
Hippocampus, Right	-39.8±15.5 p=0.012	-25.4±23.7 p=0.29	-29.3± 24.8 p=0.24	-17.6±23.9 p=0.46	-26.1±24.8 p=0.30	-28.6±23.7 p=0.23
Lesion volume	-5.8 ±128.9 p=0.97	68.2±198.9 p=0.73	78.7±209.6 p=0.71	89.0±204.3 p=0.66	27.0±206.0 p=0.90	73.0±204.8 p=0.72
	Non-anemic (n=191)					
Hippocampus, Left	-13.0±22.7 p=0.57	-8.2± 25.5 p=0.75	-11.7±25.9 p=0.65	-7.2±26.0 p=0.78	-24.1± 26.2 p=0.36	-8.5±26.2 p=0.75
Hippocampus, Right	-26.3±22.9 p=0.25	-23.5± 25.2 p=0.35	-27.5±25.5 p=0.28	-23.4±25.6 p=0.36	-34.3±26.1 p=0.19	-27.8±26.1 p=0.29
Lesion volume	25.9±146.8 p=0.86	123.2±164.1 p=0.45	134.9±166.1 p=0.42	121.8±166.6 p=0.47	121.7±171.1 p=0.48	79.0±169.5 p=0.64

Age_{v1}, age measured at HANDLS visit 1 (2004-2009); B-12, serum cobalamin; CV, coefficient of variation; ESR, erythrocyte sedimentation rate; HANDLS, Healthy Aging in Neighborhoods of Diversity Across the Life Span; HANDLS-SCAN, Brain magnetic resonance imaging scan ancillary study of HANDLS; Hb, hemoglobin; HDL, high density lipoprotein; MCH, mean cell hemoglobin; SE, standard error; v_1, visit 1 of HANDLS (2004-2009); v_2, visit 2 of HANDLS (2009-2013); v_{scan}, HANDLS-SCAN visit (2011-2015); WRAT, Wide Range Achievement Test.

a Values are adjusted linear regression coefficients β with associated SE. (N) is the sample size in each analysis. Model 2 in Table 4 was adjusted for Age_{v1}, sex, race, poverty status and time of follow-up between visit 1 and v_{scan} and selected hematological measures [i.e., Hb + other hematological measures (MCH, Serum iron, ESR)]. Volumes are expressed in mm^3.

[b] Model 1 adjusted for Age_{v1}, sex, race, poverty status, length of follow-up between v_1 and v_{scan} and total brain volume. Model 2 adjusted for other hematological measures, including MCH, ESR, serum iron, RDW at v1 (for anemia) and Hemoglobin at v1 (for RDW). Model 3 is a sensitivity analysis further adjusting Model 2 for selected nutritional/dietary factors (Healthy Eating Index-2010 total score, B-12, folate); Model 4 is a sensitivity analysis further adjusting Model 2 for selected inflammatory markers (high sensitivity C-reactive protein, albumin, White blood cells); Model 5 is a sensitivity analysis further adjusting Model 2 for selected adiposity and metabolic factors (Waist circumference, cholesterol, Cholesterol:HDL ratio, Triglycerides, Creatinine); Model 5 is a sensitivity analysis further adjusting Model 2 for other selected covariates (education, WRAT, smoking).

[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.

Supplementary Table 6. Summary of findings from sensitivity analyses (for hippocampal and lesion volume outcomes), adjusted for total brain volume as proxy to intracranial volume, by race HANDLS-SCAN 2011-2015

	Anemia at v_1					
	Model 1	Model 2	Model 3	Model 4	Model 5	Model 6
Brain volumes, mm^3	***African Americans (n=88)***					
Hippocampus, Left	-269.3±74.2 p<0.001	-294.9±91.9 p=0.002	-289.8±95.0 p=0.003	-295.2±95.4 p=0.003	-296.7±97.1 p=0.003	-268.3±93.1 p=0.005
Hippocampus, Right	-199.0±73.3 p=0.008	-242.6±90.2 p=0.009	-223.5±91.3 p=0.017	-233.4±93.8 p=0.015	-244.6±96.0 p=0.013	-219.6±92.5 p=0.020
Lesion volume	-564.5±745.3[c] p=0.45	-491.2±920.9[c] p=0.60	-640.0±954[c] p=0.50	-324.8±953.3[c] p=0.73	-787.3±986.2[c] p=0.43	-507.2±954.1[c] p=0.60
	Whites (n=125)					
Hippocampus, Left	-323.9±158.7 p=0.044	-381.8±218.9 p=0.084	-404.0±224.0 p=0.074	-403.7±223.0 p=0.073	-406.3±222.8 p=0.071	-332.3±222.9 p=0.14
Hippocampus, Right	-246.6±158.0 p=0.12	-161.1±215.2 p=0.46	-173.5±220.1 p=0.43	-175.9±219.4 p=0.42	-168.2±220.3 p=0.45	-113.1±217.4 p=0.60
Lesion volume	+3,663.6±678.0 p<0.001	+5,300.3±905.5 p<0.001	+5,353.4±925,1 p<0.001	+5,302.1±905.9 p<0.001	5,396.1±936.8 p<0.001	5,261.3±916.9 p<0.001
	RDW at v_1					
	Model 1	Model 2	Model 3	Model 4	Model 5	Model 6
	African Americans (n=88)					
Hippocampus, Left	-29.4±17.1 p=0.090	-8.2±25.5 p=0.75	-10.8±26.0 p=0.68	-5.44±26.4 p=0.84	-12.9±26.8 p=0.63	-6.67±25.7 p=0.80
Hippocampus, Right	-20.6±16.5 p=0.22	-14.8±25.0 p=0.56	-19.6±24.9 p=0.43	-11.0±25.9 p=0.67	-20.0±26.2 p=0.45	-15.7±25.5 p=0.54
Lesion volume	-117.3±162.4[c] p=0.47	+41.5±247.2[c] p=0.87	+59.8±252.4[c] p=0.81	+75.9±254.8[c] p=0.77	+104.0±260.5[c] p=0.69	+15.1±255.9[c] p=0.95
	Whites (n=125)					
Hippocampus, Left	-27.3±28.2 p=0.34	+14.1±39.7 p=0.72	+9.04±42.0 p=0.83	+17.5±40.7 p=0.67	+4.8±40.7 p=0.91	+10.2±42.4 p=0.81
Hippocampus, Right	-40.0±27.8 p=0.16	5.37±38.5 p=0.89	+1.24±0.98 p=0.98	+7.22±39.4 p=0.86	-0.80±0.98 p=0.98	+1.8±40/9 p=0.97
Lesion volume	+291.3±130.1 p=0.027	+318.8±182.8 p=0.084	+272.2±195.9 p=0.17	+298.6±184.9 p=0.11	+289.4±188.9 p=0.13	+335.1±194.6 p=0.088

Age_{v1}, age measured at HANDLS visit 1 (2004-2009); B-12, serum cobalamin; CV, coefficient of variation; ESR, erythrocyte sedimentation rate; HANDLS, Healthy Aging in Neighborhoods of Diversity Across the Life Span; HANDLS-SCAN, Brain magnetic resonance imaging scan ancillary study of HANDLS; Hb, hemoglobin; HDL, high density lipoprotein; MCH, mean cell hemoglobin; SE, standard error; v_1, visit 1 of HANDLS (2004-2009); v_2, visit 2 of HANDLS (2009-2013); v_{scan}, HANDLS-SCAN visit (2011-2015); WRAT, Wide Range Achievement Test.

[a] Values are adjusted linear regression coefficients β with associated SE. (N) is the sample size in each analysis. Model 2 in Table 4 was adjusted for Age_{v1}, sex, race, poverty status and time of follow-up between visit 1 and v_{scan} and selected hematological measures [i.e., Hb + other hematological measures (MCH, Serum iron, ESR)]. Volumes are expressed in mm^3.

[b] Model 1 adjusted for Age_{v1}, sex, poverty status, length of follow-up between v_1 and v_{scan} and total brain volume. Model 2 adjusted for other hematological measures, including MCH, ESR, serum iron, RDW at v1 (for anemia) and Hemoglobin at v1 (for RDW). Model 3 is a sensitivity analysis further adjusting Model 2 for selected nutritional/dietary factors (Healthy Eating Index-2010 total score, B-12, folate); Model 4 is a sensitivity analysis further adjusting Model 2 for selected inflammatory markers (high sensitivity C-reactive protein, albumin, White blood cells); Model 5 is a sensitivity analysis further adjusting Model 2 for selected adiposity and metabolic factors (Waist circumference, cholesterol, Cholesterol:HDL ratio, Triglycerides, Creatinine); Model 5 is a sensitivity analysis further adjusting Model 2 for other selected covariates (education, WRAT, smoking).

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

Supplementary Table 7. Summary of findings from sensitivity analyses (for total brain, Gray Matter and White Matter volumes), by race HANDLS-SCAN 2011-2015

	Anemia at v_1					
	Model 1	Model 2	Model 3	Model 4	Model 5	Model 6
Brain volumes, mm^3	***African Americans (n=88)***					
Total brain	-2,002±22,352 p=0.93	+12,157±26,984 p=0.65	+4,753±27,629 p=0.86	+10,485±28,106 p=0.71	+19,657±28,736 p=0.50	12,447±26,851 p=0.64
Gray matter	+627±12,267 p=0.96	+6,303±14,834 p=0.67	+3,298±15,220 p=0.83	5,491±15,448 p=0.72	12,170±15,788 p=0.44	+7,178±14,778 p=0.63
White matter	-2,629±11,110 p=0.81	+5,853±13,440 p=0.66	+1,456±13,743 p=0.92	4,994±13,994 p=0.72	7,487±14,332 p=0.60	5,268±13,473 p=0.70
	Whites (n=125)					
Total brain	+7,961±39,089 p=0.84	+15,907±54,306 p=0.77	+19,021±54,590 p=0.73	10,165±55,196 p=0.85	11,981±55,865 p=0.83	+18,413±55,540 p=0.74
Gray matter	-3.270±20,221 p=0.87	+376±27,992 p=0.99	4,260±28,040 p=0.88	-3,031±28,409 p=0.92	-3,739±28,665 p=0.90	+1,708±28,770 p=0.95
White matter	+11,232±20,392 p=0.58	+15,531±28,427 p=0.59	1,456±13,743 p=0.92	13,196±28,947 p=0.65	+15,720±29,319 p=0.59	+16,706±28,870 p=0.56
	RDW at v_1					
	Model 1	Model 2	Model 3	Model 4	Model 5	Model 6
	African Americans (n=88)					
Total brain	-5,147±4,802 p=0.29	-8,630±7,180 p=0.23	-7,299±7,248 p=0.32	-8,842±7,442 p=0.24	-8,525±7,548 p=0.26	-8,910±7,124 p=0.22
Gray matter	-2,273±2,642 p=0.39	-3,485±3,948 p=0.38	-2,696±3,990 p=0.50	-3,695±4,090 p=0.37	-3,630±4,164 p=0.39	-3,693±3,922 p=0.35
White matter	-2,874±2,383 p=0.23	-5,145±3,570 p=0.15	-4,602±3,604 p=0.21	-5,147±3,701 p=0.17	-4,895±3,736 p=0.19	-5,217±3,569 p=0.15
	Whites (n=125)					
Total brain	-3,663±6,844 p=0.59	-11,688±9,655 p=0.23	-11,857±10,101 p=0.24	-11,514±9,866 p=0.25	-11,265±9,966 p=0.26	-11,215±10,391 p=0.28
Gray matter	-2,887±3,534 p=0.42	-7,742±4,972 p=0.12	-7,768±5,169 p=0.14	-7,656±5,075 p=0.13	-7,850±5,112 p=0.13	-7,631±5,369 p=0.16
White matter	-776±3,578 p=0.83	-3,945±5,064 p=0.44	-4,089±5,328 p=0.44	-3,858±5,182 p=0.46	-3,416±5,244 p=0.52	-3,584±5,412 p=0.51

Age_{v1}, age measured at HANDLS visit 1 (2004-2009); B-12, serum cobalamin; CV, coefficient of variation; ESR, erythrocyte sedimentation rate; HANDLS, Healthy Aging in Neighborhoods of Diversity Across the Life Span; HANDLS-SCAN, Brain magnetic resonance imaging scan ancillary study of HANDLS; Hb, hemoglobin; HDL, high density lipoprotein; MCH, mean cell hemoglobin; SE, standard error; sMRI, structural magnetic resonance imaging; v_1, visit 1 of HANDLS (2004-2009); v_2, visit 2 of HANDLS (2009-2013); v_{scan}, HANDLS-SCAN visit (2011-2015); WRAT, Wide Range Achievement Test.

[a] Values are adjusted linear regression coefficients β with associated SE. (N) is the sample size in each analysis. Model 2 in Table 4 was adjusted for Age_{v1}, sex, race, poverty status and time of follow-up between visit 1 and v_{scan} and selected hematological measures [i.e., Hb + other hematological measures (MCH, Serum iron, ESR)]. Volumes are expressed in mm^3.

[b] Model 1 adjusted for Age_{v1}, sex, poverty status, length of follow-up between v_1 and v_{scan}. Model 2 adjusted for other hematological measures, including MCH, ESR, serum iron, RDW at v1 (for anemia) and Hemoglobin at v1 (for RDW). Model 3 is a sensitivity analysis further adjusting Model 2 for selected nutritional/dietary factors (Healthy Eating Index-2010 total score, B-12, folate); Model 4 is a sensitivity analysis further adjusting Model 2 for selected inflammatory markers (high sensitivity C-reactive protein, albumin, White blood cells); Model 5 is a sensitivity analysis further adjusting Model 2 for selected adiposity and metabolic factors (Waist circumference, cholesterol, Cholesterol:HDL ratio, Triglycerides, Creatinine); Model 5 is a sensitivity analysis further adjusting Model 2 for other selected covariates (education, WRAT, smoking).

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