Regional oxygen extraction predicts border zone vulnerability to stroke in sickle cell disease

Melanie E. Fields, MD, MSCI,* Kristin P. Williams, MD, MSCI,* Dustin K. Ragan, PhD, Michael M. Binkley, MS, Cihat Eldeniz, PhD, Yasheng Chen, DSc, Monica L. Hulbert, MD, Robert C. McKinstry, MD, PhD, Joshua S. Shimony, MD, PhD, Katie D. Vo, MD, Allan Doctor, MD, Hongyu An, DSc, Andria L. Ford, MD, MSCI,† and Jin-Moo Lee, MD, PhD†

Correspondence

Dr. Lee
leejm@neuro.wustl.edu
or Dr. Ford
forda@wustl.edu

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Abstract

Objective

To determine mechanisms underlying regional vulnerability to infarction in sickle cell disease (SCD) by measuring voxel-wise cerebral blood flow (CBF), oxygen extraction fraction (OEF), and cerebral metabolic rate of oxygen utilization (CMRO₂) in children with SCD.

Methods

Participants underwent brain MRIs to measure voxel-based CBF, OEF, and CMRO₂. An infarct heat map was created from an independent pediatric SCD cohort with silent infarcts and compared to prospectively obtained OEF maps.

Results

Fifty-six participants, 36 children with SCD and 20 controls, completed the study evaluation. Whole-brain CBF (99.2 vs 66.3 mL/100 g/min, $p < 0.001$), OEF (42.7% vs 28.8%, $p < 0.001$), and CMRO₂ (3.7 vs 2.5 mL/100 g/min, $p < 0.001$) were higher in the SCD cohort compared to controls. A region of peak OEF was identified in the deep white matter in the SCD cohort, delineated by a ratio map of average SCD to control OEF voxels. CMRO₂ in this region, which encompassed the CBF nadir, was low relative to all white matter ($p < 0.001$). Furthermore, this peak OEF region colocalized with regions of greatest infarct density derived from an independent SCD cohort.

Conclusions

Elevated OEF in the deep white matter identifies a signature of metabolically stressed brain tissue at increased stroke risk in pediatric patients with SCD. We propose that border zone physiology, exacerbated by chronic anemic hypoxia, explains the high risk in this region.

*Drs. Fields and Williams contributed equally and are designated as co-first authors.

†Drs. Ford and Lee contributed equally and are designated as co-senior authors.

From the Division of Pediatric Hematology/Oncology (M.E.F., M.L.H.), Division of Pediatric Neurology (K.P.G.), Division of Pediatric Critical Care Medicine (K.P.G., A.D.), Department of Neurology (D.K.R., Y.C., A.L.F., J.-M.L.), and Mallinckrodt Institute of Radiology (C.E., R.C.M., J.S.S., K.D.V., H.A., J.-M.L.), Washington University School of Medicine; and Department of Biomedical Engineering (M.B.M., J.-M.L.), Washington University, St. Louis, MO.

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GLOSSARY

CBF = cerebral blood flow; CMRO₂ = cerebral metabolic rate of oxygen utilization; FLAIR = fluid-attenuated inversion recovery; HbA = hemoglobin A; HbS = hemoglobin S; HbSβthal⁰ = hemoglobin S-β thalassemia null; HbSS = hemoglobin SS; MCA = middle cerebral artery; MRA = magnetic resonance angiography; OEF = oxygen extraction fraction; SCD = sickle cell disease; SCI = silent cerebral infarct; TCD = transcranial Doppler.

Sickle cell disease (SCD) is caused by an autosomal recessive mutation in the β-globin gene, resulting in abnormal hemoglobin. The mutation decreases the oxygen affinity of hemoglobin and induces polymerization under hypoxic conditions, causing chronic hemolytic anemia with downstream microcirculatory obstruction, endothelial activation, inflammation, and tissue ischemia.^{1,2} In the brain, these pathologic processes result in ischemic stroke. Historically, 10% of individuals with SCD had overt strokes by age 20.³ In the modern treatment era, 39% have silent cerebral infarcts (SCIs) by age 18 associated with cognitive decline.⁴ Strokes in patients with SCD commonly occur in the border zones, or watershed regions, even in the absence of large vessel vasculopathy.^{5–7} Mechanisms underlying this regional vulnerability are not well understood. Improved understanding may enable optimization of screening tools and primary preventive therapies for stroke in patients with SCD.

PET studies in non-SCD ischemic stroke have elucidated the compensatory responses of the brain to compromised cerebral perfusion. Autoregulatory arteriolar dilation to maintain normal cerebral blood flow (CBF) and increases in oxygen extraction fraction (OEF; the fraction of oxygen brain tissue extracts from the blood) help maintain the cerebral metabolic rate of oxygen utilization (CMRO₂) under conditions of declining perfusion pressure.^{8–10} Infarction results when these compensatory mechanisms are insufficient to meet the metabolic demands of the tissue, reflected by a decline in CMRO₂.¹¹ Elevated hemispheric OEF is a strong predictor of stroke risk in adult patients with carotid occlusive disease.¹²

Children with SCD have globally elevated CBF compared to healthy children¹³ to compensate for decreased arterial oxygen content (CaO₂) in the setting of anemia. As an additional compensatory mechanism, adults with SCD demonstrate increased whole-brain OEF compared to healthy controls,¹⁴ but regional variation of cerebral oxygen metabolism has not been studied in pediatric SCD because of technical limitations of extant MRI sequences. In this study, we used a magnetic resonance sequence measuring voxel-wise OEF in children with SCD and controls to evaluate regional OEF within territories known to be at high risk for infarction in SCD, hypothesizing that OEF may demonstrate greater elevation in these high-risk regions.

Methods

Participants 5 to 21 years of age with hemoglobin SS (HbSS) or hemoglobin S-β thalassemia null (HbSβthal⁰) and healthy sibling controls were prospectively enrolled. Siblings were

recruited to control for socioeconomic and nutritional environment, which are known to influence brain development.¹⁵ Participants with SCD were excluded if they were receiving chronic transfusion therapy at the time of MRI or had a history of overt stroke, cerebral vasculopathy, stem cell transplantation, or neurologic disorder not related to SCD. Controls were excluded for history of neurologic disorder. A board-certified neuroradiologist reviewed all MRIs, magnetic resonance angiographies (MRAs), and transcranial Dopplers (TCDs). Participants with SCD and narrowing of the distal internal carotid artery and/or proximal middle cerebral artery (MCA) on MRA were excluded. When MRA was not obtained, participants with elevated TCD velocity in the MCA, defined as a time-averaged maximum velocity >170 cm/s, were excluded. Children with SCD and SCIs, defined as lesions ≥3 mm in diameter that are seen on 2 planes (axial and coronal) of fluid-attenuated inversion recovery (FLAIR) images,¹⁶ were included to specifically evaluate this subgroup.

Presence of the hemoglobin S (HbS) trait was determined by controls' newborn screen results or hemoglobin capillary gel electrophoresis. Peripheral oxygen saturation (SpO₂) was obtained at the time of MRI. CaO₂ was calculated as 1.36 × hemoglobin (g/dL) × SpO₂. Hemoglobin used in the CaO₂ calculation was corrected by subtracting the absolute dyshemoglobin concentration (methemoglobin plus carboxyhemoglobin) from the total hemoglobin concentration because of known elevated dyshemoglobins in patients with SCD.^{17,18} Linear regression accounting for age and sex estimated hemoglobin for controls who did not undergo laboratory evaluation. Data imputation for hemoglobin estimation was calculated from a cohort including additional participants not included in this prospective analysis. The average carboxyhemoglobin and methemoglobin values for the SCD and control cohorts were used for participants (2 with SCD and 13 controls) who did not have venous hemoglobin saturation performed.

Standard protocol approvals, registrations, and patient consents

The Institutional Review Board at Washington University in St. Louis approved this study, and written informed consent was obtained from all participants or guardians of participants.

Imaging protocol and analysis

Participants underwent a brain MRI with a Siemens 3T Trim Trio (Erlangen, Germany) with a 12-channel head coil without sedation. Magnetization-prepared rapid gradient-echo T1 (echo time/repetition time = 2.94/1,800 milliseconds, TI = 1,000 milliseconds, flip angle = 8°, 1 × 1 × 1-mm voxel resolution),

FLAIR acquired in the axial and coronal planes (echo time/repetition time = 93/9,000 milliseconds, TI = 2,500 milliseconds, 0.86×0.86 -mm in-plane resolution, 5-mm slice thickness), and MRA were obtained. CBF was measured with a pseudo-continuous arterial spin labeling sequence (echo time/repetition time = 12/3,280 milliseconds, in-plane voxel resolution = 3×3 mm, slice thickness = 5 mm, 22 slices, number of averages = 80, labeling duration = 2 seconds, postlabel delay = 1,000 milliseconds).¹⁹ CBF processing did not include background suppression. Blood T1, required for CBF quantification, depends on hematocrit. Individually measuring blood T1 improves CBF reproducibility.^{20–22} Blood T1 was measured in the superior sagittal sinus with an inversion-recovery echo planar imaging sequence with an adiabatic nonselective inversion pulse. OEF was measured with an asymmetric spin echo sequence from which tissue deoxyhemoglobin was measured, permitting quantification of tissue oxygen saturation and OEF. Quantification of voxel-wise CBF and OEF has been described previously.^{23,24} Censorship of voxels exhibiting abnormal χ^2 residuals, as a metric of high error when calculating OEF, was required to prevent spurious results. An a priori nonparametric unidirectional exclusion of all data producing residuals above the 65th percentile of the white matter, as a result of the Gibbs artifact affecting primarily gray matter, was applied. We used a multiple gradient echo sequence to measure volume susceptibility difference between oxygenated and deoxygenated blood, $\Delta\chi_o$, of HbS and hemoglobin A (HbA) from 6 patients with SCD and 6 controls in a separate study. Using a multivariate generalized linear mixed model with random patient effect, we did not find a significant difference between the $\Delta\chi_o$ of HbS and HbA.²⁵ Therefore, we used a published $\Delta\chi_o$ (0.18 cgs, ppm) in the OEF calculation for all participants.²⁶ CMRO₂ within each voxel was calculated as the product of CBF $\times$ OEF $\times$ CaO₂, an equation derived from the Fick law.²⁷

For tissue-type CBF, OEF, and CMRO₂ analyses, magnetization-prepared rapid gradient-echo T1 images were segmented to classify gray and white matter with the FMRIB Automated Segmentation Tool.²⁸ Partial volume effects were minimized with 1-voxel morphologic erosion for gray and white matter. Coregistration aligned all images within a scan using the FMRIB Linear Image Registration Tool.^{29,30} SCIs were manually delineated on the FLAIR map by a board-certified vascular neurologist (A.L.F.) and removed from all analyses.

To evaluate regional variation of OEF in the SCD cohort, OEF maps from patients with SCD and controls were coregistered to the International Consortium for Brain Mapping atlas³¹ with Advanced Normalization Tools³² and averaged into 2 respective maps: an average SCD OEF map and an average control OEF map. A voxel-wise ratio of the 2 average OEF maps (SCD: control) was thresholded at >1.6, 1.7 (95th percentile of the ratio) and 1.8 to delineate the region of greatest OEF elevation in patients with SCD compared to controls. Three ratios were applied to ensure consistency of results regardless of chosen threshold. These 3 regions were applied to individual maps to calculate individual regional measures of CBF, OEF, and

CMRO₂. White matter from these regions was compared to all white matter for regional analyses because the 3 regions comprised primarily white matter (average SCD:control OEF ratio of 1.6 in participants with SCD, 88.1% [interquartile range (IQR): 87.0%–89.1%] white matter; average SCD:control OEF ratio 1.7 in participants with SCD, 89.9% [IQR: 87.6%–91.1%] white matter; average SCD:control OEF ratio 1.8 in participants with SCD, 100.0% [IQR: 95.1%–100.0%] white matter).

Infarct heat map

To define the regions at highest risk for infarction, clinically obtained brain MRIs on a 1.5T Siemens Sonata, 1.5T Siemens Avanto, or 3.0T Siemens Trio at St. Louis Children's Hospital from 2004 to 2014 in patients with HbSS or HbS β thal⁰ with SCIs were collected. Patients receiving chronic transfusion therapy at the time of MRI; those with a history of overt stroke, cerebral vasculopathy, or stem cell transplantation; or patients enrolled in the prospective cohort were excluded from heat map analysis. SCIs were manually delineated on FLAIR images with Medical Image Processing, Analysis and Visualization software (mipav.cit.nih.gov, A.F. and K.P.G.). Methods to create the infarct heat map have been described previously.⁶ The infarct heat map illustrates infarct density expressed as the percent of hemispheres with an infarct that falls in each voxel.

Statistical analysis

Data are presented as median (interquartile range). Cohort descriptors, laboratory parameters, and measures of CBF, OEF and CMRO₂ were compared with a Mann-Whitney U or Pearson χ^2 test (tables 1 and 2, sex comparison between control subgroups divided by trait status, and age comparison between control subgroups divided by acquisition of laboratory values). Sex was compared between control subgroups divided by acquisition of laboratory values with a Fisher exact test. The Kruskal-Wallis test was used to compare age, CBF, OEF, and CMRO₂ across control subgroups divided by trait status. Bivariate correlations are described with the Spearman rank correlation coefficient. Relevant clinical predictors with significant univariate correlations were entered into a multivariate linear regression model. Regional values of CBF, OEF, and CMRO₂ were compared within participant to white matter values with the Wilcoxon signed-rank test. Significance was determined with a value of $p < 0.05$ except for whole-brain and segmented analyses between cohorts and regional analyses compared to whole brain white matter, for which the Benjamini-Hochberg procedure was used to control for an overall family-wise error rate of 0.05. All statistical analyses were performed with SAS (version 9.4, SAS Institute Inc, Cary, NC).

Results

Fifty-six participants (36 children with SCD and 20 controls) underwent brain MRI (table 1). Thirty-three of the participants with SCD had HbSS disease, and 3 had HbS β thal⁰ disease. Nine controls had hemoglobin AA; 6 had HbS trait; and trait status was unknown in the remaining 5 controls. However, there was no difference in age ($p = 0.418$) or sex

Table 1 Participant characteristics

	Control cohort (n = 20)	SCD cohort, (n = 36)	p Value	SCD without SCI (n = 22)	SCD with SCI (n = 14)	p Value
Age, y	11.0 (7.5–13.5)	10.0 (7.5–11.0)	0.349	10.0 (8.0–11.0)	9.0 (7.0–11.0)	0.613
Female, n (%)	8 (40.0)	18 (50.0)	0.580	12 (54.5)	6 (42.9)	0.733
Hydroxyurea therapy, n (%)	—	21 (58.3)	—	14 (63.6)	7 (50.0)	0.499
Hemoglobin at scan, g/dL	11.7 (10.7–12.9)	8.1 (7.6–9.6)	<0.001 ^a	9.1 (7.7–9.7)	8.0 (7.4–8.3)	0.091
Hemoglobin S, %	0.0 (0.0–33.5)	77.6 (69.1–82.7)	<0.001 ^a	75.6 (66.8–80.2)	81.0 (72.6–85.4)	0.131
Hemoglobin F, %	0.0	16.7 (11.4–25.5)	<0.001 ^a	21.4 (12.9–29.1)	15.1 (8.4–21.0)	0.145
Carboxyhemoglobin, %	0.5 (0.4–0.8)	2.3 (1.8–2.9)	<0.001 ^a	2.2 (1.8–2.6)	2.6 (1.9–2.9)	0.293
Methemoglobin, %	1.1 (1.0–1.4)	2.1 (1.9–2.3)	<0.001 ^a	2.1 (1.8–2.3)	2.2 (2.1–2.4)	0.427
Spo ₂ , %	99.0 (98.5–100.0)	97.5 (94.5–99.0)	0.009 ^a	99.0 (96.0–99.0)	95.5 (94.0–99.0)	0.139

Abbreviations: SCD = sickle cell disease; SCI = silent cerebral infarct; Spo₂ = peripheral oxygen saturation.

^a Statistically significant with $p < 0.05$.

($p = 0.812$) between these subgroups of controls. Laboratory measurements were obtained in all participants with SCD and 9 controls, while hemoglobin was imputed in 11 controls. There was no difference in age ($p = 0.181$) or sex ($p = 1.000$) between controls who underwent laboratory evaluation and those who did not. MRA was obtained in 29 (80.6%) participants with SCD. The remaining 7 participants with SCD were screened with TCD for vasculopathy and had a time-averaged maximum velocity <170 cm/s in bilateral MCAs within 1.25 years of study evaluation.

Whole-brain CBF, OEF, and CMRO₂ were higher in the SCD cohort compared to controls (figure 1, A and table 2). CBF, OEF, and CMRO₂ were elevated in children with SCD in both gray and white matter. Within the SCD cohort, whole-brain and segmented OEF were higher in participants with SCIs compared to participants without SCIs, but the differences were nonsignificant. There was no difference in whole-brain or segmented measures of CBF, OEF, or CMRO₂ in controls with hemoglobin AA, HbS trait, or unknown trait status (table e-1, links.lww.com/WNL/A275).

Whole-brain CBF correlated with CaO₂ ($\rho = -0.743$, $p < 0.001$, figure 1, B) within the study population. Consistent with previous reports,²¹ we also found a correlation between age and CBF ($\rho = -0.422$, $p = 0.001$). The relationship between CBF and CaO₂ persisted after controlling for age (model $R^2 = 0.582$, $p < 0.001$; CaO₂: $\beta = -5.128$, 95% confidence interval -6.516 to -3.741 , $p < 0.001$; age: $\beta = -1.574$, 95% confidence interval -2.793 to -0.356 , $p = 0.012$). Likewise, CaO₂ and whole-brain OEF correlated ($\rho = -0.965$, $p < 0.001$) within the study population; however, in contrast to CBF, OEF did not correlate with age ($\rho = 0.136$).

A region of elevated OEF in the deep white matter was identified in individual OEF maps from children with SCD

(figure 2, A). To delineate the regions of greatest OEF elevation, we created a voxel-by-voxel ratio map calculated as: [average OEF values in patients with SCD]:[average OEF values in controls]. Figure 2, B and C illustrates the anatomic regions of the brain identified by thresholding SCD:control OEF ratios at 1.6, 1.7, and 1.8 and the relationship of these regions to CBF. In the SCD cohort, OEF was higher within these contours compared to all white matter in the brain (table 3), indicating peak white matter OEF in this region. In contrast, CBF was lower within these regions compared to all white matter, indicating that these regions represent the internal border zone. Moreover, the OEF ratio increased as regional CBF decreased, and mean CMRO₂ was lower in this region compared to all white matter.

To determine the relationship between regional OEF elevation and infarct vulnerability, we created an infarct heat map using an independent cohort of 23 children with HbSS disease and SCIs. The median age of participants included in the heat map was 16.0 (10.0–19.0) years, and 17 (74%) participants were female. Infarct density in this retrospective cohort was highest in the deep white matter and colocalized with the region of elevated OEF identified in the prospective cohort (figure 2, D). Nineteen (83%) participants included in the heat map had infarcts that fell within the region of high OEF (threshold ratio 1.6). Moreover, the cumulative infarct density, defined as total population infarct burden per volume of region (regions were delineated in the prospective cohort and applied to the infarct heat map), increased with each increment of OEF ratio (figure 2, E).

Discussion

In this study, an MRI pulse sequence measuring voxel-wise OEF²³ allowed us to examine regional variation of OEF in

Figure 1 Increased whole-brain CBF and OEF in SCD in response to decreased CaO_2

SCD. We identified a region of elevated OEF in the deep white matter in children with SCD, which overlapped with the CBF nadir, suggesting coincidence with the internal border

zone. Moreover, CMRO_2 was lower within this region compared to white matter, suggesting that oxygen supply was inadequate to meet regional metabolic demands. Indeed, the

Table 2 CBF, OEF, and CMRO_2 in control and SCD cohorts

		Control	SCD	<i>p</i> Value	SCD without SCI	SCD with SCI	<i>p</i> Value
CBF	Whole brain	66.3 (58.2–77.2)	99.2 (85.5–116.6)	<0.001 ^a	96.3 (87.2–112.1)	102.7 (78.2–120.7)	0.935
	Gray matter	83.4 (69.7–99.8)	122.9 (101.0–140.9)	<0.001 ^a	119.2 (106.0–136.0)	128.0 (88.1–146.1)	1.000
	White matter	41.7 (35.9–48.6)	60.1 (55.2–72.7)	<0.001 ^a	60.1 (54.6–72.3)	60.8 (55.8–82.4)	0.758
OEF	Whole brain	28.8 (27.3–31.2)	42.7 (37.2–45.3)	<0.001 ^a	39.3 (35.9–44.7)	43.9 (42.0–45.9)	0.077
	Gray matter	29.8 (27.9–32.3)	42.9 (37.4–46.1)	<0.001 ^a	40.1 (36.4–45.1)	44.1 (41.9–46.9)	0.089
	White matter	27.2 (25.5–29.6)	41.7 (36.2–45.5)	<0.001 ^a	37.8 (35.7–43.4)	44.1 (41.7–45.7)	0.042
CMRO₂	Whole brain	2.5 (2.2–2.8)	3.7 (3.3–4.3)	<0.001 ^a	3.7 (3.4–4.0)	3.9 (2.7–4.6)	0.973
	Gray matter	3.2 (2.9–3.7)	4.7 (4.0–5.6)	<0.001 ^a	4.7 (4.3–5.2)	4.8 (3.2–6.0)	0.973
	White matter	1.8 (1.5–2.1)	2.6 (2.2–3.1)	<0.001 ^a	2.6 (2.2–3.1)	2.6 (2.2–3.1)	0.920

Abbreviations: CBF = cerebral blood flow; CMRO_2 = cerebral metabolic rate of oxygen utilization; OEF = oxygen extraction fraction; SCD = sickle cell disease; SCI = silent cerebral infarct.

^a Statistically significant. Raw *p* values reported with significance calculated with the Benjamini-Hochberg-adjusted *p* values to maintain a family-wise error rate of 5%.

Figure 2 Increased OEF in the internal border zone predicts regional vulnerability to infarction

(A) Axial view of magnetic resonance OEF maps are shown from an 11-year-old control participant on the left and an 11-year-old participant with SCD on the right. Both global and regional (blue arrows) elevations in OEF are seen in the SCD participant. (B) Ratiometric maps of OEF were derived from the ratio of SCD to control OEF values on a voxel-wise basis using averaged maps from each cohort. Shown in the axial (left), sagittal (middle), and coronal (right) views, contours represent OEF ratios of 1.6, 1.7 (the 95th percentile of ratios) and 1.8. In children with SCD, OEF was elevated within this region compared to all white matter in the brain, while both CBF and CMRO₂ were significantly decreased. (C) Region of high OEF (threshold 1.6, outlined in blue) is overlaid on the average CBF map from the SCD cohort. (D) This region of elevated OEF (threshold 1.6, outlined in blue) also coincides with the region of highest infarct density created from an independent cohort of 23 participants with SCD. (E) Voxel-by-voxel analyses demonstrate the relationship between CBF, OEF, and infarct burden. Decreasing CBF and increasing cumulative infarct density are associated with increasing OEF, suggesting that limited blood flow within the watershed region increases oxygen stress, enhancing vulnerability to infarction. CBF = cerebral blood flow; CMRO₂ = cerebral metabolic rate of oxygen utilization; OEF = oxygen extraction fraction; SCD = sickle cell disease.

highest density of infarcts from an independent cohort of children with SCD fell within this region.

CMRO₂ is defined as the product of CBF, OEF, and CaO₂²⁷; therefore, CBF, OEF, and CaO₂ can independently adjust to maintain adequate CMRO₂. In adults with carotid occlusive disease, CBF declines in the carotid arterial territory, resulting in increased hemispheric OEF to maintain CMRO₂. However, infarction ensues if OEF is insufficient to maintain CMRO₂.¹⁰ This metabolic pattern defines ischemic

physiology caused by CBF restriction, and elevated OEF is a strong predictor of future stroke in this setting.¹² Unlike carotid occlusion, SCD results in chronically low CaO₂ (oxygen-carrying capacity) owing to hemolytic anemia. As compensatory responses, we observed increases in both whole-brain CBF and OEF in children with SCD compared to controls, similar to recent findings in adults with SCD.¹⁴ This metabolic pattern defines ischemic physiology due to CaO₂ restriction. Whether elevated OEF is a predictor of future stroke in this population remains to be determined.

Table 3 Region of high OEF in children with SCD

		White matter within region of high OEF	White matter within the whole brain	<i>p</i> Value
Threshold ratio of 1.6 in average SCD:control OEF maps	CBF, mL/100 g/min	42.2 (36.7–52.8)	60.1 (55.2–72.7)	<0.001 ^a
	OEF, %	44.3 (37.7–48.6)	41.7 (36.2–45.5)	<0.001 ^a
	CMRO ₂ , mL/100 g/min	2.0 (1.7–2.6)	2.6 (2.2–3.1)	<0.001 ^a
Threshold ratio of 1.7 in average SCD:control OEF maps	CBF, mL/100 g/min	43.9 (36.8–53.7)	60.1 (55.2–72.7)	<0.001 ^a
	OEF, %	46.1 (40.4–51.1)	41.7 (36.2–45.5)	<0.001 ^a
	CMRO ₂ , mL/100 g/min	2.2 (1.8–2.7)	2.6 (2.2–3.1)	<0.001 ^a
Threshold ratio of 1.8 in average SCD:control OEF maps	CBF, mL/100 g/min	39.0 (34.2–48.2)	60.1 (55.2–72.7)	<0.001 ^a
	OEF, %	49.7 (41.6–57.6)	41.7 (36.2–45.5)	<0.001 ^a
	CMRO ₂ , mL/100 g/min	2.3 (1.6–2.8)	2.6 (2.2–3.1)	0.001 ^a

Abbreviations: CBF = cerebral blood flow; CMRO₂ = cerebral metabolic rate of oxygen utilization; OEF = oxygen extraction fraction; SCD = sickle cell disease.
^a Statistically significant. Raw *p* values reported with significance calculated with the Benjamini-Hochberg-adjusted *p* values to maintain a family-wise error rate of 5%.

Regional changes in CBF, OEF, and CMRO₂ were apparent in the deep white matter in the SCD cohort. We believe that this pattern of regional metabolic change within the white matter—peak in OEF associated with nadir of CBF and CMRO₂—represents border zone physiology. The combination of a decrease in CaO₂ in children with SCD and restricted CBF in the border zone conspires to compromise oxygen delivery to this region of the brain. Infarct vulnerability occurs when there is mismatch between oxygen supply and metabolic demand. CMRO₂ was relatively decreased within this deep white matter region in patients with SCD, despite the elevation in OEF, suggesting a failure to meet metabolic demand. Consistent with this concept, the region we identified with a nadir of CBF, peak in OEF, and relative decrease in CMRO₂ colocalized with the region of high infarct density in an independent cohort of children with SCD. Even without prior demonstration of this border zone physiology, it has been observed that infarcts in children with SCD fall within an anatomic region consistent with the internal border zone.^{5,6} Moreover, studies using longitudinal morphometric analyses and diffusion tensor imaging have shown aberrant white matter development in SCD.^{33–35} Metabolic changes associated with vulnerability in this region may be generalizable to a variety of diseases associated with global metabolic compromise to the brain, including watershed infarction, white matter disease of the elderly, chronic pulmonary disease, congenital heart disease, and other anemias.

The region of elevated OEF appears to better align with the region of high infarct density compared to that of the CBF nadir. Indeed, there are regions of low CBF that do not overlap with high infarct density. The metabolic demand of the brain is spatially heterogeneous, as evidenced by differences in CMRO₂ between white and gray matter and between cortical and deep gray matter.^{36,37} Infarction is the ultimate

outcome of unfulfilled metabolic demand. Our data suggest that elevated OEF could be a better indicator of metabolic stress than low CBF.

We also observed global increases in CMRO₂ in children with SCD due to elevated CBF and OEF. Global elevation in CMRO₂ suggests inefficiency in oxygen metabolism resulting in increased demand. These findings are consistent with observations of higher whole-body resting metabolic rate and resting energy expenditure in children with SCD compared to controls.^{38,39} Furthermore, hypoxic challenge experienced at high altitudes also induced increased CMRO₂ in the setting of acute and sustained exposure.⁴⁰ The mechanisms underlying this increased oxygen demand in SCD are unknown. An increase in markers of inflammation and oxidative stress has been associated with increased resting energy expenditure in SCD,³⁹ and elevated erythropoietin^{e-1} (links.lww.com/WNL/A276) and enhanced inflammation^{e-2} have been proposed to increase cerebral metabolism as measured by PET in animal studies. Further studies are needed to understand why SCD increases oxygen metabolic demand.

Regional elevations in CBF and OEF, as well as declines in CMRO₂, within the internal border zone suggest enhanced vulnerability to infarction in this SCD population. In addition, compromised cerebrovascular reserve has been associated with white matter infarction in patients with carotid occlusion,^{e-3} (links.lww.com/WNL/A276) and cerebrovascular reserve has been shown to be diminished in SCD.^{e-4} Further investigation including CBF, OEF, CMRO₂, and cerebrovascular reserve will be important to better understand the phases of hemodynamic compromise in SCD. These metabolic parameters might serve as predictive biomarkers of future stroke on an individual patient basis. It is unclear if 1 or a combination of these imaging biomarkers will be predictive or how they will compare to

diagnostic tests currently used for therapeutic decision making. To this end, this cohort of patients will be followed longitudinally to address these questions.

This study has several limitations. The cross-sectional design prevents determination of whether CBF and OEF elevations occur in parallel or sequentially. We did not find differences in whole-brain or segmented CBF or OEF between controls with and those without HbS trait, but further investigations into the clinical ramifications of HbS trait are warranted. Our calculation of CaO_2 uses noninvasive SpO_2 rather than arterial oxyhemoglobin saturation because of ethical concerns about arterial blood sampling in children. Moreover, studies in children with SCD have found SpO_2 to be more accurate than arterial oxygen saturation when compared to fractional arterial oxyhemoglobin as the gold standard.^{17,18} We did not assess the extracranial cervical vasculature in this study. Stenosis of the extracranial internal carotid has been associated with increased risk for SCI in patients with SCD.⁴ Thus, we are unable to determine whether our finding of increased OEF is due, in part, to extracranial carotid disease. Finally, there are limitations to the magnetic resonance sequences used. Our pseudo-continuous arterial spin labeling sequence had a postlabel delay of 1.0 seconds, which could falsely elevate CBF measurements as a result of tagged blood not reaching capillaries and remaining within arteries at the time of the tagged image, and white matter CBF quantification using arterial spin labeling suffers from lower blood volumes and prolonged transit times compared to gray matter^{e-5} (links.lww.com/WNL/A276). However, flow velocities are increased in healthy children²¹ and further elevated in SCD,¹³ resulting in a decreased transit time, mitigating the risk of falsely increasing CBF measurement in this study population with a shortened postlabel delay, as well as improving the signal-to-noise ratio for quantification of white matter CBF. To maximize the accuracy of our CBF measurements, we used an in vivo blood T1 measurement from the sagittal sinus in our CBF calculation. While our in vivo T1 measurement accounts for the T1 dependence on age and hematocrit,^{e-6} it does not account for differences in blood oxygenation between the sagittal sinus and the capillary bed where CBF is measured.

Children with SCD demonstrate elevated CBF and OEF, which are likely compensatory responses to maintain CMRO_2 in the setting of decreased arterial oxygen content associated with severe anemia. Infarction occurs when these compensatory mechanisms fail to meet the metabolic demand of brain tissue. We conclude that regional elevation of OEF with concomitant decreases in CBF and CMRO_2 defines border zone physiology, supported by high coincidence with infarct density in a separate cohort of children with SCD. We will assess the ability of regionally elevated OEF to predict future stroke risk on an individual patient basis as we follow up this cohort longitudinally.

Author contributions

Melanie Fields designed the study, collected data, analyzed and interpreted data, performed the statistical analysis,

created figures, and wrote the manuscript. Kristin Williams collected and interpreted data. Dustin Ragan collected, processed, and interpreted data and created figures. Michael Binkley analyzed and interpreted data and performed the statistical analysis. Cihat Eldeniz collected and processed data. Yasheng Chen processed data. Monica Hulbert collected and interpreted data. Robert McKinstry interpreted data. Joshua Shimony and Katie Vo collected and interpreted data. Allan Doctor interpreted data. Hongyu An developed the asymmetric echo sequence used in the study and analyzed and interpreted data. Andria Ford designed the study, collected data, analyzed and interpreted data, and wrote the manuscript. Jin-Moo Lee designed the study, interpreted data, and wrote the manuscript. All authors critically reviewed and approved the manuscript.

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Disclosure

The authors report no disclosures relevant to the manuscript. Go to Neurology.org/N for full disclosures.

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Regional oxygen extraction predicts border zone vulnerability to stroke in sickle cell disease

Melanie E. Fields, MD, MSCI,* Kristin P. Williams, MD, MSCI,* Dustin K. Ragan, PhD, Michael M. Binkley, MS, Cihat Eldeniz, PhD, Yasheng Chen, DSc, Monica L. Hulbert, MD, Robert C. McKinstry, MD, PhD, Joshua S. Shimony, MD, PhD, Katie D. Vo, MD, Allan Doctor, MD, Hongyu An, DSc, Andria L. Ford, MD, MSCI,† and Jin-Moo Lee, MD, PhD†

Correspondence

Dr. Lee
leejm@neuro.wustl.edu
or Dr. Ford
forda@wustl.edu

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Study question

Does regional variation in cerebral oxygen metabolism explain vulnerability to infarction in children with sickle cell disease (SCD)?

Summary answer

Regional increases in oxygen extraction fraction (OEF) values were observed in the deep white matter of children with SCD, accompanied by decreases in cerebral blood flow (CBF) and the cerebral metabolic rate of oxygen utilization (CMRO₂).

What is known and what this paper adds

In children with SCD, strokes frequently occur in border zones/watershed regions, even in the absence of large vessel vasculopathy. This study defines border zone physiology, and provides evidence of its influence on stroke risk in children with SCD.

Participants and setting

Thirty-six children (age range: 5–21 years) with hemoglobin SS (HbSS) or hemoglobin S-β thalassemia null (HbSβ⁰) were prospectively enrolled. Twenty healthy siblings were recruited as controls to account for the influence of socioeconomic and nutritional environment on brain development.

Design

Brain MRI was used to measure CBF, OEF, and CMRO₂. Regional variations in OEF were delineated by a ratio map of average SCD:control OEF voxels. The region of elevated OEF was then overlaid on an average infarct heatmap created from an independent retrospective cohort of 23 children with SCD and silent cerebral infarcts (SCIs).

Main results and the role of chance

Whole-brain CBF, OEF, and CMRO₂ were higher in children with SCD than in controls. A region of elevated OEF was observed in the deep white matter of children with SCD, overlapping with the highest density of infarcts. CBF and CMRO₂ were lower in this region compared to all white matter. This pattern of high OEF, low CBF and CMRO₂ in

the deep white matter is consistent with border zone physiology, and demonstrates vulnerability to ischemia.

Bias, confounding, and other reasons for caution

The study did not assess extracranial cervical vasculature, which may influence the risk of SCIs in patients with SCD. The MR sequence used may have resulted in false elevation of CBF measurements.

Generalizability to other populations

Metabolic changes associated with vulnerability in border zones may be generalizable to a variety of diseases associated with global metabolic compromise to the brain (e.g., other anemic-hypoxic conditions, watershed infarction, white matter disease in older adults), although further studies are required to determine whether such changes predict future stroke risk.

Study funding/potential competing interests

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*These authors contributed equally to this work, and are designated as co-first authors.

†These authors contributed equally to this work, and are designated as co-senior authors.

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