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Cerebral Oxygen Metabolic Stress is Increased in Children with Sickle Cell Anemia Compared to Anemic Controls

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Abstract

Patients with sickle cell anemia (SCA) experience cerebral metabolic stress with an increase in oxygen extraction fraction (OEF) to compensate for reduced oxygen carrying capacity due to anemia. It remains unclear if anemia alone drives this metabolic stress. Using MRI, we collected voxel-wise OEF measurements to test our hypothesis that OEF would be elevated in anemic controls without SCA (AC) compared to healthy controls (HC), but OEF would be even higher in SCA compared to AC. Brain MRIs (N=159) were obtained in 120 participants (34 HC, 27 AC, 59 SCA). While hemoglobin was lower in AC versus HC ($p<0.001$), hemoglobin was not different between AC and SCA cohorts ($p=0.459$). Whole brain OEF was higher in AC compared to HC ($p<0.001$), but lower compared to SCA ($p=0.001$). Whole brain OEF remained significantly higher in SCA compared to HC ($p=0.001$) while there was no longer a difference between AC versus HC ($p=0.935$) in a multivariate model controlling for age and hemoglobin. OEF peaked within the borderzone regions of the brain in both SCA and AC cohorts, but the volume of white matter with regionally elevated OEF in AC was smaller (1.8%) than SCA (58.0%). While infarcts colocalized within regions of elevated OEF, more SCA participants had infarcts than AC

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Ethics Approval and Patient Consent: This study was approved by the Institutional Review Board at Washington University in St. Louis. Informed consent was obtained from all participants or legal guardian if the participant was less than 18 years of age upon enrollment.

(p<0.001). We conclude that children with SCA experience elevated OEF compared to AC and HC after controlling for the impact of anemia, suggesting that there are other pathophysiologic factors besides anemia contributing to cerebral metabolic stress in children with SCA.

Keywords

Sickle cell anemia; anemia; oxygen extraction fraction; stroke

Introduction

Sickle cell anemia (SCA) is an autosomal recessive disease caused by a mutation in the beta-globin gene, resulting in the production of hemoglobin S. Hemoglobin S polymerizes in the deoxygenated state, distorting the shape of the red blood cell (RBC). In addition to obstructing the microcirculation due to misshapen RBCs with aberrant rheology, hemoglobin S-containing RBCs intravascularly hemolyze, causing severe anemia, intravascular inflammation and endothelial activation throughout all organ systems.[1] Specific to the brain, complications of SCA encompass silent cerebral infarctions,[2] overt stroke,[3] intracranial vasculopathy[2,4] and cognitive decline independent of infarction or vasculopathy.[5,6]

Cerebral blood flow is increased in SCA to compensate for decreased arterial oxygen content in the setting of severe anemia.[7–9] Furthermore, our research has shown that oxygen extraction fraction (OEF), the percent of oxygen extracted from the blood into the brain tissue, increases in patients with sickle cell anemia to meet the metabolic demands of the brain tissue.[9–11] OEF peaks within the border zone of the brain, where CBF nadirs.[9,12] Furthermore, the regions with a greatest increase in OEF align with regions of diminished functional connectivity[13] and greatest risk for infarction in patients with SCA.[9,12] Primary disease modification with an increase in arterial oxygen content via transfusion of hemoglobin A laden red blood cells relieves ongoing metabolic stress with a resultant decrease in cerebral blood flow and oxygen extraction fraction in patients with SCA.[11,14]

Anemia alone, in patients without SCD, has been associated with stroke[15–17] and aberrant cognitive and brain development.[18,19] Using a variety of techniques, including positron emission tomography (PET), MRI and near infrared reflectance spectroscopy (NIRS), CBF[20–25] and OEF[20,22,24–27]have been shown to increase as hemoglobin decreases in healthy individuals and patients with anemia associated with chronic kidney disease, hepatic encephalopathy, post-operative blood loss and subarachnoid hemorrhage. It is well established that the severity of anemia is a primary risk factor for the neurocognitive complications of SCA,[2,28,29] however it remains unclear if anemia alone drives the hemodynamic compromise experienced by patients with SCA, or if additional risk factors concomitantly contribute to metabolic stress. We undertook this MR imaging study to test our hypothesis that OEF would be elevated in anemic patients without SCA compared to controls, but OEF would be higher in patients with SCA compared to patients that were anemic for reasons other than SCA.

Methods

This study was approved by the Institutional Review Board at Washington University in St. Louis. Informed consent was obtained from all participants or legal guardian if the participant was less than 18 years of age upon enrollment. Data from three subgroups of participants from St. Louis Children's Hospital (SLCH) were analyzed: healthy control (HC), anemic control (AC) and sickle cell anemia (SCA) participants. HC participants were siblings of a patient with sickle cell disease (SCD, any genotype), but concomitant enrollment of the sibling with SCD was not required for participation. AC participants were eligible if they did not have SCD and their hemoglobin was less than the lower limit of normal for age and sex. Enrollment within the SCA subgroup was limited to participants with hemoglobin SS or hemoglobin S beta thalassemia null genotypes. Participants that were greater than 21 years of age upon initial enrollment, unable to tolerate the brain MRI without sedation, had a contraindication to MRI due to metal (e.g., braces or metal implantation), were receiving chronic transfusion therapy, or had a past medical history including stem cell transplant, gene therapy, overt stroke, vasculopathy (defined as a narrowing of the internal carotid or middle cerebral arteries on magnetic resonance angiography) or neurologic condition that could impact cerebral hemodynamics were excluded from participation. Intermittent simple transfusion of red blood cells was not an exclusion criterion. These cross-sectional analyses were performed with a combined dataset from two longitudinal imaging studies. Hence, a subset of participants has multiple timepoints of data included in the analyses.

A brain MRI and laboratory evaluation, including a complete blood count and hemoglobin electrophoresis, were obtained on the same day at each study visit. Hemoglobin was not obtained at the time of MRI in 10 HC participants, and 21 participants (13 HC, 2 AC and 6 SCA) did not have a hemoglobin electrophoresis obtained. Eighteen participants (3 HC, 2 AC and 13 SCA) had a CBC drawn on a different day than the brain MRI, drawn a mean (standard deviation, SD) of 16.3 (±20.5) days and range of 1–84 days from the brain MRI. Nine participants (2 HC, 2 AC, 5 SCA) had hemoglobin electrophoresis drawn on a different day than the brain MRI, drawn a mean (SD) of 13.2 (±11.4) days and range 1–34 days from the MRI. White blood cell differential and reticulocyte counts were not collected prospectively. These laboratory values were abstracted from the medical record if available. Missing laboratory values were not imputed, and participants with missing values were excluded from multivariate analyses.

Imaging Data and Processing

Each participant underwent a brain MRI on a Siemens 3T Trio or Prisma without sedation. A three-dimensional magnetization prepared rapid acquisition gradient echo (MP-RAGE) T1 (TE/TR = 2.13/2,400ms or 2.94/1,810ms, inversion time = 1,000 ms, flip angle 8 degrees, and acquired 1×1×1 mm voxel resolution) and axial or coronal fluid attenuated inversion recovery (FLAIR, 94/9,000 ms, inversion time = 2500 ms, flip angle = 150 degrees and 3mm or 5mm slice thickness) were collected during each scan. Voxel-wise measurement of OEF was obtained with an asymmetric spine echo (ASE) sequence.[30] A board-certified neurologist (KPG or ALF) identified and delineated infarcts

on FLAIR images in anemic participants with Medical Image Processing, Analysis and Visualization software (mipav.cit.nih.gov), and voxels within infarcts were excluded from OEF processing. FLAIR images from prior clinical or research scans were used if the image acquired during the study visit was contaminated by motion artifact. Three participants (2 AC, 1 SCA) did not have a FLAIR image available, and a FLAIR image was obtained for processing from a different research or clinical scan in 15 participants with SCA.

Statistical Parametric Mapping software (SPM) was used to create T1 tissue probability maps in native (T1) space.[31,32] A T1 to OEF warp was computed for each participant using Advanced Normalization Tools (ANTs), which was subsequently applied to the T1 image and probability maps.[33,34] FSL MCFLIRT was used to motion correct the ASE series with a common reference frame.[35] The ASE data was then decomposed into contributions from each tissue type (i.e. grey matter, white matter, CSF).[36] The tissue-specific ASE signal was independently processed to compute voxel-wise OEF per participant.[13] A hematocrit value is required for OEF processing, and linear regression accounting for age and sex was used to estimate hematocrit for control participants with missing lab values. ASE frames with high motion, defined as rotation > 0.04 radians in any direction or translation > 2mm in any direction, were excluded when computing OEF. Voxels with non-physiologic values (OEF<0.05 or OEF>0.95) or with high error (i.e. error value exceeding Otsu's threshold) were excluded on a participant-specific basis, as were lesioned voxels. To create a whole brain, partial volume corrected OEF map, gray and white matter OEF maps were combined into a weighted average image, where each OEF value was weighted by the probability of the associated tissue type.

Z-score Maps with Probabilistic Threshold Free Cluster Enhancement (pTFCE)

Individual whole brain partial volume corrected OEF maps were combined to create average OEF maps for the HC, AC, and SCA cohorts. Voxels with < 80% of the participants within the cohort having a defined OEF value were excluded from the average OEF map. The AC and SCA Z-score maps were created by subtracting the mean cohort value of that voxel from the mean value of the same voxel within the HC cohort. The difference in mean OEF was divided by the standard deviation of that voxel's value within the HC cohort. pTFCE incorporated neighborhood information to create an enhanced Z-score image. Gaussian random field theory-based Z-score thresholds were computed using the publicly available pTFCE R Package (https://github.com/spisakt/pTFCE).[37,38]

Infarct Density Heatmaps

For participants with infarcts identified on FLAIR images, the individuals' FLAIR images (in native space) were aligned to an atlas space (MNI 152) through the individual's T1. The resulting warp was subsequently applied to the lesion mask to transform the mask into atlas space using ANTs.[33,34] Individual lesion masks in atlas space were combined to create the lesion density heatmap.

Statistical Analyses

Median and interquartile range were used to describe continuous variables. A Mann-Whitney *U* test or Kruskal- Wallis test was used to compare continuous variables, while

a Chi-squared test was used to compare categorical variables. The Benjamini-Hochberg procedure was used to correct for multiple comparisons.

To investigate differences in OEF between cohorts while controlling for total hemoglobin, independent variables of age, cohort, total hemoglobin, and hemoglobin-squared (due to second-order polynomial fit) were entered into a general linear mixed model predicting whole brain OEF while adjusting for repeated subject observation.

A second model was built to explore hematologic variables associated with whole brain OEF within the SCA population. Candidate variables associated with whole brain OEF (white blood cell count, absolute neutrophil count, absolute lymphocyte count, platelet, mean platelet volume, total hemoglobin, mean corpuscular volume, reticulocyte, percent hemoglobin F, percent hemoglobin S, and percent hemoglobin A) were tested with Spearman's Rho and Chi-square tests when appropriate to determine a univariate association. Age was forced into the model due to the developmental range within the study population, and hemoglobin-squared was included due to second-order polynomial fit. Variables with a univariate association of $p < 0.20$ were entered into a naïve stepwise general linear mixed model predicting whole brain OEF while adjusting for repeated subject observation.

Results

Brain MRIs were obtained in 120 participants (34 HC, 27 AC, 59 SCA). A total of 159 brain MRIs were included in the analyses, with 4 HC participants contributing 2 scans each (separated by 2.5 – 3.9 years) and 26 SCA participants contributing between 2–4 scans each (separated by 28 days – 4.5 years). Table 1 provides a description of the cohort. Hemoglobin at the time of brain MRI for baseline scans was lower in the AC subgroup compared to HC ($p < 0.001$), but there was not a significant difference in hemoglobin between the AC and SCA subgroup ($p = 0.459$, Table 1). Within the SCA cohort, 9 (9.6%) of participants received a transfusion of red blood cells at SLCH within three months of the study visit. Eighty-three percent of participants with SCA were taking hydroxyurea at a median dose of 27.9 [22.1–33.1] mg/kg/day at the time of their study visit. The AC cohort was composed of patients with heterogeneous diagnoses resulting in anemia: iron deficiency anemia (N=9), congenital dyserythropoietic anemia (N=1), aplastic anemia (N=6), hereditary spherocytosis (N=5), pyruvate kinase deficiency (N=1), loxoscelism (N=1), non-transfusion dependent beta thalassemia intermedia (N=1), hemorrhage in the setting of severe hemophilia A (N=1) and participants that were enrolled as HC participants and incidentally found to be anemic (N=2).

OEF was higher in the AC cohort compared to HC across white matter ($p < 0.001$), gray matter ($p < 0.001$) and whole brain ($p < 0.001$), but remained lower in the AC cohort compared to SCA across tissue types (white matter ($p = 0.003$), gray matter ($p = 0.001$), whole brain ($p = 0.001$), Table 1, Figure 1A). Whole brain OEF within the SCA cohort remained elevated compared to HC (parameter estimate = 3.671, 95% CI 1.486, 5.857, $p = 0.001$) while there was no longer a significant elevation in AC compared to HC ($p = 0.935$) in a multivariate model controlling for age ($p=0.251$), hemoglobin (parameter estimate =

−7.878, 95% CI −10.195, −5.561, p < 0.001), and hemoglobin-squared (parameter estimate = 0.267, 95% CI 0.148, 0.386, p < 0.001, indicating that the relationship between OEF and hemoglobin is not linear, Figure 1B).

OEF has previously been shown to peak in the border zone regions of the brain where CBF nadirs in patients with SCA compared to HC participants.[9,12] Z-score maps were created to identify the regions of white matter with significantly elevated OEF in participants with SCA versus HC and AC participants versus HC. Figure 2 illustrates that OEF peaks within the border zone regions of the brain in both the SCA and AC cohorts. However, the volume of brain with significantly elevated OEF in the AC vs HC cohorts was smaller (1.8% of white matter) than the SCA vs. HC cohorts (58.0% of white matter). Infarcts identified within the study cohort colocalized with the regions of brain with significantly elevated OEF (Figure 3). While the distribution of infarcts within the anemic cohorts co-localized with the regions of elevated OEF, a greater percentage of participants with SCA (55.2%) had infarcts identified on FLAIR imaging than anemic controls (16.0%, p < 0.001). The volume of infarction was larger in the SCA cohort (8.1 [0.0–168.4] mm^3) versus the AC cohort (0.0 [0.0–0.0] mm^3, p = 0.002, Figure 3). However, within the individuals with an infarct present, the volume of infarcted brain was not significantly different between the affected SCA participants (158.0 [26.6–351.7] mm^3) compared to the AC participants (157.3 [64.3–335.1] mm^3, p = 0.880).

As OEF remained elevated in the SCA cohort after controlling for hemoglobin, multivariate analyses were performed within the SCA cohort to explore potential hematologic variables associated with this increase in OEF. After univariate correlation (Supplemental Table 1), white blood cell count, absolute lymphocyte count, percent hemoglobin F, percent hemoglobin A, mean platelet volume and mean corpuscular volume met criteria for entry into the naive regression model. OEF increased as hemoglobin F (parameter estimate = 0.069, 95% CI 0.015, 0.123, p = 0.015) increased using mixed model linear regression to control for multiple timepoints per participants, age (p = 0.944), hemoglobin (parameter estimate = −11.525, 95% CI −16.950, −6.101, p < 0.001), and hemoglobin-squared (parameter estimate = 0.448, 95% CI 0.150, 0.746, p = 0.005). The associations between OEF, hemoglobin, hemoglobin-squared and percent hemoglobin F persisted in analyses limited to participants with SCA receiving hydroxyurea therapy (Supplemental Table 1).

Discussion

These data show that children with anemia for reasons other than SCA have increased whole brain, white matter and gray matter OEF, a marker of metabolic stress, compared to healthy controls. However, OEF is significantly higher in children with SCA compared to these anemic controls, even when there is not a significant difference in total hemoglobin between cohorts and hemoglobin is controlled for in multivariate analyses. Anatomically, the border zone regions of the brain have the highest OEF in both the SCA and AC cohorts when compared to HC, but the volume of brain with significantly elevated OEF is higher in SCA than AC.

Anemia, acute or chronic, is associated with neurocognitive complications across all ages. Acute silent cerebral infarcts detected with diffusion weighted imaging have been identified during severe anemic episodes in children[15] and there is an increased risk of overall cognitive impairment, dementia and Alzheimer's disease, in adults with anemia.[39] Silent cerebral infarcts are seen in patients with beta thalassemia, both transfusion-independent and transfusion-dependent,[40–42] and there are long-term neurocognitive consequences of iron deficiency anemia that persist after iron repletion.[43–45] The mechanism of injury for these neurocognitive complications may differ by disease state. Iron deficiency directly impacts myelination, dopamine and serotonin synthesis and signaling, epigenetic regulation of gene transcription and energy metabolism via mitochondrial proteins,[43,45] while infarction in patients with thalassemia could be in part due to the hypercoaguable state associated with thalassemia.[46–48] However, the hemodynamic compromise associated with the decreased arterial oxygen content is common across disease states, potentially contributing to the resulting neurocognitive complications. Consistent with prior literature in anemic patient populations,[20,24,26,27,49,50] our data provides evidence that pediatric patients with anemia in disease states other than SCA experience hemodynamic compromise, as measured by increased OEF. We extend these findings to show that children are impacted while at steady state and not critically ill, and that the regions of greatest OEF elevation within the AC cohort fall within the watershed distribution.

There are clinical guidelines and standards of care for treatment of patients with disorders resulting in chronic anemia,[51–53] and guidelines for transfusion of patients with acute and chronic anemia.[54] These treatment algorithms are primarily contingent upon total hemoglobin or hemoglobin isoforms and, for non-hereditary anemias, rely on measures of hemodynamic compromise such as tachycardia or hypotension. Here, we show that anemia has cerebral hemodynamic consequences that are more subtle than symptoms of imminent hemodynamic collapse. Utilization of biomarkers that directly assess oxygen delivery and metabolic demand may better inform such guidelines to improve neurological outcomes.[24,26,49] With future investigations solidifying the link between neuroimaging biomarkers and neurocognitive outcomes of interest in patients with acute or chronic anemia, our data could potentially better define the threshold for intervention in these disease states.

The relationship between OEF and hemoglobin has been shown in anemic patients with[9,10] and without SCA.[20,24,26,27,49,50] However, our results demonstrate that OEF is significantly higher in patients with SCA compared to the AC cohort after controlling for total hemoglobin. This finding suggests that there are aspects of SCA physiology aside from decreased arterial oxygen content driving the compensatory increase in OEF. Prior literature has established that CBF increases as patients become more anemic, not only in SCA,[7,8,14,55–59] but also in healthy controls[22] and disease states other than SCA with concomitant anemia.[8,20,21,24–26,56] Oxygen delivery normalizes in SCA in the setting of increased CBF,[8] suggesting there is increased demand driving the elevation in OEF in the participants with SCA compared to AC. Markers of inflammation and hemolysis have been associated with increased CBF, another biomarker of hemodynamic compromise,[23,60] and inflammation has been linked to poor cognitive outcomes.[61,62] Anemia is an established risk factor for infarction in SCA[29] and current therapies used for primary disease modification

and neuroprotection improve total hemoglobin,[63–65] however, an improved understanding of the mechanism driving metabolic demand in patients with SCA could provide the field with future therapeutic targets.

Albeit a smaller impact than hemoglobin, we report a positive relationship between hemoglobin F and OEF, which is consistent with our prior work.[66] Fetal hemoglobin is left shifted with an increased oxygen affinity compared to hemoglobins A and S,[67] but the impact of hemoglobin F alone on OEF is challenging to discern as an increase in hemoglobin F decreases polymerization of hemoglobin S and hemolysis with a resultant reduction in inflammation and increase in total hemoglobin in patients with SCA. Furthermore, the medication used to induce hemoglobin F in patients with SCA, hydroxyurea, is myelosuppressive, reducing production of white blood cells and platelets that contribute to vascular injury and endothelial activation in SCA.[68] Hence, the impact of hemoglobin F induction in SCA is complex. A recent publication by Pedrosa and Lemes showed a decreased expression of hypoxia inducible factors (HIFs) in the setting of hydroxyurea.[69] While the relationship between HIFs and cardiovascular disease is well-established, it is unclear whether induction of HIFs is beneficial or detrimental in the setting of cerebral hemodynamic compromise.[70] Ultimately, the relationship between hemoglobin F and metrics of cerebral hemodynamic stress warrants further investigation as it not only pertains to patients with SCA in the setting of current treatment with hydroxyurea, but also with novel therapeutic options that induce hemoglobin F production or modify hemoglobin's oxygen affinity.[71,72] Furthermore, this line of investigation would potentially be relevant to neonatal patient populations and patients with ineffective erythropoiesis (e.g., thalassemia, congenital dyserythropoietic anemia) that commonly have increased hemoglobin F.

We report an increase in OEF in both anemic cohorts compared to healthy controls, which is corroborated by prior work utilizing different imaging modalities in a multitude of disease states.[14,20,22,24,25,27,49,50] Contrary to our results, Vu et al. reported that OEF was significantly lower in children and adults with anemia (chronic anemias and sickle cell disease) compared to healthy controls.[73] While the direction of difference differs between studies with our report of an increase in the setting of anemia and Vu et al. reporting a decrease with anemia, the two studies are in agreement that OEF in SCA differs significantly from participants with chronic anemias of other etiologies. Furthermore, we advance this area of investigation as we report a significant difference in OEF between the AC and SCA cohorts while controlling for hemoglobin while the AC cohort evaluated by Vu et al. had a significantly higher hemoglobin than the cohort with sickle cell disease.

The differences between our results and those published by Vu et al. are most likely multifactorial. First, there is variation in study populations, as Vu et al. included a heterogeneous population ranging in age from 12 to 63 years, including all sickle cell genotypes and a large percentage of both the AC and sickle cell cohorts were chronically transfused.[73] Second, we measure voxel-wise OEF with an ASE sequence[30] while Vu et al. utilized a TRUST sequence to obtain a flow-weighted measurement of deoxyhemoglobin in the superior sagittal sinus for a single, global assessment of OEF.[74] Patients with SCA may suffer from microvascular cerebral shunting with increased arterial transit time through the microvasculature preventing adequate offloading of oxygen,[75,76] resulting in a decreased

deoxyhemoglobin measurement in the sagittal sinus and a decreased OEF measurement. The impact of microvascular shunting on OEF measurements via ASE and TRUST may differ due to differences in technique. While both ASE and TRUST have been evaluated and tested in the setting of hypercapnia[30,74] and ASE has been validated with blood gas oximetry measurements in animal studies,[77] further research will be required to best understand how the two acquisition techniques can complement each other in the study of cerebral hemodynamics in patients with SCA.

Strengths of our investigation include the inclusion of a large cohort of children with severe anemia, without a significant difference in hemoglobin between the AC and SCA cohorts, and utilization of an ASE sequence to obtain voxel-wise measurements of OEF. However, there are limitations of this study. First, the cross-sectional design limits our analyses and conclusions to understanding associations between hemoglobin and OEF. Specific markers of inflammation and hemolysis were not prospectively collected, preventing full investigation into the association of these physiologic pathways with elevated OEF. This dataset is limited to the measurement of OEF, but collection of CBF and $CMRO_2$ will ultimately be necessary to understand consequences of elevated OEF in anemic patients. Lastly, the AC cohort consisted of a heterogeneous group of patients with different pathophysiology and both acute and chronic anemia. Longitudinal follow-up will be required to understand the impact of acute versus chronic elevation of OEF on brain development and clinical outcomes, such as stroke and cognition.

We conclude that OEF is elevated in children with non-sickle cell anemias compared to unaffected children, and that anemia is primarily driving this compensatory mechanism. However, children with SCA experience significantly higher OEF compared to children with non-sickle cell anemias after controlling for the impact of anemia, suggesting that there are additional pathophysiologic factors in SCA besides anemia that influence cerebral metabolic demand. An improved understanding of the covariates driving this increase in cerebral metabolic stress in SCA could provide future therapeutic targets for neuroprotection in this vulnerable population.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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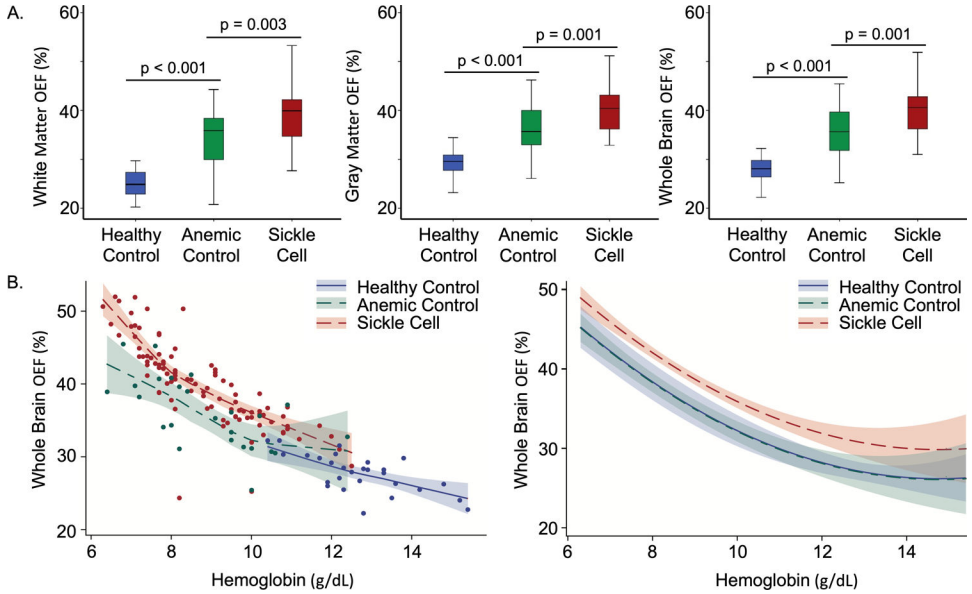

Figure 1. OEF remains elevated in the SCA cohort after controlling for hemoglobin.

A. OEF is elevated in anemic control participants (green) compared to healthy controls (blue), but significantly lower than participants with SCA (red) in white matter (left), gray matter (middle) and whole brain (right) even though there is not a significant difference in hemoglobin between the AC and SCA cohorts ($p = 0.459$). B. Whole brain OEF increases as hemoglobin decreases (Spearman's rho = −0.878, $p < 0.001$). Data is shown as a LOESS curve fit per cohort on the left. While controlling for age, hemoglobin, hemoglobin-squared and subject-specific effects, whole brain OEF remains significantly elevated in participants with SCA compared to HC ($p = 0.001$) but there is not a significant elevation in whole brain OEF in the AC participants compared to HC ($p = 0.935$) on the right. Figure displays hemoglobin effect plot computed at mean age of 12 years.

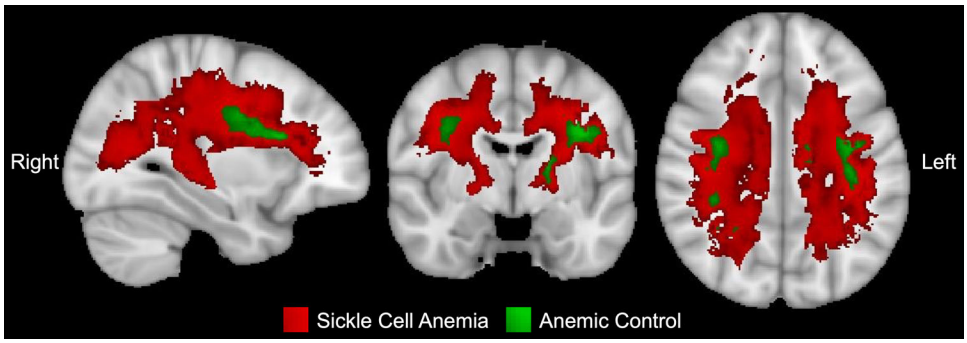

Figure 2. Regions of greatest elevation in OEF in SCA and AC compared to HC.

Regions of the white matter with significantly elevated OEF in the SCA (red) and AC (green) cohorts compared to HC ($p<0.05$ with probabilistic threshold free cluster enhancement) fall within the border zone of the brain. While the regions of elevated OEF align in both the SCA and AC cohorts, the volume of brain with elevated OEF is greater in the SCA cohort compared to AC.

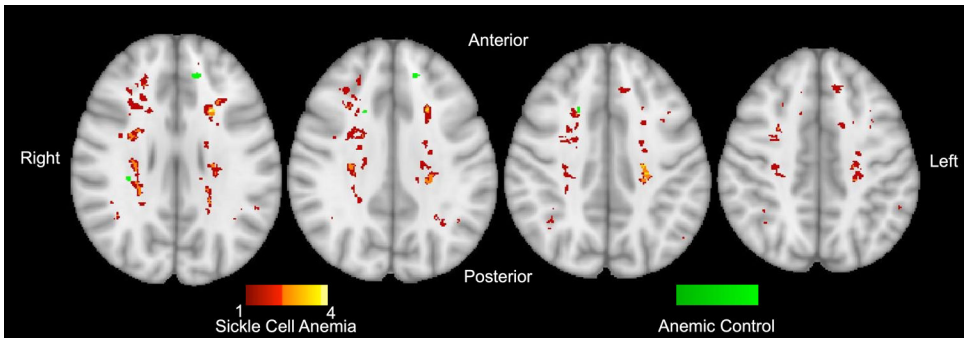

Figure 3. Infarct Burden in Anemic Cohorts.

Infarct count from the SCA cohort (red, orange, yellow) and AC cohorts (green) are overlaid on axial slices of an atlas moving inferior (left) to superior (right). In contrast to the SCA cohort, none of the infarcts in the AC cohort overlap with each other. A greater percentage of participants with SCA (55.2%) had infarcts compared to AC (16%, $p < 0.001$), and the volume of infarcted brain tissue was larger in the SCA cohort (8.1 [0.0–168.4] mm^3) versus the AC cohort (0.0 [0.0–0.0] mm^3, $p = 0.002$).

Table 1.

Cohort Description and OEF per Baseline Scan of Each Participant

	HC N = 34	AC N = 27	SCA N = 59	P-Value (HC vs. AC)	P-Value (AC vs. SCA)
Scan #	38	27	94	-	-
Age (Years)	11.0 [8.8–15.0]	14.0 [10.0–16.0]	10.0 [8.0–14.0]	0.122	0.024 *
Sex (M/F)	17/17	9/18	26/33	0.191	0.347
Hemoglobin (g/dL)	12.4 [11.9–13.2]	8.5 [7.7–10.0]	8.1 [7.7–9.6]	< 0.001 *	0.459
Hemoglobin A (%)	82.7 [60.8–97.2]	97.1 [92.7–97.5]	0.0 [0.0–0.0]	0.055	< 0.001 *
Hemoglobin F (%)	0.0 [0.0–0.2]	0.0 [0.0–0.9]	16.5 [9.5–25.1]	0.487	< 0.001 *
Hemoglobin S (%)	13.9 [0.0–35.1]	0.0 [0.0–0.0]	76.8 [68.8–83.8]	0.002 *	< 0.001 *
White Blood Cell (k/cumm)	5.7 [4.9–7.4]	5.7 [4.4–8.0]	9.9 [7.5–12.9]	0.925	< 0.001 *
Platelet (k/cumm)	285.0 [246.5–327.8]	280.0 [59.0–323.0]	396.0 [286.0–507.0]	0.509	< 0.001 *
White Matter OEF (%)	24.9 [22.8–27.4]	35.9 [29.6–38.4]	39.9 [34.5–42.2]	< 0.001 †	0.003 †
Gray Matter OEF (%)	29.6 [27.7–30.9]	35.7 [32.9–40.4]	40.4 [36.2–43.2]	< 0.001 †	0.001 †
Whole Brain OEF (%)	28.1 [26.3–29.8]	35.6 [31.3–39.7]	40.6 [36.2–42.8]	< 0.001 †	0.001 †

*Statistically significant

†Statistically significant after correction for multiple comparisons with Benjamini-Hochberg procedure