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Cerebral oxygen metabolism in adults with sickle cell disease

Lena Václavu1,2 | Jan Petr3 | Esben Thade Petersen4,5 | Henri J.M.M. Mutsaerts6 | Charles B.L. Majoie1 | John C. Wood7 | Ed VanBavel8 | Aart J. Nederveen1 | Bart J. Biemond9

1Radiology & Nuclear Medicine, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands
2C.J. Gorter Center for High Field MRI, Department of Radiology, Leiden University Medical Center, Leiden University, Leiden, The Netherlands
3Helmholtz-Zentrum Dresden-Rossendorf, Institute of Radiopharmaceutical Cancer Research, Dresden, Germany
4Danish Research Centre for Magnetic Resonance, Centre for Functional and Diagnostic Imaging and Research, Copenhagen University Hospital Hvidovre, Hvidovre, Denmark
5Center for Magnetic Resonance, Department of Health Technology, Technical University of Denmark, Kongens Lyngby, Denmark
6Radiology & Nuclear Medicine, Amsterdam UMC, Vrije Universiteit, Amsterdam, The Netherlands
7Cardiology & Radiology, Children’s Hospital of Los Angeles, Los Angeles, California
8Biomedical Engineering & Physics, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands
9Hematology, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands

Correspondence
Lena Václavu, Department of Radiology, Leiden University Medical Center, Leiden University, Leiden, The Netherlands.
Email: l.vaclavu@lumc.nl

Abstract
In sickle cell disease (SCD), oxygen delivery is impaired due to anemia, especially during times of increased metabolic demand, and cerebral blood flow (CBF) must increase to meet changing physiologic needs. But hyperemia limits cerebrovascular reserve (CVR) and ischemic risk prevails despite elevated CBF. The cerebral metabolic rate of oxygen (CMRO2) directly reflects oxygen supply and consumption and may therefore be more insightful than flow-based CVR measures for ischemic risk in SCD.

We hypothesized that adults with SCD have impaired CMRO2 at rest and that a vasodilatory challenge with acetazolamide would improve CMRO2. CMRO2 was calculated from CBF and oxygen extraction fraction (OEF), measured with arterial spin labeling and T2-prepared tissue relaxation with inversion recovery (T2-TRIR) MRI. We studied 36 adults with SCD without a clinical history of overt stroke, and nine healthy controls. As expected, CBF was higher in patients with SCD versus controls (mean ± SD: 74 ± 16 versus 46 ± 5 mL/100 g/min, P < .001), resulting in similar oxygen delivery (SCD: 377 ± 67 versus controls: 368 ± 42 μmol O2/100g/min, P = .69). OEF was lower in patients versus controls (27 ± 4 versus 35 ± 4%, P < .001), resulting in lower CMRO2 in patients versus controls (102 ± 24 versus 127 ± 20 μmol O2/100g/min, P = .002). After acetazolamide, CMRO2 declined further in patients (P < .01) and did not decline significantly in controls (P = .78), indicating that forcing higher CBF worsened oxygen utilization in SCD patients. This lower CMRO2 could reflect variation between healthy and unhealthy vascular beds in terms of dilatory capacity and resistance whereby dysfunctional vessels become more oxygen-deprived, hence increasing the risk of localized ischemia.

1 INTRODUCTION

Sickle cell disease (SCD) is caused by a genetic mutation whereby sickle hemoglobin (HbS) polymerizes in its deoxygenated form, leading to impaired oxygen transport and chronic hemolytic anemia. In SCD, acute anemia increases the risk for silent cerebral infarcts (SCI),1-3 despite elevated cerebral blood flow (CBF) to compensate anemia.4-6 Interruptions to CBF are associated with ischemic risk,7 and MRI studies have shown a high prevalence of SCI in both children and adults with SCD.8 While overt stroke is preventable with transfusion...
therapy, SCI still remain poorly understood, and advances in MR technology are becoming more instrumental in our investigation of SCI development and progression in SCD. For instance, SCIs are associated with cognitive impairment and lesion progression in adults with SCD, and indicate that the balance between oxygen demand and delivery, which serves to maintain cerebral metabolism, may be disrupted.

The cerebral metabolic rate of oxygen (CMRO₂) quantitatively describes the rate of oxygen consumption by metabolic processes in cerebral tissue. The CMRO₂ is high in healthy children to meet the metabolic demands of the developing brain, particularly between the ages of 2 and 10 years, which is also the age range in which overt stroke has the highest incidence in SCD. Hence, CBF can be particularly high in children with SCD in whom anemia and metabolic requirements play an additive role in increasing blood flow and associated increased risk of overt stroke. Note that CMRO₂ typically declines with age and CBF matches oxygen delivery to this change in metabolic demand. Oxygen extraction fraction (OEF) measured by MRI can serve as a non-invasive measure of the efficacy of oxygen supply-demand mechanisms. Studies in adult patients with SCD have varied in their methods and conclusions as to whether CMRO₂ is maintained in the presence of chronic demands on CBF to counteract anemia.

In SCD, cerebrovascular reserve is reduced, in particular, CBF may already be maximally recruited for basal oxygen demands and might even be unable to maintain the required CMRO₂ in periods of increased metabolic needs. This infers a risk of insufficient oxygen in acute anemic periods, particularly to regions where CBF is already relatively low. Recent work shows that oxygen metabolism could actually decrease if CBF increases in the presence of capillary transit time heterogeneity (CTH). A potential effect offsetting this microvascular shunting is that anemia induces a rightward shift in oxygen-dissociation, allowing more efficient oxygen diffusion in SCD. While these opposing processes are challenging to disentangle, and may cancel out, we tested the hypothesis that CMRO₂ is reduced in adult patients with SCD as a result of chronic anemia and impaired vascular reserve capacity, which may also explain the high prevalence of silent cerebral infarcts in this population. The second hypothesis was that vasodilatation can improve CMRO₂ by generating an increase in blood and oxygen flow. Accordingly, the aim of this study was to examine CMRO₂ using specialized MRI techniques in adults with SCD without a clinical history of stroke, and to measure the response to vasodilatation with acetazolamide to better understand the etiology of ischemic risk in these patients.

2 | METHODS

2.1 | Participants

This cross-sectional study was approved by the local Institutional Review Board (IRB) at the Amsterdam University Medical Centers (Amsterdam UMC, Location AMC) in The Netherlands, and carried out in accordance with the Declaration of Helsinki. This study was registered at clinicaltrials.gov under the ID: NCT02824406. All participants provided informed consent prior to study participation. Inclusion criteria were adults (>18 years) with SCD (HbSS or HbSβ₀-thalassaemia) recruited from the outpatient clinic at the Amsterdam UMC. Age-, sex-, and race-matched healthy controls were recruited from friends and relatives of the participating patients. Exclusion criteria were contraindications to MRI and acetazolamide, and a clinical history of stroke, a cerebrovascular accident or neurologic disease affecting cerebral autoregulation. Additionally, patients were in steady state at the time of the MRI examination. Steady state was defined as no infection or hospitalization for a painful crisis in the month prior to participation. The cohort has previously been described in Václavu et al. One patient was receiving blood transfusions for prevention of stroke upon detection of high TCD values while still in pediatric care, and two other patients were on transfusions for prevention of frequent hydroxyurea refractory vaso-occlusive crises/acute chest syndrome.

2.2 | Blood markers

Blood samples were drawn from an antecubital vein in all participants, and an intravenous catheter was placed at the site of cannulation directly prior to MRI for acetazolamide administration during the scan. Genotype (HbAA, HbAS, HbSS or HbSβ₀) was confirmed with high performance liquid chromatography (HPLC) and DNA analysis. The following parameters were analyzed for their association with CMRO₂: hemoglobin (Hb), hematocrit (Hct), MCV, leukocyte and platelet counts, HbS%, and fetal hemoglobin (HbF %), creatinine, ASAT, ALAT, and plasma ferritin levels. Surrogate markers for hemolysis were defined as LDH, reticulocytes, and bilirubin levels.

2.3 | MR imaging

All images were acquired on a 3 T Ingenia clinical MR system (Philips Healthcare, Best, The Netherlands) using a 32-channel receive head-coil and body-coil transmission. The MRI protocol is depicted in Figure 1A, and comprised MR angiography (MRA) for vasculopathy, fluid-attenuated inversion recovery (FLAIR) for anatomy and lesions, T₂-prepared tissue relaxation with inversion recovery (T2-TRIR) for venous blood oxygen saturation and T₁ of blood, and pseudo continuous arterial spin labelling (pCASL) for cerebral blood flow.

Lesions and brain volume were assessed using a high resolution T2-weighted 3D multi-shot TSE fluid attenuated inversion recovery (FLAIR) sequence with a FOV 250 x 250 x 180 mm, voxel size 0.98 x 0.98 x 1.12 mm, TR/TE 4800/356 ms, SENSE acceleration factor AP/FH 2.6/2, SPAIR fat suppression, inversion delay 1650 ms, flip angle 90° and scan duration of 5:11 minutes. Vasculopathy was assessed in maximum intensity projection reconstructions of right-left and feet-head projections and in magnitude images of non-contrast enhanced 3D T1 time-of-flight FFE RF spoiled gradient echo inflow angiography MRA (TR/TE 21/4 ms, FOV 200 x 200 x 90 mm, voxel size 0.39 x 0.39 x 0.5 mm, flip angle 20°, 180 axial slices, scan duration 5:45 minutes).
Venous blood $T_1$ and $T_2$ were simultaneously measured in the sagittal sinus with a $T_2$-TRIR sequence consisting of a global slab-selective frequency offset corrected inversion (FOCI) pulse and $T_2$ preparation preceding a single slice 2D single-shot FFE EPI Look-Locker read-out. The parameters of the $T_2$-TRIR were FOV 202 x 243 mm, voxel size 1.69 x 1.69 x 4 mm, 1 transverse slice placed perpendicular to the posterior sagittal sinus, TR/TE 150/24 ms, SENSE acceleration factor AP 3, and four $T_2$ weightings resulting in effective echo times (eTE) of 0, 40, 80, and 160 ms. This corresponded to 0, 4, 8 and 16 refocusing pulses in the MLEV $T_2$-preparation with a Carr-Purcell- Meiboom-Gill interpulse spacing of $\tau_{\text{CPMG}} = 10$ ms, $T_1$ 10 ms, $\Delta T_1$ 130 ms, 4 dynamic scans, a Look-Locker read-out with high flip angles (95°) to ensure brain tissue saturation, and a total scan duration of 50 seconds.

Cerebral blood flow was measured in the whole brain using a 2D gradient-echo single-shot EPI pCASL sequence with the following parameters: FOV 240 x 240 x 133 mm, voxel size 3 x 3 x 7 mm, 19 slices, labelling duration 1800 ms, post-labeling delay 1800 ms, flip angle 90°, TR/TE 4400/14 ms, and a SENSE acceleration factor AP 2.5. Additional parameters were SPIR fat suppression, two background tissue suppression pulses, 140 control-label pairs, and total...
scan duration of 20 minutes. The labelling plane was manually placed 90 mm below the middle slice of the imaging volume, and perpendicular to the brain feeding arteries visualized on a phase contrast MRA scout scan. Balanced pCASL labelling with flip angles of 27.81°, radio frequency interval and duration of 1.21 ms and 0.48 ms, and gradient strength average and maximum of 0.36 mT/m and 5.0 mT/m were used. A magnetization equilibrium (M0) scan was acquired for CBF quantification using the same parameters as pCASL, except that labelling and background suppression were switched off.

2.4 | Vasodilatory challenge

Following the baseline scans, an intravenous bolus of 16 mg/kg acetazolamide (dissolved in 20 mL saline, 0.9% NaCl) was infused at a flow rate of 0.1 mL/sec and flushed with 10 mL saline. The T2-TRIR scans were repeated 25 minutes after the start of the acetazolamide injection when vasodilation has typically reached a maximum.35,38

2.5 | Image analysis

Vessel stenosis was assessed in the circle of Willis vessels on MRA and vasculopathy was defined as >50% narrowing of a vessel or presence of comorbid Moyamoya. Presence of white matter hyper-intensities was defined as ≥2 lesions ≥5 mm in diameter, where diameter was defined as the maximum length along a major axis of a lesion in 3D as described previously.26

2.6 | SvO2, OEF, CaO2, DO2 and CMRO2 quantification

An example of the masked region of interest is shown in Figure 1C. The T1 and T2 were simultaneously fitted by a least-squares minimizing algorithm designed to find the combination of voxels in the sagittal sinus ROI resulting in the lowest residual error of fitted T1 and T2 values from the following model:

| TABLE 1 | Baseline characteristics and MRI parameters in healthy controls and patients with sickle cell disease |
|-----------------|-----------------|-----------------|-----------------|
| Baseline characterist | Controls (n = 11) | Patients with SCD (n = 38) | P-value | Groups |
| Age [yrs] | 37.36 ± 15.43 | 32.08 ± 11.14 | .31 | | |
| Sex | 55% male | 63% male | | - | |
| Genotype | HbAA (n = 9, 82%) | HbSS (n = 33, 87%) | | | |
| | HbAS (n = 2, 18%) | HbSβ0 (n = 5, 13%) | | | |
| Hydroxyurea, n [%] | - | 13 (37) | | - | |
| Average dose mg/kg | - | 15.88 ± 2.94 | | - | |
| Chronic blood transfusions, n [%] | - | 3 (9) | | - | |
| Vasculopathy (%) | - | 1 (2) | | - | |
| SCI status | 4 (45) | 31 (82) | | - | |
| Volume of lesions [mL] | 0.04 [IQR 0.54] | 0.59 [IQR 2.99] | <.001 | | |
| Hematocrit [%] | 42 ± 3 | 26 ± 4 | <.001 | | |
| Hemoglobin [g/dL] | 13.7 ± 1.3 | 8.8 ± 1.4 | <.001 | | |
| HbS% | 36.9 ± 0.4 | 80.4 ± 15.1 | <.001 | | |
| HbF% | - | 9.4 ± 7.6 | | - | |
| CaO2 [μmol O2/100mL blood] | 804 ± 73 | 515 ± 81 | <.001 | | |
| MR parameter | Baseline | Post-ACZ | Δ | Baseline | Post-ACZ | Δ |
| T1b [s] | 1.76 ± 0.09 | 1.69 ± 0.11 | 0.11 | 1.81 ± 0.10 | 1.71 ± 0.09 | <0.001 | .23 | Groups |
| T2b [ms] | 77.6 ± 10.9 | 124.5 ± 22.7 | <0.001 | 88.0 ± 13.3 | 115.4 ± 20.2 | <0.001 | .04 | |
| CBF [mL/100/min] | 45.7 ± 4.8 | 76.2 ± 9.5 | <0.001 | 73.9 ± 16.0 | 97.0 ± 17.9 | <0.001 | <.001 | |
| SvO2 [%] | 64.1 ± 3.8 | 78.7 ± 7.0 | <0.001 | 71.4 ± 4.3 | 79.4 ± 5.7 | <0.001 | <.001 | |
| DO2 [μmol O2 /100g/min] | 368.1 ± 42.3 | 616.0 ± 105.3 | <0.001 | 377.1 ± 66.8 | 499.4 ± 87.4 | <0.001 | .69 | |
| OEF [%] | 35.3 ± 3.6 | 19.7 ± 7.1 | <0.01 | 27.1 ± 4.4 | 19.0 ± 5.8 | <0.001 | <.001 | |
| CMRO2 [μmol O2/100g/min] | 127.2 ± 19.5 | 122.8 ± 43.8 | 0.78 | 101.7 ± 23.6 | 91.0 ± 29.5 | <0.01 | <.01 | |

Bold values indicate statistical significance.

Abbreviations: ACZ, acetazolamide; SCD, sickle cell disease; CaO2, Oxygen carrying capacity of blood was calculated from hemoglobin so does not represent a separate measure; HbS% and HbF% is reported for the n = 2 controls that had the HbAS genotype; CBF, whole brain cerebral blood flow; CMRO2, cerebral metabolic rate of oxygen; DO2, oxygen delivery; IQR, interquartile range; OEF, oxygen extraction fraction; SvO2, venous oxygen saturation; T1b, longitudinal relaxation time of blood; T2b, transverse relaxation time of blood; Δ, within-group change after acetazolamide.
where $M$ is the longitudinal magnetization of blood at each inversion recovery time ($T_I$), $M_0$ is the equilibrium magnetization of blood, $\varepsilon$ is the effective echo time and $IE$ is inversion efficiency. An example of the exponential $T_1$ and $T_2$ decay of blood within the sagittal sinus is shown in Figure 1D. The resulting $T_2$ values were converted to volume in L, temperature and pressure ($PV = nRT$, where $R$ is the ideal gas law constant of 0.08206 L atm/[mol K] and $T$ is 273 Kelvin). One $\mu$mol at standard chemical conditions occupies 0.0224 mL, so to substitute mL O$_2$ for $\mu$mol O$_2$ in the CaO$_2$ equation, we divided it by 44.64 mL/$\mu$mol. Then, $CMRO_2$ was calculated according to Fick’s principle:

$$CMRO_2 (\mu\text{mol O}_2/100\text{g/min}) = \text{CBF} \times \text{OEF} \times \text{CaO}_2$$

Finally, oxygen delivery ($DO_2$) was calculated by:

$$DO_2 (\mu\text{mol O}_2/100\text{g/min}) = \text{CBF} \times \text{CaO}_2$$

The $CMRO_2$ was calculated in the whole brain (calculated from $CBF$ in GM plus WM minus lesions Figure 1B) with $CBF$ quantified with a two-compartment model$^{22,29}$ for sickle cell disease patients:

$$R_2 (s^{-1}) = 70*(1 - \text{SvO}_2) + 5.75$$

and for healthy controls:

$$R_2 (s^{-1}) = 77.5 + Hct*(1 - \text{SvO}_2)^2 + 27.8*(1 - \text{SvO}_2)^2 + 6.95*Hct + 2.34$$

where $R_2 = 1/T_2$, Hct is the measured Hct. The equation was solved for venous oxygen saturation ($\text{SvO}_2$) in each subject before and after acetazolamide. Since arterial oxygenation ($\text{SaO}_2$) in the brain is similar to that in the periphery, and can be assumed to be normal in the absence of cardio-pulmonary disease, we assumed $\text{SaO}_2$ was equal to 0.98, or almost fully saturated. Subsequently, the oxygen extraction fraction (OEF) was calculated using the formula:

$$\text{OEF} (\%) = (\text{SaO}_2 - \text{SvO}_2)/\text{SaO}_2 * 100$$

Oxygen carrying capacity ($\text{CaO}_2$) was calculated from measured hemoglobin (HB, g/dL) using the formula:

$$\text{CaO}_2 (\mu\text{mol O}_2/100\text{mL blood}) = [(\text{HB} \times 1.34 \times \text{SaO}_2) + (0.003 \times pO_2)]/22.4 * 1000$$

\[1\] \[2\] \[3\] \[4\] \[5\] \[6\]

2.7 Statistical analysis

Statistical analyses were performed in R 3.4.3 (R Core Team [2017] R Foundation for Statistical Computing, Vienna, Austria). First, Shapiro-Wilk normality test was used, and subsequently Wilcoxon rank sum (significant non-normal distribution) or t test (normal distribution) for group differences. Paired tests were used to assess statistical significance of changes from baseline to post-acetazolamide conditions. $P < .05$ was considered significant. Variables were summarized by means and standard deviations. Associations between $CMRO_2$ and blood values were assessed using linear regression to determine if the slope was significantly different from zero. A Benjamini-Hochberg adjustment for family-wise error was performed to correct for multiple comparisons.

3 RESULTS

3.1 Participant characteristics

The baseline characteristics of the 38 patients and 11 healthy controls that were included are presented in Table 1. 14 patients were using hydroxyurea medication and three were receiving transfusions, of which two were also on hydroxyurea medication.

3.2 Elevated venous oxygen saturation ($\text{SvO}_2$) in SCD

In the patient group, $T_2$ fitting failed in two cases. Therefore 36 patients were included in the $T_2$, OEF, and $CMRO_2$ analysis. In the healthy control group, initially consisting of 11 healthy volunteers, two fits failed. The remaining fits were robust, as shown in the example in Figure 1D. The average $T_2$ values were higher in SCD patients compared to controls (Table 1). The corresponding $\text{SvO}_2$ was also higher in SCD patients (71.4 ± 4.2%) compared to healthy controls (64.1 ± 3.8%, $P < .001$). The average $T_1$ values did not differ between SCD patients and controls (Figure S1).

3.3 Baseline hemodynamic results

Hemoglobin concentration was lower in patients compared to controls ($8.8 ± 1.4$ versus $13.7 ± 1.3$ g/dL, $P < .001$), which lead to a lower calculated oxygen carrying capacity ($\text{CaO}_2$) in patients compared to controls ($515 ± 81$ versus $804 ± 73$ $\mu$mol O$_2$/100mL blood, $P < .001$). Oxygen delivery ($DO_2$) was similar in patients compared to controls...
(377 ± 67 versus 367 ± 42 μmol O₂ /100g/min, P = .698), due to compensatory elevated CBF in patients versus controls (74 ± 16 versus 46 ± 5 mL/100 g/min, P < .001). In spite of the similar DO₂, we observed lower OEF in patients compared to controls (27.1 ± 4.4 versus 35.3 ± 3.6%, P = .001). As a result, SCD patients had lower CMRO₂ compared to controls (101.7 ± 23.6 versus 127.2 ± 19.5 μmol O₂ /100g/min, P = .005) (Figure 2).

### 3.4 Response to acetazolamide (ACZ) challenge

In order to assess if CMRO₂ in SCD patients could be normalized by vasodilation, we repeated the measurements after acetazolamide (ACZ). We observed that there was a significant increase in venous saturation (SvO₂) in both patients with SCD (P < .001) and in healthy controls (P < .001) indicating that a greater volume of oxygenated
blood was delivered by the increased CBF after ACZ (Figure 2). Although the absolute change in CBF was similar in patients and controls ($\Delta$CBF was 23 and 30 mL/100 g/min), the relative change in CBF, called cerebrovascular reserve, was lower in patients with SCD versus controls ($43.1 \pm 12.5$ versus $67.0 \pm 20.0\%$, $P = .003$). The increase in CBF after ACZ was accompanied by a drop in OEF in patients with SCD ($P < .001$) as well as in controls ($P = .003$) (Table 1).

After ACZ, CMRO$_2$ declined significantly in SCD patients ($−8.4 \pm 16.9$, $P = .008$). No significant decline was observed in healthy controls ($−4.6 \pm 41.3$, $P = .78$), due to both the lower effect size (Cohen’s $d = 0.40$ in patients and 0.13 in controls) and sample size, however it suggests that the ACZ challenge was indeed closer to isometabolic in controls than in patients.

### 3.5 CMRO$_2$ associations with laboratory markers

In SCD patients, there was no significant difference in CMRO$_2$ between patients on hydroxyurea compared to patients not taking hydroxyurea ($P = .33$) (Figure S2). We found a moderate negative

![FIGURE 3](FIGURE 3) Laboratory correlates of cerebral metabolic rate of oxygen (CMRO$_2$) in adults with sickle cell disease A, HbF%, B, Ferritin, C, total bilirubin, D, HbS%, E, reticulocyte count, and F, lactate dehydrogenase (LDH). Non-hydroxyurea-treated patients are shown in blue, hydroxyurea-treated patients in green, and transfused patients in orange. The dotted colored lines show the fit for the non-hydroxyurea and hydroxyurea-treated groups, while the black solid line shows the linear fit through all patients’ data points. $P$-values indicate significant associations. G, HbF% was inversely associated with cerebral blood flow (CBF), H, HbF% was not associated with hemoglobin concentration, I, HbF% was inversely associated with oxygen extraction fraction (OEF) [Color figure can be viewed at wileyonlinelibrary.com]
correlation between CMRO2 and HbF% ($R^2 = 0.38$, $P < .01$), a significant but weak positive association between CMRO2 and reticulocyte count ($R^2 = 0.25$, $P < .01$), total bilirubin ($R^2 = 0.20$, $P < .01$) and ferritin ($R^2 = 0.14$, $P = .04$). There was no association with lactate dehydrogenase ($R^2 = 0.11$, $P = .07$) or HbS% ($R^2 = 0.04$, $P = .27$) (Figure 3A-F). These $P$-values remained significant after Benjamini-Hochberg family-wise error correction. Both OEF and CBF were also inversely associated with HbF% on univariate analysis (Figure 3G,I).

3.6 Brain anatomical features and whole-brain CMRO2

We found no association between CMRO2 and lesion volume in patients with SCD (Spearman’s $\rho = -0.03$, $P = .85$), and also no statistically significant correlation between OEF and lesion volume in patients with SCD (Spearman’s $\rho = -0.3$, $P = .07$). There was no difference in GM fraction (GM divided by total intracranial volume) between patients with SCD (mean $0.42 \pm 0.05$) and controls (mean $0.46 \pm 0.05$, $P = 0.10$). Also, there was no significant correlation between CMRO2 and GM fraction within the patient group ($r = -0.07$, $P = 0.70$) or within the control group ($r = 0.47$, $P = 0.21$). In patients with SCD, men had higher CMRO2 compared to women ($P < .01$). There was no significant effect of age on CMRO2 ($P = .20$).

4 DISCUSSION

The purpose of this study was to investigate ischemic risk in SCD patients by assessing CMRO2 and its response to increased flow upon vasodilation with acetazolamide. We used ASL and T2-TRIR MRI with a recently introduced SCD-specific model to non-invasively measure CBF and cerebral SvO2. Our observations support our hypothesis that SCD patients have reduced OEF and CMRO2. This indicates that in SCD, CMRO2, and therefore also neuronal function, has adapted to lower oxygen availability. Our second hypothesis was that acetazolamide would normalize CMRO2 by increasing oxygen delivery (DO2), but our observations do not support this hypothesis and instead show that DO2 was normal in SCD patients, and that OEF and CMRO2 declined after acetazolamide. The higher SvO2 after acetazolamide suggests that arterial blood shunted directly to the venous side without oxygen extraction by the brain tissue, resulting in a reduced OEF and subsequently also reduced CMRO2. Our observations suggest a potentially impaired distribution of oxygen even when blood flow is artificially increased with acetazolamide, perhaps due to differences in resistance of healthy and diseased vascular beds.

4.1 Reduced oxygen utilization despite normal oxygen delivery

Previous studies using nitrous oxide have also shown reduced cerebral oxygen utilization in SCD and other anemias.40,41 PET is the gold standard for CBF, OEF and CMRO2, and an early study in SCD patients found that CBF was appropriately elevated in the cortical MCA territory to conserve normal DO2 and average CMRO2 compared to controls.5 In the PET study, half of the patients had CMRO2 values below the lowest CMRO2 value of controls but on average the groups did not differ. We also found no difference in DO2 between patients and controls due to elevated CBF as previously reported for whole-brain and GM.23,29

4.2 Higher venous saturation

We found higher T2 in patients with SCD in our study, which denotes increased venous oxygen saturation (SvO2),62 and is in agreement with recent reports of increased SvO2 in children and adults with SCD using T2-TRUST as well as susceptibility MRI,21,22,43 but in contrast to other reports44 which differ due to differences in the choice of calibration model. Nevertheless, elevated SvO2 in SCD may indicate impaired oxygen extraction as has been reported in the peripheral tissue45 and may explain the high risk of tissue hypoxia as well as infarction in SCD.

4.3 Reduced oxygen extraction fraction

We observed reduced OEF in adults with SCD with an MR technique that measures post-capillary venous OEF,33 which is spatially and temporally different from the ASE technique which measures OEF locally in deep WM tissue and has found elevated OEF in children with SCD.27,28,46 Fields, Guilliams, Ford and colleagues consistently find increased OEF in the deep WM tissue, which, in steno-occlusive disease, reflects stage 2 hemodynamic compromise when OEF starts to compensate for critically low CBF to try to maintain DO2.47 In children with SCD, this increased OEF in WM may indicate that compensatory mechanisms are activated to maintain CMRO2. Reduced OEF in whole brain in adults with SCD may indicate that hemodynamic compromise has not reached a critical level, or that our globally measured SvO2, OEF, and CMRO2 were dominated by the metabolic activity of the GM, which may be stealing from the WM. Indeed, CBF might preferentially be distributed to maintain DO2 in the GM, where many neuronal processes must be fed, by using the cerebrovascular reserve that is still available, and hence keeping OEF normal, as others have found using global venous techniques.22,23

4.4 Homeostatic relationship between CBF and OEF after acetazolamide

If the low CMRO2 were just the result of flow limitation, acetazolamide would be expected to ameliorate the reduced metabolism in SCD patients by dilating the vessels and increasing delivery of oxygenated blood. As anticipated, CBF increased and OEF declined in both groups in response to acetazolamide.46 There was no change
detected in CMRO\textsubscript{2} in healthy controls which is supported by a previous study using susceptibility-based oximetry with hypercapnia challenge.\textsuperscript{45} Interestingly, we observed a small reduction in CMRO\textsubscript{2} in patients with SCD, indicating a mismatch between CBF and OEF. A power analysis showed that there was 95\% power to detect a reduction in the SCD group, while there was 10\% power in the control group due to the lower sample size. Normalizing CBF and increasing oxygen carrying capacity with transfusions could shed light on a potential mechanism explaining this mismatch. However, studies on children with SCD addressing acute effects of transfusions on global or regional OEF and CMRO\textsubscript{2} have not found significant changes in CMRO\textsubscript{2}, possibly due to sufficiently elevated compensatory CBF before transfusion.\textsuperscript{46,50} An alternative explanation for the drop in CMRO\textsubscript{2} is that carbonic anhydrase inhibition with acetazolamide may influence the Hb-O\textsubscript{2} dissociation curve due to the increased acidotic environment, leading to tighter Hb-O\textsubscript{2} binding and lower OEF and CMRO\textsubscript{2}.

4.5 | Heterogeneous oxygen distribution after acetazolamide

The ability of vessels to dilate will govern their vascular resistance which will determine the favorable dispersal of blood towards healthier vascular beds. This would suggest that there exists heterogeneity in the blood flow distributed to different vascular beds, and could explain the preferential localization of ischemic lesions in specific low-flow areas.\textsuperscript{27} It is known that blood arrival times are heterogeneous due to the anatomic architecture of the cerebral vessels,\textsuperscript{51} but downstream capillary transit times may also reflect certain regional differences. For instance, recent work has shown that heterogenous blood distribution can lead to reduced OEF and CMRO\textsubscript{2} in the presence or introduction of increased transit time heterogeneity,\textsuperscript{20,52} which could be an explanation for why we found lower OEF and CMRO\textsubscript{2} after acetazolamide. Acetazolamide would induce dilation only in healthy vessels which are able to dilate, leading to lower resistance and more blood flow. For diseased vessels, acetazolamide would have little effect, leading to no or little change in blood flow and hence no change in oxygen delivery. The surplus oxygen is delivered to healthy vascular beds due to lower resistance, but have neither the additional oxygen requirement nor a sufficient oxygen diffusion gradient for oxygen to unload, so oxygen flows to the venous side (we found increased SvO\textsubscript{2}). Hence, reduced CMRO\textsubscript{2} might only occur if there exists a heterogeneity in the ability of different vascular beds to dilate. We cannot exclude the possibility that chronically anemic patients may also lose capillary surface area through vascular remodeling; but the imaging used in this study cannot quantify vascular surface area.

4.6 | CMRO\textsubscript{2} and ischemic risk

In this study there was no association between CMRO\textsubscript{2} and lesion volume contrary to what has been reported in patients with multiple sclerosis.\textsuperscript{53} We observed a higher prevalence of lesions in the WM where CBF is low, and recent studies show that although OEF may be elevated in children in these locations,\textsuperscript{27,28} CMRO\textsubscript{2} is primarily high in more physiologically active GM.\textsuperscript{54} Global CMRO\textsubscript{2} measurements may not reflect local changes in metabolism, so regional measurements may better identify areas of particular risk for SCIs. Hence, the lack of association between global CMRO\textsubscript{2} and lesion volumes in WM can be explained by the high CBF in GM, masking the reduced CMRO\textsubscript{2} in areas with lesions. The role of oxygenation in the development and progression of lesions is particularly challenging to study, because lesions are not detected at the time they occur, and can therefore represent periods of acute anemia experienced a long time before lesion detection with MRI. So, the lack of association between CMRO\textsubscript{2} and lesions in our study does not necessarily mean that there is no relationship, but rather that our cross-sectional design was poorly suited to detect it, and longitudinal studies following young patients into adulthood would be insightful in this respect.

4.7 | CMRO\textsubscript{2} and HbF

We found that CMRO\textsubscript{2} was inversely associated with HbF, which was not found by Herold et al.\textsuperscript{5} In univariate analyses, Fields et al. recently found that SCD patients with higher HbF levels also had lower OEF in a comparison between patients treated with hydroxyurea and not treated with hydroxyurea.\textsuperscript{55} This is not surprising if OEF elevations are related to disease severity as they suggest, but would be surprising if OEF elevations are related to improved oxygen extraction. If higher HbF is associated with low oxygen extraction fraction, then this could suggest that HbF has a tighter Hb-O\textsubscript{2} binding affinity, leading to less efficient oxygen unloading. Finally, the inverse relationship between CBF and HbF is supported by literature in sickle cell mice,\textsuperscript{56} which Cui et al.\textsuperscript{56} suggest may be related to lower sickling, reduced oxidative stress, improved NO activity or improved oxygen carrying capacity and delivery. Thus, the balance between oxygen delivery and extraction may be perturbed in the presence of HbF whereby more oxygen is delivered than can be extracted. Furthermore, as discussed above, variations in capillary transit time and shunting may mean that there is less time and insufficient oxygen diffusion gradient to unload oxygen resulting in a lower global OEF and subsequently lower global CMRO\textsubscript{2}. Together, our findings suggest that reduced CMRO\textsubscript{2} could partly be mediated by the effect of higher HbF levels on both OEF and CBF. However, the HbF correlation with CMRO\textsubscript{2} was from univariate analysis, and future studies with larger cohorts will likely show that the effects of HbF on CMRO\textsubscript{2} are mitigated by covariation with HbA, so the driving factor in predicting CMRO\textsubscript{2} requires further investigation.

4.8 | Limitations

A general limitation of MRI-derived CMRO\textsubscript{2} is error propagation of the individual measurements of CBF, OEF and CaO\textsubscript{2} due to multiplication.\textsuperscript{57} While there is currently no direct MRI-CMRO\textsubscript{2} measurement,
we attempted to reduce errors in CBF by correcting for differences in T1 of blood, arterial transit time and labeling efficiency.26 Another limitation of CMRO2 calculation from T2-TRIR MRI is the reliance on a model to obtain OEF values. A recent study has highlighted the importance of model choice in calculating SvO2 (and subsequent OEF), which can explain the observed differences of elevated,21,28,44,46,50 reduced,22 or unchanged21,23 CMRO2 in patients with SCD. Until recently, two models were available for T2-derived SvO2; the first was calibrated in bovine blood in a wide range of Hct (35-55%) considered to be a healthy range,58,59 and the second was calibrated in human sickle cell blood in a slightly smaller but more appropriate range of Hct for anemia of 24-40%.22 The bovine model shows higher OEF in SCD (lower SvO2) compared to controls,21,44 but it should be noted that the model is biased at low Hct. On the other hand, the sickle cell model previously showed lower OEF in SCD (higher SvO2) compared to controls,22 a finding that is supported here and also by an independent susceptibility MRI study showing higher SvO2 in SCD compared to controls.43 The main difference between the sickle-cell model and healthy human blood model is that the sickle-cell model does not show dependence on Hct which is most probably due to the narrow range of Hct used for calibration or that Hct is too low to influence the measured T2 value. A recent approach has used subject-specific calibration resulting in OEF similar to the sickle-cell model.23 Although validation of the models in SCD is still warranted by comparison with PET, which is the current gold standard for OEF and CMRO2 measurements, our choice for the sickle cell model is justified by the fact that it was calibrated over a relevant hematocrit range for our population. We assumed arterial oxygen saturation to be 0.98 which may have been inaccurate in SCD patients, but given the median saturation of 0.97 measured clinically in two studies including more than 100 children and 89 adults with SCD in steady-state respectively60,61 we expect that any resulting error in SaO2 and CaO2 will be small, particularly because we did not include patients with proven pulmonary hypertension or severe cardiomyopathy, both of which may result in low arterial saturation.

5 | CONCLUSION

In this study, we used T2-TRIR and ASL MRI to measure reduced CMRO2 in adults with SCD. Overall, oxygen delivery to the brain was normal in SCD patients. However, specific capillary beds may have adapted to chronic low oxygen delivery due to anemia as differences in dilatory capacity and resistance may arise depending on metabolic requirements. We conclude firstly that CMRO2 does not improve in response to transient changes in oxygen availability induced by acetazolamide in SCD patients, despite the increase in CBF, and secondly, that this worsening CMRO2 in SCD patients may be due to increased capillary transit time heterogeneity exacerbated by high flow.30,52

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CONFLICT OF INTEREST

The authors declare no competing financial interests.

AUTHOR CONTRIBUTIONS

L.V. and B.J.B. recruited and scanned the participants, and wrote the manuscript. E.T.P. developed and implemented the T2-TRIR sequence and analysis software to fit the T2 data. H.J.M.M.M. wrote the analysis software for the ExploreASL toolbox. J.P. performed preprocessing analysis of ASL images to remove an artifact using principal components analysis. C.B.M. performed radiologic assessment of MRA and FLAIR data. E.T.P., E.vB., A.J.N., and B.J.B. analyzed and interpreted the data. All authors reviewed the manuscript.

ORCID

Lena Václavů https://orcid.org/0000-0001-8617-7752

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