The Journal of Nutrition
Nutritional Epidemiology

Red Cell Distribution Width Is Directly Associated with Poor Cognitive Performance among Nonanemic, Middle-Aged, Urban Adults

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ABSTRACT

Background: Epidemiological evidence suggests that both anemia and elevated red cell distribution width (RDW) are associated with cognitive impairment. However, the interplay between these 2 predictors has been understudied.

Objectives: We examined sex- and anemia-specific associations between RDW and cognitive performance among urban adults in the United States.

Methods: Data from the Healthy Aging in Neighborhoods of Diversity Across the Life Span Study (Baltimore, MD; participants aged 30–65 y at baseline, ~59% African-American, 45% men) were used. Participants were selected based on the completion of 11 cognitive tasks at baseline (2004–2009) and follow-up (2009–2013) visits (mean time between visits: 4.64 ± 0.93 y) and availability of exposure and covariate data, yielding a sample of between 1526 and 1646 adults out of the initial 3720 adults recruited at baseline. Multiple linear mixed-effects regression models were conducted with RDW as the main exposure of interest and anemia/sex as the key effect modifiers.

Results: Overall, high RDWs were linked to poorer baseline performance on the California Verbal Learning Test (CVLT) List A (per 1 unit increase in RDW %, main effect: $\gamma_{01} = -0.369 \pm 0.114$; $P = 0.001$) and to slower rates of decline on the CVLT Delayed Free Recall (per 1 unit increase in RDW %, RDW × time: $\gamma_{11} = +0.036 \pm 0.013$; $P = 0.007$). Among nonanemic participants, RDWs were consistently associated with poorer baseline performance on the Trailmaking Test, Part A ($\gamma_{01} = +3.11 \pm 0.89$; $P < 0.001$) and on the CVLT List A ($\gamma_{01} = -0.560 \pm 0.158$; $P < 0.001$). Moreover, RDWs were associated with poorer baseline performance on the Brief Test of Attention in the total population ($\gamma_{01} = -0.123 \pm 0.039$; $P = 0.001$) and among men ($\gamma_{01} = -0.221 \pm 0.068$; $P = 0.001$). We did not detect an association between hemoglobin (Hb) and baseline cognitive performance or changes over time.

Conclusions: Elevated RDW had a consistent cross-sectional association with poor cognitive performance in the domains of verbal memory and attention among the nonanemic group in a sample of middle-aged, urban adults. Anemia and Hb concentrations were not associated with cognition. More longitudinal studies are needed to replicate our findings. *J Nutr* 2020;150:128–139.

Keywords: red cell distribution width, anemia, cognitive performance, urban adults; aging

Introduction

Population aging will lead to a rise in the prevalence of chronic health conditions, including diabetes, hypertension, and dementia (1). The prevalence of Alzheimer's disease (AD), the primary cause of dementia, is expected to reach 115.4 million worldwide by 2050 (2). Both AD and undiagnosed anemia are common conditions in nursing homes, with prevalences of around 45% (3). In fact, anemia affects 33% of the world's population, and about half the cases are due to iron deficiency (4). The epidemiological evidence thus far indicates that anemia, defined by the WHO as a low blood hemoglobin (Hb) concentration (<13 g/dL among men and <12 g/dL among women) (5), increases in prevalence with age and is an independent risk factor for mortality and reduced qualities of life, health, and physical functions (1). Moreover, anemia is featured among biomarkers with high diagnostic accuracy for AD (6) and has been associated with poor cognition, as well as the dementia incidence (7–17), with the WHO definition being the most commonly used measure for anemia (18). In fact, compared to the healthy population, AD patients were reported

Manuscript received March 28, 2019. Initial review completed May 8, 2019. Revision accepted July 15, 2019.
First published online September 5, 2019; doi: https://doi.org/10.1093/jn/nxz182.

to have significantly lower concentrations of Hb, mean cell Hb concentrations, and packed cell volumes. Moreover, anemia triggered by deficiency of micronutrients, including iron and vitamin B-12, may also be associated with cognitive impairment and dementia (19).

The link between anemia and cognitive decline was primarily attributed to reduced oxygen access by obligate aerobic cortical brain tissue (20). For instance, a lower oxygen-carrying capacity in the blood may trigger brain hypoperfusion, leading to oxidative stress and inflammation, which can contribute to neurodegeneration (21). Both anemia and elevated Hb, which have been implicated in cerebral hypoxia (12, 22), are patterned by age, such that older compared to younger individuals face increased risks (23). Although treatment may reduce the symptomology of anemia and elevated Hb, the extent to which treatment may affect cognitive abilities and the dementia risk remains unknown (22).

Additionally, elevated red cell distribution width (RDW), a novel marker of variations in red blood cell sizes (i.e., anisocytosis), was shown to independently and directly predict cardiovascular and other chronic disease morbidity and mortality (24–28). In fact, prior to anemia development, normal and abnormal RBC coexist (small in iron deficiency and large in folate deficiency) in the circulation. In early iron deficiency and during recovery, the full blood count shows the Hb as near the lower limit of normal, with a low mean cell volume (MCV) and a high RDW CV, expressed as a percentage. For folate deficiency, which is often brief and may not cause anemia, a similar pattern has been observed, though with higher MCV values. Folate deficiency is often brief and may not cause anemia. In contrast, an elevated RDW CV can persist for many months (29).

The association between RDW and cardiovascular disease was particularly strong among individuals who were free from anemia, indicating that RDW is capturing a pathway for mortality risk that is independent of low Hb concentrations (28). Similarly, it was shown that RDW was associated with prevalent dementia among the elderly, and specifically those who were anemia-free (30). To date, however, the observed association between RDW and anemia has not been replicated in a sample of middle-aged adults in the context of normal cognitive aging. Low Hb concentrations may predict incident cognitive impairments in older populations, and this association may be stronger among men than women, lending support to the differential effect of iron status on brain functions by sex (31). It is worth noting that both RDW and mean corpuscular Hb have been correlated with iron deficiency anemia, as well as with cognitive outcomes, indicating a possible deficit in heme synthesis or iron metabolism as an underlying trait of cognitive aging (32). Despite a lack of trials to determine a causal pathway involving iron deficiency anemia, iron metabolism is disrupted in cortical neurons and the beta-amyloid protein precursor has shown ferroxidase activity in mouse models (33), while a correlation between iron biochemistry and amyloid beta has also been described (34).

To expand on the existing literature, we conducted a longitudinal, epidemiologic study of RDW in relation to cognitive performance and changes over time in a bi-racial cohort of urban, community-dwelling, middle-aged adults, while further examining heterogeneity in these associations by sex and anemia status. We further examined the non-linear relationships between Hb and cognitive performance and changes over time, in models stratified by sex.

Methods

Database

The Healthy Aging in Neighborhoods of Diversity Across the Life Span (HANDLS) Study is a prospective cohort study aimed at examining disparities in cardiovascular disease and cognitive aging. HANDLS recruited an ethnically and socioeconomically diverse sample of African-American (AA) and white urban, middle-aged adults (baseline age, 2004–2009, 30–64 y) and included an extensive cognitive battery standardized across time points [follow-up time between baseline and first follow-up (2009–2013) visits: 4.64 ± 0.93 y], in addition to clinical biomarkers spanning multiple physiological systems. The primary sampling units were 13 Baltimore, MD, neighborhoods, using an area probability sampling strategy (35). The study protocol was approved by the National Institute on Environmental Health Sciences' Institutional Review Board of the NIH.

Study subjects

The original HANDLS cohort included 3720 participants (30–65 y, AAs and whites, Phase I, Visit 1). During Phase II of Visit 1 [also known as the medical research vehicle (MRV) baseline visit], in-depth examinations were conducted, including a fasting blood draw, an in-depth physical examination, a cognitive assessment, and two 24-hr dietary recalls completed 3–10 d apart between Phase I and Phase 2. The average of the 2 dietary recalls was used to assess dietary intakes. A sample from the fasting blood draw was used to assess several measures, including RDW. Since HANDLS is a closed cohort, some participants who did not show up at Visit 2 were potentially examined again at the third visit. Our analysis of the total sample of 3720 between Visits 1, 2, and 3 indicated the following: 124 participants died between Visits 1 and 2 (aka Wave 3); 1321 were not examined at Visit 2 (aka Wave 3), while 2275 were examined. At Visit 3 (not included in this study, aka Wave 4), 2147 individuals were in common with Visit 2: 1861 were seen at the MRV, while 286 were not seen.

In this study, we included participants of all ages who had complete and valid cognitive test scores at Visits 1 and/or 2. Thus, when excluding invalid cognitive test scores (mainly due to literacy-related or physical/sensory limitations), samples sizes were 2719 for Visit 1 and 2207 for Visit 2 (see **Supplemental Figure 1**, Samples 2a and 2b). Baseline RDW data were available for 2744 of all HANDLS participants. This sample was reduced to 2581 for individuals with Visit 1 cognitive data and 1934 for those with Visit 2 cognitive data. We further excluded participants with missing covariate data: dietary intake, depressive symptom scores, self-reported chronic conditions, use of non-steroidal anti-inflammatory drugs (NSAIDs), BMI, and the Wide Range Achievement Test (WRAT) for literacy. As detailed in Supplemental Figure 1 (Sample 5a–5k), samples varied by cognitive

This study was entirely supported by the National Institute on Aging, Intramural Research Program.
Author disclosures: MAB, SH, HAB, DS, JW, MKE, and ABZ, no conflicts of interest.
The views expressed in this article are those of the authors and do not reflect the official policy of the Department of the Army/Navy/Air Force, Department of Defense, or the US Government.
Supplemental Figure 1, Supplemental Methods 1, and Supplemental Table 1 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/jn/.
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Abbreviations used: AA, African-American; AD, Alzheimer's disease; AF, Animal Fluency Test; BTA, Brief Test of Attention; BVRT, Benton Visual Retention Test; CDT, Clock Drawing Test; CVLT, California Verbal Learning Test; DFR, Delayed Free Recall; ESR, erythrocyte sedimentation rate; HANDLS, Healthy Aging in Neighborhoods of Diversity Across the Life Span; Hb, hemoglobin; HEI-2010, Healthy Eating Index, 2010 version; HS, high school; hsCRP, high-sensitivity C-reactive protein; MCI, mild cognitive impairment; MCV, mean cell volume; MMSE, Mini-Mental State Examination; MRV, medical research vehicle; NSAID, nonsteroidal anti-inflammatory drug; RDW, red cell distribution width; WRAT, Wide Range Achievement Test.

test, with 1666 at Visit 1 and 1,219 at Visit 2. Compared to those excluded from the initial HANDLS sample, the final analytic sample (i.e., $n = 2885$ observations) was older and more likely to have more than a high school (HS) level of education. This sample selectivity was accounted for in our primary analysis using a 2-stage Heckman selection procedure (see Statistical analysis section).

Cognitive assessment

There were 7 cognitive tests, which yielded 11 test scores [the Mini-Mental State Examination (MMSE), California Verbal Learning Test (CVLT) Immediate (List A) and Delayed Free Recall (DFR), Digit Span Forward and Backwards Tests, Benton Visual Retention Test (BVRT), Animal Fluency Test (AF), Brief Test of Attention [BTA], Trailmaking test A and B, and the Clock Drawing Test (CDT])] representing 7 distinctive cognitive domains (global, attention, learning/memory, executive function, visuo-spatial/visuo-construction ability, psychomotor speed, and language/verbal). Most cognitive test scores were in the direction of a higher score indicating better performance, with the exception of BVRT, reflecting the number of errors, and the 2 parts of the Trailmaking test, A and B (number of seconds to completion). All participants were able to complete informed consent after being probed for understanding the protocol. All participants were screened using the MMSE as a global mental status test in lieu of a formal dementia diagnosis. Participants who scored <24 points on the MMSE (~6.6% at Visit 1 and 1.9% at Visit 2) were believed to have poor literacy rather than exhibit early signs of dementia. because earlier findings in HANDLS had emphasized the importance of considering reading levels when interpreting performance on cognitive tasks (36, 37). Low scores on the MMSE are not always due to poor literacy. However, it was apparent in our study that participants with poor scores were cognitively intact and functional community-dwelling adults, based on in-depth interviews with trained psychometricians.

Cognitive tests are described in detail in **Supplemental Methods 1**.

Red cell distribution width, anemia, and other iron status measures

All laboratory tests selected for this study were done at Quest Diagnostics, Chantilly, VA, following a fasting blood draw, after which participants were offered breakfast.

Red cell distribution width.

RDW was measured by an automated Coulter DXH 800 hematology analyzer as part of the peripheral complete blood count (Beckman Coulter) and was expressed as CV (%) of red blood cell volume distribution. Regular calibration was performed every 3 mo on the hematology analyzer and quality control was performed according to the manufacturer's recommendations (38). There are usually 2 RDW measurements used for clinical purposes: namely, the RDW CV (unit: %), which we used in this study, and the RDW SD (unit: fL) from which RDW CV is derived. In fact, RDW CV = RDW SD × 100/MCV. The normal range for RDW CV is 11.0–15.0%. Thus, the RDW CV depends on both the width of the distribution (normal range: 40–55 fL) curve and the MCV (39).

Hemoglobin concentrations and anemia.

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 was refrigerated up to 6 d (Quest Diagnostics). We followed the WHO's definition of anemia as Hb <13 g/dl for men and <12 g/dl for women (5). This was the main anemia definition used in this study, as it was the most common definition in most previous studies. Based on CDC recommendations (40), cutoffs for Hb as suggested by the WHO may need to be modified according to ethnic ancestry and smoking status, whereby African-Americans are anemic for a 1 g/dL cutoff less than their white counterparts, while current smokers are considered anemic for an additional +0.30 g/dL to the cutoff, compared to their non-smoker counterparts. Nevertheless, most previous studies have used the WHO definition, which only takes gender into account. Moreover, Hb was further categorized into tertiles to represent low, medium, and high concentrations. This categorization was used to assess whether low and high, compared to medium, concentrations of Hb were risk factors for cognitive impairment and/or declines over time. Given the inverse moderate correlation between Hb and RDW and between Hb and erythrocyte sedimentation rate (ESR; Pearson's $r = -0.46$ to -0.47), and the direct association between Hb, MCV, ferritin, and serum iron (Pearson's r range: 0.20–0.39), we included all these measures as potential confounders when examining 1 or more as key exposures, with cognitive performance/change as the main outcome of interest.

Other iron status markers.

Ferritin. Ferritin is decreased in iron deficiency anemia and increased in iron overload. It is measured with an immunoassay, with reference ranges of 20–380 ng/mL among men and 10–232 ng/mL among women (41).

Erythrocyte sedimentation rate. Using 5 mL of refrigerated whole blood stored in lavender-top EDTA tubes, the ESR was tested within 24 h of the blood draw. This test used automated modified Westergren photochemical capillary stopped flow kinetic analysis (38, 42). The Mayo clinic reports a reference of 0–22 mm/hr for men and 0–29 mm/hr for women (43) and is considered a proxy measure for serum fibrinogen (44).

Serum iron. We collected 0.5–1 mL of fasting serum, which was transported at room temperature (with heparin added) and refrigerated or frozen subsequently. Serum iron was measured with spectrophotometry (45, 46), with reference ranges for men aged ≥30 y set at 50–180 μg/dL; for women 20–49 y set at 40–190 μg/dL; and for women 50+ y set at 45–160 μg/dL (46).

Mean cell volume. 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 of 1 mL whole blood in an EDTA (lavender-top) tube was transported at room temperature to the laboratory facility (38).

Covariates

Covariates were selected for their well-established associations with cognitive declines (47–49). Sociodemographic characteristics included ageFootnotes/callouts in Tables 2 and 3 were reordered per style. Ask author to check. (measured at baseline), sex, race (white vs. AA), marital status (married vs. not), educational attainment (<HS; HS; >HS), poverty status (<125% of federal poverty line for "below poverty"), employment status (employed vs. not), and WRAT score (50), a test of verbal knowledge used as a proxy for literacy and educational quality. Participants were asked to pronounce a list of 50 words that increased in difficulty. If the criterion of the first 5 words being correctly pronounced was not reached, a letter reading was administered. The tan form was administered according to standard instructions and the score was the total number of words correctly pronounced. We also included several lifestyle and health-related characteristics: BMI (kg/m^2); dietary quality; baseline chronic conditions; NSAIDs use over the past 2 weeks (as prescription and over-the-counter); opiate, marijuana, or cocaine use (current vs. never or former); and smoking status (current vs. never or former). The Center for Epidemiological Studies-Depression (CES-D) (51) is a 20-item measure of depressive symptoms. Participants self-reported the frequency and severity of symptoms over the prior week. Scores range from 0 to 60; scores of 16 and higher indicate significant depressive symptomology, and scores of 20 and higher indicate significant clinically depressive symptomology. Our analyses included the total CES-D score as a key covariate. Dietary quality was operationalized using the total score on the Healthy Eating Index (HEI-2010), obtained by taking the mean of two 24-h recalls that were administered at the baseline visit (Visit 1). The computational procedures for the HEI-2010 total score and each of its 12 components have been outline previously (52, 53). Baseline, chronic conditions included a self-reported history of type 2 diabetes, hypertension, dyslipidemia, cardiovascular disease, and inflammatory

disease. A sensitivity analysis was also conducted, whereby serum folate, vitamin B-12, and high-sensitivity C-reactive protein were added to the model [see previous studies for measurement details (54–57)]. Given their lower correlations with the main exposures of interest (RDW and Hb; $r < 0.20$ in absolute value), they were excluded from the main analysis. In a second sensitivity, folate, vitamin B-12, and C-reactive protein were retained in the model, while all serum iron covariates except for Hb were eliminated. In a third sensitivity analysis, we redefined anemia status, while simultaneously taking into account sex, race, and smoking status (40), and examined the association of RDW on cognitive performance and changes by anemia status with this new definition, using only the covariates entered into the main analysis.

Statistical methods

Stata release 15.0 was used for all analyses (58). First, population means and proportions were estimated with appropriate sampling weights, whereby sex and anemia status differences were tested using a design-based F-test and Wald test (svy: tab and svy: reg). Second, we compared cognitive performance by sex within each wave, and subsequently compared the wave-specific performance on each test, using a design-based Wald test and accounting for sampling weights. Third, we estimated a series of mixed-effects regression models in our primary analysis for each of the 11 continuous cognitive test score outcomes and the main exposure variable of RDW, stratifying by sex and by anemia status separately. Each model included years elapsed between visits (time), exposures/covariate main effects, and 2-way interaction terms between time and exposures/covariates. We assumed the unavailability of outcomes to be missing at random (59). Effect modifications by sex and anemia statuses were also tested for an effect of RDW on baseline cognitive performance (2-way interaction terms) and on cognitive changes over time (3-way interaction terms). These models were adjusted for covariates (listed under Covariates section) that included socio-demographics, lifestyle factors, and health-related factors, in addition to other iron status measures, including Hb, ferritin, MCV, ESR, and serum iron. We included age, sex, race, marital status, employment status, poverty status, education, WRAT score, BMI, HEI-2010 score, smoking, drug use, several chronic conditions, depressive symptoms, and NSAID medications, along with a number of iron status measures. Fourth, in similar linear, mixed-effects regression models adjusted for the same covariates (plus RDW) that were stratified by sex, Hb, expressed as tertiles, was the main exposure of interest. Taking the middle Hb tertile as the referent, low and high concentrations were tested against baseline cognitive performance and cognitive changes over time, adjusting for key confounders. Heterogeneity in these relationships were tested using 2-way and 3-way interaction terms with sex. All continuous predictors in the models were mean-centered.

To aid in the interpretation of our findings, we plotted our results over time (in years) and stratified by RDW standardized z-score levels (-1 = mean $-$ 1 SD; 0 = mean; $+1$ = mean + 1 SD). Models were presented for the overall eligible sample and stratified by sex or anemia status, while adding in separate models, 2- and 3-way interaction terms between exposure, the effect modifier, and time.

Selection bias, triggered by systematic differences in key characteristics between selected and excluded samples of the target population, can occur. To account for this bias, we conducted a 2-stage Heckman selection process, applied to the main mixed-effects regression models. At the first stage, a binary selection variable (selected = 1 vs. unselected = 0) was predicted with a probit model by age, sex, race, and poverty status, along with educational attainment. The conditional predicted probability of selection was then utilized to calculate an inverse Mills ratio, which, at the second stage, was entered into the main causal models as a covariate (60).

We considered 0.05 and 0.10 as Type I errors for main effects and interaction terms, respectively (61), prior to the multiple testing adjustment. Adjustment was done using a family-wise Bonferroni approach, while accounting for cognitive test multiplicity and assuming that each exposure was a distinctive substantive hypothesis (62). Thus, significance levels for main effects were $P < 0.0045$ (0.05/11), with P values of 0.10/11 = 0.0090 for 2-way interaction terms and P values of 0.05 for 3-way interaction terms. This approach was similar to other published works (63).

Results

Table 1 displays baseline characteristics of the analytic sample, overall and stratified by sex and anemia status. Men had higher incomes and were more likely to be smokers and current drug users, compared to women. Women had higher BMIs than men. Depressive symptom severities and inflammatory conditions were both more elevated among women. The hypertension prevalence was found to be higher in women (vs. men). Both means of ESR and RDW were higher in women, while the reverse was true for the remaining iron status measures, which were more elevated in men. Based on the WHO definition (18), the anemia prevalence was estimated at 15% (main definition), while the CDC's more restrictive definition yielded a prevalence of 7% (data not shown) (40). In fact, ESR and RDW were also higher among anemic participants (vs. the nonanemic group), while the anemic group was characterized by lower serum concentrations of ferritin and iron and a reduced MCV (vs. the nonanemic group). In addition, compared to the nonanemic group, anemic individuals were more likely to be African-American (87.1% vs. 62.0%) and unmarried (75.5% vs. 63.5%), but less likely to have >HS education (30.1% vs. 44.1%). Their mean WRAT-3 score was lower (41.4 vs. 43.4), they were less likely to be currently employed (45.8% vs. 56.2%), they had a higher mean BMI (31.2 vs. 29.2), and they had a higher prevalence of hypertension (48.0% vs. 35.0%). A similar pattern of association was found between baseline characteristics and Hb tertiles.

Supplemental Table 1 reports sex and anemia status differentials in cognitive performance within each data time point, in addition to variations across time points. Overall, declines in cognitive performance were observed for the CVLT List A, CVLT DFR, and BVRT. Sex differences were observed in cross-sectional cognitive performance at both visits, specifically in the case of the CVLT List A (Visit 1) and DFR (Visit 1), reflecting better performance among women. The reverse was true for BVRT (Visits 1 and 2), AF (Visits 1 and 2), and the CDT (Visit 1). Anemia was linked with poorer performance on several tests reflecting visual memory, attention, verbal fluency, and psychomotor speed at 1 or 2 visits.

A series of mixed-effects linear regression models adjusting for key confounders and multiple testing were conducted to test cross-sectional and longitudinal associations between the baseline RDW and cognitive performance (Table 2). In the total selected sample, RDW was found to be related to poorer baseline performance on the CVLT List A (RDW main effect: $\gamma_{01} = -0.369 \pm 0.114$; $P = 0.001$), an association that was significant only among the nonanemic group (RDW main effect: $\gamma_{01} = -0.560 \pm 0.091$; $P < 0.001$). Similarly, among nonanemic participants, RDW was consistently associated with poorer baseline performance on the Trailmaking test, Part A (RDW main effect: $\gamma_{01} = +3.11 \pm 0.89$; $P < 0.001$) and on the CVLT List A (RDW main effect: $\gamma_{01} = -0.560 \pm 0.158$; $P < 0.001$). Those 2 latter findings are illustrated in Figures 1 and 2, contrasting them with the patterns and trajectories found in the anemic group. Moreover, RDW was associated with poorer baseline performance on the BTA in the total population (RDW main effect: $\gamma_{01} = -0.123 \pm 0.039$; $P = 0.001$) and among men (RDW main effect: $\gamma_{01} = -0.221 \pm 0.068$; $P = 0.001$). Nevertheless, RDW was also linked to slower declines over

TABLE 1 Selected baseline (Visit 1) study participant characteristics by sex and anemia status[1]

	All ($n = 1666$)	Women (52.0% ± 2.1%) ($n = 907$)	Men (48.0% ± 2.1%) ($n = 759$)	Nonanemic (84.4% ± 1.5%) ($n = 1400$)	Anemic (15.6% ± 1.5%) ($n = 266$)	P_{sex}[2] 0.041[3]	P_{anemia}[2] 0.041[3]
Age at baseline, y	46.9 ± 0.38	46.9 ± 0.53	46.9 ± 0.55	46.8 ± 0.42	47.6 ± 0.87	1.00	0.39
African-American, %	65.9 ± 1.80 ($n = 1597$)	66.1 ± 2.50 ($n = 870$)	65.8 ± 2.60 ($n = 727$)	62.0 ± 2.10 ($n = 1361$)	87.1 ± 2.60 ($n = 235$)	0.92	<0.001
Married, %	35.6 ± 2.10	32.9 ± 2.80	38.6 ± 3.00	37.5 ± 2.30	24.5 ± 4.10	0.17	0.01
Education, %							
<HS	4.2 ± 0.60	4.3 ± 0.90	4.1 ± 0.80	4.2 ± 0.70	4.1 ± 1.60	0.88	0.03
HS	53.8 ± 2.10	54.0 ± 3.00	53.7 ± 3.00	51.6 ± 2.30	65.8 ± 4.90	—	—
>HS	41.9 ± 2.10	41.6 ± 3.00	42.1 ± 3.00	44.1 ± 2.30	30.1 ± 4.80	—	—
Missing	0.1 ± 0.10 ($n = 1656$)	0.1 ± 0.10 ($n = 902$)	0.0 ± 0.00 ($n = 754$)	0.1 ± 0.10 ($n = 1392$)	0.0 ± 0.00 ($n = 264$)	—	—
Literacy (WRAT score)	43.1 ± 0.30	43.3 ± 0.40	42.9 ± 0.50	43.4 ± 0.30	41.4 ± 0.80	0.45	0.02
PIR <125%, %	19.3 ± 1.20	22.2 ± 2.00	16.1 ± 1.40	18.3 ± 1.30	24.3 ± 3.30	0.01	0.08
Employment, %							
Yes	54.6 ± 2.10	51.0 ± 3.00	58.6 ± 2.90	56.2 ± 2.30	45.8 ± 5.20	0.15	0.01
Missing	14.6 ± 1.30	15.2 ± 2.00	14.0 ± 1.70	15.3 ± 1.50	10.8 ± 3.10	—	—
Current smoking status, %							
Currently smoking	43.2 ± 2.10	37.9 ± 3.00	48.8 ± 3.00	43.9 ± 2.30	39.3 ± 5.10	0.15	0.19
Missing	1.7 ± 0.80	2.0 ± 1.30	1.3 ± 1.10	1.9 ± 1.00	0.3 ± 0.20	—	—
Current use of illicit drugs, %							
Used any type	51.3 ± 2.10	38.6 ± 3.00	65.0 ± 2.70	52.3 ± 2.30	45.8 ± 5.30	<0.001	0.43
Missing	1.7 ± 0.50	2.6 ± 0.10	0.7 ± 0.30	1.6 ± 0.60	2.1 ± 1.10	—	—
BMI, kg/m^2	29.5 ± 0.40	31.0 ± 0.60	27.8 ± 0.40	29.2 ± 0.40	31.2 ± 1.00	<0.001	0.04
HEI-2010 total score	44.2 ± 0.45	44.7 ± 0.67	43.7 ± 0.60	44.5 ± 0.50	42.5 ± 0.94	0.28	0.05
Depressive symptoms	($n = 1665$)	($n = 907$)	($n = 759$)	($n = 1400$)	($n = 266$)	—	—
CES-D score	13.9 ± 0.44	14.9 ± 0.68	12.8 ± 0.56	13.7 ± 0.48	15.0 ± 1.19	0.01	0.30
Diabetes, %	11.8 ± 1.20 ($n = 1665$)	12.3 ± 1.70	11.3 ± 1.80	11.4 ± 1.30	14.0 ± 3.10	0.68	0.42
Hypertension, %	37.1 ± 2.00	41.7 ± 3.00	32.1 ± 2.70	35.0 ± 2.20	48.0 ± 5.20	0.02	0.02
Dyslipidemia, %	23.3 ± 1.70	24.6 ± 2.40	22.0 ± 2.30	23.9 ± 1.90	20.5 ± 3.70	0.45	0.43
Cardiovascular disease,[4] %	11.0 ± 1.10	12.6 ± 1.60	9.4 ± 1.50	10.4 ± 1.20 ($n = 1399$)	14.5 ± 3.30	0.16	0.19
Inflammatory conditions,[5] %	12.9 ± 1.40	16.2 ± 2.20	9.3 ± 1.50	12.3 ± 1.50	16.1 ± 3.40	0.01	0.27
NSAIDs,[6] %	20.0 ± 1.70	20.2 ± 2.40	19.8 ± 2.30	19.3 ± 1.80	23.8 ± 4.90	0.89	0.36
RDW, %	14.0 ± 0.06	14.2 ± 0.08	13.8 ± 0.08	13.8 ± 0.05	15.3 ± 0.22	0.003	<0.001
Serum Hb, g/dL	13.6 ± 0.06	12.9 ± 0.06	14.5 ± 0.07	14.0 ± 0.06	11.5 ± 0.07	<0.001	<0.001
T1: 4.5–13 g/dL	35.6 ± 2.20	56.7 ± 2.90	12.7 ± 2.10	23.6 ± 2.20	100.0 ± 0.00	<0.001	<0.001
T2: 13.1–14.3 g/dL	32.4 ± 2.00	34.4 ± 2.70	30.2 ± 2.90	38.4 ± 2.20	0.0 ± 0.00	—	—
T3: 14.4–17.9 g/dL	32.0 ± 1.90	8.9 ± 1.40	57.1 ± 3.00	38.0 ± 2.20	0.0 ± 0.00	—	—
Serum iron, μg/dL	82.9 ± 1.38	76.6 ± 1.86	89.7 ± 2.04	87.1 ± 1.45 ($n = 1399$)	60.1 ± 3.37	<0.001	<0.001
Erythrocyte sedimentation rate, mm/h	17.3 ± 0.93	21.1 ± 0.96	13.3 ± 1.61	15.3 ± 1.04	28.2 ± 2.13	<0.001	<0.001
Mean cell volume, fL	89.6 ± 0.30	89.0 ± 0.40	90.2 ± 0.04	90.4 ± 0.30	85.5 ± 0.80	0.03	<0.001
Serum ferritin, ng/mL	119 ± 6.17	64.2 ± 3.76	178 ± 10.60	125 ± 7.00	87.5 ± 10.1	<0.001	0.002

[1] Data are for HANDLS participants with complete and reliable cognitive data, RDW, and covariate data. Values are shown as weighted mean ± SEM or % ± SE of the %. Largest sample size is $n = 1666$. CES-D, Center for Epidemiologic Studies-Depression; HANDLS, Healthy Aging in Neighborhoods of Diversity Across the Life Span; Hb, hemoglobin; HEI-2010, Healthy Eating Index, 2010 version; HS, high school; NSAID, nonsteroidal anti-inflammatory drug; PIR, poverty-income ratio; RDW, red cell distribution width; T, tertile; WRAT, Wide Range Achievement Test.

[2] *P* value was based on linear regression models when row variable is continuous and with sex and anemia status and design-based F-test when row variable is categorical.

[3] *P* value associated with design-based F-test for independence of anemia status and sex.

[4] Cardiovascular disease included a self-reported stroke, congestive heart failure, non-fatal myocardial infarction, or atrial fibrillation.

[5] Inflammatory conditions included multiple sclerosis, systemic lupus, gout, rheumatoid arthritis, psoraiasis, thyroid disorder, and Crohn's disease.

[6] NSAIDs include over-the-counter and prescription drugs in that category.

time on CVLT DFR in the total population (RDW × time effect: $\gamma_{11} = +0.036 \pm 0.013$; $P = 0.007$), though without showing any association within gender or anemia status strata. In our first sensitivity analysis, which further adjusted for serum folate, serum vitamin B-12, and serum high-sensitivity C-reactive protein (hsCRP), our key results were not markedly altered, despite the association of serum folate with both baseline CVLT List A and its change over time. In a second sensitivity analysis—in which serum folate, serum vitamin B-12, and serum hsCRP were retained in the model while all serum iron covariates were eliminated, with the exception of Hb—the results also remained unaltered, as was the case in the final sensitivity analysis, whereby we replaced the WHO definition of anemia with the CDC definition that incorporated race and

TABLE 2 Cognitive performance test scores by red cell distribution width, stratified by sex and anemia status[1]

	All	Men	Women	Nonanemic	Anemic
Mini-Mental State Exam, total score	$n = 1633$; k = 1.6	$n = 738$; k = 1.6	$n = 895$; k = 1.7	$n = 1371$; k = 1.6	$n = 262$; k = 1.6
Intercept	+26.6 ± 0.20[2]	+25.6 ± 0.33[2]	+26.1 ± 0.97[2]	+26.7 ± 0.22[2]	+25.9 ± 0.70[2]
Time	+0.13 ± 0.06[2]	+0.2 ± 0.09	+0.04 ± 0.07	+0.1 ± 0.06	+0.10 ± 0.20
RDW	−0.049 ± 0.03	+0.003 ± 0.06	−0.04 ± 0.04	−0.08 ± 0.04	+0.000 ± 0.05
RDW × Time	+0.003 ± 0.01	−0.005 ± 0.02	−0.003 ± 0.01	+0.01 ± 0.01	−0.02 ± 0.01
CVLT, List A	$n = 1559$; k = 1.6	$n = 695$; k = 1.5	$n = 864$; k = 1.6	$n = 1309$; k = 1.6	$n = 250$; k = 1.5
Intercept	+24.7 ± 0.71[2]	+21.3 ± 1.06[2]	+26.1 ± 0.97[2]	+24.9 ± 0.77[2]	+22.7 ± 2.37[2]
Time	−1.41 ± 0.18[2]	−1.6 ± 0.27[2]	−1.60 ± 0.23[2]	−1.50 ± 0.19[2]	+0.20 ± 0.57
RDW	−0.36 ± 0.11[2,3]	−0.2 ± 0.21	−0.40 ± 0.15[2]	−0.60 ± 0.16[2,3,4]	+0.07 ± 0.19
RDW × Time	+0.04 ± 0.03	+0.04 ± 0.05	+0.03 ± 0.04	+0.02 ± 0.04	+0.1 ± 0.05[2]
CVLT, Delayed Free Recall	$n = 1526$; k = 1.5	$n = 675$; k = 1.5	$n = 851$; k = 1.6	$n = 1282$; k = 1.5	$n = 244$; k = 1.5
Intercept	+7.8 ± 0.34[2]	+6.5 ± 0.50[2]	+8.3 ± 0.46[2]	+7.8 ± 0.37[2]	+7.3 ± 1.15[2]
Time	−0.45 ± 0.08[2]	−0.5 ± 0.14[2]	−0.50 ± 0.11[2]	−0.50 ± 0.09[2]	−0.1 ± 0.29
RDW	−0.14 ± 0.06[2]	−0.07 ± 0.09	−0.1 ± 0.07	−0.2 ± 0.08[2]	−0.05 ± 0.09
RDW × Time	+0.04 ± 0.01[2]	+0.03 ± 0.03	+0.02 ± 0.02	+0.04 ± 0.02	+0.05 ± 0.02
BVRT	$n = 1639$; k = 1.7	$n = 744$; k = 1.6	$n = 895$; k = 1.7	$n = 1376$; k = 1.7	$n = 263$; k = 1.6
Intercept	+9.5 ± 0.54[2]	+8.9 ± 0.79[2]	+9.2 ± 0.73[2]	+9.8 ± 0.58[2]	+6.5 ± 1.94[2]
Time	+0.3 ± 0.13[2]	+0.5 ± 0.20[2]	+0.3 ± 0.18	+0.3 ± 0.14	+0.9 ± 0.46[2]
RDW	+0.1 ± 0.09	+0.1 ± 0.15	+0.1 ± 0.11	+0.1 ± 0.12	+0.04 ± 0.15
RDW × Time	+0.003 ± 0.02	+0.001 ± 0.04	−0.000 ± 0.03	−0.02 ± 0.03	+0.06 ± 0.04
Brief Test of Attention	$n = 1580$; k = 1.6	$n = 713$; k = 1.5	$n = 867$; k = 1.6	$n = 1328$; k = 1.6	$n = 252$; k = 1.5
Intercept	+6.5 ± 0.25[2]	+5.9 ± 0.37[2]	+6.7 ± 0.34[2]	+6.6 ± 0.27[2]	+6.3 ± 0.86[2]
Time	−0.09 ± 0.07	−0.06 ± 0.11	−0.20 ± 0.08	−0.10 ± 0.07	+0.09 ± 0.25
RDW	−0.12 ± 0.04[2,3]	−0.20 ± 0.07[2,3]	−0.08 ± 0.05	−0.10 ± 0.05[2]	−0.10 ± 0.07
RDW × Time	+0.004 ± 0.01	+0.02 ± 0.02	−0.007 ± 0.01	+0.01 ± 0.01	−0.02 ± 0.02
Animal fluency	$n = 1646$; k = 1.7	$n = 747$; k = 1.7	$n = 899$; k = 1.7	$n = 1382$; k = 1.7	$n = 264$; k = 1.6
Intercept	+17.7 ± 0.58[2]	17.5 ± 0.90[2]	+19.2 ± 0.75[2]	+18.0 ± 0.63[2]	+16.5 ± 1.81[2]
Time	−0.09 ± 0.13	−0.30 ± 0.17	−0.10 ± 0.16	−0.20 ± 0.14	+0.30 ± 0.39
RDW	−0.18 ± 0.09[2]	−0.30 ± 0.17	−0.05 ± 0.11	−0.10 ± 0.13	−0.09 ± 0.14
RDW × Time	+0.04 ± 0.02[2]	+0.03 ± 0.04	+0.03 ± 0.02	+0.04 ± 0.03	+0.03 ± 0.03
Digits Span, Forward	$n = 1638$; k = 1.6	$n = 741$; k = 1.6	$n = 897$; k = 1.7	$n = 1375$; k = 1.7	$n = 263$; k = 1.6
Intercept	+7.1 ± 0.23[2]	+7.2 ± 0.35[2]	+6.9 ± 0.31[2]	+7.2 ± 0.25[2]	+6.6 ± 0.81[2]
Time	−0.009 ± 0.05	−0.006 ± 0.08	−0.03 ± 0.07	−0.03 ± 0.05	+0.1 ± 0.18
RDW	−0.02 ± 0.04	−0.007 ± 0.07	−0.02 ± 0.05	−0.006 ± 0.05	+0.008 ± 0.06
RDW × Time	+0.007 ± 0.01	+0.001 ± 0.02	+0.008 ± 0.01	+0.01 ± 0.01	−0.02 ± 0.015
Digits Span, Backward	$n = 1642$; k = 1.6	$n = 744$; k = 1.6	$n = 898$; k = 1.7	$n = 1380$; k = 1.6	$n = 262$; k = 1.6
Intercept	+7.1 ± 0.23[2]	5.3 ± 0.33[2]	+6.0 ± 0.29[2]	+5.8 ± 0.24[2]	+5.2 ± 0.71[2]
Time	−0.009 ± 0.05	−0.08 ± 0.08	−0.2 ± 0.07[2]	−0.1 ± 0.06[2]	−0.04 ± 0.19
RDW	−0.17 ± 0.04	+0.04 ± 0.06	+0.02 ± 0.04	+0.02 ± 0.01	+0.06 ± 0.06
RDW × Time	+0.007 ± 0.01	+0.01 ± 0.01	−0.009 ± 0.01	−0.005 ± 0.01	+0.009 ± 0.02
Clock, Command	$n = 1642$; k = 1.7	$n = 742$; k = 1.6	$n = 900$; k = 1.7	$n = 1379$; k = 1.7	$n = 263$; k = 1.6
Intercept	+8.7 ± 0.14[2]	+8.6 ± 0.21[2]	+8.9 ± 0.19[2]	+8.7 ± 0.14[2]	+8.3 ± 0.48[2]
Time	−0.05 ± 0.04	+0.001 ± 0.06	−0.1 ± 0.05[2]	−0.05 ± 0.042	−0.01 ± 0.16
RDW	−0.04 ± 0.02	−0.04 ± 0.04	−0.02 ± 0.03	−0.04 ± 0.030	−0.04 ± 0.04
RDW × Time	+0.005 ± 0.006	+0.005 ± 0.01	+0.002 ± 0.008	+0.001 ± 0.008	+0.02 ± 0.01
Trailmaking Test, Part A	$n = 1612$; k = 1.7	$n = 712$; k = 1.6	$n = 893$; k = 1.7	$n = 1360$; k = 1.7	$n = 252$; k = 1.6
Intercept	+36.5 ± 4.22[2]	41.6 ± 5.48[2]	+34.3 ± 5.73[2]	+37.5 ± 4.61[2]	+33.5 ± 6.75[2]
Time	+1.90 ± 1.29	+1.50 ± 2.21	+2.50 ± 1.72[2]	+0.70 ± 1.44	+12.4 ± 4.09[2]
RDW	+1.20 ± 0.84	+0.60 ± 1.01	+1.7 ± 0.82[2]	+3.1 ± 0.89[2,3,4]	−0.90 ± 0.52
RDW × Time	+0.30 ± 0.20	−0.04 ± 0.41	−0.50 ± 0.26	−0.50 ± 0.28	+0.02 ± 0.34

(*Continued*)

smoking status in addition to gender ($P < 0.004$ for RDW main effect).

Finally, as shown in **Table 3**, tests of the non-linear relationships of Hb concentrations were expressed as tertiles and cognitive performance and changed over time. Overall, there were non-linear relationships between tertiles of Hb and baseline performance on a measure of verbal fluency (AF) in the total population, whereby both low and high concentrations were associated with poorer performance. Elevated Hb was also linked to poorer performance on tests of verbal learning and memory (CVLT List A and DFR). However, this association did not hold after correction for multiple testing.

TABLE 2 (*Continued*)

	All	Men	Women	Nonanemic	Anemic
Trailmaking Test, Part B	$n = 1602$; k = 1.6	$n = 713$; k = 1.6	$n = 889$; k = 1.7	$n = 1352$; k = 1.6	$n = 250$; k = 1.6
Intercept	+213.9 ± 16.25[2]	+306.6 ± 25.40[2]	+169.0 ± 21.10[2]	+211.1 ± 17.2[2]	+225.0 ± 59.6[2]
Time	−1.60 ± 3.65	−1.80 ± 5.35	+0.50 ± 4.93	−1.60 ± 3.92	+7.10 ± 13.57
RDW	+2.3 ± 2.48	+7.4 ± 4.69	+0.4 ± 3.01	+7.0 ± 3.33[2]	−3.7 ± 4.6
RDW × Time	−0.40 ± 0.54	−0.30 ± 0.89	−0.20 ± 0.72	−0.70 ± 0.73	+0.80 ± 1.08

[1]Data are for HANDLS participants. Values are fixed effects regression coefficients from mixed-effects linear regression models ($\gamma \pm$ SE). Most cognitive test scores were in the direction of a higher score indicating better performance, except for BVRT (total errors), and both parts of the Trailmaking Test (expressed in seconds). Models were controlled for socio-demographic factors: namely, age (centered at 50 y), sex, race, poverty status, education, marital status, literacy (WRAT score, centered at 40), and employment status and the inverse Mills ratio. Other covariates were also included: namely, current smoking status, current drug use, BMI (centered at 30), CES-D total score (centered at 15), HEI-2010 (centered at 40), self-reported diabetes, hypertension, high cholesterol, cardiovascular disease, inflammatory conditions, NSAIDs, and several iron status indices: serum Hb (centered at 13.6), serum ferritin (centered at 125.9), ESR (centered at 16.5), serum iron (centered at 84.2), and mean cell volume (centered at 89.5). All covariates interacted with time. RDW was centered at the mean of 14.2. All inverse Mills ratios were centered at 0. Time was measured in years and RDW in percentages. BVRT, Benton Visual Retention Test; CES-D, Center for Epidemiologic Studies-Depression; CVLT, California Verbal Learning Test; ESR, erythrocyte sedimentation rate; HANDLS, Healthy Aging in Neighborhoods of Diversity Across the Life Span; Hb, hemoglobin; HEI-2010, Healthy Eating Index, 2010 version; k, number of observations/participant; NSAID, nonsteroidal anti-inflammatory drug; RDW, red cell distribution width; WRAT, Wide Range Achievement Test.
[2]$P < 0.05$ for null hypothesis that $\gamma = 0$.
[3]$P < 0.004$ for null hypothesis that $\gamma = 0$ for main effect RDW.
[4]$P < 0.05$ for null hypothesis of no difference by sex or anemia status, based on 2-way and 3-way interaction terms with RDW and time.
[5]$P < 0.009$ for null hypothesis that $\gamma = 0$ for interaction between RDW and time.

Discussion

In this study of 1526–1646 community-dwelling, urban adults, elevated RDW was linked cross-sectionally and longitudinally with poor cognitive performance (verbal memory and attention) among nonanemic individuals, after adjustments for socio-demographic, lifestyle, and health-related factors, as well as key iron status biomarkers. Our findings replicated those of a previous study whose outcomes were prevalent dementia and AD (30), since we observed consistent cross-sectional associations between RDW and tests of verbal memory and attention in the nonanemic group. To our knowledge, ours is the first study to examine these associations among middle-aged adults in the context of normal cognitive aging, adding to the evidence that RDW is an important risk factor for cognitive function that may affect this outcome in the absence of anemia.

In fact, the large body of evidence regarding iron statuses and cognitive outcomes focuses on Hb concentrations (10, 11, 13, 20, 22, 31, 32, 64–66), anemia (1, 9, 10, 12, 14–17, 20, 22, 67–70), MCV (20, 32, 71), serum ferritin or iron (72), transferrin saturation (72), and ESR (20). RDW is rarely included among measures of iron status and is often considered among putative confounders, as opposed to key exposures (30, 32). Cognitive outcomes included cognitive impairment at 1 point in time, including prevalent AD/dementia or mild cognitive impairment (MCI) (1, 9, 14, 20, 31, 32, 65, 70); progression from MCI to overt dementia (17); combined cognitive and physical decline (67); poor performance and/or decline on a global measure of cognition (11–14, 17, 22, 64, 66, 68, 72); incidence of dementia, AD, or cognitive impairment (12, 16, 17, 31); or poor performance and/or decline on specific domains, such as episodic memory, psychomotor speed,

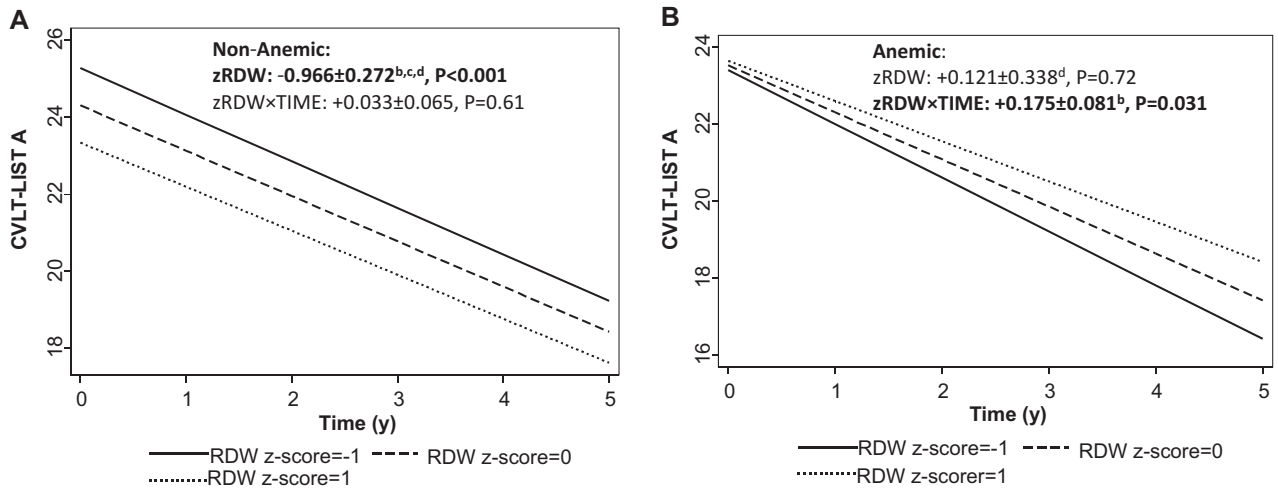

FIGURE 1 Predictive margins of CVLT-List A by standardized value of RDW over time among HANDLS participants in the non-anemic (n = 1309, k = 1.6; A) and anemic (n = 250, k = 1.5; B) groups: mixed effect linear regression model [a]zRDW: Effect of the standardized RDW exposure (per SD increase) on baseline cognitive performance; zRDW × Time: Effect of standardized RDW exposure (per SD increase) on annual rate of change in cognitive performance. [b]$P < 0.05$ for null hypothesis that $\gamma = 0$; [c]$P < 0.004$ for null hypothesis that $\gamma = 0$ for main effect RDW or anemia; [d]$P < 0.05$ for null hypothesis of no difference by sex or anemia status, based on 2-way and 3-way interaction terms with RDW and TIME.

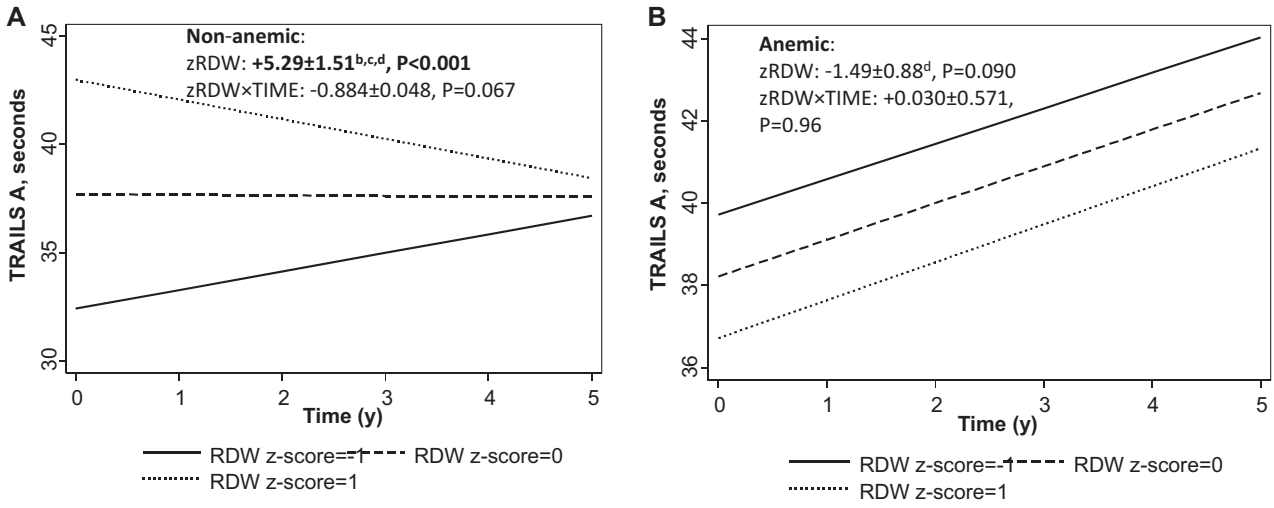

FIGURE 2 Predictive margins of TRAILS A by standardized value of RDW over time among HANDLS participants in the non-anemic ($n = 1,360$, k = 1.7; A) and anemic ($n = 252$, k = 1.6; B) groups: mixed effect linear regression model [a]zRDW: Effect of the standardized RDW exposure (per SD increase) on baseline cognitive performance; zRDW × Time: Effect of standardized RDW exposure (per SD increase) on annual rate of change in cognitive performance. [b]$P < 0.05$ for null hypothesis that $\gamma = 0$; [c]$P < 0.004$ for null hypothesis that $\gamma = 0$ for main effect RDW or anemia; [d]$P < 0.05$ for null hypothesis of no difference by sex or anemia status, based on 2-way and 3-way interaction terms with RDW and TIME.

and executive function (1, 10, 11, 15, 22, 32, 64, 69, 71). Among prospective cohort studies with anemia as the key exposure, using the Short Portable Mental Status Questionnaire, a study by Denny et al. (14) ($n = 1744$, 65% women, mean age 74.4 y at follow-up) observed that older adults who were anemic at baseline were more cognitively impaired at follow-up than the nonanemic group, adjusting for age, sex, race, education, BMI, estimated glomerular filtration rate, and comorbidities. Using domain-specific analysis, Deal et al. (15) found a positive association between anemia and declines in episodic memory and executive functions among community-dwelling women ($n = 374$, median age 74 y, 9 y of follow-up) (15). In another large cohort study of community-dwelling older adults ($n = 881$, 75% women, mean age: 80.6 y, follow-up: 5 y), Shah et al. (12) detected non-linear relationships between Hb concentration and several cognitive outcomes, whereby the lowest incidence of AD and slowest rate of cognitive decline (global) was found at an Hb concentration of 13.7 g/dL. Similarly, Atti et al. (16) and Stephan et al. (17) found that anemia was a significant predictor of incident dementia (OR = 13.5, 95% CI: 2.6, 71.3) and progression from MCI to dementia (HR = 1.6, 95% CI: 1.1, 2.4), respectively, among older adults. This relationship was only found for combined physical and cognitive declines in another study by Atkinson et al. (67) involving 558 community-dwelling, older adults with a 3-y average follow-up. Nevertheless, at least 1 large cohort study did not detect relationships between anemia and declines on 6 tests of cognitive performance (69). In the Atherosclerosis Risk in Communities Study (ARIC), cross-sectionally, anemia overall was linked to poorer global cognition and poorer performance on a test of psychomotor speed, the digits symbol substitution test. The same study found no notable longitudinal relationships (22). In a prospective cohort of 1227 cognitively intact, elderly subjects at baseline, a lower Hb concentration was not associated with MMSE performance. However, after 4 y of follow-up, the lowest tertile of Hb was linked to a 60% higher risk of cognitive impairment among men (95% CI: 1.06, 2.41; $P = 0.02$), but not among women (31).

A strong relationship between AD and anemia was also found in at least 1 case-control study (OR = 2.43, 95% CI: 1.31, 4.54) (20). This study indicated that a lower MCV, mean cell Hb concentrations, and higher ESR were important predictors of this cognitive outcome (20). In another recent, cross-sectional study, anemia was specifically associated with poorer verbal memory and executive functions (1). Other cross-sectional studies found no associations between anemia or Hb concentrations and various cognitive outcomes (66, 70).

Importantly, several studies have indicated that an elevated RDW is linked with poorer cognitive performance or with prevalent dementia. Most notably, Weuve et al. (30) reported that among 2556 community-dwelling adults, elevated RDW was associated with increased odds of having dementia, after adjusting for age, sex, race, and education, particularly among nonanemic individuals. This replicated our key finding, which was also based on cross-sectional cognitive performance rather than cognitive declines over time. More recently, the AddNeuroMed study ($n = 738$) demonstrated that various red blood cell indices, including anemia, mean corpuscular Hb concentration, and RDW, were associated with cognitive performance and AD status (32). Specifically, they showed a greater decline in RBC indices for AD patients, when compared to controls (32). Moreover, RDW among others was shown to be associated with 4 cognitive function tests, including for reaction time and reasoning (32).

While our study did not directly detect any associations between anemia or Hb concentrations and cognitive outcomes, we found that RDW was associated with poorer cross-sectional performances in verbal learning (CVLT List A) and attention (Trailmaking Test A), particularly among nonanemic individuals. In addition, and particularly among men, RDW was linked with poorer performance in another test of attention (BTA).

The pathways through which anemia may influence cognition remain unclear. However, several mechanisms have been proposed, including that: *1*) the chronic reduction of cerebral oxygenation could be secondary to decreased oxygen-carrying capacity of the blood (1, 7, 16, 68, 72–74); *2*) anemia could be

TABLE 3 Cognitive performance test scores by tertiles of hemoglobin concentration, stratified by sex and anemia status[1]

	All	Men	Women
Mini-Mental State Exam, total score	$n = 1633$; k = 1.6	$n = 738$; k = 1.6	$n = 895$; k = 1.7
Intercept	+26.5 ± 0.21[2]	+25.5 ± 0.34[2]	+27.08 ± 0.26[2]
Time	+0.1 ± 0.06[2]	+0.2 ± 0.09	+0.07 ± 0.07
T1	+0.09 ± 0.11	+0.5 ± 0.25	−0.003 ± 0.13
T1 × Time	−0.01 ± 0.03	−0.09 ± 0.07	−0.001 ± 0.04
T3	−0.03 ± 0.12	−0.2 ± 0.16	+0.4 ± 0.18
T3 × Time	+0.002 ± 0.03	+0.04 ± 0.04	−0.06 ± 0.05
CVLT, List A	$n = 1559$; k = 1.6	$n = 695$; k = 1.5	$n = 864$; k = 1.6
Intercept	+25.3 ± 0.74[2]	+21.6 ± 1.11[2]	+26.1 ± 1.00[2]
Time	−1.5 ± 0.18[2]	−1.6 ± 0.28[2]	−1.5 ± 0.24[2]
T1	−0.6 ± 0.41	−0.7 ± 0.81	−0.5 ± 0.50
T1 × Time	+0.09 ± 0.1	+0.065 ± 0.21	+0.1 ± 0.12
T3	−1.03 ± 0.42[2]	−1.3 ± 0.53	+0.08 ± 0.73
T3 × Time	+0.1 ± 0.10	+0.2 ± 0.13	−0.04 ± 0.17
CVLT, Delayed Free Recall	$n = 1526$; k = 1.5	$n = 675$; k = 1.5	$n = 851$; k = 1.6
Intercept	+7.9 ± 0.35[2]	+6.5 ± 0.52[2]	+8.2 ± 0.47[2]
Time	−0.5 ± 0.09[2]	−0.5 ± 0.14[2]	−0.5 ± 0.11[2]
T1	+0.08 ± 0.19	+0.1 ± 0.39	+0.2 ± 0.24
T1 × Time	−0.05 ± 0.05	−0.1 ± 0.11	−0.08 ± 0.06
T3	−0.4 ± 0.19[2]	−0.4 ± 0.25	−0.03 ± 0.34
T3 × Time	+0.08 ± 0.05	+0.1 ± 0.07	−0.09 ± 0.08
BVRT	$n = 1639$; k = 1.7	$n = 744$; k = 1.6	$n = 895$; k = 1.7
Intercept	+9.5 ± 0.56[2]	+8.9 ± 0.83[2]	+9.3 ± 0.75[2]
Time	+0.3 ± 0.14[2]	+0.5 ± 0.21[2]	+0.3 ± 0.18
T1	+0.09 ± 0.32	−0.3 ± 0.61	+0.04 ± 0.37
T1 × Time	+0.04 ± 0.08	+0.3 ± 0.15	+0.02 ± 0.09
T3	+0.09 ± 0.3	+0.1 ± 0.39	−0.3 ± 0.53
T3 × Time	−0.02 ± 0.08	−0.08 ± 0.09	+0.09 ± 0.13
Brief Test of Attention	$n = 1580$; k = 1.6	$n = 713$; k = 1.5	$n = 867$; k = 1.6
Intercept	+6.6 ± 0.23[2]	+5.8 ± 0.38[2]	+6.7 ± 0.35[2]
Time	−0.1 ± 0.07	−0.07 ± 0.11	−0.1 ± 0.08
T1	−0.1 ± 0.14	−0.07 ± 0.27	−0.09 ± 0.17
T1 × Time	+0.01 ± 0.04	+0.01 ± 0.08	+0.03 ± 0.04
T3	+0.04 ± 0.14	+0.01 ± 0.18	+0.1 ± 0.25
T3 × Time	+0.03 ± 0.04	+0.04 ± 0.05	−0.02 ± 0.06
Animal fluency	$n = 1646$; k = 1.7	$n = 747$; k = 1.7	$n = 899$; k = 1.7
Intercept	+18.2 ± 0.59[2]	+17.84 ± 0.94[2]	+19.3 ± 0.76[2]
Time	−0.09 ± 0.13	−0.11 ± 0.22	−0.07 ± 0.16
T1	−0.77 ± 0.33[2]	−0.43 ± 0.68	−0. ± 0.37
T1 × Time	+0.01 ± 0.07	−0.11 ± 0.15	−0.01 ± 0.08
T3	−0.69 ± 0.33[2]	−0.73 ± 0.45	−0.06 ± 0.54
T3 × Time	−0.04 ± 0.07	−0.05 ± 0.09	−0.2 ± 0.11
Digits Span, Forward	$n = 1638$; k = 1.6	$n = 741$; k = 1.6	$n = 897$; k = 1.7
Intercept	+7.1 ± 0.24[2]	+7.2 ± 0.37[2]	+6.9 ± 0.31[2]
Time	−0.01 ± 0.06	−0.003 ± 0.09	−0.02 ± 0.07
T1	−0.01 ± 0.13	−0.09 ± 0.26	+0.02 ± 0.15
T1 × Time	−0.003 ± 0.023	+0.03 ± 0.06	−0.004 ± 0.04
T3	+0.1 ± 0.13	−0.12 ± 0.18	+0.2 ± 0.22
T3 × Time	+0.003 ± 0.03	+0.02 ± 0.04	−0.02 ± 0.05
Digits Span, Backward	$n = 1642$; k = 1.6	$n = 744$; k = 1.6	$n = 898$; k = 1.7
Intercept	+5.6 ± 0.23[2]	+5.2 ± 0.35[2]	+5.9 ± 0.29[2]
Time	−0.09 ± 0.05	−0.08 ± 0.09	−0.1 ± 0.07
T1	+0.10 ± 0.12	+0.2 ± 0.25	+0.1 ± 0.15
T1 × Time	−0.04 ± 0.03	−0.005 ± 0.06	−0.04 ± 0.03
T3	+0.12 ± 0.13	+0.06 ± 0.17	+0.1 ± 0.21
T3 × Time	−0.03 ± 0.03	−0.007 ± 0.04	−0.07 ± 0.05
Clock, Command	$n = 1642$; k = 1.7	$n = 742$; k = 1.6	$n = 900$; k = 1.7
Intercept	+8.8 ± 0.14[2]	+8.7 ± 0.22[2]	+8.9 ± 0.19[2]
Time	−0.05 ± 0.04	−0.007 ± 0.07	−0.1 ± 0.05[2]

(*Continued*)

TABLE 3 (*Continued*)

	All	Men	Women
T1	−0.06 ± 0.08	−0.09 ± 0.16	+0.02 ± 0.09
T1 × Time	−0.006 ± 0.02	−0.03 ± 0.05	+0.007 ± 0.03
T3	−0.07 ± 0.08	−0.1 ± 0.11	+0.06 ± 0.13
T3 × Time	+0.003 ± 0.02	+0.05 ± 0.03	−0.04 ± 0.04
Trailmaking Test, Part A	n = 1612; k = 1.7	n = 712; k = 1.6	n = 893; k = 1.7
Intercept	+37.6 ± 4.36[2]	+43.3 ± 5.67[2]	+34.3 ± 5.86[2]
Time	+1.9 ± 1.34	+1.8 ± 2.29	+2.3 ± 1.77
T1	−2.2 ± 2.32	−3.4 ± 4.02	−1.5 ± 2.81
T1 × Time	+0.1 ± 0.72	−1.2 ± 1.67	+0.4 ± 0.87
T3	−2.2 ± 2.35	−3.6 ± 2.58	−0.7 ± 4.07
T3 × Time	+0.2 ± 0.73	−0.7 ± 1.05	+0.7 ± 1.24
Trailmaking Test, Part B	n = 1602; k = 1.6	n = 713; k = 1.6	n = 889; k = 1.7
Intercept	−1.1 ± 3.76[2]	+301.9 ± 26.31[2]	+171.1 ± 21.54[2]
Time	−3.0 ± 8.91	−0.3 ± 5.49	+1.1 ± 5.07
T1	+0.38 ± 1.91	−2.3 ± 18.75	−1.6 ± 10.31
T1 × Time	+0.5 ± 9.03	−0.3 ± 3.65	−0.4 ± 2.35
T3	−2.2 ± 1.91	+12.9 ± 12.06	−2.7 ± 14.98
T3 × Time	+214.9 ± 16.75	−4.5 ± 2.26	−3.2 ± 3.34

[1] Data are for HANDLS participants. Values are fixed effects regression coefficients from mixed-effects linear regression models ($\gamma \pm$ SE). Most cognitive test scores were in the direction of a higher score indicating better performance, except for BVRT (total errors) and both parts of the Trailmaking Test (expressed in seconds). Models were controlled for socio-demographic factors: namely, age (centered at 50 y), sex, race, poverty status, education, marital status, literacy (WRAT score, centered at 40), and employment status and the inverse Mills ratio. Additional covariates were also included: namely, current smoking status, current drug use, BMI (centered at 30), CES-D total score (centered at 15), HEI-2010 (centered at 40), self-reported diabetes, hypertension, high cholesterol, cardiovascular disease, inflammatory conditions, NSAIDs, and several iron status indices: RDW (centered at the mean of 14.2), serum ferritin (centered at 125.9), ESR (centered at 16.5), serum iron (centered at 84.2), and mean cell volume (centered at 89.5). All covariates interacted with time. All inverse Mills ratios were centered at 0. Time was measured in years and T2 is the referent category.
BVRT, Benton Visual Retention Test; CES-D, Center for Epidemiologic Studies-Depression; CVLT, California Verbal Learning Test; ESR, erythrocyte sedimentation rate; HEI-2010, Healthy Eating Index, 2010 version; k, number of observations/participant; NSAID, nonsteroidal anti-inflammatory drug; RDW, red cell distribution width; T, tertile; WRAT, Wide Range Achievement Test.
[2] $P < 0.05$ for null hypothesis that $\gamma = 0$.

a marker of processes, such as ischemia, which increase the risk of neuronal degeneration (1, 12, 19, 65); *3*) iron dysregulation could be associated with increased brain oxidative stress; *4*) chronic cerebral hypoperfusion may be present in the prefrontal cortex or inflammatory neurodegenerative processes; *5*) anemia could negatively affect cardiorespiratory fitness by causing a decrease in the brain-derived neurotrophic factor or reduced efficiency of the prefrontal neural function (8, 69); and *6*) anemia could lower the threshold capacity, such that an otherwise silent cerebrovascular accident, like a small stroke or transient ischemic attack, has a greater impact on subsequent cognition (7). In addition, anemia has been associated with an increased risk of white matter hyperintensities progression, especially in older adults with high blood pressure (19). Thus, anemia may influence changes in the brain tissue and vessels (1).

Elevated hemoglobin, when primary, has been labelled as polycythemia. Examples are polycythemia vera or myeloproliferative diseases, which are genetic/cancerous diseases, each having their own modalities. Polycythemia can be secondary (also known as erythrocytosis) and, therefore, can result from dehydration, any disorder that causes low oxygen concentrations/hypoxia, smoking, and carbon monoxide exposure, in addition to a list of multiple cardiac, renal, and pulmonary conditions. Primary and secondary polycythemia are treated differently. For instance, dehydration is treated with intraveneous fluids, hypoxia is treated with supplemental oxygen, smoking is treated with cessation, and carbon monoxide toxicity is treated by removing the offending agent and using high oxygen delivery systems with supportive therapy (75, 76).

Furthermore, the most common cause of elevated RDW in older adults is the "anemia of chronic diseases." Elevated RDW also has been associated with systemic inflammation; indices of oxidative stress; increased blood viscosity, as indicated by elevated fibrinogen; diabetes; a history of coronary heart disease; and a history of stroke. Each of these factors appears also to be associated with poor cognition, cognitive declines, dementia, or dementia pathology (30).

Our study had several strengths, including stratified analyses by key potential effect modifiers and measures of a wide range of cognitive tasks with follow-ups, allowing us to elucidate temporal associations across multiple cognitive domains. Finally, to study the independent effects of RDW on cognitive performance and changes over time, we adjusted for numerous iron status measures among key potential confounders.

Nevertheless, our findings should be interpreted with caution in light of several limitations. First, residual confounding cannot be ruled out, given the observational nature of the study. Second, cognitive measures were only available at 2 time points. Third, at the time of the study, genotyping had only been completed for a sub-sample of African-American participants, which prevented us from including known genetic risk factors for AD, such as apolipoprotein E4.

In sum, we found a consistent, cross-sectional association between elevated RDW and poor cognitive performance on tests that mainly reflected the domains of verbal memory and attention among the nonanemic group and among men. Overall, RDW had opposing associations with baseline performance (risk factor) and longitudinal changes (protective factor) on a test of verbal memory. We did not detect any association between Hb and cognitive performance or changes over time. More longitudinal studies are needed to replicate our findings, particularly for outcomes of incident dementia and AD. Interventions to reduce RDW should be tailored and tested

for their effectiveness in slowing age-related cognitive declines among nonanemic, middle-aged adults.

Acknowledgments

The authors thank Megan Williams and Nicolle Mode (National Institute on Aging, Intramural Research Program) for internally reviewing the manuscript.

The authors' contributions were as follows—MAB: contributed to study concept, planned analysis, conducted data management and statistical analysis, conducted literature review, wrote and revised the manuscript; SH: 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; DS: conducted literature search and review, wrote parts of the manuscript, revised the manuscript; JW: assisted in statistical analysis, wrote parts of the manuscript, revised the manuscript; MKE: acquired data, wrote and revised parts of the manuscript; ABZ: acquired data, planned analysis, wrote and revised parts of the manuscript; and all authors: read and approved the final manuscript.

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Supplemental Method 1: Cognitive Assessment

Mini-Mental State Examination (MMSE): The MMSE (1) is a brief mental status test and global cognitive functioning measuring orientation, concentration, immediate and delayed memory, language and constructional praxis. Scores range from 0 to 30, with higher scores indicating better cognitive performance.

California Verbal Learning Test (CVLT): The CVLT (2) is a 16-item shopping list measuring verbal learning and memory. A modified version of the CVLT was used with three, rather than five, list A learning trials. Cued recall was not administered. Variables of interest in this study were total correct for List A sum across trials 1-3 and List A long-delay free recall. Scores ranged from 0 to 48 for List A sum and 0 to 16 for List A long-delay free recall. Higher scores indicate better verbal memory. The CVLT is described in detail elsewhere (2).

Benton Visual Retention Test (BVRT): The BVRT (3) is a test of short-term figural memory and visuo-constructional abilities. Administration A, Form D was used. Two trained examiners independently scored the BVRT using a modified error scoring system, based on the BVRT Manual scoring. A consensus was achieved for discrepancies in scoring. If a consensus between the two examiners could not be reached, MKT, a research psychologist assigned the score. Scores were total errors, such that higher values indicate poorer visual memory.

Brief Test of Attention (BTA): The BTA (4) is a measure of divided auditory attention. An examiner administered 10 trials where increasing longer lists of letters and numbers (containing 4-18 items) were read. Participants were instructed to keep track of how many numbers were read during each trial, disregarding the number of letters, and were told to keep their hands in fists to

discourage counting on their fingers. Only the numbers portion of the test was administered. The total score was the total number of trials correct out of 10.

Animal Fluency: Animal fluency, (5, 6) a measure of semantic verbal fluency, requires participants to generate as many animals as possible for 60 seconds. Higher scores indicate better verbal fluency, with the total number of words, minus intrusions and perseverations analyzed.

Digit Span Forward and Backward (DS-F and DS-B): The Wechsler Adult Intelligence Scale, Revised(7) Digit Span Forward and Backward are tests of attention and executive functioning, specifically working memory. They were administered according to standard instructions, and the total score was the total number correct for each test.

Clock Drawing Test – Clock to Command (CDT): The Clock Drawing Test (8) is a test of visuo-spatial and visuo-constructional abilities. Participants are asked to draw a clock, put in all of the numbers and set the hands for 10 after 11. Scores are assessed for the clock face (0-2), numbers (0-4) and hands (0-4), with a range from 0 to 10, with higher scores indicating more accurate clock drawing. Participants who did not score a 10 on the command version of the test were asked to copy a clock with the time set to 10 after 11.

Trail Making Tests A and B (Trails A and Trails B) : Trailmaking test A and B (9) are tests of attention and executive functioning, respectively, specifically cognitive control and visuo-motor scanning/processing speed. Participants were instructed to draw lines between consecutive numbers (Trails A) or alternate between numbers and letter (Trails B) as fast as they could while a stop watch recorded time. When errors were committed the participant corrected the error by returning to his/her last correct response and continued from there. The stop-watch ran while

corrections were made. Scores reflected time to completion (in seconds) separately for Trails A and B. Higher scores indicate poorer performance.

Supplemental References

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SUPPLEMENTARY DATA

Supplemental Table S1. Cognitive performance test scores at baseline (Visit 1), follow-up (Visit 2), and change between visits, by sex, for HANDLS participants with complete and reliable baseline and/or follow-up cognitive scores and complete data on RDW, anemia status and covariates

	All	Women	Men	Non-anemic	Anemic
Mini-Mental State Exam, total score					
Visit 1	27.9±0.09	27.9±0.12	27.8±0.13	27.9±0.09	27.7±0.28
	(n=1,607)	*(n=883)*	*(n=724)*	*(n=1,350)*	*(n=257)*
Visit 2	28.0±0.08	28.1±0.09	27.9±0.13	28.0±0.09	27.9±0.17
	(n=1,080)	*(n=636)*	*(n=444)*	*(n=918)*	*(n=162)*
P(Visit2-Visit1)	*0.21*	*0.47*	*0.33*	*0.29*	*0.50*
California Verbal Learning Test (CVLT), List A					
Visit 1	24.9±0.31	26.2±0.38 [2]	23.4±0.47	25.1±0.34	24.0±0.71
	(n=1,335)	*(n=741)*	*(n=594)*	*(n=1,116)*	*(n=219)*
Visit 2	19.2±0.42	19.8±0.61	18.4±0.57	19.4±0.45	17.9±1.10
	(n=1,076)	*(n=633)*	*(n=443)*	*(n=914)*	*(n=162)*
P(Visit2-Visit1)	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*
CVLT, free delayed recall					
Visit 1	7.40±0.14	7.8±0.17 [2]	6.9±0.23	7.5±0.16	6.9±0.28
	(n=1,314)	*(n=732)*	*(n=582)*	*(n=1,097)*	*(n=217)*
Visit 2	5.5±0.17	5.7±0.23	5.2±0.25	5.5±0.18	5.0±0.39
	(n=1,076)	*(n=633)*	*(n=443)*	*(n=914)*	*(n=162)*
P(Visit2-Visit1)	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*
Benton Visual Retention Test					
Visit 1	5.3±0.20	5.8±0.31 [2]	4.8±0.26	5.3±0.23	5.6±0.43
	(n=1,603)	*(n=880)*	*(n=723)*	*(n=1,347)*	*(n=256)*
Visit 2	7.4±0.23	7.8±0.32 [2]	6.9±0.32	7.2±0.25 [2]	8.6±0.49
	(n=1,075)	*(n=632)*	*(n=443)*	*(n=914)*	*(n=161)*
P(Visit2-Visit1)	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*
Brief Test of Attention					
Visit 1	6.6±0.11	6.6±0.17	6.5±0.14	6.7±0.12 [2]	5.9±0.28
	(n=1,398)	*(n=774)*	*(n=624)*	*(n=1,175)*	*(n=223)*
Visit 2	6.8±0.10	6.9±0.15	6.8±0.14	6.9±0.11	6.4±0.24
	(n=993)	*(n=583)*	*(n=410)*	*(n=850)*	*(n=143)*
P(Visit2-Visit1)	*0.11*	*0.31*	*0.21*	*0.26*	*0.17*
Animal Fluency					
Visit 1	19.3±0.25	18.4±0.35 [2]	20.3±0.33	19.6±0.28 [2]	17.6±0.44
	(n=1,590)	*(n=874)*	*(n=716)*	*(n=1,338)*	*(n=252)*
Visit 2	19.4±0.30	18.7±0.39 [2]	20.5±0.45	19.6±0.34 [2]	18.3±0.52
	(n=1,073)	*(n=634)*	*(n=439)*	*(n=912)*	*(n=161)*

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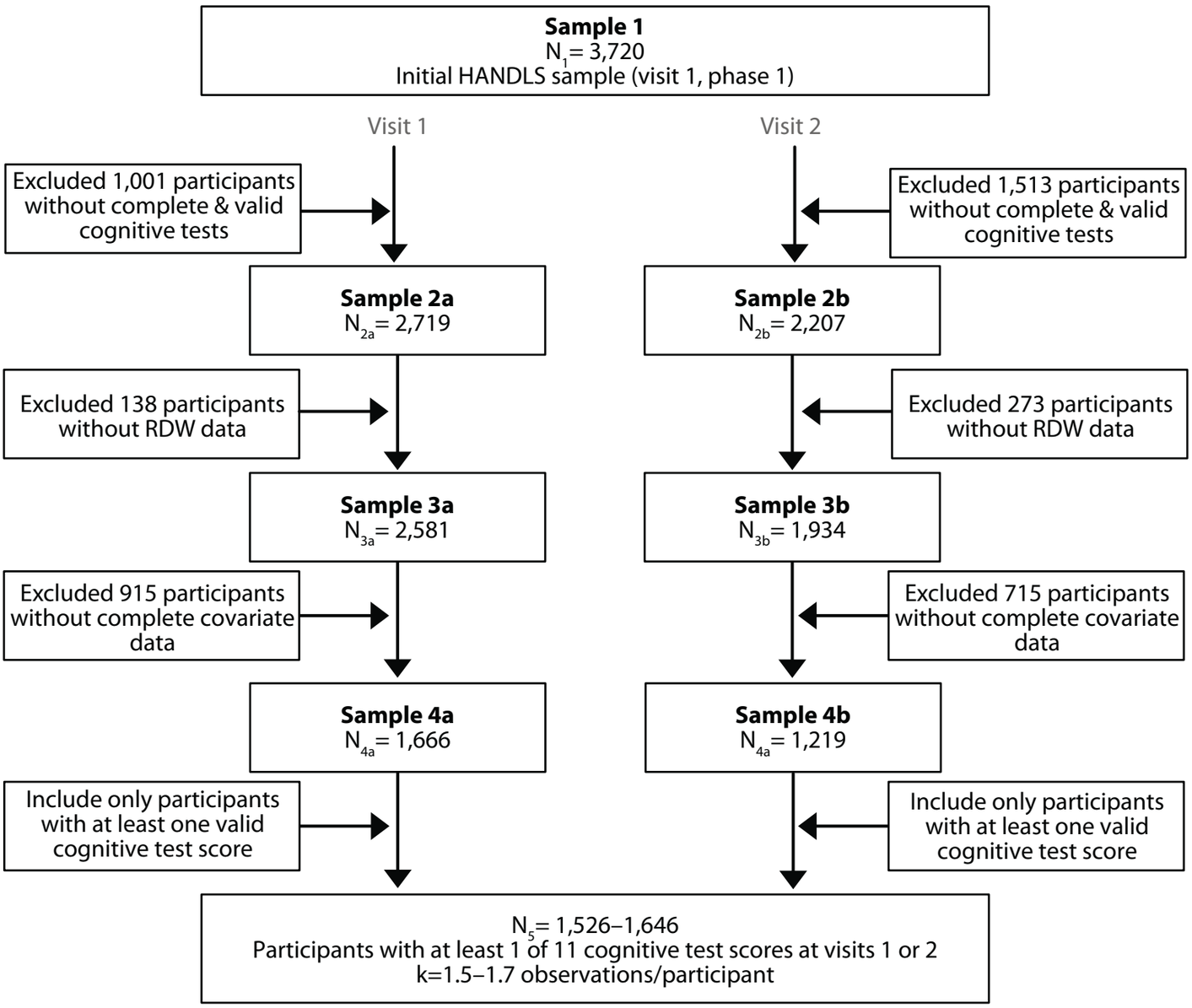
P(Visit2-Visit1)	*0.74*	*0.67*	*<0.001*	*0.96*	*0.35*
Digits Span, Forward					
Visit 1	7.4±0.09	7.4±0.13	7.5±0.13	7.5±0.10 [2]	6.9±0.24
	(n=1,571)	*(n=863)*	*(n=708)*	*(n=1,321)*	*(n=250)*
Visit 2	7.6±0.11	7.4±0.15	7.8±0.16	7.6±0.12	7.2±0.27
	(n=988)	*(n=581)*	*(n=406)*	*(n=843)*	*(n=145)*
P(Visit2-Visit1)	*0.29*	*0.79*	*0.16*	*0.44*	*0.47*
Digits Span, Backward					
Visit 1	5.8±0.09	5.8±0.12	5.8±0.13	5.9±0.09 [2]	5.2±0.21
	(n=1,564)	*(n=856)*	*(n=708)*	*(n=1,317)*	*(n=247)*
Visit 2	5.9±0.10	5.9±0.13	6.1±0.17	6.1±0.11 [2]	5.4±0.25
	(n=986)	*(n=581)*	*(n=405)*	*(n=842)*	*(n=144)*
P(Visit2-Visit1)	*0.14*	*0.65*	*0.10*	*0.23*	*0.49*
Clock, command					
Visit 1	8.8±0.06	8.7±0.08 [2]	8.9±0.08	8.8±0.06	8.6±0.15
	(n=1,599)	*(n=878)*	*(n=721)*	*(n=1,342)*	*(n=257)*
Visit 2	8.8±0.06	8.8±0.09	8.9±0.08	8.9±0.07 [2]	8.4±0.06
	(n=1,065)	*(n=622)*	*(n=436)*	*(n=906)*	*(n=159)*
P(Visit2-Visit1)	*0.53*	*0.38*	*0.95*	*0.28*	*0.20*
Trailmaking test, Part A					
Visit 1	34.9±0.73	35.0±1.08	34.9±0.98	34.3±0.78	38.4±2.09
	(n=1,591)	*(n=872)*	*(n=719)*	*(n=1,339)*	*(n=252)*
Visit 2	35.9±1.35	33.1±0.87	39.7±2.97	36.0±1.54	35.3±3.51
	(n=1,070)	*(n=632)*	*(n=438)*	*(n=910)*	*(n=160)*
P(Visit2-Visit1)	*0.53*	*0.17*	*0.12*	*0.33*	*0.24*
Trailmaking test, Part B					
Visit 1	148.0±6.63	140.0±7.6	156.8±11.10	144.5±7.37	167.5±14.84
	(n=1,590)	*(n=872)*	*(n=718)*	*(n=1,338)*	*(n=252)*
Visit 2	137.1±6.92	135.2±7.6	139.6±12.70	136.5±7.77	140.6±12.48
	(n=1,069)	*(n=632)*	*(n=437)*	*(n=909)*	*(n=160)*
P(Visit2-Visit1)	*0.25*	*0.66*	*0.31*	*0.46*	*0.17*

Key: CES-D=Center for Epidemiologic Studies-Depression; HANDLS=Healthy Aging in Neighborhoods of Diversity Across the Life Span; MMSE=Mini-Mental State Examination; *n*=number of participants; PIR=poverty income ratio; WRAT=Wide Range Achievement Test.

SUPPLEMENTARY DATA

[1] Values are Mean±SEM for cognitive test performance at each visit and P-value from regression model with outcome being cognitive test score and predictor being visit (Visit 2 vs. Visit 1). Most cognitive test scores were in the direction of higher score=better performance, except for BVRT (total errors), and Trailmaking Test both parts (expressed in seconds).

[2] $P<0.05$ for null hypothesis of no difference in means of cognitive test scores by sex or anemic status (referent categories: Women; Non-anemic) within each visit. Wald test from svy:reg command.


Sample 1
N1= 3,720
Initial HANDLS sample (visit 1, phase 1)
Visit 1
Visit 2
Excluded 1,001 participants without complete & valid cognitive tests
Excluded 1,513 participants without complete & valid cognitive tests
Sample 2a
N2a= 2,719
Sample 2b
N2b= 2,207
Excluded 138 participants without RDW data
Excluded 273 participants without RDW data
Sample 3a
N3a= 2,581
Sample 3b
N3b= 1,934
Excluded 915 participants without complete covariate data
Excluded 715 participants without complete covariate data
Sample 4a
N4a= 1,666
Sample 4b
N4a= 1,219
Include only participants with at least one valid cognitive test score
Include only participants with at least one valid cognitive test score
N5= 1,526–1,646
Participants with at least 1 of 11 cognitive test scores at visits 1 or 2
k=1.5–1.7 observations/participant