Neurobiology of Aging 105 (2021) 229–240

Contents lists available at ScienceDirect

Neurobiology of Aging

journal homepage: www.elsevier.com/locate/neuaging.org

Red cell distribution width, anemia and their associations with white matter integrity among middle-aged urban adults

May A. Beydoun[a,†,*], Danielle Shaked[b], Sharmin Hossain[a], Jordan Weiss[c], Hind A. Beydoun[d], Ana I. Maldonado[a,e], Leslie I. Katzel[f,g], Christos Davatzikos[h], Rao P. Gullapalli[i], Stephen L. Seliger[j], Guray Erus[h], Michele K. Evans[a], Alan B. Zonderman[a], Shari R. Waldstein[e,f,g]

[a] Laboratory of Epidemiology and Population Sciences, NIA/NIH/IRP, Baltimore, MD, USA
[b] Department of Psychology, VA Boston Healthcare System, Boston, MA, USA
[c] Department of Demography, University of California, Berkeley, Berkeley, CA, USA
[d] Department of Research Programs, Fort Belvoir Community Hospital, Fort Belvoir, VA, USA
[e] Department of Psychology, University of Maryland Baltimore County, Catonsville, MD, USA
[f] Geriatric Research Education and Clinical Center, Baltimore VA Medical Center, Baltimore, MD, USA
[g] Division of Gerontology & Geriatric Medicine, Department of Medicine, University of Maryland School of Medicine, Baltimore, MD, USA
[h] Artificial Intelligence in Biomedical Imaging Lab, Center for Biomedical Image Computing and Analytics (CBICA), Department of Radiology, University of Pennsylvania, Philadelphia, PA, USA
[i] Department of Diagnostic Radiology, University of Maryland School of Medicine, Baltimore, MD, USA
[j] Division of Nephrology, Department of Medicine, University of Maryland School of Medicine, Baltimore, MD, USA

ARTICLE INFO

Article history:
Received 21 December 2020
Revised 3 May 2021
Accepted 4 May 2021
Available online 21 May 2021

Keywords:
Red cell distribution width
Anemia
White matter integrity
Aging

ABSTRACT

Anemia (blood hemoglobin [Hb] <13 g/dL among males; <12 g/dL among females) and elevated red cell distribution width (RDW) are potential risk factors for reduced brain white matter integrity (WMI), reflected by lower fractional anisotropy or increased mean diffusivity. Cross-sectional data with exposure-outcome lag time was used, whereby hematological exposures (RDW and Hb) and covariates were compiled from the Healthy Aging in Neighborhoods of Diversity across the Life Span (HANDLS) study with available visit 1 (v_1; 2004–2009) and/or v_2 (2009–2013) data; while diffusion tensor magnetic resonance imaging (dMRI) outcome data were collected at HANDLS SCAN visit (v_{scan}: 2011–2015, n = 214, mean follow-up from v_1 ±SD: 5.6 ± 1.8 year). Multivariable-adjusted linear regression analyses were conducted, overall, stratifying by sex, and further restricting to the nonanemic for RDW exposures in part of the analyses. Among males, $RDW_{(v1)}$ was linked with lower global mean fractional anisotropy (standardized effect size b = −0.30, p= 0.003, q < 0.05; basic model), an association only slightly attenuated with further covariate adjustment. Anemia was not a risk factor for poor WMI, independently of RDW. Ultimately, pending further longitudinal evidence, initial RDW appears to be associated with poorer WMI among males.

Published by Elsevier Inc.

Abbreviations: AP, Above Poverty; BP, Below Poverty; C-TRIM, Core for Translational Research in Imaging @ Maryland; CV, Coefficient of Variation; dMRI, Diffusion Tensor Magnetic Resonance Imaging; DXA, Dual X-ray absorptiometry; FA, Fractional Anisotropy; FDR, False Discovery Rate; FLAIR, Fluid-Attenuated Inversion Recovery; FOV, Field of View; GM, Gray Matter; Hb, Hemoglobin; (HANDLS) study, Healthy Aging in Neighborhoods of Diversity across the Life Span; HEI-2010, Healthy Eating Index 2010 release; Hcy, Homocysteine; HS, High School; LASSO, Least absolute shrinkage and selection operator; MCH, Mean Cell Hemoglobin; MCV, Mean Cell Volume; MD, Mean Diffusivity; MP-RAGE, Magnetization prepared rapid gradient echo; MRI, Magnetic Resonance Imaging; MRV, Medical Research Vehicle; MICO, Multiplicative intrinsic component optimization; MUSE, Multi-atlas region Segmentation utilizing Ensembles; RBC, Red Blood Cells; RDW, Red Cell Distribution Width; ROI, Regions of Interest; SA, Sensitivity Analysis; SD, Standard Deviation; SE, Standard Error; sMRI, Structural MRI; US, United States; WRAT-3, Wide Range Achievement Test, version 3; WM, White Matter; WMI, White Matter Integrity; WML, White Matter Lesion; WMLV, White Matter Lesion Volume; WHO, World Health Organization.

* Corresponding author at: NIH Biomedical Research Center, National Institute on Aging, IRP, 251 Bayview Blvd., Suite 100, Room #: 04B118, Baltimore, MD 21224, USA. Fax: 410-558-8236.
E-mail address: baydounm@mail.nih.gov (M.A. Beydoun).

† MAB had full access to the data used in this manuscript and completed all the statistical analyses.

0197-4580/$ – see front matter Published by Elsevier Inc.
https://doi.org/10.1016/j.neurobiolaging.2021.05.004

1. Introduction

A consequence of a progressively aging population is an increase in the number of older adults living with chronic conditions like dementia, many of which have no cure and require a large unmet medical need (Winchester et al., 2018). Consequently, researchers have investigated multiple pathways through which progression to dementia can be slowed by delaying cognitive impairment. In addition to several structural brain neuroimaging markers [e.g., reduced brain volumes and increased white matter lesion volume (WMLV)], reduced white matter integrity (WMI) is associated with decrements in various cognitive abilities and may be used as an early marker for dementia risk (Cavedo et al., 2012; Glymour et al., 2012; Hsu et al., 2018; Louapre et al., 2016; Muller et al., 2020; Walter et al., 2019).

In recent years, anemia—defined by the World Health Organization (WHO) as reduced blood hemoglobin (Hb) <13 g/dL among males and <12 g/dL among females (World Health Organization, 1972)—has been identified as an important and understudied direct, mechanistic pathway to age-related cognitive decline (Chung et al., 2014; Hong et al., 2020; Park et al., 2019; Schneider et al., 2016; Sousa et al., 2018; Taniguchi et al., 2019; Trevisan et al., 2016; Weuve et al., 2014; Winchester et al., 2018; Wolters et al., 2019). It is also linked to numerous other markers of poor health and physical function and potentially triggered by other nutritional insufficiencies (e.g., folate and vitamin B-12; Dlugaj et al., 2016). Prior work examining anemia's relationship with cognitive decrements suggest several mechanisms including reduced blood oxygen-carrying capacity resulting in brain hypoperfusion, as well as heightened oxidative stress and inflammation, both of which can contribute to neurodegeneration (Faux et al., 2014; Zlokovic, 2011).

Relatedly, red cell distribution width (RDW) is a marker for variation in red blood cell sizes, known as anisocytosis. It has been associated with chronic disease morbidity and mortality (Hoffmann, 2012; Li et al., 2017; Patel et al., 2010; Perlstein et al., 2009; Tajuddin et al., 2017), and recent research demonstrated that RDW was related to reduced performance on tests of verbal memory, attention and higher prevalence of dementia (Beydoun et al., 2020; Ozturk et al., 2013; Wan et al., 2020; Weuve et al., 2014; Winchester et al., 2018). For example, a recent study observed independent associations of RDW and anemia with lower cognitive health (Winchester et al., 2018).

To date, few mechanistic studies in humans have examined associations of anemia or RDW with brain imaging markers (Dagistan and Cosgun, 2019; Jonassaint et al., 2014; Lee et al., 2016). The few studies that have been conducted indicate an association between anemia and smaller total and gray matter (GM) brain volumes, as well as a positive association between RDW and white matter lesion volumes (WMLV; Dagistan and Cosgun, 2019; Jonassaint et al., 2014; Lee et al., 2016). This limited research to date has not investigated sex differences in those associations. This is an important consideration as prior work has reported that females face higher prevalence of anemia compared with males (Beydoun et al., 2020). Moreover, researchers have observed a correlation between RDW and anemia (Beydoun et al., 2020). Sex-based differences in brain MRI outcomes are well established (Cosgrove et al., 2007), and are thought to often be independent of other socio-demographic characteristics. Furthermore, elevated RDW has been linked to poorer cognitive performance, particularly within the attention domain, in a sex-specific manner (Beydoun et al., 2020). Taken together, the literature suggests a relationship between RDW/anemia and worse neurocognitive outcomes, which may vary across population strata, including sex. Given that RDW and anemia have been shown to have adverse effects on other neurobiological endpoints (e.g., GM, WMLV), perhaps they also negatively impact WMI, captured by reduced global and regional fractional anisotropy (FA) and increased mean diffusivity (MD), and in a sex-specific manner.

We build on the extant literature by examining associations of anemia and RDW status—and change therein—with global and regional cortical WMI. We use a unique sample of urban adults that varies in balanced manner with respect to sex, socioeconomic position, and race. Our primary hypothesis is that baseline anemia and RDW, and change in RDW over time, would be associated with reduced WMI and may vary with respect to sex.

2. Methods

2.1. Database

We used data from the Healthy Aging of Neighborhoods of Diversity across the Life Span (HANDLS) study, an ongoing prospective cohort study among a socio-demographically diverse sample of middle-aged White and African American urban adults aged 30–65 year at baseline in 2004–2009. Interviews were completed among participants who were identified by random sampling of addresses within every census tract. Participants were eligible and invited when meeting the following eligibility criteria: (1) age range: 30–64 year; (2) not pregnant; (3) no active cancer treatment within 6 months; (4) without an AIDS diagnosis; (5) capable of written informed consent, and (6) capable of producing valid government-issued identification and verifiable address. Details on sampling strategy and inclusion criteria are described elsewhere (Evans et al., 2010).

In visit 1, HANDLS researchers collected information on study participants in 2 phases. In Phase 1, in-home interviews were conducted during which researchers collected information on demographic characteristics, psychosocial health, and completed a dietary interview. Phase 2 was conducted on Medical Research Vehicles (MRVs), which allowed researchers to collect a wide range of physical and mental health metrics as well as anthropometrics and biological samples. These included, for example, Dual X-ray Absorptiometry (DXA) for bone mineral density and body composition, an electrocardiogram, intima-media thickness by ultrasound, physical examination by a physician, neuropsychological tests, and inventories to assess psychiatric symptoms (Evans et al., 2010). Comparable information was collected on MRVs during a second visit (v_2: 2009-2013). Diffusion tensor magnetic resonance imaging (dMRI) measures were collected at a follow-up visit, as part of the HANLS SCAN ancillary study (Waldstein et al., 2017). Both v_1 and v_2 data were collected prior to the HANDLS SCAN visit (v_{scan}: 2011–2015) for all participants. The current study utilized hematological data (anemia and RDW) from visit 1 (v_1: 2004–2009) and change in RDW ascertained from v_1 and v_2 (2009–2013) among a sub-sample of $N_{max} = 240$ participants within the HANDLS SCAN sub-study (v_{scan}: 2011–2015; Evans et al., 2010; Waldstein et al., 2017).

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 also approved by IRBs of the University of Maryland School of Medicine and the University of Maryland, Baltimore County. Written informed consent was obtained for all participants.

2.2. Study participants

The baseline HANDLS sample was comprised of 3720 participants (30–65 y, African Americans and Whites, Phase 1, v_1). Of the

initial 3,720 participants, we retained individuals with RDW data at either v_1 or v_2 (N = 3017), among whom v_1 Hb was complete for 2744 participants. We further restricted our sample to those who participated in the HANDLS SCAN sub-study (the largest available HANDLS SCAN sample was n = 240). This yielded an analytic sample of 214 participants with complete dMRI parameters of interest, RDW at either visit, and v_1 Hb. Comparing the final sample (N = 214) with the remaining excluded participants from the initial sample (n = 3720), the final sample had higher proportions of Whites (59% vs. 40%, $p< 0.05$) and individuals living above poverty (67% vs. 58%, $p< 0.05$). Sample selectivity for the non-anemic group at v_1 (n=190) was similar with respect to race ($p < 0.05$), but not with respect to poverty status ($p\geq0.05$), while no socio-demographic differences were detected for the non-anemic group at either visit (i.e., v_1 and/or v_2: n = 182) vs. those excluded from the initial sample (n = 3720). In the main final analytic sample (n = 214), the length of follow-up time between v_1 and v_{scan} had a mean±SD of 5.6 ± 1.8 year (range: 2.3–10.0 year).

2.3. Brain dMRI

dMRI was assessed using multi-band spin echo EPI sequence with a multi-band acceleration factor of 3 (Supplemental method 1). FA and trace (TR, also known as mean diffusivity, MD) images were evaluated from tensor images, with higher FA values indicating healthier WMI. Summation of eigenvalues for diffusion tensor yields MD, with higher values suggesting poorer WMI (Jones, 2008). Computed FA and MD images were aligned to a common template space using deformable registration with a standard dMRI template (i.e., EVE (Wakana et al., 2004)). We calculated the average of right and left FA and MD values for each ROI corresponding to WM regions (See Supplemental Table 1 for list of ROIs). Global FA and MD were computed as the average across all WM ROIs. Selection of cortical WM sub-regions that comprised the bilateral (Left/Right) larger brain regions (Frontal, Temporal, Parietal, Occipital) was similar to previous studies (see Roalf et al., 2015; Shaked et al., 2019).

2.4. RDW at v_1 and δRDW

RDW was determined by an automated Coulter DXH 800 hematology analyzer as part of peripheral complete blood count (Beckman Coulter, Brea, CA). The main exposure of interest was RDW-CV (coefficient of variation, expressed in %), computed using RDW-Standard Deviation (SD, unit: fL) as a percent of mean cell volume (MCV), whereby RDW-CV = RDW-SD × 100/MCV. In addition to RDW(v_1), annual rate of change in RDW between v_1 and v_2 (aka δRDW) was also of interest (see Supplemental method 2), to assess the relationships between longitudinal change in this exposure and follow-up outcome measures.

2.5. Hb and anemia

Hb was determined from 1 ml of blood sampled from participants after an 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 (World Health Organization, 1972). Non-anemic participants were selected out for a secondary analysis for RDW exposures. Specifically, for RDW(v_1), absence of anemia was defined only for v_1, whereas in the case of δRDW, non-anemic reflected v_1, v_2 or both. V_1 anemia was the primary exposure of interest, while v_1 Hb was considered a secondary exposure.

2.6. Covariates

Covariates were selected for their potential association with the exposures and/or outcome. We included age (y) at baseline, sex (male, female), self-reported race (African American, White), poverty status determined by self-reported household income either <125% or ≥125% of the 2004 Health and Human Services poverty guidelines (Department of Health and Human Services, 2004) and time (days) between v_1 MRV visit and v_{scan}. We adjusted for additional covariates after identifying them as associated with anemia and/or RDW exposures with machine learning techniques [Least absolute shrinkage and selection operator (LASSO) models], followed by backward elimination. Additional information is provided in **online supplemental method 3**. It is worth noting that even though MCV is a major hematological measure linked to RDW, it was dropped from covariate selection, given its high correlation with other measures such as mean cell hemoglobin (MCH), (Pearson's r > 0.90). While sex was the main effect modifier in our analyses, other secondary stratifying variables included poverty status (defined as such by design at first-visit), race (self-identified), and age group categorized to distinguish younger middle-aged with older middle-aged adults (30–49 at v_1 vs. 50–66 at v_1). In the final selected sample (n = 214), the breakdown by socio-demographic factors was as follows: sex (males: n = 97; females: n = 117); age group (<50 years: n = 127; ≥50 years: n = 87); race (Whites: n = 127; African-Americans: n = 87); poverty status (Below poverty: n = 70; Above poverty: n = 144).

2.7. Statistical analysis

We used Student's t and chi-square tests as appropriate to compare sample characteristics by sex. We further tested for sex differences in sample characteristics after covariate adjustment (age, race, and poverty status) using multivariable-adjusted models. We additionally examined sample characteristics across anemia and RDW_{v1}. To test our main hypotheses, we fit a series of covariate-adjusted ordinary least square (OLS) regression models, overall and stratified by sex, using dMRI as the outcome. These models produced coefficient estimates indicating the association between each of the three primary exposures of interest (i.e. v_1 anemia, RDW and δRDW) to dMRI outcomes. We obtained standardized coefficient estimates which were interpreted as the fraction of 1 SD change in dMRI outcome per 1 SD change in a continuous exposure (i.e., RDW and δRDW). Estimates >0.20 were considered moderate-to-strong; estimates between 0.10 and 0.20 were considered weak-to-moderate.

We conducted our analysis in 2 stages. *Analysis A* included global mean FA and mean MD. In addition to stratifying by sex, an additional analysis was carried out (*Analysis A'*), which considered age group (<50 years vs. ≥50 years), race and poverty status as alternate stratifying variables. *Analysis B* included measures of FA and MD for the frontal, temporal, parietal, and occipital cortical WM regions (left and right) yielding a total of 16 models. *Analysis B* was conducted on a case-by-case basis if at least one exposure-outcome (global mean FA/mean MD) association was statistically significant from *Analysis A* ($P_{uncorr} < 0.05$), considering each main exposure separately (i.e., anemia at v_1, RDW at v_1 and δRDW), within each stratum (i.e., overall or within males and females) and each adjusted model.

Secondary outcomes were bilateral means of FA/MD at each independent ROI (Supplemental Table 1, 51 WM-related ROIs highlighted in red). Volcano plots were used to visualize select findings from these models (R Foundation for Statistical Computing, 2013). These plots display Log_{10} (p-values) for each set of models against

standardized beta coefficients (*b*) on the X-axis, highlighting findings with larger *b*. Only ROIs with uncorrected *p* value <0.05 are presented. Visualization of ROI-specific *b* with standard brain images was carried out using FSLeyes software (Jenkinson et al., 2002; Jenkinson and Smith, 2001) applied to these dMRI results (URL: https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSLeyes).

We corrected for multiple testing using the false discovery rate (FDR, q-value) while treating the overall and sex-stratified analyses as separate hypotheses. Our correction for multiple testing was only applied to the minimally adjusted models (i.e., Model 1, explained below) for each of *Analysis A* and *Analysis B*. In Model 1, we adjusted for age, sex, race, poverty status, and follow-up time between v_1 and v_{scan}; FDR q-values were only presented 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. We considered a q-value<0.10 indicative of a trend. The results for *Analysis A'* were shown only for the minimally adjusted model without correction for multiple testing, though additional adjustment for other hematological factors was explored.

In sensitivity analyses, we used a data set where only the additional covariates were imputed (k = 5 imputations, 10 iterations, chained equations) to obtain the largest possible sample size with complete exposure and outcome data. This sensitivity analysis adjusted for those additional covariates which were selected using a multi-step process as described in Supplemental method 3. The initial pool of selected and imputed covariates were associated with hematological measures and/or cognitive outcomes as identified in prior work (Beydoun et al., 2014).

Overall, our modeling approach consisted of: a model with limited covariate adjustment fit on the unimputed data (Model 1) with a sensitivity analysis on the imputed data; Model 2 built on Model 1 by adding hematological measures that are considered to be the most important potential confounders in the main associations, aside from socio-demographic factors; Model 3 built on Model 2 by adding nutritional/dietary characteristics (i.e., Healthy Eating Index-2010 total score, serum B-12 and serum folate), considered as potential confounders, after other hematological factors are explained away; Model 4 built on Model 2 through the addition of an alternate set of potential confounders, namely inflammatory conditions (high sensitivity C-reactive protein, albumin, White blood cells); Model 5 built on Model 2 with measures of adiposity and metabolic disturbance factors, as a third alternate set of confounders (waist circumference, cholesterol, cholesterol:HDL ratio, triglycerides, creatinine); and Model 6 built on Model 2 with additional covariate adjustment, which may act as additional confounders to the main socio-demographic and hematological factors (e.g., education, Wide Range Achievement Test [WRAT], smoking). In sensitivity analyses for these models, we tested for effect modification by including 2-way interaction terms between the exposure and sex with a type I error of 0.10 used for 2-way interaction terms due to reduced statistical power (Selvin, 2004).

We replicated all analyses in the subsample of participants who were non-anemic at v_1 for the $RDW_{(v1)}$ and the non-anemic at v_1 and v_2 for δRDW (See Fig. 1). We also conducted a secondary analysis for visit 1 Hb (as a continuous exposure), overall and stratified by sex. We did not account for multiple testing in these analyses nor in models 2-6. All analyses were conducted using Stata version 16.0 (STATA, 2019) or R version 3.6.1.

3. Results

3.1. Sample characteristics

Sample characteristics included in this study, overall and stratified by sex, are presented in Table 1. The selected sample consisted of 97 males and 117 females, with v_1 ages of mean ± SD 47.7 ± 9.1 year, 40.7% of whom were African American and 67.3% living above poverty. Males had a higher proportion than females with household incomes above 125% of the federal poverty line (aka "above poverty"), lower mean RDW_{v1} (13.7 vs. 14.2, $p = 0.007$; overall SD = 1.55) and had a reduced $anemia_{(v1)}$ prevalence (6.1% vs. 14.0%, $p = 0.034$). Mean follow-up times did not differ significantly by sex ($p = 0.75$, *t*-test), nor did they differ significantly by anemia status at v_1 or v_1/v_2 ($p > 0.05$, data not shown). Nevertheless, follow-up times were longer on average by 0.8y among African Americans vs. Whites, and by 1.3y among individuals living below poverty vs. those above poverty at v_1 (data not shown). While mean FA did not differ by sex, mean MD was higher among males, suggesting reduced WMI compared to females ($p< 0.010$). However, within cortical regions, both FA and MD regional measures showed reduced WMI among males (i.e., lower FA and higher MD). 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). Importantly, v_1 Hb was significantly more elevated among males compared with females ($p< 0.001$).

Supplemental Table 2 presents those same study characteristics across anemia and $RDW_{(v1)}$ tertile groups, in the total sample, as well as stratified by sex. Generally, African Americans were more represented in the anemic group (vs. non-anemic) and in the uppermost RDW tertile vs. the lower 2 tertiles exhibiting a dose-response relationship. RDW was similarly more elevated in the $anemia_{(v1)}^{+}$ vs. $anemia_{(v1)}^{-}$ groups. Overall and among females, anemia and RDW tertiles were generally not associated with FA or MD global or cortical regional measures, with few exceptions. However, among males, there was a linear dose-response relationship between $RDW_{(v1)}$ tertiles and mean global FA as well as FA in specific regions, mainly frontal, parietal and occipital, reflecting poorer WMI (lower FA), with higher $RDW_{(v1)}$. Similar patterns were observed among males with respect to the association of FA measures with $anemia_{(v1)}$ groups, suggesting that anemia is linked to poorer WMI (lower FA) among males. Due to reduced statistical power (only $n = 3$ were anemic), with few exceptions, no significant associations were found among males across anemia status at v_1/v_2, with respect to means of FA and MD global or cortical regional measures.

3.2. Anemia, Hb and RDW's associations with global and cortical WMI: sex-specific findings

Tables 2 and 3 and supplemental Tables 3 and 4 test our main hypotheses, namely the associations of anemia and/or RDW exposures with WMI markers, in covariate-adjusted linear regression models and accounting for multiple testing. All results are represented in the total sample, among males and females separately and in the non-anemic group for RDW exposures. $Anemia_{(v1)}$ was not associated with WMI measures (FA or MD) as shown in Table 2. In a sensitivity analysis, $Hb_{(v1)}$ was directly associated with global FA among males in the minimally adjusted model, reflecting a potential beneficial effect on WMI, particularly in frontal and occipital regions. This association, was, however, markedly attenuated in Model 2, upon adjustment for other hematological measures, including $RDW_{(v1)}$.

However, based on Table 3 findings, $RDW_{(v1)}$ was linked to lower global WMI in males, particularly in terms of reduced mean FA (Table 3, Model 1, *Analysis A*, standardized effect size b = −0.30, $p= 0.003$, q < 0.05). In region-specific analyses, (*Analysis B*), associations of greater RDW with lower WMI was

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

	Total (N = 214)	Females (N = 117)	Males (N = 97)	P_{sex}
Demographic factors				
Sex, % males	45.3	—	—	
Age_{v1}	47.7 ± 9.1	47.6 ± 0.9	47.8 ± 0.9	0.85
Race, % African American	40.7	41.9	39.2	0.69
% above poverty	67.3	60.7	75.3	0.024
Follow-up time (v_1 through v_{scan}), years	5.6 ± 1.8	5.6 ± 0.2	5.5 ± 0.2	0.75
Imputed covariates, % or Mean ± SE				
Education, years				
<High School	7.1	7.4	6.8	0.88
High School	54.4	54.2	54.8	—
> High School	38.4	38.5	38.4	0.96
WRAT-3 score	43.7 ± 0.50	43.7 ± 0.6	43.9 ± 0.8	0.83
Current smoker, % yes	45.3	48.2	42.0	0.36
HEI-2010 total score	42.3 ± 0.8	43.6 ± 1.2	40.8 ± 1.1	0.10
Serum vitamin B-12, pg/mL	517.4 ± 16.8	534.3 ± 25.9	497.1 ± 20.0	0.27
Serum folate, ng/mL	15.2 ± 0.4	15.0 ± 0.6	15.3 ± 0.6	0.88
C-reactive protein, mg/L	4.3 ± 0.6	5.7 ± 1.0	2.7 ± 0.5	0.013
Albumin, g/dL	4.34 ± 0.02	4.29±0.03	4.42 ± 0.03	<0.001
White blood cell, count*10^9/L	6.7 ± 0.2	6.9 ± 0.2	6.4 ± 0.2	0.088
Waist size, cm	99.0 ± 1.1	99.1 ± 1.6	99.1 ± 1.5	0.98
Total cholesterol, mg/dL	190.7 ± 3.1	192.4 ± 4.4	188.7 ± 4.4	0.55
Cholestrol:HDL-Cholesterol ratio	3.9 ± 0.1	3.6 ± 0.1	4.1 ± 0.2	0.023
Triglycerides, mg/dL	124.6 ± 5.0	113.9 ± 5.0	137.5 ± 8.9	0.018
Creatinine, mg/dL	0.89 ± 0.03	0.79 ± 0.04	1.02 ± 0.03	<0.001
Other hematological measures at v_1				
Imputed covariates, Mean ± SE				
Mean Cell Hemoglobin, pg	30.3 ± 0.2	30.0 ± 0.3	30.7 ± 0.2	0.028
Serum iron, μg/dL	87.1 ± 2.6	78.7 ± 3.4	97.3 ± 3.7	<0.001
Erythrocyte Sedimentation Rate, mm/hr	13.7 ± 0.8	17.2 ± 1.2	9.5 ± 0.9	<0.001
RDW (v_1)				
CV (%), Mean ± SD/SE	13.98 ± 1.55	14.2 ± 0.2	13.7 ± 0.1	0.0065
Median	13.6	13.8	13.5	
IQR	13.1;14.3	13.1;14.6	13.1;14.1	
RDW (v_2-v_1, annual), δRDW				
CV (%)	+0.054 ± 0.068	+0.057 ± 0.007	+0.052 ± 0.005	0.62
Hemoglobin (v_1), g/dL, *Mean±SD/SE*	13.93 ± 1.46	13.19 ± 0.13	14.82 ± 0.10	<0.001
Anemia (v_1)				
Yes, %	11.21	15.4	6.2	0.034
Anemia (v_1 and v_2)	*(N = 196)*	*(N = 107)*	*(N = 89)*	
Yes, %	7.14	10.3	3.4	0.061
dMRI measures, *Mean±SD/SE*	*(N = 214)*	*(N = 117)*	*(N = 97)*	
Global WMI measures	***mean ± SD***	***mean ± SE***	***mean ± SE***	
Mean FA	+0.299 ± 0.017	+0.300 ± 0.0014	+0.298 ± 0.002	0.25
Mean MD, mm^2/sec	+0.00254 ± 0.0016	+0.00252 ± 0.00002	+0.00258 ± 0.00002	0.0018
Regional cortical WMI measures	***mean ± SD***	***mean ± SE***	***mean ± SE***	
Left Brain				
Frontal FA	+0.234 ± 0.002	+0.236 ± 0.002	+0.232 ± 0.002	0.054
Frontal MD, mm^2/sec	+0.00239 ± 0.00014	+0.00236 ± 0.00001	+0.00244 ± 0.00002	<0.0001
Temporal FA	+0.240 ± 0.016	+0.243 ± 0.001	+0.237 ± 0.002	0.012
Temporal MD, mm^2/sec	+0.00243 ± 0.00014	+0.00240 ± 0.00001	+0.00247 ± 0.00001	0.0026
Parietal FA	+0.230 ± 0.016	+0.232 ± 0.001	+0.228 ± 0.0017	0.043
Parietal MD, mm^2/sec	+0.00266 ± 0.00020	+0.00260 ± 0.00002	+0.00273 ± 0.00002	<0.0001
Occipital FA	+0.203 ± 0.015	+0.204 ± 0.001	+0.200 ± 0.001	0.039
Occipital MD, mm^2/sec	+0.00248 ± 0.00016	+0.00251 ± 0.00001	+0.00251 ± 0.00002	0.0058
Right Brain				
Frontal FA	+0.234 ± 0.015	+0.236 ± 0.001	+0.231 ± 0.002	0.017
Frontal MD, mm^2/sec	+0.00237 ± 0.00014	+0.00233 ± 0.000012	+0.00240 ± 0.00002	0.0001
Temporal FA	+0.248 ± 0.016	+0.251 ± 0.001	+0.246 ± 0.002	0.020
Temporal MD, mm^2/sec	+0.00234 ± 0.00014	+0.00232 ± 0.00001	+0.00237 ± 0.00001	0.0196
Parietal FA	+0.230 ± 0.017	+0.229 ± 0.001	+0.223 ± 0.002	0.005
Parietal MD, mm^2/sec	+0.00271 ± 0.00022	+0.00264 ± 0.00002	+0.00280 ± 0.00002	<0.0001

(*continued on next page*)

Table 1 (*continued*)

	Total (N = 214)	Females (N = 117)	Males (N = 97)	P_{sex}
Occipital FA	+0.204 ± 0.015	+0.205 ± 0.001	+0.202 ± 0.002	0.073
Occipital MD, mm^2/sec	+0.00256 ± 0.00018	+0.00252 ± 0.00002	+0.00259 ± 0.00002	0.0058

Abbreviations: Age_{v1}, age measured at HANDLS visit 1 (2004–2009); CV, Coefficient of Variation; IQR, Interquartile Range; dMRI, Diffusion Magnetic Resonance Imaging; δRDW, Red Cell Distribution Width annualized change between visits 1 and 2; FA, Fractional Anisotropy; 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; HDL, High Density Lipoprotein; HEI-2020, Healthy Eating Index, 2010 release; IQR, Interquartile range (25th–75th percentile); MCH, Mean Cell Hemoglobin; MD, Mean Diffusivity; RDW, Red Cell Distribution Width; v_1, visit 1 of HANDLS (2004–2009); v_2, visit 2 of HANDLS (2009–2013); v_{scan}, HANDLS-SCAN visit (2011–2015); WMI, White Matter Integrity; WRAT-3, Wide Range Achievement Test, 3rd version.

[a] Values are Mean±SD for totals and Mean ± SE for stratum-specific, or %. For continuous imputed covariates, values are Mean±SE for all 3 columns. For RDW, medians and inter-quartile ranges (IQR) were also provided. Psex 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.

observed specifically in frontal and parietal FAs and occipital FAs and MDs. These associations among males remained statistically significant upon further adjustment for hematological measures (Table 3, Model 2), and our further analyses indicated statistically significant sex differences in those associations (Table 3, Model 2, sex × $RDW_{(v1)}$ $P<0.05$ in non-stratified models). When further adjustment was made for additional covariates, these relationships remained largely unaltered (Supplemental Table 3). One exception was Model 6 for $RDW_{(v1)}$ vs. mean FA among males, suggesting potential confounding effects by education, literacy and smoking status, factors known for their associations with various neuro-cognitive outcomes (Beydoun et al., 2014). Another exception was Model 5 for $RDW_{(v1)}$ vs. mean MD among males, suggesting potential confounding by adiposity and metabolic disturbance factors (Beydoun et al., 2008). There were no significant associations of FA/MD measures with $RDW_{(v1)}$ among females or in the non-anemic group (Models 1 and 2, Table 3); as well as between the δRDW exposure and

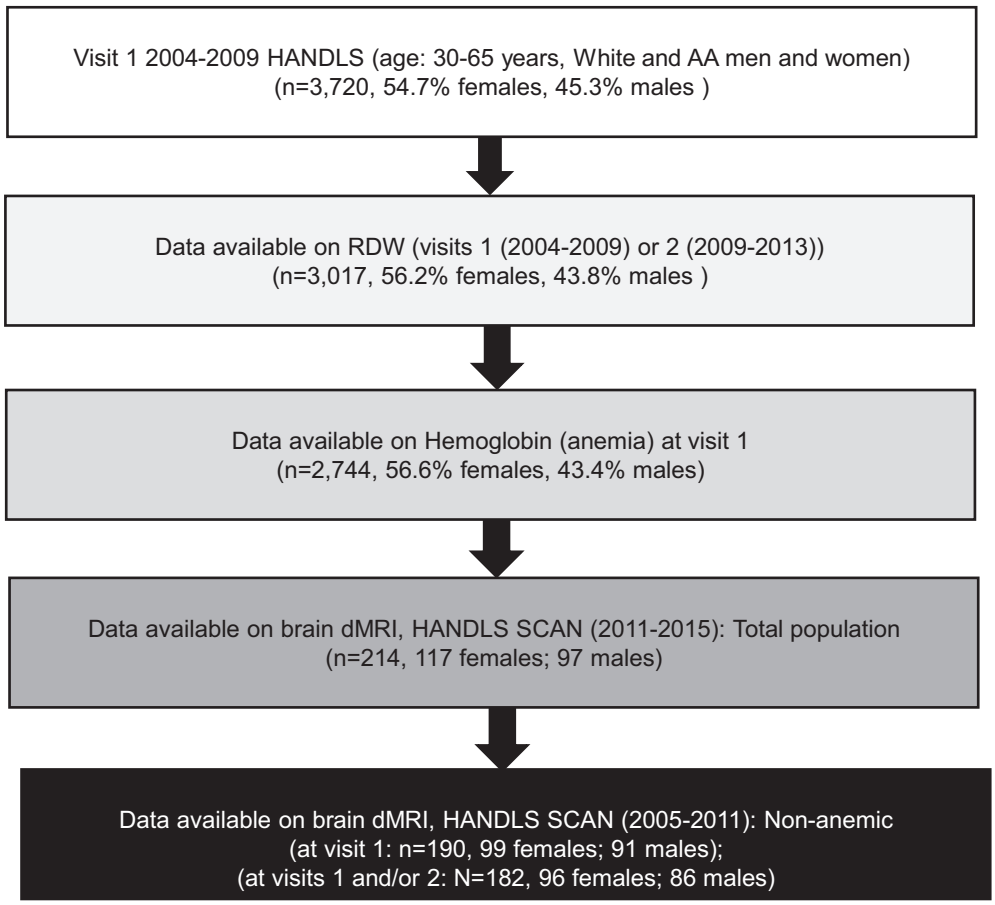


Fig. 1. Study participant schematic: HANDLS 2004–2013 and HANDLS-SCAN 2011–2015.[a] *Abbreviations:* HANDLS, Healthy Aging in Neighborhoods of Diversity Across the Life Span; HANDLS-SCAN, HANDLS brain MRI ancillary study; MRI, Magnetic Resonance Imaging. [a]Visit 1 refers to HANDLS 2004-2009; Visit 2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

Table 2
Anemia analyses: Minimally and hematological measure adjusted associations from analyses A (global FA/MD), B (Regional cortical FA/MD, Left/Right) vs. visit 1 Anemia (overall and stratified by sex) and continuous Hb level: 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]			
Visit 1 Anemia								
Total sample (N = 214)	*β*	*(SE)*	*P*	*q-value*	*β*	*(SE)*	*P*	*P for Interaction of Anemia$_{(v1)}$ by sex*
dMRI, Analysis A								
Mean FA	−0.0048	(0.004)	0.19	__	−0.0037	(0.004)	0.40	0.21
Mean MD	+0.0000297	(0.000032)	0.35	__	+0.0000202	(0.0000385)	0.60	0.30
Males (N = 97)								
dMRI, Analysis A								
Mean FA	−0.0108	(0.00759)	0.16	__	−0.00418	0.0085242	0.63	__
Mean MD	+0.0000936	(0.0000654)	0.16	__	+0.0000445	(0.0000744)	0.55	__
Females (N = 117)								
dMRI, Analysis A								
Mean FA	−0.002265	(0.003989)	0.57	__	−0.00307	(0.00498)	0.54	__
Mean MD	+7.78e − 06	(0.0000362)	0.83	__	+0.000016	(0.000045)	0.72	__
Visit 1 Hb								
Total sample (N = 214)	*β*	*(SE)*	*P*	*q-value*	*β*	*(SE)*	*P*	*P for Interaction By of Hb$_{(v1)}$ sex*
dMRI, Analysis A								
Mean FA	+0.0018	(0.0009)	0.060	SA	+0.0023	(0.0012)	0.070	0.15
Mean MD	−0.0000104	(2.28E-06)	0.21	SA	−0.0000122	(0.000011)	0.27	0.052
Males (N = 97)								
dMRI, Analysis A								
Mean FA	+0.0040	(0.0018)	0.028	SA	+0.0034	(0.0020)	0.098	__
Mean MD	−0.000029	(0.000015)	0.061	SA	−0.000023	(0.000018)	0.20	__
dMRI, Analysis B								
Left Brain								
Frontal FA	+0.00278	(0.00165)	0.095	SA	__	__	__	__
Frontal MD	−0.000017	(0.000015)	0.26	SA	__	__	__	__
Temporal FA	+0.00264	(0.00166)	0.12	SA	__	__	__	__
Temporal MD	−0.000021	(0.000014)	0.14	SA	__	__	__	__
Parietal FA	+0.00251	(0.0016)	0.13	SA	__	__	__	__
Parietal MD	−1.51E − 07	(0.000019)	0.99	SA	__	__	__	__
Occipital FA	+0.0032	(0.0015)	0.033	SA	__	__	__	__
Occipital MD	−0.000028	(0.000016)	0.075	SA	__	__	__	__
Right Brain								
Frontal FA	+0.0040	(0.0016)	0.013	SA	__	__	__	__
Frontal MD	−0.000023	(0.0000014)	0.098	SA	__	__	__	__
Temporal FA	+0.0032	(0.0018)	0.062	SA	__	__	__	__
Temporal MD	−0.000010	(0.000013)	0.42	SA	__	__	__	__
Parietal FA	+0.0025	(0.0018)	0.18	SA	__	__	__	__
Parietal MD	5.24E − 06	(0.00002)	0.80	SA	__	__	__	__
Occipital FA	+0.0034	(0.0017)	0.050	SA	__	__	__	__
Occipital MD	−0.000031	(0.000017)	0.066	SA	__	__	__	__
Females (N=117)								
dMRI, Analysis A								
Mean FA	+0.00076	(0.0011)	0.48	SA	+0.0019	(0.0016)	0.23	__
Mean MD	−2.85E − 06	(9.64E-06)	0.77	SA	−8.92E − 06	(0.000014)	0.53	__

Abbreviations: Age$_{v1}$, age measured at HANDLS visit 1 (2004–2009); CV, Coefficient of Variation; dMRI, Diffusion Magnetic Resonance Imaging; ESR, Erythrocyte Sedimentation Rate; FA, Frational anisotropy; FDR, False Discovery Rate; 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; MD, Mean Diffusivity; RDW, Red Cell Distribution Width; SA, Sensitivity Analysis; 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).

[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, __ otherwise. Model 1 was adjusted for age, sex, race, poverty status and time of follow-up between visit 1 and v_{scan}. MD is measured in mm^2/sec.

[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 Supplemental methods 3).

the main outcomes of interest (Supplemental Table 4, Models 1–2).

3.3. RDW's association with ROI-specific WMI among males

Fig. 2 displays the findings from the minimally adjusted models among males with respect to the association of RDW$_{(v1)}$ with regional FAs and MDs. As shown in the volcano plots, all associations were in the expected direction whereby a higher RDW$_{(v1)}$ was associated with lower regional FAs and higher regional MDs. Among significant associations, some of the strongest effects sizes (b < -0.30) for RDW$_{(v1)}$ vs. FA were concentrated in the parietal region (e.g., supramarginal WM, pre-cuneus WM, precentral WM, and angular WM), while others were found in occipital (e.g., lingual WM),

Table 3
RDW analyses: Minimally and hematological measure adjusted associations from analyses A (global FA/MD), B (Regional cortical FA/MD, Left/Right) vs. visit 1 RDW (overall and stratified by sex; and among nonanemic 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 = 214)	β	(SE)	b	P	q-value	β	(SE)	P	P for Interaction of $RDW_{(v1)}$by sex
dMRI, Analysis A									
Mean FA	−0.00091	(0.00075)	−0.085	0.23	__	−0.00131	(0.00106)	0.22	__
Mean MD	+4.50e − 06	(6.68e − 06)	+0.043	0.50	__	+3.14e − 06	(9.49e − 06)	0.91	__
Males (N = 97)									
dMRI, Analysis A									
Mean FA	−0.00593	(0.00196)	−0.30	0.003	0.038	−0.006473	(0.002433)	0.009	0.004
Mean MD	+4.28e − 05	(1.71e − 05)	+0.24	0.014	0.086	+0.000045	(0.000021)	0.038	0.029
dMRI, Analysis B									
Left Brain									
Frontal FA	−0.005704	(0.001796)	−0.31	0.002	0.037	−0.0066874	(0.002241)	0.004	0.011
Frontal MD	+0.0000316	(0.000017)	+0.18	0.066	__	+0.0000351	(0.0000213)	0.10	0.028
Temporal FA	−0.0046025	(0.001839)	−0.26	0.014	0.14	−0.0043301	(0.0023103)	0.064	0.022
Temporal MD	+0.0000257	(0.000016)	+0.17	0.11	__	+0.000021	(0.00002)	0.31	0.17
Parietal FA	−0.005925	(0.001778)	−0.33	0.001	0.030	−0.007343	(0.002212)	0.001	0.003
Parietal MD	+0.0000345	(0.0000209)	+0.16	0.10	__	+0.000061	(0.000026)	0.021	0.20
Occipital FA	−0.0051361	(0.001637)	−0.32	0.002	0.037	−0.0056694	(0.0020237)	0.006	0.003
Occipital MD	+0.0000507	(0.0000173)	+0.28	0.004	0.051	+0.0000619	(0.0000213)	0.005	0.020
Right Brain									
Frontal FA	−0.005948	(0.0017423)	−0.33	0.001	0.028	−0.006399	(0.002151)	0.004	0.001
Frontal MD	+0.0000286	(0.0000152)	+0.18	0.064	__	+0.000030	(0.000019)	0.12	0.036
Temporal FA	−0.004967	(0.001916)	−0.27	0.011	0.12	−0.005407	(0.002380)	0.026	0.011
Temporal MD	+0.000027	(0.0000139)	+0.19	0.058	__	+0.0000331	(0.0000175)	0.061	0.14
Parietal FA	−0.0067969	(0.001986)	−0.34	0.001	0.030	−0.0085593	(0.002467)	0.001	0.001
Parietal MD	+0.0000388	(0.0000232)	+0.16	0.097	__	+0.0000702	(0.0000286)	0.016	0.18
Occipital FA	−0.0065511	(0.0018366)	−0.36	0.001	0.028	−0.0076675	(0.0022795)	0.001	0.001
Occipital MD	+0.0000534	(0.0000182)	+0.29	0.004	0.051	+0.0000628	(0.0000225)	0.006	0.040
Females (N = 117)									
dMRI, Analysis A									
Mean FA	+0.0001416	(0.0007934)	+0.017	0.86	__	−0.0003437	(0.0012155)	0.78	__
Mean MD	−2.99e − 06	(7.18e-06)	−0.037	0.68	__	−7.01e − 06	(0.0000111)	0.53	__
Nonanemic (N = 190)									
dMRI, Analysis A									
Mean FA	−0.0005986	(0.0011114)	−0.041	0.59	SA	−0.0008299	(0.0012592)	0.51	__
Mean MD	−4.42e − 06	(0.0000104)	−0.030	0.67	SA	−6.08e − 06	(0.0000118)	0.61	__

Abbreviations: Age_{v1}, age measured at HANDLS visit 1 (2004-2009); CV, Coefficient of Variation; dMRI, Diffusion Magnetic Resonance Imaging; ESR, Erythrocyte Sedimentation Rate; FA, Fractional Anisotropy; FDR, False Discovery Rate; 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; MD, Mean Diffusivity; RDW, Red Cell Distribution Width; SA, Sensitivity Analysis; 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).

[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, __ otherwise. Model 1 was adjusted for age, sex, race, poverty status and time of follow-up between visit 1 and v_{scan}. MD is measured in mm^2/sec.

[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 Supplemental methods 3).

frontal (e.g., middle fronto-orbital WM) and temporal cortical regions (e.g., inferior temporal WM). Nevertheless, the strongest association was found in the cingulate WM among males with respect to FA (b = −0.35), and other associations with b <-0.30 included $RDW_{(v1)}$ vs. FA in the splenium of the corpus callosum, cerebellar, and middle cerebellar peduncle. However, with respect to MD, only three ROIs had effect sizes b >+0.30, namely lingual WM (occipital), medial lemniscus and the fornix. Thus, poor lingual WM integrity was associated with higher $RDW_{(v1)}$ among males, consistently in terms of lower FA and higher MD, with a strong effect size.

3.4. Anemia and RDW's associations with global and cortical WMI: age-, race- and poverty status-specific findings

Fig. 3 presents the findings from the minimally adjusted models with all exposures and global FA/MD as outcomes, stratifying by age group, race and poverty status (*Analysis A'*). The results indicate that anemia was associated with lower mean FA in the older group only (≥50: $\beta \pm$ SE: −0.0152 ± 0.00070, $p = 0.033$), as well as among individuals living above poverty (AP: $\beta \pm$ SE: −0.00886 ± 0.00445, $p = 0.048$). These findings were, however, markedly attenuated and did not retain statistical significance ($p > 0.05$) with further adjustment for other hematological factors, including $RDW_{(v1)}$, (i.e., Model 2, data not shown). In contrast, $RDW_{(v1)}$ was linked with lower FA among individuals living above poverty in the minimally adjusted model (AP: $\beta \pm$ SE: −0.00209 ± 0.00089, $p = 0.020$), an association that remained statistically significant in Model 2, with adjustment for other hematological factors including blood Hb (AP: $\beta \pm$ SE: −0.00391 ± 0.00130, $p = 0.004$). No racial differences were detected in the main associations between anemia/RDW exposures and global FA/MD outcomes. No stratum-specific associ-

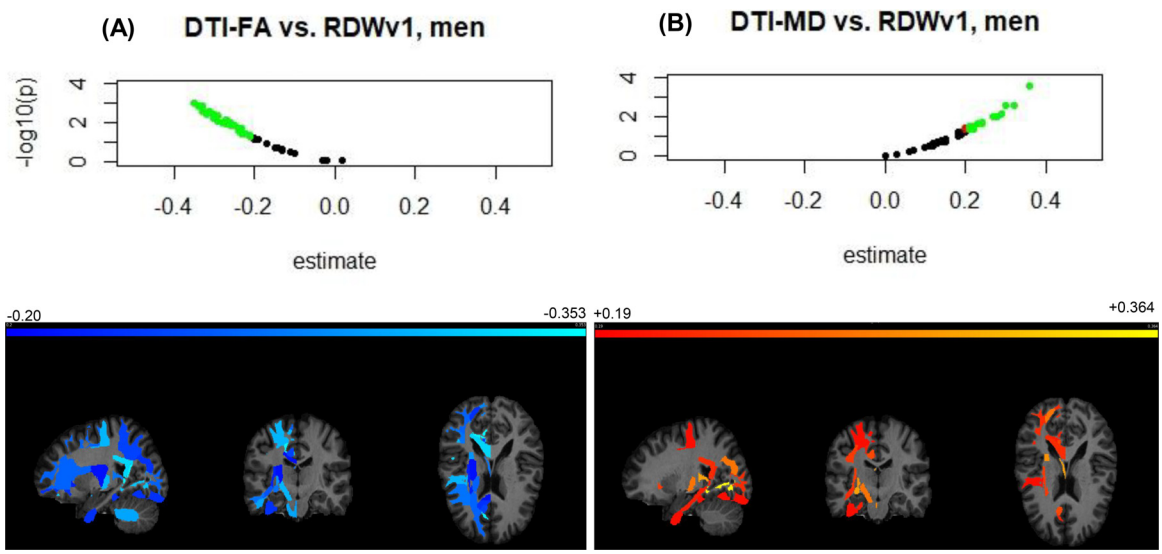


Fig. 2. Volcano plots and brain images of FA and MD vs. RDW(v_1) among males: HANDLS 2004-2013 and HANDLS-SCAN 2011–2015.[a,b,c] Abbreviations: bayes1RDW, δRDW; DTI, Diffusion Tensor Imaging; FA, Fractional Anisotropy; HANDLS, Healthy Aging in Neighborhoods of Diversity Across the Life Span; HANDLS-SCAN, HANDLS brain MRI ancillary study; MD, Mean Diffusivity; MRI, Magnetic Resonance Imaging; TR, Tracts. [a] In both the volcano plot and the brain images: Values are effect sizes from adjusted linear regression models restricted to males only with RDW(v_1) as the exposure and outcomes being alternately regional bilateral FA and regional bilateral MD. The multiple linear models were adjusted for age, race, poverty status and time of follow-up between visit 1 and v_{scan}. [b]The volcano plot represents the results of the 2 models among men, with outcomes being regional FA and MD (A) RDW(v_1) vs. DTI-FA; (B) RDW(v_1) vs. DTI-MD. "Estimate" refers to the standardized beta coefficient or effect size; -Log10(p) is the associated negative Log base 10 of the p-value for each regional association. Red dots represent $p < 0.05$ with b $<+0.20$ for positive associations or b >-0.20 for negative associations; Green dots represent $p < 0.05$ with b $> +0.20$ for positive associations or b <-0.20 for negative associations. [c]The brain images represent the same results of the 2 models among men, using FSLEYES software for visualizing effect sizes. Those effect sizes were selected for regions with $p < 0.05$ and are presented at a threshold of 0.20 up to the maximum effect size, using a color gradient. Colder (blue) colors are for negative associations (expected for DTI-FA) and warmer colors (red through yellow) are for positive associations (expected for DTI-MD). Lighter colors indicate stronger effect sizes.

ations with global FA/MD outcomes were found for the δRDW exposure.

4. Discussion

This study is among a few to examine the association between anemia and WMI and to our knowledge, the first to link RDW with WMI, particularly in a racially and socio-economically diverse sample of middle-aged urban adults. Among key findings, greater RDW_{v1} was consistently associated with poorer WMI among males. Most notably, in males, $RDW_{(v1)}$ was linked with lower global mean FA (standardized effect size b = −0.30, p= 0.003, q < 0.05) in the basic model adjusted for socio-demographic factors, an association that remained statistically significant upon adjustment for other hematological measures and other potential confounders and detected in all cortical regions except the temporal lobe. No such associations were found for anemia, and δRDW (overall or sex-specific), or for RDW exposures among females and the non-anemic group.

Before our study, there was no evidence for relationships between anemia or RDW and WMI in humans. Most prior studies examined relations between Hb or anemia and other brain MRI outcomes (e.g., volumetric markers) and only a few tested associations of those various brain MRI outcomes with RDW exposures (Dagistan and Cosgun, 2019; Jonassaint et al., 2014; Lee et al., 2016). For instance, one study reported an association between lower Hb and smaller GM and intracranial volumes, with a trend observed towards reduced WM volume (Jonassaint et al., 2014). Given the link between anemia, RDW and cardiovascular disease (Mozos, 2015), it has been opined that among brain outcomes, these variables may be especially related to cerebrovascular markers (Wolters et al., 2019). Indeed, studies have found relations between Hb, RDW, and anemia with key markers of cerebrovascular health, including vascular brain disease, global cerebral perfusion (Wolters et al., 2019), and WM lesion burden (Dagistan and Cosgun, 2019; Lee et al., 2016). This is consistent with our findings given that WMI is also a measure of cerebrovascular health (Burgmans et al., 2010; Falvey et al., 2013; Kennedy and Raz, 2009).

Our findings further the literature by demonstrating the deleterious effect of RDW on WMI among males, above and beyond the contribution of Hb and other hematological factors. Our results on the positive relation between Hb and FA, particularly among males, held only in reduced models that did not adjust for RDW and other variables. It is our understanding that our sex-specific findings are novel as this is the first study to examine these differential relations of dMRI outcomes across sex groups. These findings are consistent with results of our previous study indicating that RDW was associated with poorer baseline performance on the Brief Test of Attention in the total population ($\gamma = -0.123 \pm 0.039$; p= 0.001), a relationship detected mostly among men ($\gamma = -0.221 \pm 0.068$; p= 0.001; Beydoun et al., 2020). Given the potential clinical relevance of these findings (e.g., males may be uniquely susceptible to poorer neurological outcomes as a function of these hematologic variables), it would be prudent for other studies to attempt to replicate our finding, which would provide clarity on the validity and generalizability of these results.

Elevated RDW may reflect iron deficiency early before MCV changes and when multiple nutritional deficiencies are present that would lead to opposing directional changes in MCV. Although the exact pathophysiological mechanism linking elevated RDW and severity of white matter hyperintensity (leukoaraiosis) is unclear, Jonassaint and colleagues suggested that RDW reflects an underlying inflammation due to its positive correlation with various inflammatory markers (Lee et al., 2016). Inflammation induces ineffective erythropoiesis and enables immature RBCs to enter the circulation, which leads to anisocytosis. Recent studies have sug-

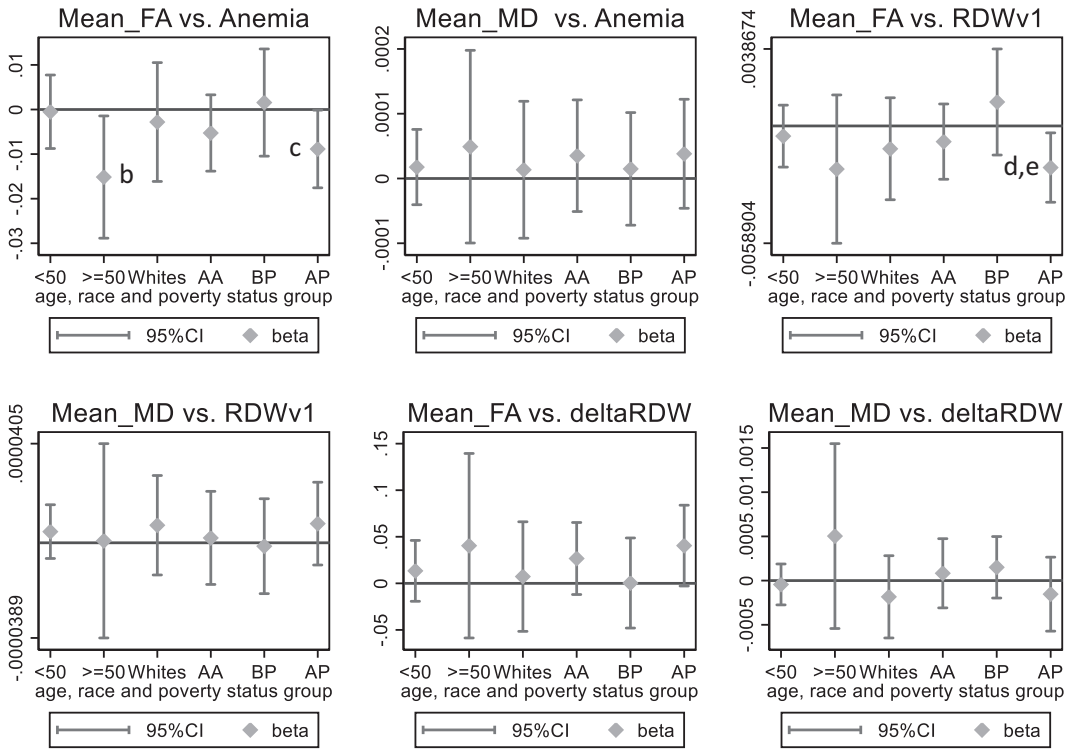


Fig. 3. Associations of mean FA and MD with the 3 main exposures (Anemic(v_1), RDW(v_1) and δRDW) by age group, race and poverty status, minimally adjusted model (Model 1): HANDLS 2004–2013 and HANDLS-SCAN 2011–2015.[a] Abbreviations: AA, African Americans; AP, Above Poverty; BP, Below Poverty; ESR, Erythrocyte Sedimentation Rate; FA, Fractional Anisotropy; HANDLS, Healthy Aging in Neighborhoods of Diversity Across the Life Span; HANDLS-SCAN, HANDLS brain MRI ancillary study; Hb, Hemoglobin; MCH, Mean Cell Hemoglobin; MD, Mean Diffusivity; MRI, Magnetic Resonance Imaging. [a]Values are adjusted linear regression coefficients β with associated 95% CI. All stratified multiple linear models with outcomes being global Mean FA and global Mean MD and the 3 exposures entered alternately, were adjusted for age, sex, race, poverty status and time of follow-up between visit 1 and v_{scan} (*Analysis A'*) The models presented are stratified by age group (<50 years: group 1, n = 127; ≥ 50 years: group 2, n = 87); race (Whites: group 3, n = 127; African-Americans: group 4, n = 87); and poverty status (Below poverty: group 5, n = 70; Above poverty: group 6, n = 144). δRDW is the empirical bayes estimator from mixed-effects regression model using the full HANDLS cohort with 2 repeats, over time, representing annual rates of change at the individual-level in RDW. Anemia (using sex-specific WHO criteria) and RDW(v_1) are measured at visit 1. Model 2 was conducted on selected findings, further adjusting Model 1 by RDW (for anemia), Hb (for RDW), ESR, serum iron and MCH. [b]Mean FA vs. anemia at v_1 among older adults (aged ≥50 years) in Model 1: $\beta = -0.0152$, SE = 0.00070, $p = 0.033$. $p > 0.05$ in Model 2. [c]Mean FA vs. anemia at v_1 among individuals living above poverty in Model 2: $\beta = -0.0088$, SE = 0.0044, $p = 0.048$. $p > 0.05$ in Model 2. [d]Mean FA vs. RDW at v_1 among individuals living above poverty in Model 1: $\beta = -0.0021$, SE = 0.0009, $p = 0.020$. [e]Mean FA vs. RDW at v_1 among individuals living above poverty in Model 2: $\beta \pm$ SE: -0.00391 ± 0.00130, $p = 0.004$.

gested that elevated RDW might also reflect impairment of microcirculation (Patel et al., 2013). Over their life, RBCs gradually lose cell membrane deformability and eventually rupture and are eliminated in the spleen. Elevated RDW is strongly associated with reduced RBC deformability (Patel et al., 2013). As chronic ischemic disease and inadequate blood supply in the cerebral microcirculation are important mechanisms for the development and progression of leukoaraiosis, it is plausible that reduced RBC deformability impairs the cerebral microcirculation and subsequently leads to the development of leukoaraiosis in people with elevated RDW level. This mechanism might be extended to the effect of RDW on WMI, given the inverse association between white matter lesion volume and WMI in our cohort (Absolute value of Pearson's correlation, |r| between 0.29 and 0.33 for both mean FA and mean MD), although further studies are needed. The mechanisms behind sex differences in the effects of RDW on WMI, particularly the stronger effect on FA among males are unknown, despite the well-known fact that anemia and elevated RDW are both more common among females, triggered mainly by menstruation during the pre-menopausal period. In contrast, among males, gastro-intestinal bleeding and cancer are some of the main causes of anemia. Pending replication of our findings, animal and other mechanistic studies are needed to uncover pathways behind those sex differences. It is also possible that the main causes for elevated RDW among men underlies the link with WMI, rather than RDW itself. Thus, triggers for gastro-intestinal bleeding should be studied in relation to WMI and their association with WMI measures compared between men and women. Such triggers may include but are not limited to certain infections that may be conducive to anemia of inflammation and elevated RDW (e.g., *H. pylori* infection; Haile and Timerga, 2021). In fact, *H. pylori* infection is more prevalent and can lead to atrophic gastritis to a greater extent among men than among women (Ferro et al., 2019) and was shown recently to be associated with incident AD mostly among men (Beydoun et al., 2018). Nevertheless, more studies are needed to examine those complex relationships.

Our findings also indicate that RDW may affect mostly WMI in the occipital and parietal regions of the brain, with a strong effect consistently found with respect to FA and MD in the lingual WM (|b| >0.30). The latter small region located in the occipital lobe is known to be involved in visual processing pathways including color perception, visual word processing and analyzing complex features of visual forms (Szeszko et al., 2005).

Our novel examination of the associations between anemia-related biomarkers with brain structural dMRI measures—reflecting global and regional white matter integrity, potentially underlying various neuropathologies—are among our study's key strengths. Despite our cross-sectional design, our study provided 5–6 years of latency between exposure (RDW_{v1} and anemia) and outcome (brain MRI measures) while accounting for longitudinal changes

in RDW as an additional exposure of interest. Our study sample provided sufficient power (power >0.80 for an effect size of 0.30; power >0.68 for an effect size of 0.25 for both male and female strata) to estimate sex-stratified models, an important consideration given the reported associations between sex, anemia, and cognitive impairment. Other stratified models were also well-powered, with the possible exception of the "below poverty" group. Our analytic approach and data sample adds to the strength of the current study. We were able to test our hypotheses in our overall sample, and separately for males and females while correcting for multiple testing and adjusting for a wide range of potential confounders, including socio-demographic, lifestyle and health-related characteristics including hematological measures, and additional nutritional biomarkers. Those potential confounders were selected using machine learning (i.e., LASSO) and backward elimination to reduce bias without that being at the expense of statistical efficiency.

Despite the strengths of our study, it is not without limitations. Due to the cross-sectional design, our findings are only speculative in nature despite the 5–6 year latency period. Further, despite our inclusion of several potential confounders previously identified for their association with the exposure and/or outcome, we are not able to rule out residual confounding. Finally, our final sample differed significantly with the initially recruited HANDLS sample, particularly with respect to distribution according to poverty status, affecting generalizability to Baltimore city or other similar urban settings across the United States. However, the differences were minor when comparing the initial largest available HANDLS SCAN sample (n = 240) with the final sample selected based on exposure variable availability.

In conclusion, initial RDW was consistently associated with poorer WMI among males. Further longitudinal studies should be directed at elucidating the mechanisms that mediate or link higher RDW with poor white matter integrity, particularly among males.

Disclosure statement

This work was supported in part by the Intramural research Program of the NIH, National institute on Aging. This work was also supported by the National Institutes of Health, R01-AG034161 to S.R.W, ZIA–AG000513 to M.K.E. and A.B.Z., and The University of Maryland Claude D. Pepper Older Americans Independence Center (NIH grant P30 AG028747).

The authors declare no conflict of interest.

Acknowledgements

The authors would like to thank participants, staff and investigators of HANDLS and HANDLS SCAN and the internal reviewers of the manuscript at NIA/NIH/IRP. We wish to acknowledge the Core for Translational Research in Imaging @ Maryland (C-TRIM) which is part of the Center for Innovative Biomedical Resources at the University of Maryland School of Medicine.

Author contributions

MAB contributed to study concept, planned analysis, conducted data management and statistical analysis, conducted literature review, wrote and revised the manuscript. DS planned analysis, assisted in statistical analysis, wrote parts of the manuscript, revised the manuscript. SH planned analysis, conducted data management, conducted literature review, wrote and revised parts of the manuscript. JW planned analysis, conducted literature search and review, assisted in statistical analysis, wrote parts of the manuscript, revised the manuscript. HAB planned the analysis, conducted literature review, wrote parts of the manuscript, revised the manuscript. AM planned analysis, assisted in statistical analysis, wrote parts of the manuscript, revised the manuscript. LIK acquired data, wrote and revised parts of the manuscript. CD acquired data, wrote and revised parts of the manuscript. RPG acquired data, wrote and revised parts of the manuscript. SLS acquired data, wrote and revised parts of the manuscript. GE acquired data, planned analysis, assisted in data management and statistical analysis, wrote and revised parts of the manuscript. MKE acquired data, wrote and revised parts of the manuscript. ABZ acquired data, planned analysis, wrote and revised parts of the manuscript. SW acquired data, planned analysis, wrote and revised parts of the manuscript. All authors read and approved the final version of the paper.

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 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.

Disclaimer

The views expressed in this article are those of the authors and do not necessarily reflect the official policy or position of Fort Belvoir Community Hospital, the Defense Health Agency, Department of Defense, or U.S. Government. Reference to any commercial products within this publication does not create or imply any endorsement by Fort Belvoir Community Hospital, the Defense Health Agency, Department of Defense, or U.S. Government.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.neurobiolaging.2021.05.004.

References

Beydoun, M.A., Beydoun, H.A., Elbejjani, M., Dore, G.A., Zonderman, A.B., 2018. Helicobacter pylori seropositivity and its association with incident all-cause and Alzheimer's disease dementia in large national surveys. Alzheimers Dement 14 (9), 1148–1158.

Beydoun, M.A., Beydoun, H.A., Gamaldo, A.A., Teel, A., Zonderman, A.B., Wang, Y., 2014. Epidemiologic studies of modifiable factors associated with cognition and dementia: systematic review and meta-analysis. BMC Public Health 14, 643.

Beydoun, M.A., Beydoun, H.A., Wang, Y., 2008. Obesity and central obesity as risk factors for incident dementia and its subtypes: a systematic review and meta–analysis. Obes Rev 9 (3), 204–218.

Beydoun, M.A., Hossain, S., Beydoun, H.A., Shaked, D., Weiss, J., Evans, M.K., Zonderman, A.B., 2020. Red cell distribution width is directly associated with poor cognitive performance among nonanemic, middle-aged, urban adults. J Nutr 150 (1), 128–139.

Burgmans, S., van Boxtel, M.P., Gronenschild, E.H., Vuurman, E.F., Hofman, P., Uylings, H.B., Jolles, J., Raz, N., 2010. Multiple indicators of age-related differences in cerebral white matter and the modifying effects of hypertension. Neuroimage 49 (3), 2083–2093.
Cavedo, E., Galluzzi, S., Pievani, M., Boccardi, M., Frisoni, G.B., 2012. Norms for imaging markers of brain reserve. J Alzheimers Dis 31 (3), 623–633.
Chung, S.D., Sheu, J.J., Kao, L.T., Lin, H.C., Kang, J.H., 2014. Dementia is associated with iron-deficiency anemia in females: a population-based study. J Neurol Sci 346 (1-2), 90–93.
Cosgrove, K.P., Mazure, C.M., Staley, J.K., 2007. Evolving knowledge of sex differences in brain structure, function, and chemistry. Biol Psychiatry 62 (8), 847–855.
Dagistan, E., Cosgun, Z., 2019. Could hemogram parameters be predictors of dementia in elderly patients? Aging Male 22 (3), 192–197.
Department of Health and Human Services, O.o.t.A.S.f.P.a.E., 2004. The 2004 HHS Poverty Guidelines https://aspe.hhs.gov/2004-hhs-poverty-guidelines.
Dlugaj, M., Winkler, A., Weimar, C., Durig, J., Broecker-Preuss, M., Dragano, N., Moebus, S., Jockel, K.H., Erbel, R., Eisele, L.Heinz Nixdorf Recall Study Investigative, G., 2016. Anemia and mild cognitive impairment in the german general population. J Alzheimers Dis 49 (4), 1031–1042.
Evans, M.K., Lepkowski, J.M., Powe, N.R., LaVeist, T., Kuczmarski, M.F., Zonderman, A.B., 2010. Healthy aging in neighborhoods of diversity across the life span (HANDLS): overcoming barriers to implementing a longitudinal, epidemiologic, urban study of health, race, and socioeconomic status. Ethn Dis 20 (3), 267–275.
Falvey, C.M., Rosano, C., Simonsick, E.M., Harris, T., Strotmeyer, E.S., Satterfield, S., Yaffe, K., Health, A.B.C.S., 2013. Macro- and microstructural magnetic resonance imaging indices associated with diabetes among community-dwelling older adults. Diabetes Care 36 (3), 677–682.
Faux, N.G., Rembach, A., Wiley, J., Ellis, K.A., Ames, D., Fowler, C.J., Martins, R.N., Pertile, K.K., Rumble, R.L., Trounson, B., Masters, C.L., Group, A.R., Bush, A.I., 2014. An anemia of Alzheimer's disease. Mol Psychiatry 19 (11), 1227–1234.
Ferro, A., Morais, S., Pelucchi, C., Dierssen-Sotos, T., Martin, V., Lopez-Carrillo, L., Malekzadeh, R., Tsugane, S., Hamada, G.S., Hidaka, A., Hernandez-Ramirez, R.U., Lopez-Cervantes, M., Zaridze, D., Maximovitch, D., Pourfarzi, F., Zhang, Z.F., Yu, G.P., Pakseresht, M., Ye, W., Plymoth, A., Leja, M., Gasenko, E., Derakhshan, M.H., Negri, E., La Vecchia, C., Peleteiro, B., Lunet, N., 2019. Sex differences in the prevalence of Helicobacter pylori infection: an individual participant data pooled analysis (StoP Project). Eur J Gastroenterol Hepatol 31 (5), 593–598.
Glymour, M.M., Chene, G., Tzourio, C., Dufouil, C., 2012. Brain MRI markers and dropout in a longitudinal study of cognitive aging: the Three-City Dijon Study. Neurology 79 (13), 1340–1348.
Haile, K., Timerga, A., 2021. Evaluation of hematological parameters of helicobacter pylori-infected adult patients at Southern Ethiopia: a comparative cross-sectional study. J Blood Med 12, 77–84.
Hoffmann, J.J., 2012. Red cell distribution width and mortality risk. Clin Chim Acta 413 (7-8), 824–825.
Hong, C.T., Hsieh, Y.C., Liu, H.Y., Chiou, H.Y., Chien, L.N., 2020. Association between anemia and dementia-a nationwide, population-based cohort study in Taiwan. Current Alzheimer research.
Hsu, F.C., Sink, K.M., Hugenschmidt, C.E., Williamson, J.D., Hughes, T.M., Palmer, N.D., Xu, J., Smith, S.C., Wagner, B.C., Whitlow, C.T., Bowden, D.W., Maldjian, J.A., Divers, J., Freedman, B.I., 2018. Cerebral structure and cognitive performance in African Americans and European Americans with Type 2 diabetes. J Gerontol A Biol Sci Med Sci 73 (3), 407–414.
Jenkinson, M., Bannister, P., Brady, M., Smith, S., 2002. Improved optimization for the robust and accurate linear registration and motion correction of brain images. Neuroimage 17 (2), 825–841.
Jenkinson, M., Smith, S., 2001. A global optimisation method for robust affine registration of brain images. Med Image Anal 5 (2), 143–156.
Jonassaint, C.R., Varma, V.R., Chuang, Y.F., Harris, G.C., Yasar, S., Polinder-Bos, H., Carlson, M.C., 2014. Lower hemoglobin is associated with poorer cognitive performance and smaller brain volume in older adults. J Am Geriatr Soc 62 (5), 972–973.
Jones, D.K., 2008. Studying connections in the living human brain with diffusion MRI. Cortex 44 (8), 936–952.
Kennedy, K.M., Raz, N., 2009. Pattern of normal age-related regional differences in white matter microstructure is modified by vascular risk. Brain Res 1297, 41–56.
Lee, H.B., Kim, J., Oh, S.H., Kim, S.H., Kim, H.S., Kim, W.C., Kim, S., Kim, O.J., 2016. Red blood cell distribution width is associated with severity of leukoaraiosis. PLoS One 11 (2), e0150308.
Li, N., Zhou, H., Tang, Q., 2017. Red blood cell distribution width: a novel predictive indicator for cardiovascular and cerebrovascular diseases. Dis Markers 2017, 7089493.
Louapre, C., Govindarajan, S.T., Gianni, C., Madigan, N., Nielsen, A.S., Sloane, J.A., Kinkel, R.P., Mainero, C., 2016. The association between intra- and juxta-cortical pathology and cognitive impairment in multiple sclerosis by quantitative T2* mapping at 7 T MRI. Neuroimage Clin 12, 879–886.
Mozos, I., 2015. Mechanisms linking red blood cell disorders and cardiovascular diseases. Biomed Res Int 2015, 682054.
Muller, T., Payton, N.M., Kalpouzos, G., Jessen, F., Grande, G., Backman, L., Laukka, E.J., 2020. Cognitive, genetic, brain volume, and diffusion tensor imaging markers as early indicators of dementia. J Alzheimers Dis 77 (4), 1443–1453.
Ozturk, Z.A., Unal, A., Yigiter, R., Yesil, Y., Kuyumcu, M.E., Neyal, M., Kepekci, Y., 2013. Is increased red cell distribution width (RDW) indicating the inflammation in Alzheimer's disease (AD)? Arch Gerontol Geriatr 56 (1), 50–54.
Park, S., Kim, D.K., Myung, W., Yoo, J.H., Shin, S.J., Na, D.L., Kim, S.Y., Lee, J.H., Kim, S.Y., Han, S.H., Choi, S.H., Shin, J., 2019. Risk factors of behavioral and psychological symptoms in patients with alzheimer disease: the clinical research of dementia of South Korea Study. Korean J Fam Med 40 (1), 16–21.
Patel, K.V., Mohanty, J.G., Kanapuru, B., Hesdorffer, C., Ershler, W.B., Rifkind, J.M., 2013. Association of the red cell distribution width with red blood cell deformability. Adv Exp Med Biol 765, 211–216.
Patel, K.V., Semba, R.D., Ferrucci, L., Newman, A.B., Fried, L.P., Wallace, R.B., Bandinelli, S., Phillips, C.S., Yu, B., Connelly, S., Shlipak, M.G., Chaves, P.H., Launer, L.J., Ershler, W.B., Harris, T.B., Longo, D.L., Guralnik, J.M., 2010. Red cell distribution width and mortality in older adults: a meta-analysis. J Gerontol A Biol Sci Med Sci 65 (3), 258–265.
Perlstein, T.S., Weuve, J., Pfeffer, M.A., Beckman, J.A., 2009. Red blood cell distribution width and mortality risk in a community-based prospective cohort. Arch Intern Med 169 (6), 588–594.
R Foundation for Statistical Computing, 2013. R: A Language and Environment for Statistical Computing http://www.R-project.org/.
Roalf, D.R., Gur, R.E., Verma, R., Parker, W.A., Quarmley, M., Ruparel, K., Gur, R.C., 2015. White matter microstructure in schizophrenia: associations to neurocognition and clinical symptomatology. Schizophr Res 161 (1), 42–49.
Schneider, A.L., Jonassaint, C., Sharrett, A.R., Mosley, T.H., Astor, B.C., Selvin, E., Coresh, J., Gottesman, R.F., 2016. Hemoglobin, anemia, and cognitive function: the atherosclerosis risk in communities study. J Gerontol A Biol Sci Med Sci 71 (6), 772–779.
Selvin, S., 2004. Statistical analysis of epidemiologic data, 3rd ed. Oxford University Press ed..
Shaked, D., Leibel, D.K., Katzel, L.I., Davatzikos, C., Gullapalli, R.P., Seliger, S.L., Erus, G., Evans, M.K., Zonderman, A.B., Waldstein, S.R., 2019. Disparities in diffuse cortical white matter integrity between socioeconomic groups. Front Hum Neurosci 13, 198.
Sousa, N.D.S., Menezes, T.N., Silva, N.A., Eulalio, M.D.C., Paiva, A.A., 2018. Prevalence of anemia and correlation between the concentration of hemoglobin and cognitive factors among the elderly. Cien Saude Colet 23 (3), 935–944.
STATA, 2019. Statistics/Data Analysis: Release 16.0. Stata Corporation, Texas.
Szeszko, P.R., Ardekani, B.A., Ashtari, M., Malhotra, A.K., Robinson, D.G., Bilder, R.M., Lim, K.O., 2005. White matter abnormalities in obsessive-compulsive disorder: a diffusion tensor imaging study. Arch Gen Psychiatry 62 (7), 782–790.
Tajuddin, S.M., Nalls, M.A., Zonderman, A.B., Evans, M.K., 2017. Association of red cell distribution width with all-cause and cardiovascular-specific mortality in African American and white adults: a prospective cohort study. J Transl Med 15 (1), 208.
Taniguchi, Y., Kitamura, A., Kaito, S., Yokoyama, Y., Yokota, I., Shinozaki, T., Seino, S., Murayama, H., Matsuyama, Y., Ikeuchi, T., Fujiwara, Y., Shinkai, S., 2019. Albumin and hemoglobin trajectories and incident disabling dementia in community-dwelling Older Japanese. Dement Geriatr Cogn Disord 47 (4-6), 233–242.
Trevisan, C., Veronese, N., Bolzetta, F., De Rui, M., Maggi, S., Zambon, S., Musacchio, E., Sartori, L., Perissinotto, E., Crepaldi, G., Manzato, E., Sergi, G., 2016. Low hemoglobin levels and the onset of cognitive impairment in older people: the PRO.V.A. study. Rejuvenation Res 19 (6), 447–455.
Wakana, S., Jiang, H., Nagae-Poetscher, L.M., van Zijl, P.C., Mori, S., 2004. Fiber tract-based atlas of human white matter anatomy. Radiology 230 (1), 77–87.
Waldstein, S.R., Dore, G.A., Davatzikos, C., Katzel, L.I., Gullapalli, R., Seliger, S.L., Kouo, T., Rosenberger, W.F., Erus, G., Evans, M.K., Zonderman, A.B., 2017. Differential associations of socioeconomic status with global brain volumes and white matter lesions in African American and White Adults: the HANDLS SCAN study. Psychosom Med 79 (3), 327–335.
Walter, S., Dufouil, C., Gross, A.L., Jones, R.N., Mungas, D., Filshtein, T.J., Manly, J.J., Arpawong, T.E., Glymour, M.M., 2019. Neuropsychological test performance and MRI markers of dementia risk: reducing education bias. Alzheimer Dis Assoc Disord 33 (3), 179–185.
Wan, J., Luo, P., Du, X., Yan, H., 2020. Preoperative red cell distribution width predicts postoperative cognitive dysfunction after coronary artery bypass grafting. Biosci Rep 40 (4), 1–9.
Weuve, J., Mendes de Leon, C.F., Bennett, D.A., Dong, X., Evans, D.A., 2014. The red cell distribution width and anemia in association with prevalent dementia. Alzheimer Dis Assoc Disord 28 (2), 99–105.
Winchester, L.M., Powell, J., Lovestone, S., Nevado-Holgado, A.J., 2018. Red blood cell indices and anaemia as causative factors for cognitive function deficits and for Alzheimer's disease. Genome Med 10 (1), 51.
Wolters, F.J., Zonneveld, H.I., Licher, S., Cremers, L.G.M., Ikram, M.K., Koudstaal, P.J., Vernooij, M.W., Ikram, M.A.Heart Brain Connection Collaborative Research, G., 2019. Hemoglobin and anemia in relation to dementia risk and accompanying changes on brain MRI. Neurology 93 (9), e917–e926.
World Health Organization, 1972. Nutritional Anaemias, Technical Report Series 503. WHO, Geneva.
Zlokovic, B.V., 2011. Neurovascular pathways to neurodegeneration in Alzheimer's disease and other disorders. Nat Rev Neurosci 12 (12), 723–738.

Red cell distribution width, anemia and their associations with white matter integrity among middle-aged urban adults

Beydoun et. al.

ONLINE SUPPLEMENTARY MATERIAL

Supplemental Method 1. Brain structural (s) and diffusion (d) MRI detailed description:

sMRI

T1-weighted MP-RAGE images at a thickness of 1.2 mm for 160 sagital slices (TR/TE/TI=2300/2.9/900 ms; FOV 25.6cm) were completed for the entire brain. Then, in order to allow for comparison, these images were converted to axial sections.

The Artificial Intelligence in Biomedical Imaging Lab, Center for Biomedical Image Computing and Analytics (CBICA), Department of Radiology at the University of Pennsylvania, preprocessed structural MRI scans with techniques developed in-house. A multi-atlas registration method(Doshi et al., 2013) was used to remove the extra-cranial material on the T1-weighted images. A multiplicative intrinsic component optimization (MICO) method(Li et al., 2014) was used to correct for bias. Multi-atlas region Segmentation utilizing Ensembles (MUSE) grouped the pre-processed images into a set of anatomical regions of interest (ROIs) (Doshi et al., 2016). MUSE integrates a broad ensemble of labeled templates by using a number of warping algorithms, regularization atlases and parameters (Doshi et al., 2016).

dMRI

Isotropic resolution images were obtained with an in-plane resolution of 2x2 mm and 2 mm slice thickness over a 22.4 cm FOV. A total of 66 slices at a TE = 122ms, TR = 3300ms, and flip angle = 90° were used. Eddy current effects were reduced by using bipolar diffusion. Diffusion-weighting scheme was a 2-shell (b = 1000, 2500), optimized for uniform sampling of each shell and non-overlapping diffusion directions of 60 and 120, respectively, and 6 b0 volumes. Image acquisition time was ten minutes.

Joint Linear Minimum Mean Squared Error software, (jLMMSE; Tristan-Vega and Aja-Fernandez, 2010) was used to de-noise the raw DWI data. The DT images were reconstructed by fitting the de-noised DWI data using multivariate linear fitting. Motion correction was conducted with FSL' s "eddycorrect" tool (Andersson and Sotiropoulos, 2016).

Fractional Anisotropy (FA) is a widely established method for quantifying WMI that is sensitive to the degree of myelination, density, and organization of WM. FA was used to determine directionality of water diffusion in the brain. FA measures the degree of anisotropy of the diffusion at the voxel level. This is derived from the variance of the average of the three eigenvalues of the diffusion tensor that are used to compute FA values (0 - 1; 0 = completely unrestricted diffusion, 1 = completely restricted diffusion). Computing the sum of the eigenvalues of the diffusion tensor yields the TR or mean diffusivity (MD), with a higher value indicative of poorer WMI. (Jones, 2008).

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 several quality control measures in place to ensure the highest level of quality and safety. For example, as mandated by the American College of Radiology(Mulkern et al., 2008), the scanner routine undergoes quality data assurance. In addition, the AD Neuroimaging Initiative phantom assesses weekly signal-to-noise ratio and monthly structural distortions(Gunter et al., 2009). Finally, the reliability of diffusion data is periodically checked with the National Institutes of Standards and Technology diffusion phantom. This ensures that the measurements from diffusion MRI are stable(phantom)

Table S1: Regions of Interest (ROI) used for dMRI measures: Fractional anisotropy (FA) and trace (TR)[1]

LEFT BRAIN			GM vs. WM	REGION
1	SPG_L	Superior Parietal Gyrus Left	GM	
2	CingG_L	Cingulate Gyrus Left	GM	
3	SFG_L	Superior Frontal Gyrus Left	GM	
4	MFG_L	Middle Frontal Gyrus Left	GM	
5	IFG_L	Inferior Frontal Gyrus Left	GM	
6	PrCG_L	Precentral Gyrus Left	GM	
7	PoCG_L	Postcentral Gyrus Left	GM	
8	AG_L	Angular Gyrus Left	GM	
9	PrCu_L	Pre-Cuneus Left	GM	
10	Cu_L	Cuneus Left	GM	
11	LG_L	Lingual Gyrus Left	GM	
12	Fu_L	Fusiform Gyrus Left	GM	
13	PHG_L	Parahippocampal Gyrus Left	GM	
14	SOG_L	Superior Occipital Gyrus Left	GM	
15	IOG_L	Inferior Occipital Gyrus	GM	
16	MOG_L	Middle Occipital Gyrus	GM	
17	ENT_L	Entorhinal Area	GM	
18	STG_L	Superior Temporal Gyrus	GM	
19	ITG_L	Inferior Temporal Gyrus	GM	
20	MTG_L	Middle Temporal Gyrus	GM	
21	LFOG_L	Lateral Fronto-Orbital Gyrus	GM	
22	MFOG_L	Middle Fronto-Orbital Gyrus	GM	
23	SMG_L	Supramarginal Gyrus	GM	
24	RG_L	Gyrus Rectus	GM	
25	Ins_L	Insular	GM	
26	Amyg_L	Amygdala	GM	
27	Hippo_L	Hippocampus	GM	
28	Cerebrellum_L	Cerebellum	GM	
29	CST_L	Corticospinal Tract Left	WM	
30	ICP_L	Inferior Cerebellar Peduncle Left	WM	
31	ML_L	Medial Lemniscus Left	WM/GM	
32	SCP_L	Superior Cerebellar Peduncle Left	WM	
33	CP_L	Cerebellar Peduncle Left	WM	
34	ALIC_L	Anterior Limb of Internal Capsule Left	WM	
35	PLIC_L	Posterior Limb of Internal Capsule Left	WM	
36	PTR_L	Posterior Thalamic Radiation (Include Optic Radiation) Left	WM	
37	ACR_L	Anterior Corona Radiata Left	WM	

38	SCR_L	Superior Corona Radiata Left	WM	
39	PCR_L	Posterior Corona Radiata Left	WM	
40	CGC_L	Cingulum (Cingulate Gyrus) Left	WM	
41	CGH_L	Cingulum (Hippocampus) Left	WM	
42	Fx/ST_L	Fornix (Cres) / Stria Terminalis (Can Not Be Resolved With Current Resolution) Left	WM	
43	SLF_L	Superior Longitudinal Fasciculus Left	WM	
44	SFO_L	Superior Fronto-Occipital Fasciculus (Could Be A Part of Anterior Internal Capsule) Left	WM	
45	IFO_L	Inferior Fronto-Occipital Fasciculus Left	WM	
46	SS_L	Sagittal Stratum (Include Inferior Longitidinal Fasciculus And Inferior Fronto-Occipital Fasciculus) Left	WM	
47	EC_L	External Capsule Left	WM	
48	UNC_L	Uncinate Fasciculus Left	WM	
49	PCT_L	Pontine Crossing Tract (A Part of Mcp) Left	WM	
50	MCP_L	Middle Cerebellar Peduncle Left	WM	
51	FX_L	Fornix (Column And Body of Fornix) Left	WM	
52	GCC_L	Genu of Corpus Callosum Left	WM	
53	BCC_L	Body of Corpus Callosum Left	WM	
54	SCC_L	Splenium of Corpus Callosum Left	WM	
55	RLIC_L	Retrolenticular Part of Internal Capsule Left	WM	
56	REDNC_L	Red Nucleus Left	GM	
57	SNIGRA_L	Substancia Nigra Left	GM	
58	TAP_L	Tapatum Left	GM	
59	Caud_L	Caudate Nucleus Left	GM	
60	Put_L	Putamen Left	GM	
61	Thal_L	Thalamus Left	GM	
62	GP_L	Globus Pallidus Left	GM	
63	Midbrain_L	Midbrain Left	GM	
64	Pons_L	Pons Left	WM	
65	Medulla_L	Medulla Left	WM/GM	
66	SPWM_L	Superior Parietal WM Left	WM	Parietal
67	Cingwm	Cingulum WM Left	WM	Cingulum
68	SFWM_L	Superior Frontal WM Left	WM	Frontal
69	MFWM_L	Middle Frontal WM Left	WM	Frontal
70	IFWM_L	Inferior Frontal WM Left	WM	Frontal
71	PrCWM_L	Precentral WM Left	WM	Frontal
72	PoCWM_L	Postcentral WM Left	WM	Parietal
73	AWM_L	Angular WM Left	WM	Parietal
74	PrCuWM_L	Pre-Cuneus WM Left	WM	Parietal
75	CuWM_L	Cuneus WM Left	WM	Occipital
76	LWM_L	Lingual WM Left	WM	Occipital

77	Fu_WM_L	Fusiform WM Left	WM	Occipital
78	SOWM_L	Superior Occipital WM Left	WM	Occipital
79	IOWM_L	Inferior Occipital WM Left	WM	Occipital
80	MOWM_L	Middle Occipital WM Left	WM	Occipital
81	STwm_L	Superior Temporal WM Left	WM	Temporal
82	ITWM_L	Inferior Temporal WM Left	WM	Temporal
83	MTWM_L	Middle Temporal WM Left	WM	Temporal
84	LFOWM_L	Lateral Fronto-Orbital WM Left	WM	Frontal
85	MFOWM_L	Middle Fronto-Orbital WM Left	WM	Frontal
86	SMWM_L	Supramarginal WM Left	WM	Parietal
87	RGWM_L	Rectus WM Left	WM	Frontal
88	Cerebrellumwm_L	Cerebellum WM Left	WM	
RIGHT BRAIN				
89	SPG_R	Superior Parietal Gyrus Right	GM	
90	CingG_R	Cingulate Gyrus Right	GM	
91	SFG_R	Superior Frontal Gyrus Right	GM	
92	MFG_R	Middle Frontal Gyrus Right	GM	
93	IFG_R	Inferior Frontal Gyrus Right	GM	
94	PrCG_R	Precentral Gyrus Right	GM	
95	PoCG_R	Postcentral Gyrus Right	GM	
96	AG_R	Angular Gyrus Right	GM	
97	PrCu_R	Pre-Cuneus Right	GM	
98	Cu_R	Cuneus Right	GM	
99	LG_R	Lingual Gyrus Right	GM	
100	FuG_R	Fusiform Gyrus Right	GM	
101	PHG_R	Parahippocampal Gyrus Right	GM	
102	SOG_R	Superior Occipital Gyrus Right	GM	
103	IOG_R	Inferior Occipital Gyrus Right	GM	
104	MOG_R	Middle Occipital Gyrus Right	GM	
105	ENT_R	Entorhinal Area Right	GM	
106	STG_R	Superior Temporal Gyrus Right	GM	
107	ITG_R	Inferior Temporal Gyrus Right	GM	
108	MTG_R	Middle Temporal Gyrus Right	GM	
109	LFOG_R	Lateral Fronto-Orbital Gyrus Right	GM	
110	MFOG_R	Middle Fronto-Orbital Gyrus Right	GM	
111	SMG_R	Supramarginal Gyrus Right	GM	
112	RG_R	Gyrus Rectus Right	GM	
113	Ins_R	Insular Right	GM	
114	Amyg_R	Amygdala Right	GM	
115	Hippo_R	Hippocampus Right	GM	
116	Cerebellum_R	Cerebellum Right	GM	

117	CST_R	Corticospinal Tract Right	WM	
118	ICP_R	Inferior Cerebellar Peduncle Right	WM	
119	ML_R	Medial Lemniscus Right	WM/GM	
120	SCP_R	Superior Cerebellar Peduncle Right	WM	
121	CP_R	Cerebellar peduncle, Right		
122	ALIC_R	Anterior Limb of Internal Capsule Right	WM	
123	PLIC_R	Posterior Limb of Internal Capsule Right	WM	
124	PTR_R	Posterior Thalamic Radiation (Include Optic Radiation) Right	WM	
125	ACR_R	Anterior Corona Radiata Right	WM	
126	SCR_R	Superior Corona Radiata Right	WM	
127	PCR_R	Posterior Corona Radiata Right	WM	
128	CGC_R	Cingulum (Cingulate Gyrus) Right	WM	
129	CGH_R	Cingulum (Hippocampus) Right	WM	
130	Fx/ST_R	Fornix (Cres) / Stria Terminalis (Can Not Be Resolved With Current Resolution) Right	WM	
131	SLF_R	Superior Longitudinal Fasciculus Right	WM	
132	SFO_R	Superior Fronto-Occipital Fasciculus (Could Be A Part of Anterior Internal Capsule) Right	WM	
133	IFO_R	Inferior Fronto-Occipital Fasciculus Right	WM	
134	SS_R	Sagittal Stratum (Include Inferior Longitidinal Fasciculus And Inferior Fronto-Occipital Fasciculus) Right	WM	
135	EC_R	External Capsule Right	WM	
136	UNC_R	Uncinate Fasciculus Right	WM	
137	PCT_R	Pontine Crossing Tract (A Part of MCP) Right	WM	
138	MCP_R	Middle Cerebellar Peduncle Right	WM	
139	FX_R	Fornix (Column And Body of Fornix) Right	WM	
140	GCC_R	Genu of Corpus Callosum Right	WM	
141	BCC_R	Body of Corpus Callosum Right	WM	
142	SCC_R	Splenium of Corpus Callosum Right	WM	
143	RLIC_R	Retrolenticular Part of Internal Capsule Right	WM	
144	REDNC_R	Red Nucleus Right	GM	
145	SNIGRA_R	Substancia Nigra Right	GM	
146	TAP_R	Tapatum Right	GM	
147	Caud_R	Caudate Nucleus Right	GM	
148	Put_R	Putamen Right	GM	
149	Thal_R	Thalamus Right	GM	
150	GP_R	Globus Pallidus Right	GM	
151	Midbrain_R	Midbrain Right	GM	
152	Pons_R	Pons Right	WM	
153	Medulla_R	Medulla Right	WM/GM	
154	SPwm_R	Superior Parietal WM Right	WM	Parietal
155	Cingwm_R	Cingulum WM Right	WM	Cingulum
156	SFWM_R	Superior Frontal WM Right	WM	Frontal

157	**MFWM_R**	**Middle Frontal WM Right**	**WM**	**Frontal**
158	**IFWM_R**	**Inferior Frontal WM Right**	**WM**	**Frontal**
159	**PrCWM_R**	**Precentral WM Right**	**WM**	**Frontal**
160	**PoCWM_R**	**Postcentral WM Right**	**WM**	**Parietal**
161	**AWM_R**	**Angular WM Right**	**WM**	**Parietal**
162	**PrCuWM_R**	**Pre-Cuneus WM Right**	**WM**	**Parietal**
163	**CuWM_R**	**Cuneus WM Right**	**WM**	**Occipital**
164	**LWM_R**	**Lingual WM Right**	**WM**	**Occipital**
165	**Fuwm_R**	**Fusiform WM Right**	**WM**	**Occipital**
166	**SOWM_R**	**Superior Occipital WM Right**	**WM**	**Occipital**
167	**IOWM_R**	**Inferior Occipital WM Right**	**WM**	**Occipital**
168	**MOWM_R**	**Middle Occipital WM Right**	**WM**	**Occipital**
169	**STWM_R**	**Superior Temporal WM Right**	**WM**	**Temporal**
170	**ITWM_R**	**Inferior Temporal WM Right**	**WM**	**Temporal**
171	**MTWM_R**	**Middle Temporal WM Right**	**WM**	**Temporal**
172	**LFOWM_R**	**Lateral Fronto-Orbital WM Right**	**WM**	**Frontal**
173	**MFOWM_R**	**Middle Fronto-Orbital WM Right**	**WM**	**Frontal**
174	**SMWM_R**	**Supramarginal WM Right**	**WM**	**Parietal**
175	**RGWM_R**	**Rectus WM Right**	**WM**	**Frontal**
176	**Cerebrellumwm_R**	**Cerebellum WM Right**	**WM**	

[1]Right and Left measures of FA and TR were averaged out before analyses C and D was carried out. This resulted in 100 measures in total, 50 for FA and 50 for TR, when excluding measures with missing data. Measures included in the analysis are bolded and in red font. All others are excluded. In addition, cerebellum wm TR (Right and Left) were only available for 85 subjects, as was the case for SNIGRA FA/TR (Right and Left). TR is also known as mean diffusivity or MD.

Supplemental 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 vs. **Composite models**	
Eq.		
1.1-1.4	$Y_{ij} = \pi_{0i} + \pi_{1i} Time_{ij} + \varepsilon_{ij}$	$\pi_{0i} = \gamma_{00} + \gamma_{0a} X_{aij} + \sum_{k=1}^{l} \gamma_{0k} Z_{ik} + \zeta_{0i}$
		$\pi_{1i} = \gamma_{10} + \gamma_{1a} X_{aij} + \sum_{m=1}^{n} \gamma_{1m} Z_{im} + \zeta_{1i}$
		$Y_{ij} = \gamma_{00} + \gamma_{0a} X_{aij} + \sum_{k=1}^{l} \gamma_{0k} Z_{ik} + \gamma_{10} Time_{ij} + \gamma_{1a} X_{aij} Time_{ij} + \sum_{m=1}^{n} \gamma_{1m} Z_{im} Time_{ij} + (\zeta_{0i} + \zeta_{1i} Time_{ij} + \varepsilon_{ij})$

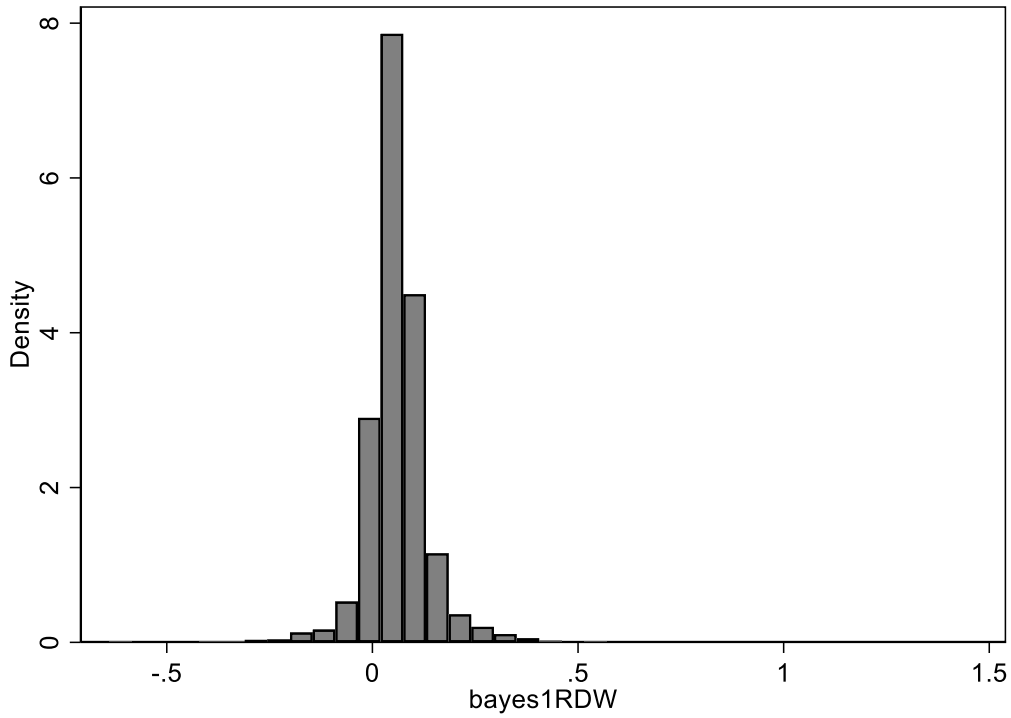
Where Y_{ij} is the outcome (RDW) for each individual "i" and visit "j"; π_{0i} is the level-1 intercept for individual i; π_{1i} is the level-1 slope for individual i; γ_{00} is the level-2 intercept of the random intercept π_{0i}; γ_{10} is the level-2 intercept of the slope π_{1i}; Z_{ik} is a vector 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.(Blackwell et al., 2006) 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 vs. brain MRI markers. Below are the results of the mixed effects regression models for each of the RDW exposure:

	RDW (n=3,017, k=1.7)
Intercept (γ_{00} ±SE)	14.09±0.18***
Time (γ_{10} ±SE)	+0.02±0.04
Age(v1) γ_{01} ±SE	-0.000±0.003
Age(v1)×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

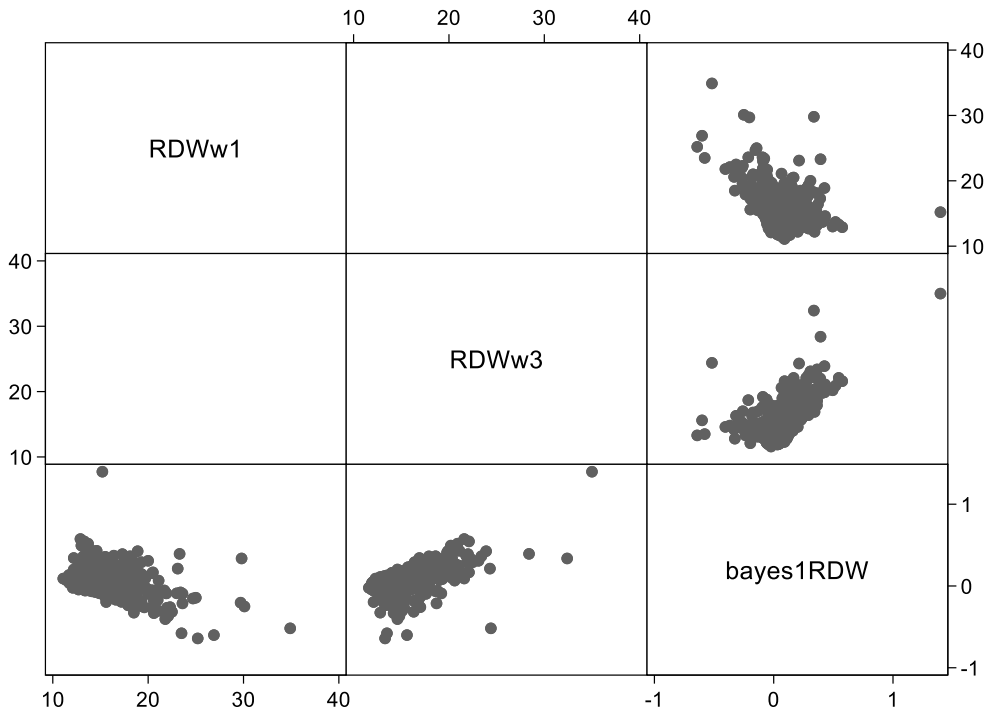
Below are distributional graphs for each of the 3 empirical Bayes estimators of the slope, which are estimated as follows: $\gamma_{10} + \gamma_{11}\times$ Age + $\gamma_{12}\times$ Sex + $\gamma_{13}\times$ Race + $\gamma_{14}\times$ Poverty + ζ_{1i}

RDW annual rate of change



Below is a scatter plot of the empirical Bayes estimators against visits 1 and 2 values.

Baseline and annual rates of change in RDW



Abbreviations: 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.

Supplemental 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) (Beydoun et al., 2018b).

A.2. Lifestyle

Smoking and drug use
Current use of opiates, marijuana or cocaine ("current" *vs*. "never or former") and smoking status ("current" vs. "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-hr 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*.(Mellen et al.). 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 (Mellen et al.).

Mean Adequacy Ratio (MAR)
Diet quality was also assessed using Nutrient Adequacy Ratio (NAR) and Mean Adequacy Ratio (MAR) scores(Fanelli Kuczmarski et al., 2013; Murphy et al., 2006). 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(Murakami et al., 2019). 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(Fanelli Kuczmarski et al., 2018). 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.(Centers for Disease Control and Prevention, 2007) 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(Beydoun et al., 2018b). 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.(Radloff, 1977) A score of ≥16 on the CES-D is reflective of elevated depressive symptoms (EDS), (Ramos et al., 2004) and predicts clinical depression based on the Diagnostic and Statistical Manual, fourth edition (DSM-IV) criteria.(Myers and Weissman, 1980) Four CES-D sub-domains exhibiting an invariant factor structure between The National Health and Nutrition Examination Survey I and pilot HANDLS data (Nguyen et al., 2004) 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.(Nguyen et al., 2004)

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. (Hisamatsu et al., 2014; Manickam et al., 2011; Nair et al., 2009)

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(Beydoun et al., 2016b; Beydoun et al., 2019).

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(Beydoun et al., 2016a). 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 hours 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 (Beydoun et al., 2018a; Diagnostics), and previously validated against other automated methods with coefficient of variation (CV) < 10% (Ispir et al., 2015; Owen and Roberts, 2003).

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 (Powe et al., 2013). 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 (Beydoun et al., 2010a; Beydoun et al., 2018b; Beydoun et al., 2010b).

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.(Diagnostics)

Erythrocyte Sedimentation Rate (ESR): Using 5 mL of refrigerated whole blood stored in lavender-top EDTA tubes, the ESR was tested within 24 hr of blood draw. This test used automated modified Westergren photochemical capillary-stopped flow kinetic analysis.(Diagnostics; Larsson and Hansson, 2004) The Mayo Clinic reports a reference of 0-22 mm/hr for men and 0-29 mm/hr for women(Mayo Clinic, 2017) and is considered a proxy measure for serum fibrinogen.(Yin et al., 2017)

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,

(Diagnostics; Samarina and Proskurnin, 2015) with reference ranges for men aged ≥30y set at 50-180 μg/dL, and for women: 20-49y (40-190 μg /dL) and 50+y(45-160 μg /dL). (Diagnostics)

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.(Diagnostics)

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 model 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.(Zou, 2006) 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.(Zou, 2006) 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.(Zou, 2006) 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.

	Selected covariates[1]			
	cvLASSO	**Min BIC LASSO**	**Adaptive LASSO**	**Reduced model**
RDW (v_1)	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 (v_2-v_1, 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 (v_1)	ESR, RDW, MCH, Albumin, Serum iron, race, WBC, age, WRAT total score, Cholesterol, Folate, B12,	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**	**ESR, RDW, MCH, Albumin, Serum iron, race, WBC, age, Cholesterol, Folate,**

	Inflammatory conditions, education, WC, married, diagnosed hypertension, vitamin supplements, current drugs, WHR, Triglycerides, 25(OH)D, poverty status, sex.	**conditions, education, WC, poverty status, sex.**	**B12, education, WC, poverty status, sex.**

Abbreviations: 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(v_1): RDW(v_1), 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(v_1) and RDW (v_2-v_1, annual): Hb(v_1), 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 measrues+ 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 **supplemental 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 (Selvin, 2004).

Supplemental References:

Andersson, J.L.R., Sotiropoulos, S.N., 2016. An integrated approach to correction for off-resonance effects and subject movement in diffusion MR imaging. Neuroimage 125, 1063-1078.

Beydoun, M.A., Beydoun, H.A., Mode, N., Dore, G.A., Canas, J.A., Eid, S.M., Zonderman, A.B., 2016a. Racial disparities in adult all-cause and cause-specific mortality among us adults: mediating and moderating factors. BMC Public Health 16(1), 1113.

Beydoun, M.A., Canas, J.A., Dore, G.A., Beydoun, H.A., Rostant, O.S., Fanelli-Kuczmarski, M.T., Evans, M.K., Zonderman, A.B., 2016b. Serum Uric Acid and Its Association with Longitudinal Cognitive Change Among Urban Adults. J Alzheimers Dis 52(4), 1415-1430.

Beydoun, M.A., Fanelli-Kuczmarski, M.T., Canas, J.A., Beydoun, H.A., Evans, M.K., Zonderman, A.B., 2018a. Dietary factors are associated with serum uric acid trajectory differentially by race among urban adults. Br J Nutr 120(8), 935-945.

Beydoun, M.A., Fanelli Kuczmarski, M.T., Beydoun, H.A., Shroff, M.R., Mason, M.A., Evans, M.K., Zonderman, A.B., 2010a. The sex-specific role of plasma folate in mediating the association of dietary quality with depressive symptoms. J Nutr 140(2), 338-347.

Beydoun, M.A., Hossain, S., Fanelli-Kuczmarski, M.T., Beydoun, H.A., Canas, J.A., Evans, M.K., Zonderman, A.B., 2018b. Vitamin D Status and Intakes and Their Association With Cognitive Trajectory in a Longitudinal Study of Urban Adults. J Clin Endocrinol Metab 103(4), 1654-1668.

Beydoun, M.A., Obhi, H.K., Weiss, J., Canas, J.A., Beydoun, H.A., Evans, M.K., Zonderman, A.B., 2019. Systemic inflammation is associated with depressive symptoms differentially by sex and race: a longitudinal study of urban adults. Mol Psychiatry.

Beydoun, M.A., Shroff, M.R., Beydoun, H.A., Zonderman, A.B., 2010b. Serum folate, vitamin B-12, and homocysteine and their association with depressive symptoms among U.S. adults. Psychosom Med 72(9), 862-873.

Blackwell, E., de Leon, C.F., Miller, G.E., 2006. Applying mixed regression models to the analysis of repeated-measures data in psychosomatic medicine. Psychosom Med 68(6), 870-878.

Centers for Disease Control and Prevention, 2007. National Health and Nutrition Examination Surveys 2007-2008: https://wwwn.cdc.gov/nchs/nhanes/continuousnhanes/default.aspx?BeginYear=2007. https://wwwn.cdc.gov/nchs/nhanes/continuousnhanes/default.aspx?BeginYear=2007.

Diagnostics, Q., Ferritin. https://www.questdiagnostics.com/testcenter/TestDetail.action?ntc=457&searchString=8272. (Accessed May 13sth 2019).

Diagnostics, Q., Hemogram. https://www.questdiagnostics.com/testcenter/BUOrderInfo.action?tc=7008&labCode=DAL. (Accessed May 13sth 2019).

Diagnostics, Q., Iron, Total and Total Iron Binding Capacity. https://www.questdiagnostics.com/testcenter/BUOrderInfo.action?tc=7573&labCode=SEA. (Accessed May 13sth 2019).

Diagnostics, Q., Vitamin B-12 (cobalamin) and folate panel. https://testdirectory.questdiagnostics.com/test/test-detail/7065/vitamin-b12-cobalamin-and-folate-panel-serum?cc=MASTER. (Accessed October 21st 2019).

Doshi, J., Erus, G., Ou, Y., Gaonkar, B., Davatzikos, C., 2013. Multi-atlas skull-stripping. Acad. Radiol. 20(12), 1566-1576.

Doshi, J., Erus, G., Ou, Y., Resnick, S.M., Gur, R.C., Gur, R.E., Satterthwaite, T.D., Furth, S.,

Davatzikos, C., Alzheimer's Neuroimaging, I., 2016. MUSE: MUlti-atlas region Segmentation utilizing Ensembles of registration algorithms and parameters, and locally optimal atlas selection. Neuroimage 127, 186-195.

Fanelli Kuczmarski, M., Bodt, B.A., Stave Shupe, E., Zonderman, A.B., Evans, M.K., 2018. Dietary Patterns Associated with Lower 10-Year Atherosclerotic Cardiovascular Disease Risk among Urban African-American and White Adults Consuming Western Diets. Nutrients 10(2).

Fanelli Kuczmarski, M., Mason, M.A., Beydoun, M.A., Allegro, D., Zonderman, A.B., Evans, M.K., 2013. Dietary patterns and sarcopenia in an urban African American and White population in the United States. J Nutr Gerontol Geriatr 32(4), 291-316.

Gunter, J.L., Bernstein, M.A., Borowski, B.J., Ward, C.P., Britson, P.J., Felmlee, J.P., Schuff, N., Weiner, M., Jack, C.R., 2009. Measurement of MRI scanner performance with the ADNI phantom. Med Phys 36(6), 2193-2205.

Hisamatsu, T., Fujiyoshi, A., Miura, K., Ohkubo, T., Kadota, A., Kadowaki, S., Kadowaki, T., Yamamoto, T., Miyagawa, N., Zaid, M., Torii, S., Takashima, N., Murakami, Y., Okamura, T.,

Horie, M., Ueshima, H., Group, S.R., 2014. Lipoprotein particle profiles compared with standard lipids in association with coronary artery calcification in the general Japanese population. Atherosclerosis 236(2), 237-243.

Ispir, E., Serdar, M.A., Ozgurtas, T., Gulbahar, O., Akin, K.O., Yesildal, F., Kurt, I., 2015. Comparison of four automated serum vitamin B12 assays. Clin Chem Lab Med 53(8), 1205-1213.
Jones, D.K., 2008. Studying connections in the living human brain with diffusion MRI. Cortex 44(8), 936-952.

Larsson, A., Hansson, L.O., 2004. Analysis of inflammatory response in human plasma samples by an automated multicapillary electrophoresis system. Clin Chem Lab Med 42(12), 1396-1400.

Li, C., Gore, J.C., Davatzikos, C., 2014. Multiplicative intrinsic component optimization (MICO) for MRI bias field estimation and tissue segmentation. Magn. Reson. Imaging 32(7), 913-923.

Manickam, P., Rathod, A., Panaich, S., Hari, P., Veeranna, V., Badheka, A., Jacob, S., Afonso, L., 2011. Comparative prognostic utility of conventional and novel lipid parameters for cardiovascular disease risk prediction: do novel lipid parameters offer an advantage? Journal of clinical lipidology 5(2), 82-90.
Mayo Clinic, 2017. Sed rate (erythrocyte sedimentation rate). https://www.mayoclinic.org/tests-procedures/sed-rate/about/pac-20384797. (Accessed May 10th 2019).

Mellen, P.B., Gao Sk Fau - Vitolins, M.Z., Vitolins Mz Fau - Goff, D.C., Jr., Goff, D.C., Jr., Deteriorating dietary habits among adults with hypertension: DASH dietary accordance, NHANES 1988-1994 and 1999-2004. (0003-9926 (Print)).

Mulkern, R.V., Forbes, P., Dewey, K., Osganian, S., Clark, M., Wong, S., Ramamurthy, U., Kun, L., Poussaint, T.Y., 2008. Establishment and results of a magnetic resonance quality assurance program for the pediatric brain tumor consortium. Acad Radiol 15(9), 1099-1110.

Murakami, K., Livingstone, M.B.E., Sasaki, S., 2019. Diet quality scores in relation to metabolic risk factors in Japanese adults: a cross-sectional analysis from the 2012 National Health and Nutrition Survey, Japan. Eur J Nutr 58(5), 2037-2050.

Murphy, S.P., Foote, J.A., Wilkens, L.R., Basiotis, P.P., Carlson, A., White, K.K., Yonemori, K.M., 2006. Simple measures of dietary variety are associated with improved dietary quality. Journal of the American Dietetic Association 106(3), 425-429.

Myers, J.K., Weissman, M.M., 1980. Use of a self-report symptom scale to detect depression in a community sample. Am J Psychiatry 137(9), 1081-1084.

Nair, D., Carrigan, T.P., Curtin, R.J., Popovic, Z.B., Kuzmiak, S., Schoenhagen, P., Flamm, S.D., Desai, M.Y., 2009. Association of total cholesterol/ high-density lipoprotein cholesterol ratio with proximal coronary atherosclerosis detected by multislice computed tomography. Preventive cardiology 12(1), 19-26.

Nguyen, H.T., Kitner-Triolo, M., Evans, M.K., Zonderman, A.B., 2004. Factorial invariance of the CES-D in low socioeconomic status African Americans compared with a nationally representative sample. Psychiatry research 126(2), 177-187.

Owen, W.E., Roberts, W.L., 2003. Comparison of five automated serum and whole blood folate assays. Am J Clin Pathol 120(1), 121-126.
phantom, N.d.

Powe, C.E., Evans, M.K., Wenger, J., Zonderman, A.B., Berg, A.H., Nalls, M., Tamez, H., Zhang, D., Bhan, I., Karumanchi, S.A., Powe, N.R., Thadhani, R., 2013. Vitamin D-binding protein and vitamin D status of black Americans and white Americans. N Engl J Med 369(21), 1991-2000.

Radloff, L., 1977. The CES-D scale: a self-report depression scale for research in the general population. Applied Psychological Measurement 1(385-401).

Ramos, M.I., Allen, L.H., Haan, M.N., Green, R., Miller, J.W., 2004. Plasma folate concentrations are associated with depressive symptoms in elderly Latina women despite folic acid fortification. Am J Clin Nutr 80(4), 1024-1028.

Samarina, T., Proskurnin, M., 2015. Rapid assessment of iron in blood plasma and serum by spectrophotometry with cloud-point extraction. F1000Res 4, 623.

Selvin, S., 2004. Statistical analysis of epidemiologic data, 3rd ed. ed. Oxford University Press.

Tristan-Vega, A., Aja-Fernandez, S., 2010. DWI filtering using joint information for DTI and HARDI. Med Image Anal 14(2), 205-218.

Yin, W., Xu, Z., Sheng, J., Xie, X., Zhang, C., 2017. Erythrocyte sedimentation rate and fibrinogen concentration of whole blood influences the cellular composition of platelet-rich plasma obtained from centrifugation methods. Exp Ther Med 14(3), 1909-1918.

Zou, H., 2006. The adaptive Lasso and it oracle properties. Journal of the American Statistical Association 101(476), 1418-1428.

Table S2. Descriptive analyses by anemia status and RDW tertiles: Study sample characteristics of eligible study sample by Anemia (v_1 and v_1/v_2) status and by RDW(v_1) tertiles, overall, among males and among females ; HANDLS 2004-2009 and HANDLS-SCAN 2011-2015[a]

	Anemia status at v_1		*Anemia status at v_1/v_2*		*RDW at v_1, tertiles*		
	Non-anemic	*Anemic*	*Non-anemic (v1 or v2)*	*Anemic (v1 and v2)*	*T1*	*T2*	*T3*
Total sample	*(N=190)*	*(N=24)*	*(N=182)*	*(N=14)*	*(N=74)*	*(N=70)*	*(N=70)*
Demographic factors							
Sex, % males	47.8[b]	25[b]	47.3	21.4	46	52.9	37.1
Age$_{v1}$	48±8.8	45±11.2	48.3±8.6[b]	44.1±13.1[b]	47.4±9.4	48.3±8.4	47.2±9.5
Race, % AA	36.3[b]	75[b]	39	64.2	29.7[c]	37.1[c]	55.7[c]
% above poverty	68.42	58.3	70.3	57.1	67.6	71.4	62.9
RDW (v_1)							
CV (%)	13.7±1.1[b]	16.2±2.7[b]	13.7±1.2[b]	15.69±2.9[b]	12.8±.36[c]	13.7±.22[c]	15.5±1.8[c]
Median	13.5	16.1	13.5	14.1	12.9	13.7	14.9
IQR	13;14.1	13.8;18	13;14.2	13.7;18.3	12.6;13.1	13.5;13.9	14.3;15.8
dMRI measures	*(N=190)*	*(N=24)*	*(N=182)*	*(N=14)*	*(N=74)*	*(N=70)*	*(N=70)*
dMRI, Analysis A							
Mean FA	+0.30±0.02	+0.29±0.02	+0.30±0.02	+0.30±.01	+0.3±0.01	+0.3±0.02	+0.30±0.02
Mean MD	2.546E-03±1.563E-04	2.545E-03±1.918E-04	2.546E-03±1.586E-04	2.476E-03±1.280E-04	2.541E-03±1.669E-04	2.557E-03±1.604E-04	2.540E-03±1.542E-04
dMRI, Analysis B							

Left Brain							
Frontal FA	+0.23±0.02	+0.23±0.02	+0.23±0.01	+0.24±0.02	+0.24±0.02 [c]	+0.23±0.02 [c]	+0.23±0.01 [c]
Frontal MD	2.394E-03±1.421E-04	2.397E-03±1.902E-04	2.393E-03±1.445E-04	2.341E-03±1.619E-04	2.382E-03±1.235E-04	2.408E-03±1.724E-04	2.392E-03±1.453E-04
Temporal FA	+0.24±0.02	+0.24±0.02	+0.24±0.02	+0.24±0.01	+0.24±0.01	+0.24±0.02	+0.24±0.01
Temporal MD	2.432E-03±1.468E-04	2.435E-03±1.379E-04	2.431E-03±1.470E-04	2.413E-03±1.253E-04	2.440E-03±1.662E-04	2.429E-03±1.442E-04	2.428E-03±1.237E-04
Parietal FA	+0.23±0.02	+0.23±0.02	+0.23±0.02	+0.24±0.01	+0.23±0.01	+0.23±0.02	+0.23±0.02
Parietal MD	2.665E-03±1.922E-04	2.637E-03±2.433E-04	2.664E-03±1.963E-04	2.583E-03±1.762E-04	2.663E-03±1.957E-04	2.663E-03±1.810E-04	2.659E-03±2.188E-04
Occipital FA	+0.2±0.01	+0.2±0.01	+0.2±0.01	+0.21±0.01	+0.21±0.01	+0.2±0.02	+0.2±0.02
Occipital MD	2.475E-03±1.532E-04	2.509E-03±1.965E-04	2.478E-03±1.561E-04	2.422E-03±1.182E-04	2.484E-03±1.593E-04	2.462E-03±1.424E-04	2.491E-03±1.730E-04
Right Brain							
Frontal FA	+0.23±0.01	+0.23±0.02	+0.23±0.01	+0.24±0.01	+0.24±0.01	+0.23±0.02	+0.23±0.02
Frontal MD	2.366E-03±1.326E-04	2.372E-03±1.850E-04	2.366E-03±1.348E-04	2.308E-03±1.422E-04	2.367E-03±1.283E-04	2.376E-03±1.486E-04	2.357E-03±1.410E-04
Temporal FA	+0.25±0.02	+0.25±0.02	+0.25±0.02	+0.25±0.01	+0.25±0.01	+0.25±0.02	+0.25±0.02
Temporal MD	2.344E-03±1.401E-04	2.344E-03±1.341E-04	2.341E-03±1.383E-04	2.321E-03±1.171E-04	2.344E-03±1.628E-04	2.353E-03±1.330E-04	2.335E-03±1.178E-04
Parietal FA	+0.23±0.02	+0.22±0.02	+0.23±0.02	+0.23±0.02	+0.23±0.01 [c]	+0.22±0.02 [c]	+0.22±0.02 [c]
Parietal MD	2.720E-03±2.129E-04	2.671E-03±2.439E-04	2.717E-03±2.160E-04	2.608E-03±1.934E-04	2.722E-03±2.110E-04	2.709E-03±2.074E-04	2.714E-03±2.336E-04
Occipital FA	+0.2±0.01	+0.2±0.02	+0.2±0.01	+0.21±0.01	+0.21±0.01	+0.2±0.02	+0.2±0.02
Occipital MD	2.554E-03±1.718E-04	2.569E-03±2.028E-04	2.553E-03±1.713E-04	2.522E-03±1.409E-04	2.554E-03±1.900E-04	2.556E-03±1.623E-04	2.556E-03±1.734E-04
Males	*(N=91)*	*(N=6)*	*(N=86)*	*(N=3)*	*(N=34)*	*(N=37)*	*(N=26)*
Demographic factors							
Age_{v1}	47.7±8.8	49.3±8.8	48.5±8.5	47.6±12.9	47.2±9.6	47.4±8	49.1±9
Race, % AA	35.2 [b]	50.0	36.0 [b]	100.0 [b]	26.4	40.5	53.9
% above poverty	75.8	66.7	77.9	66.7	76.5	78.4	69.2

RDW (v_1)							
CV (%)	13.6±.77 [b]	15.3±1.3	13.5±0.77 [b]	14.3±0.72 [b]	12.8±0.34 [c]	13.6±0.21 [c]	14.8±0.74 [c]
Median	13.5	15.4	13.5	14.1	12.9	13.6	14.7
IQR	13;14	14.1;16.6	13;14	13.7;15.1	12.6;13.1	13.5;13.9	14.2;15.2
dMRI measures	*(N=91)*	*(N=6)*	*(N=86)*	*(N=3)*	*(N=34)*	*(N=37)*	*(N=26)*
dMRI, Analysis A							
Mean FA	+0.30±0.02 [b]	+0.28±0.04 [b]	+0.30±0.02	+0.31±0.02	+0.30±0.01 [c]	+0.30±0.02 [c]	+0.29±0.02 [c]
Mean MD	2.576E-03±1.463E-04 [b]	2.696E-03±3.072E-04 [b]	2.579E-03±1.494E-04	2.451E-03±1.937E-04	2.549E-03±1.007E-04	2.597E-03±1.866E-04	2.609E-03±1.817E-04
dMRI, Analysis B							
Left Brain							
Frontal FA	+0.23±0.02 [b]	+0.22±0.03 [b]	+0.23±0.02	+0.24±0.01	+0.24±0.01 [c]	+0.23±0.02 [c]	+0.23±0.02 [c]
Frontal MD	2.434E-03±1.556E-04	2.529E-03±2.675E-04	2.438E-03±1.593E-04	2.320E-03±1.801E-04	2.405E-03±1.276E-04	2.453E-03±1.904E-04	2.466E-03±1.647E-04
Temporal FA	+0.24±0.02	+0.23±0.03	+0.24±0.01	+0.25±0.01	+0.25±0.01	+0.24±0.02	+0.23±0.02
Temporal MD	2.462E-03±1.349E-04	2.523E-03±2.137E-04	2.464E-03±1.364E-04	2.381E-03±2.266E-04	2.453E-03±1.027E-04	2.460E-03±1.733E-04	2.490E-03±1.319E-04
Parietal FA	+0.23±0.01 [b]	+0.22±0.03 [b]	+0.23±0.01 [b]	+0.24±0.01 [b]	+0.23±0.01 [c]	+0.23±0.02 [c]	+0.22±0.02 [c]
Parietal MD	2.731E-03±1.775E-04	2.804E-03±3.935E-04	2.739E-03±1.803E-04	2.519E-03±2.542E-04	2.717E-03±1.565E-04	2.734E-03±1.806E-04	2.762E-03±2.552E-04
Occipital FA	+0.2±0.01 [b]	+0.19±0.02 [b]	+0.20±0.01	+0.20±0.01	+0.2±0.01 [c]	+0.2±0.01 [c]	+0.16±0.02 [c]
Occipital MD	2.502E-03±1.434E-04 [b]	2.662E-03±3.361E-04 [b]	2.507E-03±1.486E-04	2.410E-03±2.432E-04	2.487E-03±1.101E-04	2.498E-03±1.607E-04	2.563E-03±2.129E-04
Right Brain							
Frontal FA	+0.23±0.01 [b]	+0.21±0.03 [b]	+0.23±0.01	+0.24±0.01	+0.24±0.01 [c]	+0.23±0.02 [c]	+0.23±0.02 [c]
Frontal MD	2.398E-03±1.323E-04 [b]	2.521E-03±2.798E-04 [b]	2.404E-03±1.356E-04	2.310E-03±2.218E-04	2.380E-03±9.660E-05	2.417E-03±1.695E-04	2.425E-03±1.640E-04

Temporal FA	+0.25±0.02 [b]	+0.23±0.03 [b]	+0.25±0.01	+0.26±0.01	+0.25±0.01	+0.24±0.02	+0.24±0.02
Temporal MD	2.365E-03±1.210E-04	2.425E-03±1.994E-04	2.364E-03±1.221E-04	2.294E-03±1.761E-04	2.347E-03±9.870E-05	2.380E-03±1.440E-04	2.380E-03±1.332E-04
Parietal FA	+0.22±0.02	+0.21±0.04	+0.22±0.02 [b]	+0.24±0.02 [b]	+0.23±0.01 [c]	+0.23±0.02 [c]	+0.22±0.02 [c]
Parietal MD	2.799E-03±2.021E-04	2.833E-03±4.089E-04	2.807E-03±2.055E-04	2.550E-03±3.464E-04	2.782E-03±1.861E-04	2.796E-03±2.125E-04	2.831E-03±2.619E-04
Occipital FA	+0.2±0.02 [b]	+0.19±0.03 [b]	+0.2±0.02	+0.21±0.02	+0.21±0.01 [c]	+0.2±0.02 [c]	+0.2±0.02 [c]
Occipital MD	2.584E-03±1.483E-04	2.699E-03±3.660E-04	2.588E-03±1.512E-04	2.464E-03±2.689E-04	2.552E-03±1.054E-04	2.608E-03±1.820E-04	2.620E-03±2.078E-04
Females	*(N=99)*	*(N=18)*	*(N=96)*	*(N=11)*	*(N=40)*	*(N=33)*	*(N=44)*
Demographic factors							
Age_{v1}	48.3±8.7 [b]	43.5±11.8 [b]	48.1±8.81 [b]	43.2±13.6 [b]	47.7±9.3	49.4±8.8	46.07±9.8
Race, % AA	37.4 [b]	66.7 [b]	41.2	54.6	32.5 [c]	33.3 [c]	56.8 [c]
% above poverty	61.6	55.6	63.5	54.6	60.0	63.6	59.1
RDW (v_1)							
CV (%)	13.8±1.3 [b]	16.5±3 [b]	14±1.5 [b]	16.1±3.2 [b]	12.8±.38 [c]	13.7±.22 [c]	16±2 [c]
Median	-13.7	17.1	13.7	14	12.8	13.7	15.2
IQR	13;14.3	13.7;18.3	13;14.5	13.6;19.7	12.6;13.1	13.5;13.9	14.4;17.5
dMRI measures	*(N=99)*	*(N=18)*	*(N=96)*	*(N=11)*	*(N=40)*	*(N=33)*	*(N=44)*
dMRI, Analysis A							
Mean FA	+0.3±0.02	+0.3±0.01	+0.3±0.02	+0.3±0.01	+0.3±0.01	+0.3±0.02	+0.3±0.01
Mean MD	2.519E-03±1.609E-04	2.495E-03±1.067E-04	2.517E-03±1.615E-04	2.482E-03±1.171E-04	2.534E-03±2.085E-04	2.513E-03±1.117E-04	2.499E-03±1.200E-04
dMRI, Analysis B							

Left Brain							
Frontal FA	+0.24±0.02	+0.24±0.01	+0.24±0.02	+0.24±0.01	+0.24±0.02	+0.23±0.02	+0.23±0.01
Frontal MD	2.357E-03±1.176E-04	2.353E-03±1.403E-04	2.352E-03±1.166E-04	2.347E-03±1.655E-04	2.363E-03±1.182E-04	2.358E-03±1.357E-04	2.348E-03±1.131E-04
Temporal FA	+0.24±0.02	+0.24±0.01	+0.24±0.02	+0.24±0.01	+0.24±0.02	+0.24±0.02	+0.24±0.01
Temporal MD	2.406E-03±1.528E-04	2.406E-03±9.290E-05	2.402E-03±1.508E-04	2.421E-03±9.880E-05	2.430E-03±2.062E-04	2.396E-03±9.390E-05	2.391E-03±1.034E-04
Parietal FA	+0.23±0.02	+0.23±0.01	+0.23±0.02	+0.23±0.01	+0.23±0.02	+0.23±0.02	+0.23±0.01
Parietal MD	2.604E-03±1.859E-04	2.581E-03±1.462E-04	2.598E-03±1.868E-04	2.601E-03±1.608E-04	2.617E-03±2.151E-04	2.583E-03±1.468E-04	2.599E-03±1.701E-04
Occipital FA	+0.2±0.01	+0.21±0.02	+0.2±0.02	+0.21±0.01	+0.21±0.02	+0.2±0.02	+0.21±0.01
Occipital MD	2.451E-03±1.586E-04	2.458E-03±8.890E-05	2.453E-03±1.590E-04	2.425E-03±7.940E-05	2.481E-03±1.930E-04	2.422E-03±1.075E-04	2.448E-03±1.288E-04
Right Brain							
Frontal FA	+0.24±0.02	+0.24±0.01	+0.24±0.01	+0.24±0.01	+0.24±0.01	+0.23±0.02	+0.24±0.01
Frontal MD	2.336E-03±1.263E-04	2.322E-03±1.130E-04	2.333E-03±1.254E-04	2.308E-03±1.283E-04	2.357E-03±1.505E-04	2.329E-03±1.050E-04	2.317E-03±1.091E-04
Temporal FA	+0.25±0.02	+0.25±0.01	+0.25±0.02	+0.25±0.01	+0.25±0.02	+0.25±0.02	+0.25±0.01
Temporal MD	2.325E-03±1.538E-04	2.318E-03±9.790E-05	2.319E-03±1.489E-04	2.328E-03±1.065E-04	2.341E-03±2.033E-04	2.324E-03±1.141E-04	2.309E-03±9.990E-05
Parietal FA	+0.23±0.02	+0.23±0.02	+0.23±0.02	+0.23±0.02	+0.23±0.01	+0.23±0.02	+0.23±0.02
Parietal MD	2.649E-03±1.976E-04	2.618E-03±1.379E-04	2.637E-03±1.931E-04	2.623E-03±1.529E-04	2.671E-03±2.195E-04	2.611E-03±1.524E-04	2.644E-03±1.854E-04
Occipital FA	+0.21±0.01	+0.21±0.01	+0.21±0.01	+0.21±0.01	+0.21±0.02	+0.2±0.01	+0.21±0.01
Occipital MD	2.526E-03±1.872E-04	2.526E-03±9.090E-05	2.522E-03±1.827E-04	2.538E-03±1.003E-04	2.556E-03±2.411E-04	2.498E-03±1.137E-04	2.519E-03±1.389E-04

Abbreviations: Age_{v1}=age measured at HANDLS visit 1 (2004-2009); CV=Coefficient of Variation; dMRI=Diffusion Magnetic Resonance Imaging; δRDW=Red Cell Distribution Width annualized change between visits 1 and 2; FA=Fractional Anisotropy; 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 (25^{th}-75^{th} percentile); MD=Mean Diffusivity; RDW=Red Cell Distribution Width; 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).

[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. MD is measured in mm^2/sec.

[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.

Table S3. RDW analyses (Cont'd): Hematological measures and other covariate-adjusted associations from analyses A (global FA/MD), B (regional FA/MD) vs. 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=214)	*β*	*(SE)*	*β*	*(SE)*	*β*	*(SE)*	*β*	*(SE)*
dMRI, Analysis A								
Mean FA	-0.0013072	(0.0010711)	-0.0011523	(0.0010678)	-0.001326	(0.001088)	-0.0010872	(0.0010995)
Mean MD	+1.27e-06	(9.57e-06)	+2.55e-06	(9.69e-06)	+4.00e-06	(9.58e-06)	+2.53e-06	(9.75e-06)
Males (N=97)								
dMRI, Analysis A								
Mean FA	-0.0061189	(0.0024086)[d,f]	-0.0068095	(0.0024779)[e,f]	-0.0064143	(0.0025614)[d,f]	-0.0053811	(0.0025583)[d,f]
Mean MD	+0.0000417	(0.0000211)[c,f]	+0.0000498	(0.0000217)[d,f]	+0.0000367	(0.0000222)	+0.0000389	(0.0000224)[c,f]
dMRI, Analysis B								
Left Brain								
Frontal FA	-0.0064265	(0.0022481)[e,f]	-0.007076	(0.0022551)[e,f]	-0.0066959	(0.0023443)[e,f]	-0.0059463	(0.0023719)[d,f]
Frontal MD	+0.000031	(0.0000207)[f]	+0.0000393	(0.0000215)[c,f]	+0.0000326	(0.0000225)[f]	+0.0000289	(0.0000222)[f]
Temporal FA	-0.0039687	(0.002306)[c,f]	-0.0043248	(0.0023592)[c,f]	-0.003822	(0.0024024)[f]	-0.0026346	(0.0023733)
Temporal MD	+0.0000186	(0.0000202)	+0.0000229	(0.0000204)	8.13e-06	(0.0000206)	9.98e-06	(0.0000209)
Parietal FA	-0.0069908	(0.0021851)[e,f]	-0.0078526	(0.002206)[e,f]	-0.0072938	(0.0023132)[e,f]	-0.0060713	(0.0022953)[d,f]
Parietal MD	+0.0000576	(0.0000258)[d]	+0.0000657	(0.0000265)[d]	+0.0000524	(0.0000269)[c]	+0.0000549	(0.0000269)[d]
Occipital FA	-0.0054071	(0.0019033)[e,f]	-0.0059088	(0.002073)[e,f]	-0.0050007	(0.0020933)[d,f]	-0.0045708	(0.0021247)[d,f]
Occipital MD	+0.0000602	(0.0000213)[e,f]	+0.0000642	(0.000022)[e,f]	+0.0000478	(0.0000212)[d]	+0.0000584	(0.0000226)[d,f]
Right Brain								
Frontal FA	-0.0059632	(0.0021011)[e,f]	-0.0068167	(0.0021735)[e,f]	-0.0067503	(0.0022576)[e,f]	-0.0054727	(0.0022344)[d,f]
Frontal MD	+0.000026	(0.0000181)[f]	+0.0000345	(0.0000191)[c,f]	0.0000314	(0.0000197)[f]	+0.0000249	(0.0000199)[f]
Temporal FA	-0.0051441	(0.0023779)[d,f]	-0.0057939	(0.0024318)[e,f]	-0.0051369	(0.0024906)[d,f]	-0.0047414	(0.0025071)[c,f]
Temporal MD	+0.0000305	(0.0000173)	+0.0000368	(0.0000174)[d]	+0.0000333	(0.0000185)[c]	+0.0000302	(0.000018)
Parietal FA	-0.0082193	(0.0024504)[e,f]	-0.0089263	(0.0025193)[e,f]	-0.0082897	(0.0026023)[e,f]	-0.0080125	(0.0026049)[e,f]

Parietal MD	+0.0000658	(0.0000282)[d]	+0.0000736	(0.0000294)[d]	+0.0000602	(0.0000298)[d]	+0.0000697	(0.0000296)d
Occipital FA	-0.0073574	(0.0022346)[e,f]	-0.0079766	(0.0023243)[e,f]	-0.0073366	(0.0023859)[e,f]	-0.0070161	(0.0024197)[e,f]
Occipital MD	+0.0000595	(0.0000221)[e]	+0.0000671	(0.000023)[e,f]	+0.0000524	(0.0000226)[d]	+0.000057	(0.0000236)[d,f]
Females (N=117)								
dMRI, Analysis A								
Mean FA	-0.0007666	(0.0012518)	-0.0001301	(0.0012146)	-0.0001499	(0.0012589)	-0.0004769	(0.0012667)
Mean MD	-7.83e-06	(0.0000115)	-9.25e-06	(0.0000114)	-5.93e-06	(0.0000115)	-4.92e-06	(0.0000113)

Abbreviations: Age_{v1}=age measured at HANDLS visit 1 (2004-2009); B-12=serum cobalamin; CV=Coefficient of Variation; dMRI=Diffusion Magnetic Resonance Imaging; ESR=Erythrocyte Sedimentation Rate; FA=Fractional Anisotropy; FDR=False Discovery Rate; 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; MD=Mean Diffusivity; RDW=Red Cell Distribution Width; SA=Sensitivity Analysis; 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 3 was adjusted for age, 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)]. MD is measured in mm^2/sec.

[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 disturbance factors (Waist circumference, cholesterol, Cholesterol:HDL ratio, Triglycerides, Creatinine); Model 6 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.

Table S4. δRDW analyses: Minimally and hematological measure adjusted associations from analyses A (global FA/MD), B (Regional cortical FA/MD, Left/Right) vs. 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=214)	*β*	*(SE)*	*b*	*P*	*q-value*	*β*	*(SE)*	*P*	*Interaction of* δRDW *by sex*
dMRI, Analysis A									
Mean FA	+0.0021	(0.016)	+0.088	0.18	__	+0.020	(0.016)	0.21	__
Mean MD	-0.000011	(0.0014)	+0.004	0.94	__	7.87e-06	(0.00014)	0.96	__
Males (N=97)									
dMRI, Analysis A									
Mean FA	+0.027	(0.036)	+0.08	0.45	__	+0.009	(0.038)	0.81	0.79
Mean MD	-0.00007	(0.00031)	-0.02	0.83	__	+0.0001	(0.0003)	0.75	0.67
Females (N=117)									
dMRI, Analysis A									
Mean FA	+0.021	(0.018)	+0.11	0.23	__	+0.023	(0.017)	0.19	__
Mean MD	+4.12e-06	(0.0002)	+0.002	0.97	__	-3.83E-06	(0.00016)	0.98	__
Non-Anemic (N=182)									
dMRI, Analysis A									
Mean FA	+0.018	(0.016)	+0.078	0.28	__	+0.012	(0.0017)	0.51	__
Mean MD	+0.00002	(0.0002)	+0.009	0.89	__	+0.0001	(0.0002)	0.68	__

Abbreviations: Age_{v1}=age measured at HANDLS visit 1 (2004-2009); CV=Coefficient of Variation; dMRI=Diffusion Magnetic Resonance Imaging; ESR=Erythrocyte Sedimentation Rate; FA=Fractional Anisotropy; FDR=False Discovery Rate; 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; MD=Mean Diffusivity; RDW=Red Cell Distribution Width; SA=Sensitivity Analysis; 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).

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}.

[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 Supplemental methods 3). MD is measured in mm^2/sec.

[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.