Hemoglobin Concentration and Risk of Incident Stroke in Community-Living Adults

Bhupesh Panwar, MD; Suzanne E. Judd, PhD; David G. Warnock, MD; William M. McClellan, MD, MPH; John N. Booth, III, MS; Paul Muntner, PhD; Orlando M. Gutiérrez, MD, MMSc

Background and Purpose—In previous observational studies, hemoglobin concentrations have been associated with an increased risk of stroke. However, these studies were limited by a relatively low number of stroke events, making it difficult to determine whether the association of hemoglobin and stroke differed by demographic or clinical factors.

Methods—Using Cox proportional hazards analysis and Kaplan–Meier plots, we examined the association of baseline hemoglobin concentrations with incident stroke in the Reasons for Geographic and Racial Differences in Stroke (REGARDS) study, a cohort of black and white adults aged ≥45 years.

Results—A total of 518 participants developed stroke over a mean 7±2 years of follow-up. There was a statistically significant interaction between hemoglobin and sex (P=0.05) on the risk of incident stroke. In Cox regression models adjusted for demographic and clinical variables, there was no association of baseline hemoglobin concentration with incident stroke in men, whereas in women, the lowest (<12.4 g/dL) and highest (>14.0 g/dL) quartiles of hemoglobin were associated with higher risk of stroke when compared with the second quartile (12.4–13.2 g/dL; quartile 1: hazard ratio, 1.59; 95% confidence interval, 1.09–2.31; quartile 2: referent; quartile 3: hazard ratio, 0.91; 95% confidence interval, 0.59–1.38; quartile 4: hazard ratio, 1.59; 95% confidence interval, 1.08–2.35). Similar results were observed in models stratified by hemoglobin and sex and when hemoglobin was modeled as a continuous variable using restricted quadratic spline regression.

Conclusions—Lower and higher hemoglobin concentrations were associated with a higher risk of incident stroke in women. No such associations were found in men. (Stroke. 2016;47:2017-2024. DOI: 10.1161/STROKEAHA.116.013077.)

Key Words: anemia • cohort studies • hematocrit • hemoglobins • renal insufficiency, chronic • stroke

Observational studies have reported an independent association between hemoglobin concentration and risk of developing stroke.1–4 This association has been shown to follow a J- or a U-shaped curve with increased risk of developing a stroke in the lower and higher end of the hemoglobin spectrum.1,2,4,5 Some studies have also reported that this association differs by sex. For example, a follow-up study of the Framingham cohort described a U-shaped relationship between hematocrit and incident stroke among older women, whereas no such association was present among men.3 However, the level of evidence remains weak because of the relatively few number of stroke events in these studies.2,4,3

The clinical relevance of the association between hemoglobin and stroke has more recently been emphasized by the findings of large randomized controlled trials in individuals with chronic kidney disease (CKD) that showed an increased incidence of cardiovascular events,8–10 including increased incidence of ischemic strokes,10,11 in individuals treated with erythropoietin-stimulating agents to a higher hemoglobin target. Whether attaining a higher hemoglobin or direct effect of erythropoietin-stimulating agents is responsible for the increased incidence of stroke in these studies is not clear.

Several observational studies have linked the presence of anemia to a higher incidence of stroke.2,4,7 One previous study reported that the association of anemia with stroke risk was substantially higher in individuals with CKD than in to those without CKD.3 However, this finding was based on relatively few stroke events and was based on a definition of CKD determined by creatinine clearance alone. This is important in that we have previously shown that CKD definitions that incorporate albuminuria are more strongly associated with stroke risk than creatinine-based estimates of glomerular filtration rate alone.12

To address current uncertainties in the relationship between hemoglobin and stroke, we examined the association of
hemoglobin concentration with incident stroke events among participants enrolled in the Reasons for Geographic and Racial Differences in Stroke (REGARDS) study, a prospective cohort of black and white adults aged at least 45 years.

Methods
The REGARDS study is a population-based investigation of stroke incidence in black and white US adults aged ≥45 years. Details of the study design have been reviewed elsewhere. Briefly, the study was designed to provide approximately equal representation of men and women and oversampled individuals who were black and individuals living in the US stroke belt/buckle. Trained interviewers conducted computer-assisted telephone interviews to obtain information including participants’ sociodemographics, cardiovascular risk factors, and self-reported use of antihypertensive, antihyperglycemic, and cholesterol-lowering medication. After this interview, health professionals conducted an in-home study visit that included an ECG recording, blood pressure, height and weight measurements, inventory of medications taken during the previous 2 weeks and collection of blood and urine samples. Overall, 30,239 individuals were enrolled between January 2003 and October 2007 (42% black and 55% women). The REGARDS study protocol was approved by the institutional review boards governing research in human subjects at each participating center.

Primary Exposure
The exposure of interest was hemoglobin concentrations measured in baseline blood samples using automated cell counting on a Beckman Coulter LH 755 Hematology Workcell (Beckman Coulter, Inc, Fullerton, CA) with an interassay coefficient of variation of 3.0%. Hemoglobin was not collected in REGARDS participants before May 2004. Therefore, hemoglobin data are not available for ≈10,000 REGARDS participants enrolled before this date.

Outcome of Interest
The outcome of interest was incident stroke through October 31, 2013. Suspected strokes were reported via telephone follow-up with participants every 6 months. Medical records were requested for stroke events and reviewed by members of a committee of stroke experts to validate and classify potential strokes. Stroke events were defined based on symptoms according to the World Health Organization definition of stroke. Events not meeting this definition but characterized by symptoms lasting <24 hours, with neuroimaging consistent with acute ischemia or hemorrhage were classified as clinical strokes and included as stroke events. Cases in which adjudicators agreed that the event was likely a stroke or stroke-related death but information was incomplete for World Health Organization or clinical classification were classified as probable strokes and also included as stroke events.

Covariates of Interest
Age, race, sex, smoking history, annual family income, educational attainment, and aspirin use were determined by self-report. Systolic and diastolic blood pressures were defined as the average of 2 seated measures taken after a 5-minute rest. Body mass index was calculated as weight in kilograms divided by height in meters squared. Waist circumference was measured during the in-home visit using a tape measure positioned midway between the lowest rib and the iliac crest with the participant standing. History of coronary heart disease (CHD) and left ventricular hypertrophy (LVH) were determined by self-report or by detection in ECG recordings obtained during the baseline study visit. LVH was classified using ECG criteria. Serum creatinine was calibrated to an international isotope dilution mass spectroscopic standard, measured by colorimetric reflectance spectrophotometry. Estimated glomerular filtration rate was determined from serum creatinine measurements using the Chronic Kidney Disease (CKD) Epidemiology Collaboration equation. Urine albumin was measured by the BNII ProSpec nephelometer (Siemens AG), and urine creatinine measured by the rate Jäff method (Roche/Hitachi, Basel, Switzerland) were used to calculate urine albumin:creatinine ratio (ACR). Spot urine ACR was calculated in mg/g, and albuminuria was defined as an ACR ≥30 mg/g. High-sensitivity C-reactive protein was analyzed by particle-enhanced immunonephelometry with the BNII nephelometer according to manufacturer’s instructions (N High Sensitivity CRP, Dade Behring, Deerfield, IL). REGARDS did not collect information on pulmonary conditions, and therefore we defined chronic lung disease as participants with chronic bronchitis or emphysema (modeled with respiratory medications including beta-agonists, leukotriene inhibitors, inhaled corticosteroids, combination inhalers, and other pulmonary medications such as ipratropium, cromolyn, aminophylline, and theophylline).

Statistical Analysis
Descriptive statistics were used to present participant characteristics across quartiles of baseline hemoglobin. Crude rates of incident stroke were calculated by sex-specific hemoglobin quartiles. The Kaplan–Meier method was used to calculate cumulative incidence of stroke by hemoglobin quartile and sex. Next, proportional hazards analysis was used to examine associations of baseline hemoglobin concentrations with incident stroke in sequential models. Model 1 was adjusted for age, race, age×race interaction term (because associations of race with stroke are greater at younger ages, as previously reported), sex, and region of residence. Model 2 was adjusted for variables in model 1 plus established stroke risk factors (body mass index, waist circumference, dyslipidemia, aspirin use, estimated glomerular filtration rate, ACR, diabetes mellitus, atrial fibrillation, current smoking, income, education, systolic blood pressure, coronary heart disease, chronic pulmonary disease, high-sensitivity C-reactive protein, and LVH) and factors that had plausible biological links to risk of stroke. Given differences in the distribution of hemoglobin values by sex, we modeled hemoglobin using sex-specific quartiles. As previous studies have shown a J- or U-shaped association of hemoglobin with risk of stroke, we chose the second quartile to serve as the referent group. Cox regression models were also used to estimate the hazard ratio (HR) for incident stroke as a function of sex and baseline hemoglobin quartiles in sequentially adjusted models with women in the second quartile of hemoglobin serving as the referent group. Cox regression models with restricted quadratic splines were used to examine the association of baseline hemoglobin (modeled as a continuous variable) with risk of incident stroke stratified by sex. Because previous studies showed that the association of hemoglobin with health outcomes (stroke and mortality) was modified by CKD status and sex, we tested the statistical significance (P<0.10) of multiplicative interaction terms (hemoglobin×sex; hemoglobin×CKD) in separate models. A 2-tailed P value of <0.05 was considered statistically significant, except for the interaction analyses. All analyses were conducted using SAS software version 9.4 (SAS Institute, Cary, NC).

Results
After excluding 10,302 participants without data on baseline hemoglobin concentration, 546 participants lost to follow-up, and 978 participants who reported a history of stroke at baseline, 18,413 participants were included in the final analyzed sample (Figure 1). Table 1 depicts the baseline characteristics of the study sample by quartiles of baseline hemoglobin. When compared with participants with higher hemoglobin, individuals with lower
hemoglobin were more likely to be women, black, nonsmokers; live in the US stroke belt/buckle; be overweight or obese; have lower annual income and educational achievement; have higher systolic blood pressure; have a higher prevalence of diabetes mellitus, atrial fibrillation, LVH; a lower prevalence of chronic pulmonary disease; have higher ACR; and lower estimated glomerular filtration rate.

**Associations of Baseline Hemoglobin With Incident Stroke**

A total of 518 participants developed a stroke over a mean 7 (SD 2) years of follow-up. There was significant interaction between hemoglobin and sex \((P=0.05)\) on the risk of incident stroke, but not between hemoglobin and CKD \((P=0.31)\).

There were no statistically significant associations of sex-specific hemoglobin quartiles with risk of incident stroke in men (Figure 2A; \(P\) for log-rank test=0.09). In contrast, the highest and lowest quartiles of hemoglobin were associated with higher cumulative incidence of stroke in women (Figure 2B; \(P\) for log-rank test <0.001).

Table 2 depicts sex-specific incidence rates (IRs) for stroke per 1000 person-years of follow-up and multivariable-adjusted HRs of incident stroke by categories of baseline hemoglobin using sex-specific quartiles. The absolute risk of stroke was higher in men than in women in each sex-specific hemoglobin quartile. However, when compared across quartiles of hemoglobin within each sex, the absolute risk of stroke was 2× higher in the first than in the second quartile in women (IR, 5.59; 95% confidence interval [CI], 4.86–7.08 versus IR, 2.76; 95% CI, 2.10–3.62), whereas it was 1.4× higher in men (IR, 7.14; 95% CI, 5.63–9.04 versus IR, 5.15; 95% CI, 3.97–6.67). Similarly, the absolute risk of stroke was ≈1.5× higher in the fourth than in the second quartile in women (IR, 4.04; 95% CI, 3.23–5.04 versus IR, 2.76; 95% CI, 2.10–3.62), whereas it was lower in the fourth than in the second quartile in men (IR, 4.68; 95% CI, 3.10–6.05 versus IR, 5.15; 95% CI, 3.97–6.67).

In women, when compared with the second quartile of hemoglobin (12.4 and 13.2 g/dL), hemoglobin concentrations in the lowest (<12.4 g/dL) and highest (>14.0 g/dL) quartiles were associated with a higher hazard of incident stroke in models adjusted for age, race, age×race interaction, and geographic region of residence. The associations were attenuated and remained statistically significant after further adjustment for education, income, current cigarette smoking, a history of diabetes mellitus, atrial fibrillation, LVH, or chronic pulmonary disease, systolic blood pressure, coronary heart disease, body mass index, waist circumference, dyslipidemia, aspirin use, log-transformed high-sensitivity C-reactive protein, log-transformed ACR, and estimated glomerular filtration rate (quartile 2: referent; quartile 1: HR, 1.59; 95% CI, 1.09–2.31; quartile 3: HR, 0.91; 95% CI, 0.59–1.38; quartile 4: HR, 1.59; 95% CI, 1.08–2.35). In contrast, there were no statistically significant associations of hemoglobin with incident stroke in any multivariable-adjusted Cox regression model among men. Similarly, when examined on a continuous scale stratified by sex, unlike women, there were no discernible associations of hemoglobin with incident stroke risk in fully adjusted models in men (Figures I and II in the online-only Data Supplement).

HRs for incident stroke by sex and hemoglobin quartiles are shown in Table 3. In fully adjusted models, similar to the results depicted in Table 2, a U-shaped relationship between baseline hemoglobin and risk of stroke was noted among women. In addition, when compared with the women in the second quartile of hemoglobin, men in the second, third, and fourth quartiles of hemoglobin had higher risk of developing stroke in fully adjusted models.

**Discussion**

In this cohort of community-dwelling adults, we found that the association of hemoglobin with incident stroke risk differs by sex. There was a U-shaped association among women such that the lower and higher extremes of hemoglobin concentration were associated with increased risk of stroke.
Independently of traditional stroke risk factors. In contrast, there was no association of hemoglobin and stroke among men in unadjusted or multivariable-adjusted models.

Previous studies have examined whether the association between hemoglobin concentration and stroke risk differed by sex, but the results have been inconsistent. Kannel et al.¹ examined the association between hemoglobin concentration and incident stroke in the Framingham cohort. They reported an increased risk of stroke with increasing hemoglobin concentration within the reported normal range of hemoglobin. However, when stratified by sex, higher hemoglobin concentrations were statistically significantly associated with higher stroke risk in men but not in women in univariate analysis. This association did not remain statistically significant when adjusted for traditional risk factors for stroke in multivariate analysis.² A 34-year follow-up study was later published in the same cohort examining the association of baseline hematocrit with cardiovascular events.² A U-shaped association was reported between hematocrit and stroke especially among older women (≥65 years) but not among younger (<65 years) women or men from any age group.² Among older women the multivariable adjusted odds ratio for incident stroke comparing first (25%–42%) to the third (45%–46%), hematocrit quintile was 1.92, P=0.01, and comparing fifth (46% to 65%) to the third quintile was 1.60, P=0.03.² A report from the Hisayama Study showed that lower and higher extremes of hemoglobin were associated with higher risk of ischemic stroke in multivariable adjusted analyses, with no evidence of heterogeneity by sex.¹⁰ A study published in 1986 by Kiyohara et al.⁷ reported sex differences in the association between hematocrit and stroke with a higher risk of stroke among women in the low and high hematocrit group versus the normal hematocrit group although the results were only statistically significant when comparing the low with normal hematocrit groups in multivariable-adjusted models. Similar to our findings, no consistent relationship was found between hematocrit and stroke among men. The current study adds to this literature by showing a robust U-shaped relationship between hemoglobin concentration and stroke risk in women even after adjustment for established stroke risk factors, underscoring the importance of deviations in hemoglobin concentrations from the normal range as a risk factor for stroke in women.

### Table 1. Baseline Characteristics of Study Participants by Quartiles of Hemoglobin

<table>
<thead>
<tr>
<th>Hemoglobin Quartile</th>
<th>Quartile 1 (&lt;12.7 mg/dL)</th>
<th>Quartile 2 (12.7–13.5 mg/dL)</th>
<th>Quartile 3 (13.6–14.5 mg/dL)</th>
<th>Quartile 4 (&gt;14.5 mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>4191</td>
<td>4400</td>
<td>4963</td>
<td>4859</td>
</tr>
<tr>
<td>Age, y, mean (SD)</td>
<td>64.8 (10.2)</td>
<td>64.0 (9.9)</td>
<td>63.8 (9.4)</td>
<td>62.7 (9.2)</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>3589 (85.6)</td>
<td>3510 (79.8)</td>
<td>3144 (63.4)</td>
<td>1259 (25.9)</td>
</tr>
<tr>
<td>Black, n (%)</td>
<td>2609 (62.3)</td>
<td>1985 (45.1)</td>
<td>1589 (32.0)</td>
<td>1061 (21.8)</td>
</tr>
<tr>
<td>Region, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Belt</td>
<td>1493 (35.6)</td>
<td>1547 (35.2)</td>
<td>1756 (35.4)</td>
<td>1653 (34.0)</td>
</tr>
<tr>
<td>Buckle</td>
<td>1094 (26.1)</td>
<td>1140 (25.9)</td>
<td>1141 (23.0)</td>
<td>1114 (22.9)</td>
</tr>
<tr>
<td>Nonbelt</td>
<td>1604 (38.3)</td>
<td>1713 (38.9)</td>
<td>2066 (41.6)</td>
<td>2091 (43.0)</td>
</tr>
<tr>
<td>Body mass index, kg/m², mean (SD)</td>
<td>30.6 (7.2)</td>
<td>29.4 (6.6)</td>
<td>28.9 (6.0)</td>
<td>28.8 (5.3)</td>
</tr>
<tr>
<td>Waist circumference, cm, mean (SD)</td>
<td>95.5 (16.7)</td>
<td>92.9 (16.4)</td>
<td>93.9 (15.4)</td>
<td>97.7 (14.1)</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg, mean (SD)</td>
<td>127.3 (17.1)</td>
<td>125.6 (16.5)</td>
<td>125.8 (16.1)</td>
<td>126.3 (15.4)</td>
</tr>
<tr>
<td>Less than high school education, n (%)</td>
<td>663 (15.8)</td>
<td>470 (10.7)</td>
<td>449 (9.1)</td>
<td>370 (7.6)</td>
</tr>
<tr>
<td>Annual income &lt;$20,000 per y, n (%)</td>
<td>957 (22.8)</td>
<td>760 (17.3)</td>
<td>711 (14.3)</td>
<td>523 (10.8)</td>
</tr>
<tr>
<td>Comorbidities</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current smoking, n (%)</td>
<td>376 (9.0)</td>
<td>491 (11.2)</td>
<td>727 (14.7)</td>
<td>1011 (20.9)</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>1300 (31.2)</td>
<td>798 (18.3)</td>
<td>771 (15.6)</td>
<td>661 (13.7)</td>
</tr>
<tr>
<td>Atrial fibrillation, n (%)</td>
<td>414 (10.2)</td>
<td>337 (7.8)</td>
<td>352 (7.2)</td>
<td>389 (8.2)</td>
</tr>
<tr>
<td>Coronary heart disease, n (%)</td>
<td>693 (16.9)</td>
<td>610 (14.1)</td>
<td>752 (15.4)</td>
<td>818 (17.1)</td>
</tr>
<tr>
<td>Left ventricular hypertrophy, n (%)</td>
<td>461 (11.2)</td>
<td>394 (9.1)</td>
<td>372 (7.6)</td>
<td>320 (6.7)</td>
</tr>
<tr>
<td>Chronic pulmonary disease, n (%)</td>
<td>431 (10.3)</td>
<td>393 (8.9)</td>
<td>430 (8.7)</td>
<td>428 (8.8)</td>
</tr>
<tr>
<td>eGFR, mL/min per 1.73 m², mean (SD)</td>
<td>82.8 (25.3)</td>
<td>87.9 (19.7)</td>
<td>87.9 (17.7)</td>
<td>87.7 (16.2)</td>
</tr>
<tr>
<td>UACR, mg/g, median (IQR)</td>
<td>8.4 (13.7)</td>
<td>7.2 (9.1)</td>
<td>6.9 (8.7)</td>
<td>6.4 (8.5)</td>
</tr>
<tr>
<td>hsCRP, mg/L, median (IQR)</td>
<td>3.1 (1.3–7.3)</td>
<td>2.4 (1.0–5.5)</td>
<td>2.0 (0.9–4.5)</td>
<td>1.6 (0.8–3.7)</td>
</tr>
</tbody>
</table>

eGFR indicates estimated glomerular filtration rate; hsCRP, high-sensitivity C-reactive protein; IQR, interquartile range; and UACR, urine albumin:creatinine ratio.
incidence could potentially include increased blood viscosity reducing cerebral circulation, increased peripheral platelet activation, and increased oxidative stress from increased iron accumulation. At the other end of the hemoglobin spectrum, anemia could potentially lead to myocardial ischemia and LVH ultimately predisposing to development of stroke. It is also possible that anemia is a good marker of general illness related to chronic disease states that may predispose to the development of stroke.

The reason why hemoglobin was differentially associated with stroke risk by sex is unclear. Middle-aged women have a lower cardiovascular disease and stroke risk than age-matched men. Because of declining estrogen levels, the risk of stroke nearly doubles in women within 10 years post menopause and continues to increase exponentially such that older postmenopausal women have similar stroke rates as age-matched men. Estrogen deficiency is thought to promote cardiovascular disease by promoting structural and functional changes in the blood vessels and via effects on multiple elements of the coagulation pathway. It is plausible that estrogen deficiency among postmenopausal women could potentiate the pathological mechanisms behind the increased risk of stroke related to anemia and higher hemoglobin concentrations (discussed above), potentially explaining why both low and high hemoglobin concentrations were strongly associated with increased risk of stroke in women but not in men. Unfortunately, because >90% of women in this study fell in the postmenopausal age group, we did not have enough power to detect pre- and postmenopausal differences in stroke risk. In addition, a previous study suggested that physiological differences in oxygen-carrying capacity may make women less tolerant of lower hemoglobin concentrations than men with respect to stroke recovery, providing a possible explanation for why
lower hemoglobin was associated with higher risk of stroke in the current study.

In the Atherosclerosis Risk in Communities (ARIC) cohort, Abramson et al5 showed that the presence of CKD is associated with a higher incidence of stroke events, and that this association was modified by the presence of anemia such that individuals with CKD who were anemic had a higher risk of developing stroke when compared with individuals with CKD who were not anemic. However, in our study, the presence or absence of CKD did not modify the association between hemoglobin and stroke. This could potentially be explained by differences in the study populations. The ARIC study cohort was younger, had a lower prevalence of black individuals, and had a lower prevalence of major comorbidities such as coronary heart disease, diabetes mellitus, obesity, hypertension, and the use of antihypertensive medications than the REGARDS study, all of which may have attenuated effect modification of CKD (a CVD-equivalent condition) on the association of lower hemoglobin with stroke. Furthermore, stroke events in the ARIC study were captured from the mid-1980s to mid-1990s, a period of time in which cardiovascular disease treatment and prevention practices were much different from the period of time in which stroke events were captured in REGARDS (mid-2000s).31

Importantly, evidence from recent large randomized clinical trials suggests treating anemia of CKD with erythropoietin to a higher hemoglobin target is potentially related to worse outcomes in terms of stroke and other cardiovascular events.8–11 Based on the results of above trials, over the past decade, the target hemoglobin for treatment of anemia of CKD has been reduced. It is not entirely clear whether high doses of erythropoietin versus achieving a higher hemoglobin concentration itself in CKD leads to worse cardiovascular

### Table 2. Hazard Ratios (95% Confidence Interval) and Incidence Rates per 1000 Person-Years of Follow-Up (95% Confidence Intervals) of Incident Stroke According to Sex-Specific Quartiles (Q) of Baseline Hemoglobin

<table>
<thead>
<tr>
<th>Quartile</th>
<th>Men</th>
<th></th>
<th></th>
<th>Women</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Events</td>
<td>IR (95% CI)</td>
<td>Model 1, HR (95% CI)*</td>
<td>Model 2, HR (95% CI)†</td>
<td></td>
</tr>
<tr>
<td>Q1 (Men: &lt;13.7 g/dL; Women: &lt;12.4 g/dL)</td>
<td>1621</td>
<td>69</td>
<td>7.14 (5.64–9.04)</td>
<td>1.18 (0.83–1.68)</td>
<td>1.00 (0.68–1.49)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ref</td>
<td>Ref</td>
<td></td>
</tr>
<tr>
<td>Q2 (Men: 13.7–14.6 g/dL; Women: 12.4–13.2 g/dL)</td>
<td>1690</td>
<td>57</td>
<td>5.15 (3.97–6.68)</td>
<td>1.13 (0.79–1.62)</td>
<td>1.18 (0.80–1.73)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ref</td>
<td>Ref</td>
<td></td>
</tr>
<tr>
<td>Q3 (Men: 14.7–15.4 g/dL; Women: 13.3–14.0 g/dL)</td>
<td>1751</td>
<td>63</td>
<td>5.46 (4.27–6.99)</td>
<td>1.02 (0.70–1.48)</td>
<td>0.96 (0.64–1.44)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ref</td>
<td>Ref</td>
<td></td>
</tr>
<tr>
<td>Q4 (Men: &gt;15.4 g/dL; Women: &gt;14.0 g/dL)</td>
<td>1849</td>
<td>58</td>
<td>4.68 (3.61–6.05)</td>
<td>1.02 (0.70–1.48)</td>
<td>0.96 (0.64–1.44)</td>
<td></td>
</tr>
</tbody>
</table>

CI indicates confidence interval; HR, hazard ratio; and IR, incidence rate.

*Adjusted for age, race, and age×race interaction, region of residence.

†Adjusted for variables in model 1 plus estimated glomerular filtration rate, urine albumin:creatinine ratio, history of diabetes mellitus, atrial fibrillation, coronary heart disease, left ventricular hypertrophy and chronic pulmonary disease, current smoking, income, education, systolic blood pressure, body mass index, waist circumference, dyslipidemia, aspirin use, and log-transformed C-reactive protein.

### Table 3. Hazard Ratios (95% Confidence Interval) of Incident Stroke by Sex and Hemoglobin Categories

<table>
<thead>
<tr>
<th>Quartile</th>
<th>Men</th>
<th></th>
<th></th>
<th>Women</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Events</td>
<td>IR (95% CI)</td>
<td>Model 1*</td>
<td>Model 2†</td>
<td></td>
</tr>
<tr>
<td>Q1 (Men: &lt;13.7 g/dL; Women: &lt;12.4 g/dL)</td>
<td>1621</td>
<td>69</td>
<td>7.14 (5.64–9.04)</td>
<td>1.78 (1.29–2.45)</td>
<td>1.69 (1.09–2.31)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ref</td>
<td>Ref</td>
<td></td>
</tr>
<tr>
<td>Q2 (Men: 13.7 to 14.6 g/dL; Women: 12.4 to 13.2 g/dL)</td>
<td>1690</td>
<td>57</td>
<td>5.15 (3.97–6.68)</td>
<td>2.01 (1.23–3.28)</td>
<td>1.59 (1.09–2.31)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ref</td>
<td>Ref</td>
<td></td>
</tr>
<tr>
<td>Q3 (Men: 14.7 to 15.4 g/dL; Women: 13.3 to 14.0 g/dL)</td>
<td>1751</td>
<td>63</td>
<td>5.46 (4.27–6.99)</td>
<td>2.19 (1.55–3.09)</td>
<td>0.91 (0.59–1.38)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ref</td>
<td>2.15 (1.22–3.86)</td>
<td>0.91 (0.59–1.38)</td>
</tr>
<tr>
<td>Q4 (Men: &gt;15.4 g/dL; Women: &gt;14.0 g/dL)</td>
<td>1849</td>
<td>58</td>
<td>4.68 (3.61–6.05)</td>
<td>2.19 (1.55–3.09)</td>
<td>1.59 (1.08–2.35)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ref</td>
<td>Ref</td>
<td></td>
</tr>
</tbody>
</table>

*Adjusted for age, race, and age×race interaction, region of residence.

†Adjusted for variables in model 1 plus estimated glomerular filtration rate, urine albumin:creatinine ratio, history of diabetes mellitus, atrial fibrillation, coronary heart disease, left ventricular hypertrophy and chronic pulmonary disease, current smoking, income, education, systolic blood pressure, body mass index, waist circumference, dyslipidemia, aspirin use, and log-transformed C-reactive protein.
outcomes. Although a direct causal relationship could not be established, findings from our and other observational studies point toward a likely role of high hemoglobin concentration as an independent risk factor for poor cardiovascular outcomes in women.

Our study has several limitations. Because of the observational study design, we cannot make any causal inferences. Moreover, residual confounding could potentially influence our results. We had only 1 baseline measurement of hemoglobin and so were not able to determine whether longitudinal changes in hemoglobin were associated with stroke risk. Strengths of our study include a large cohort of black and white individuals, prospective study design, and well-documented and standardized data collection instruments, physician adjudicated stroke events, and a relatively large number of stroke events to power the overall study and sex-specific analyses.

In summary, in the current study, there was an independent, U-shaped association of hemoglobin with risk of incident stroke in women but not in men. Additional studies are needed to clarify potential reasons for these findings.

Acknowledgments

We thank the other investigators, the staff, and the participants of the Reasons for Geographic and Racial Differences in Stroke (REGARDS) study for their valuable contributions. A full list of participating REGARDS investigators and institutions can be found at http://www.regardsstudy.org.

Sources of Funding

This study was supported by a cooperative agreement U01 NS041588 and by R01NS080850 from the National Institute of Neurological Disorders and Stroke (NINDS), National Institutes of Stroke. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NINDS or the NIH. Additional funding was provided by an investigator-initiated grant-in-aid from Amgen Corporation. Amgen did not have any role in the design and conduct of the study or the preparation of the article. Dr Gutiérrez was further supported by P30DK079337.

Disclosures

None.

References

22. Di Tullio MR, Zwas DR, Sacco RL, Sciacca RR, Homma S. Left ventricular mass and geometry and the risk of ischemic stroke. Stroke. 2003;34:2380–2384. doi: 10.1161/01.STR.0000089680.77236.60.


Hemoglobin Concentration and Risk of Incident Stroke in Community-Living Adults
Bhupesh Panwar, Suzanne E. Judd, David G. Warnock, William M. McClellan, John N. Booth III, Paul Muntner and Orlando M. Gutiérrez

Stroke. 2016;47:2017-2024; originally published online July 5, 2016;
doi: 10.1161/STROKEAHA.116.013077

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2016 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/47/8/2017

Data Supplement (unedited) at:
http://stroke.ahajournals.org/content/suppl/2016/07/07/STROKEAHA.116.013077.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org//subscriptions/
ONLINE SUPPLEMENT

Hemoglobin concentration and risk of incident stroke in community-living adults

Bhupesh Panwar, MD, 1 Suzanne E. Judd, PhD, 2 David G. Warnock, MD, 1 William M. McClellan, MD, MPH, 4 John N. Booth III, MS, 1, 3 Paul Muntner, PhD, 1, 3 and Orlando M. Gutiérrez, MD, MMSc1, 3

Departments of 1Medicine, 2Biostatistics, and 3Epidemiology, University of Alabama at Birmingham, Birmingham, AL; 4Departments of Epidemiology and Medicine, Emory University, Atlanta, GA

Cover Title: Hemoglobin and Stroke in REGARDS

Supplemental Tables: 0 Supplemental Figures: 2

Keywords: hemoglobin, stroke, anemia, ischemic stroke, chronic kidney disease, cohort study, hematocrit

Subject Terms: Cerebrovascular Disease/Stroke, Ischemic Stroke, Epidemiology, Clinical Studies

Corresponding author: Orlando M. Gutiérrez, MD, MMSc, University of Alabama at Birmingham, ZRB 638, 1720 2nd AVE S, Birmingham, AL  35294-0006

phone: 205-996-2736    fax: 205-996-6465    email: ogutierr@uab.edu
SUPPLEMENTAL METHODS

Cox regression models with restricted quadratic splines were used to examine the association of baseline hemoglobin (modeled as a continuous variable) with risk of incident stroke stratified by sex. A two-tailed P value < 0.05 was considered statistically significant. All analyses were conducted using SAS software version 9.4 (SAS Institute, Cary, NC).
Supplemental Figure I. Hazard ratios of incident stroke as a function of baseline hemoglobin concentration in men. Hemoglobin was modeled as a continuous variable and fitted in a Cox proportional hazard model using restricted quadratic spline regression adjusted for age, race, age x race interaction, region of residence, eGFR, ACR, history of diabetes, atrial fibrillation, CHD, LVH and chronic pulmonary disease, current smoking, income, education, systolic blood pressure, body mass index, waist circumference, dyslipidemia, aspirin use and hsCRP. Knots for the spline were placed at the 10th, 50th and 90th percentiles of hemoglobin distribution for each sex, and the reference point was a hemoglobin of 14.2 g/dL for men. Dashed lines correspond to reference values. Shaded areas represent 95% confidence intervals for hazard ratios. Histograms present distributions of hemoglobin in study participants.
Supplemental Figure II. Hazard ratios of incident stroke as a function of baseline hemoglobin concentration in women. Hemoglobin was modeled as a continuous variable and fitted in a Cox proportional hazard model using restricted quadratic spline regression adjusted for age, race, age x race interaction, region of residence, eGFR, ACR, history of diabetes, atrial fibrillation, CHD, LVH and chronic pulmonary disease, current smoking, income, education, systolic blood pressure, body mass index, waist circumference, dyslipidemia, aspirin use and hsCRP. Knots for the spline were placed at the 10th, 50th and 90th percentiles of hemoglobin distribution for each sex, and the reference point was a hemoglobin of 12.8 g/dL for women. Dashed lines correspond to reference values. Shaded areas represent 95% confidence intervals for hazard ratios. Histograms present distributions of hemoglobin in study participants.