Predictors of cerebral blood flow in patients with and without anemia

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Borzage MT, Bush AM, Choi S, Nederveen AJ, Václavík L, Coates TD, Wood JC. Predictors of cerebral blood flow in patients with and without anemia. J Appl Physiol 120: 976–981, 2016. First published January 21, 2016; doi:10.1152/japplphysiol.00994.2015.—Sickle cell disease (SCD) is the most common cause of stroke in childhood and results primarily from a mismatch of cerebral oxygen supply and demand rather than arterial obstruction. However, resting cerebral blood flow (CBF) has not been examined in the general African American population, in whom obesity, hypertension, cerebrovascular disease, and diminished cerebrovascular reserve capacity are common. To better understand the underlying physiological substrate upon which SCD is superimposed, we measured CBF in 32 young (age 28 ± 10 yr), asymptomatic African American subjects with and without sickle cell trait (n = 14). To characterize the effects of chronic anemia, in isolation of sickle hemoglobin we also studied a cohort of 13 subjects with thalassemia major (n = 10), dyserythropoetic anemia (n = 1), or spherocytosis (n = 2). Blood was analyzed for complete blood count, hemoglobin electrophoresis, cell free hemoglobin, and lactate dehydrogenase. Multivariate regression analysis showed that oxygen content was the strongest predictor of CBF (r² = 0.33, P < 0.001). CBF declined rapidly in the second and third decades of life, but this drop was explained by reductions in cerebral gray matter. However, age effects persisted after correction for brain composition, possibly representing microvascular impairment. CBF was independent of viscosity, hemoglobin S%, and body mass index. Hyperoxia resulted in reduced CBF by 12.6% (P = 0.0002), and CBF changes were proportional to baseline oxygen content (r² = 0.16, P = 0.02). These data suggest that these hemoglobin subtypes do not alter the normal CBF regulation of the balance of oxygen supply and demand.

African Americans; anemia; cerebral blood flow; hyperoxia; stroke

SICKLE CELL DISEASE (SCD) is the most common cause of stroke in the pediatric population. In patients with SCD, abnormal red cell rheology, hemolysis, anemia, oxygen-dissociation curve changes, and endothelial dysfunction impair oxygen delivery, whereas rapid changes in the maturing brain affect oxygen consumption. More than 95% of patients with SCD are also African American, in whom factors such as obesity and hypertension are known cerebrovascular risk factors. In fact, African Americans develop cerebrovascular disease at twice the rate of white Americans (15) and have significantly diminished cerebrovascular reserve capacity even in apparently healthy young adults (18), and more than 1.5% of all children born in the United States and 7.3% of African Americans have sickle cell trait (SCT); despite these observations, resting cerebral blood flow (CBF) in African Americans has not been systematically examined.

Therefore, the primary goal of this study was to determine the predictors of CBF in asymptomatic young African American subjects with and without SCT to establish reference values for patients with SCD. To thoroughly characterize the effect of anemia on CBF independent of sickle hemoglobin it was necessary to recruit subjects with thalassemia major, dyserythropoetic anemia, and spherocytosis. This was necessitated because of a lack of appropriate African American subjects with severe, chronic anemia (Hb <10) and without either sickle hemoglobin or other vasculopathy in the age group required. Our overarching hypotheses were that 1) the presence of SCT (one sickle cell gene) would not affect CBF, and 2) blood oxygen content would be the largest determinant of CBF in our study population, overwhelming any effect of blood viscosity, blood pressure, hemolysis, and body mass index.

MATERIALS AND METHODS

This study was approved by the Children’s Hospital Los Angeles Committee on Clinical Investigations (CCI 11-00083) and informed consent was obtained from all patients. Two ethnically distinct cohorts were recruited. The first cohort consisted of African Americans older than 12 yr of age (n = 32). Exclusion criteria included prior history of neurologic insult; developmental delay; seizures; learning disability; or serious, chronic illness requiring daily medications. The second cohort consisted of patients with chronic anemia, excluding sickle cell anemia, who were otherwise healthy and age-matched to the first cohort. The etiology of anemia consisted of thalassemia major (n = 10), congenital dyserythropoetic anemia (n = 1), and spherocytosis (n = 2). Exclusion criteria included prior neurologic insult, developmental delay, seizures, learning disability, or major medical problems in addition to chronic anemia.

Patients underwent measurement of vital signs and phlebotomy. Complete blood count, reticulocyte total, quantitative hemoglobin electrophoresis, and lactate dehydrogenase (LDH) and cell free hemoglobin levels were analyzed in the clinical laboratory. A 10-ml aliquot of each sample was heparanized, equilibrated with humidified room air using a hollow tube gas exchanger (Living Systems Instrumentation, Burlington, VT), and analyzed for viscosity at a controlled temperature of 37°C (Rheolog Health Vector, Pennsauken, NJ).
MRI was performed using an eight-element phased-array head coil on a 3T Philips Achieva. A large field-of-view magnetic resonance angiogram was reformatted into coronal and sagittal images to better localize the phase-contrast imaging plane approximately 1 cm above the carotid bifurcation and orthogonal to both carotids. Vertebral arteries exhibited obliquity of up to 15 degrees in ~10% of the patients. Image parameters for the phase-contrast examination were as follows: repetition time, 12.3 ms; echo time, 7.5 ms; field of view, 260 mm; thickness, 5 mm; signal averages, 10; acquisition matrix, 204 × 201; reconstruction matrix, 448 × 448; bandwidth, 244 Hz/pixel; and velocity encoding gradient, 200 cm/s.

Initially, the within-session stability of phase-contrast measurements was unknown. As a result, phase-contrast measurements were interleaved (without relocating) with the other imaging experiments.

After baseline anatomic and phase-contrast imaging, patients were fitted with a custom rebreathing apparatus with a 4-liter inspiratory reservoir to provide low-resistance tidal breathing. The reservoir was refreshed with 10-15 l/min of inflowing gas, which was typically 21% oxygen/79% nitrogen. Patients breathed through a SCUBA mouthpiece coupled via a three-way valve to the breathing reservoir. Nasal plugs blocked nasal inflow. Phase-contrast imaging was subsequently repeated with patients breathing a mixture of 21% oxygen and 79% nitrogen. The patients then underwent two blood oxygen level-dependent (BOLD) imaging experiments (not reported in this manuscript). During the first BOLD experiment, five breaths of pure nitrogen were administered to transiently reduce patient oxygen saturations to 80–85%. During the second BOLD experiment, 100% oxygen was administered for 4 min to increase blood oxygen content. Phase-contrast measurements were repeated before and after both oxygen challenges. Arterial blood oxygen content was estimated using the following equation:

\[ \text{O}_2\text{sat}\% = 100 \times \frac{\text{PO}_2}{\text{PO}_2 + 0.003 \times \text{Hb}} \]

where \( \text{Hb} \) represents the hemoglobin from chronic transfusion therapy. The two patients with spherocytosis exhibited polycythemia (Hb 17.5), although the other 11 non-African American patients had hemoglobin levels of 10.2 ± 0.7; patients with spherocytosis were retained in the regression analyses because they provided additional dynamic range to assess the effect of hemoglobin on CBF.

A representative phase-contrast acquisition with vessel boundaries and time course of CBF measurements is shown in Figure 1. Within-session stability of the CBF measurements was assessed in 21 patients (17 with normal hemoglobin and 4 with anemia). An average of 4.7 ± 1.1 (range, 2–7) CBF measurements were performed across 30.3 ± 9.5 (range 4–50) min. The coefficient of variation was 4.6% ± 3.0% (range, 0.6–12.4) with a median of 3.5%.

Not surprisingly, the strongest predictor of CBF normalized to brain weight was oxygen content (\( r^2 = 0.33, P < 0.001, \) Fig. 2). After removing the contribution from oxygen content, the dominant predictors of CBF are summarized in Table 2. Age was a negative correlate of CBF. Systolic and diastolic blood pressures were also negatively associated with CBF. CBF was equally correlated with gray matter volume and cortical surface area, and less strongly associated with total brain volume. Notably, CBF was independent of white matter volume and cortical thickness.

The observed age-related reduction in CBF during adolescence and early adulthood could be explained by normal brain maturation. There was a dramatic decrease in gray matter volume as a function of patient age up to 25 yr of age (Fig. 3, open circles), paralleling the changes in CBF corrected for oxygen content (Fig. 3, diamonds). Because gray matter consumes more energy than white matter, we entered gray matter volume into the multivariate model before entering age. Age remained a powerful independent predictor of CBF, but no effect was observed until after 30 years of age. After inclusion of oxygen content, gray matter volume, and age, CBF was independent of other patient demographics, vital signs, blood viscosity, and all clinical laboratory variables except LDH. The multivariate model for CBF log-transformed both CBF and all the following parameters. The resulting model used log-transformed parameter values as follows: log oxygen content (ml \( \text{O}_2 \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1} \), -0.542 (\( r^2 = 0.35, P < 0.001 \); log total gray matter (mm\(^3\)), 0.337 (\( r^2 = +0.16, P < 0.001 \); log age (years), -0.245 (\( r^2 = +0.08, P = 0.008 \)), and log LDH, -0.322 (\( r^2 = +0.07, P = 0.007 \)). The total model \( r^2 \) was 0.67.
Following baseline CBF assessments, patients were given the breathing apparatus, and CBF was remeasured while they breathed room air. Breathing through the circuit resulted in a CBF decrease from 752 to 695 ml/min (standard error 15 ml/min, P < 0.001). After the brief exposure to 100% nitrogen and subsequent 5-min recovery, CBF was unchanged (P = 0.85). Following the 5-min exposure to 100% oxygen, CBF was measured again (Fig. 4). CBF declined 12.6% (P < 0.001) and response magnitude was inversely proportional to hemoglobin level (Fig. 4, right; r² = 0.19, P = 0.01). The CBF reduction with 100% oxygen administration was exactly balanced by the improved oxygen content provided by the dissolved oxygen, such that oxygen delivery was unchanged by exogenous oxygen administration.

**DISCUSSION**

Our data are the first in humans to systematically explore the relative importance of oxygen content, brain weight and composition, age, and viscosity in determining CBF in a predominantly African American population. These data were in excellent agreement with a large historical cohort of Caucasians (8) and reinforce the hypothesis that CBF is controlled to maintain a stable oxygen delivery to the brain.

The inverse relationship between CBF and oxygen content at baseline was expected, but the reduction in CBF with 100% oxygen has not been universally observed (8, 9, 25, 28, 32, 35, 36). Some authors attribute the decreased CBF to reductions in cerebral metabolic rate (35), others dispute this (36) and attribute the response to the stimulatory effect of oxygen on ventilation (9, 25, 35). However, the larger CBF decrease observed in patients with anemia cannot be attributed to hyperventilation. Instead, our observations suggest that the brain is adjusting CBF to maintain oxygen delivery, and the dissolved oxygen fraction is a much larger fraction of total oxygen carrying capacity in patients with anemia, producing a greater effect on CBF. This study cannot identify how local oxygen demand in the brain is transduced and regulated. Partial pressure of oxygen has been proposed as the regulatory stimulus (7, 13, 24). We speculate that if this is true, the oxygen-sensing apparatus must be sufficiently distal in the microvascular distribution network that dissolved oxygen fraction is reflective of supply-demand balance.

The second striking observation was that the sharp reduction in CBF we observed in the second and third decades of life was explained by the reduction in gray matter volume and surface area that occurs during normal brain maturation (6, 12, 33). The independence of CBF from white matter volume under-
Interestingly, gray matter volume and cortical surface area (CSA) were equally important in determining CBF, but CBF was independent of cortical thickness. Gray matter volume is dependent on both CSA and cortical thickness, but these parameters account for different morphological traits of the neocortex. The radial unit hypothesis explains that CSA is affected by the number of neuronal columns, whereas cortical thickness is determined by the number of neurons in each column (29). Expansion of CSA increases the number of columns, thereby increasing the complexity of neuronal networks (17), and hence increasing energy consumption. Determinants of cortical thickness on the other hand are not as straightforward. Cortical thickening is a normal developmental process, cortical thinning is typically the result of myelination and synaptic pruning (20), and later in adulthood brain atrophy due to normal aging and disease further thins the cortex.

Systolic and diastolic blood pressures were negatively associated with CBF after controlling for oxygen content. However, systolic and diastolic blood pressures both rise sharply through childhood and adolescence, so we believe that blood pressure associations were merely surrogates for patient age.

![Fig. 1. A: phase-contrast imaging plane referenced to a coronal angiogram. B: magnitude image. C: phase image depicting the voxels selected by the vessel-selection algorithm. D: representative time course from our youngest control subject (12.6 yr of age).](image)

![Fig. 2. Plot of cerebral blood flow (CBF) normalized to per 100 g of total brain weight vs. oxygen content. Large dots indicate our experimental data. Plus signs (+) represent controls described by Brown et al. (8).](image)

Table 2. Significant predictors of first residual of cerebral blood flow and O2 content

<table>
<thead>
<tr>
<th>Parameter</th>
<th>$r^2$, Direction</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td>Age</td>
<td>0.20, ↓</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total brain volume</td>
<td>0.12, ↑</td>
<td>0.008</td>
</tr>
<tr>
<td>Gray matter volume</td>
<td>0.14, ↑</td>
<td>0.003</td>
</tr>
<tr>
<td>Cortical surface area</td>
<td>0.14, ↑</td>
<td>0.003</td>
</tr>
<tr>
<td>Cortical thickness</td>
<td>0.02, ↓</td>
<td>0.28</td>
</tr>
<tr>
<td>White matter volume</td>
<td>0.02, ↓</td>
<td>0.23</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>0.1, ↓</td>
<td>0.01</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>0.12, ↓</td>
<td>0.007</td>
</tr>
<tr>
<td>Lactate dehydrogenase</td>
<td>0.17, ↓</td>
<td>0.01</td>
</tr>
</tbody>
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All variables were log-transformed in the analysis.
After accounting for age, blood pressure disappeared as a predictor of CBF.

After correcting for changes in brain size and composition, further age-related CBF decline remained apparent in the fourth and fifth decades of life, which could not be accounted for by cortical atrophy. Similar findings have been described in positron emission tomography, computed tomographic perfusion, magnetic resonance arterial spin labeling, and phase-contrast MRI studies (2, 19, 19a, 27, 31). Recent work by Lu and colleagues (22) suggests that CBF reductions with age are not the result of declining cerebral metabolic rate, but instead they reflect microvascular disease.

This is particularly interesting given the perplexing relationship between CBF, LDH, and absolute neutrophil count (ANC). We could find no precedent for these associations in the literature. LDH can reflect intravascular hemolysis in patients with SCD, but neither reticulocyte count nor cell free hemoglobin (which are better hemolytic markers) correlated with CBF. We speculate that the LDH may serve as a nonspecific marker of endothelial damage. ANC could represent a surrogate of systemic inflammation; we did not measure high-sensitivity C-reactive protein, which would have been a better surrogate of endothelial damage. ANC could represent a surrogate of systemic inflammation; we did not measure high-sensitivity C-reactive protein, which would have been a better surrogate of systemic inflammation. Regardless, the CBF associations with LDH and ANC should be independently repeated in a larger cohort before too much weight is placed on these observations.

A number of negative findings were noteworthy. After controlling for hemoglobin and gray matter volume, sex differences in CBF disappeared. CBF was also independent of hemoglobin electrophoresis results, despite the known effect of SCT on the hemoglobin dissociation curve (4). Blood viscosity had no detected effect on CBF after correction for differences in oxygen carrying capacity. This does not imply that blood viscosity is unimportant in mediating changes in CBF in response to stimuli (8, 30), but that it plays no apparent role in establishing the “set point” for baseline CBF (7). CBF was also independent on BMI over a fairly broad range. This is interesting given accumulating evidence that obesity directly promotes vascular inflammation via adipose-based synthesis of cytokines and accelerates cerebral microvascular disease (21). However, to observe this, an older cohort of individuals with higher BMI may have been required.

Our study had limitations. Our patients with chronic anemia had Asian or Middle Eastern ancestry, whereas the primary control cohort was of African descent. This was necessitated by design because we wanted to study the effect of anemia alone without the confounding influences of SCD (e.g., vaso-occlusion, intravascular hemolysis, and stroke, which usually occur in patients with SCD). We measured CBF in a single axial plane rather than four acquisitions optimized to vessel angle as advocated by some because the temporal variation in any one vessel is higher than the variability in their sum. We estimated pressure of arterial oxygen rather than directly measuring it via arterial blood gases. Although this would have little effect on our regression analyses, patient-specific differences in alveolar-arterial oxygen gradient could affect results of the 100% oxygen challenge. Our study would have benefited from continuous measurements of CO2. We speculate that the reduction in CBF that occurred when subjects transitioned from breathing room air freely to breathing room air through a breathing apparatus was caused by mild hyperventilation. However, without an MRI-compatible transcutaneous CO2 measuring device, we cannot confirm this hypothesis.

In summary, we demonstrated that oxygen-demand principles dominate resting CBF and its response to inhaling 100% oxygen. Increased CBF in patients with chronic anemia is sufficient to preserve brain oxygen delivery. Marked changes in CBF observed in the second and third decades of life were entirely explained by normal brain maturation (33). After correction for oxygen content and gray matter volume, no effect of sex, sickle cell hemoglobin, or blood viscosity was evident. Age-related decline in CBF was still observed starting around 30 yr of age. Our results in this predominantly African American cohort provide key normative data for studying impaired oxygen delivery in patients with sickle cell anemia.

GRANTS

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Fig. 3. First residual of CBF, after removing the contribution from oxygen content plotted as a function of age (solid diamonds). Gray matter volume as a function of age (open circles).

Fig. 4. Left: plot of CBF and cerebral oxygen delivery when breathing room air and 100% oxygen through nonbreathing ventilatory circuit. CBF decreased 7.6% when patients breathed oxygen, roughly balancing the projected increase in oxygen carrying capacity. As a result, cerebral oxygen delivery is completely neutral to inhaled oxygen. Right: percent reduction in CBF with 100% oxygen treatment as a function of hemoglobin level.
DISCLOSURES

J. Wood is a consultant for AMAG Pharmaceuticals, ISIS Pharmaceuticals, ApoPharma, Pfizer, Celgene, WorldCare Clinical, and BiomedInformatics. He also receives support-in-kind from Philips Healthcare. T. Coates is a consultant for Acceleron, ApoPharma, Celgene, ISIS Pharmaceuticals, and Novartis. None of the others authors have commercial affiliations.

AUTHOR CONTRIBUTIONS


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5. Bernaudin F, Verlhac S, Arnaud C, Kamdem A, Chevret S, Hau I, J. Wood is a consultant for AMAG Pharmaceuticals, ISIS Pharmaceuticals, ApoPharma, Pfizer, Celgene, WorldCare Clinical, and BiomedInformatics. He also receives support-in-kind from Philips Healthcare. T. Coates is a consultant for Acceleron, ApoPharma, Celgene, ISIS Pharmaceuticals, and Novartis. None of the others authors have commercial affiliations.

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