Developmental Changes in Resting and Functional Cerebral Blood Flow and Their Relationship to the BOLD Response

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Abstract: Our understanding of cerebral blood flow (CBF) in the healthy developing brain has been limited due to the invasiveness of methods historically available for CBF measurement. Clinically based studies using radioactive tracers with children have focused on resting state CBF. Yet potential age-related changes in flow during stimulation may affect the blood oxygenation level dependent (BOLD) response used to investigate cognitive neurodevelopment. This study used noninvasive arterial spin labeling magnetic resonance imaging to compare resting state and stimulus-driven CBF between typically developing children 8 years of age, 12 years of age, and adults. Further, we acquired functional CBF and BOLD images simultaneously to examine their relationship during sensory stimulation. Analyses revealed age-related CBF differences during rest; the youngest group showed greater CBF than 12-year-olds or adults. During stimulation of the auditory cortex, younger children also showed a greater absolute increase in CBF than adults. However, the magnitude of CBF response above baseline was comparable between groups. Similarly, the amplitude of the BOLD response was stable across age. The combination of the 8 year olds' elevated CBF, both at rest and in response to stimulation, without elevation in the BOLD response suggests that additional physiological factors that also play a role in the BOLD effect, such as metabolic processes that are also elevated in this period, may offset the increased CBF in these children. Thus, CBF measurements reveal maturational differences in the hemodynamics underlying the BOLD effect in children despite the resemblance of the BOLD response between children and adults. *Hum Brain Mapp* 35:3188–3198, 2014. © 2013 Wiley Periodicals, Inc.

Key words: perfusion; hemodynamic response; arterial spin labeling; ASL; development; FMRI; children; auditory cortex

INTRODUCTION

Magnetic resonance imaging (MRI)-based techniques have been used to examine and quantify many different aspects of the structure and function of the developing brain in order to understand typical growth patterns and their underlying mechanisms. One process central to brain function that has not yet received full attention in the healthy, typically developing brain is cerebral blood flow (CBF), the rate at which oxygenated blood is delivered to the cerebrum. While CBF is fundamental for brain function and it contributes directly to the blood oxygenation level dependent (BOLD) signal measured in a rapidly increasing

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number of functional MRI (fMRI) studies conducted with children, our knowledge of CBF in the developing brain is limited.

Our current understanding of developmental change in CBF stems mainly from radionuclide tracing techniques such as positron emission tomography (PET) or single photon emission (computed) tomography (SPET, SPECT), which have been the primary means of measuring CBF. PET and SPECT entail the intravenous administration of a radioactive isotope. The tracer emits gamma rays that are detected and used to measure CBF. Pediatric PET and SPECT studies have reported changes in CBF from birth to adulthood. CBF is below adult levels in infancy, with a rapid increase in the first years of life [Chiron et al., 1992, Rubinstein et al., 1989, Takahashi et al., 1999, Wintermark et al., 2004]. Children show peak rates between 4 and 10 years of age when their rates surpass adults by 70% or more [Barthel et al., 1997, Chiron et al., 1992, Ogawa et al., 1989, Takahashi et al., 1999, Wintermark et al., 2004]. In teenage years, CBF gradually slows to adult levels [Barthel et al., 1997, Chiron et al., 1992, Ogawa et al., 1989, Takahashi et al., 1999]. This trajectory occurs in concert with, and likely supports, the characteristic pattern of early exuberant growth and subsequent pruning evident in multiple facets of brain development such as synaptic density [Huttenlocher, 1990], dendritic density [Purpura, 1975], gray matter volume [Giedd et al., 1999], and in levels of cerebral glucose [Chugani, 1987] and oxygen [Takahashi et al., 1999] metabolism. Given the invasive nature of these CBF measurement techniques, the PET and SPECT studies with infants and children are clinically based, retrospective studies of diagnostic data. As clinical studies, they have measured CBF only during resting state, often accompanied by sedation. Whether or not typically developing children display a similar pattern at rest and during activity remains to be determined.

Further, the maturational differences in CBF point to possible age-related differences in the BOLD signal that may reflect differences in blood flow, instead of developmental differences in neural or cognitive processes. Rather than a direct measure of neural activity, the BOLD signal reflects changes in the state of blood oxygenation. Multiple physiological mechanisms function together to give rise to the BOLD effect. During resting state, arterial blood containing oxyhemoglobin flows to the capillary bed where oxygen is extracted and metabolized. The deoxygenated blood leaves through the venous system. In a magnetic environment, the paramagnetic property of deoxyhemoglobin generates local magnetic field gradients that disrupt the magnetic resonance signal. In contrast, during a state of neural activity local CBF increases, cerebral blood volume (CBV) increases, though to a lesser degree, and the cerebral metabolic rate of oxygen (CMRO₂) increases only slightly. Consequently, the net oxygen extraction fraction (OEF) decreases, and more oxygenated blood passes into the veins. The resultant decrease in deoxyhemoglobin concentration reduces the local field inhomogeneity and

increases the magnetic resonance signal, which is known as the BOLD effect [Ogawa et al., 1990, 1992]. Thus CBF plays a central role in the complex set of events that culminates in the BOLD signal. These mechanisms suggest that the different CBF rates seen in children during rest and possibly during activity could contribute to corresponding differences in the amplitude of the BOLD response. While the relationship between these mechanisms of the BOLD effect has been examined in the mature brain, potential differences in the immature brain have not been explored in children to the same extent (see Harris et al., 2011 for a review of potential physiological confounds to the BOLD response in the developing brain).

An increasing number of developmental studies use fMRI to study age- and performance-related changes in the neural mediation of a range of functions with an approach of comparing the sites and extents of BOLD activation in children and adults. Some studies include reports of positive correlations between the percent of BOLD signal change and age [e.g., Klingberg et al., 2002]. Other studies suggest principles of developmental change in the pattern and extent of activation in cross-sectional [e.g., Brown et al., 2005, Durston et al., 2006] and longitudinal designs [Szaflarski et al., 2006]. However few studies have been conducted to specifically examine the BOLD effect and its underlying physiology during childhood. Those that have done so designed their studies to control for possible developmental differences in task performance by implementing a sensory or simple motor task or by eliminating a task condition from the experiment. Kang et al. [2003] compared the time course of the BOLD response in children 7–8 years of age with adults in a sensorimotor task and found comparable responses between the two groups. The hemodynamic responses have also been found to remain similar during both transient and sustained stimulation [Wenger et al., 2004]. Richter and Richter [2003] examined the time course of the BOLD response to a visual checkerboard in a broad age range from 7 to 61 years of age. Their analysis revealed that the latency for the rise of the hemodynamic response and the time to the peak were similar with age, but the latency for the post-stimulus decline of the signal was shorter for children than for younger and older adults. Another study investigated vascular reactivity and the BOLD response to breath holding (hypercapnia) [Thomason et al., 2005]. Breath holding triggers a BOLD response at rest that is similar to a response during stimulation and neural activity so that it provides an opportunity to examine vascular responsiveness outside of the context of cognitive or sensory tasks. Thomason and colleagues found that children 7–12 years of age showed a greater percent BOLD signal change than adults during breath holding, which suggests age-based differences in the underlying vascular dynamics.

Arterial spin labeling (ASL), a newer MRI technique, allows for noninvasive quantification of CBF in healthy children during rest and stimulation and provides an

opportunity to examine the relationship between CBF and the BOLD effect. ASL applies a magnetic tag to arterial blood with an inversion pulse. The tagged blood flows to the cerebrum where tag images are acquired. In alternation, control images are acquired in the same slices without the presence of the tag in the blood. A quantitative measure of CBF is derived from the difference between the control and tag images, which is proportional to the flow of blood. ASL can be used to measure resting state CBF or in a functional paradigm for a quantitative assessment of the hemodynamic response.

Investigators have begun to apply ASL to study resting state CBF in children. The first studies to date that have examined neurologically normal children during states of rest or sedation in clinical settings demonstrate the feasibility of using ASL with young children [Wang and Licht, 2006, Wang et al., 2003] and examine large age ranges whose profiles concur with the previously reported profile of CBF during development [Biagi et al., 2007]. More recently ASL has also been used to assess resting perfusion in healthy infants [Miranda et al., 2006, Wang et al., 2008] and children [Taki et al., 2011]. A study of 202 typical children from 5 to 18 years of age shows an arching trajectory of CBF increase and decline across childhood [Taki et al., 2011]. A single experimental study combined ASL and FMRI techniques to examine the role of CBF in the inverse BOLD response observed in infancy and early development [e.g., Anderson et al., 2001, Born et al., 1996, Martin et al., 1999, Yamada et al., 1997]. Born et al. [2002] found decreases in both CBF and the BOLD signal in participants from 4 months to 6 years of age in response to passive visual stimulation during sedation or sleep. To our knowledge, no one has investigated CBF and BOLD hemodynamic responses in typical, middle school-age children.

The goal of the current study was to measure CBF in typically developing, nonsedated children to examine possible age-related changes during both rest and in response to sensory stimulation. Further, this study examined the CBF stimulus response in direct relationship to the BOLD response to determine whether or not elevated CBF in childhood corresponds to greater amplitude in the BOLD response. We used ASL to compare CBF in three age groups, 8 year olds, 12 year olds, and adults, during resting state and in response to auditory sensory stimulation. These age periods were selected to capture a period of peak CBF (8 years of age), an early phase of CBF decline (12 years of age), and mature rates of CBF in adulthood. Further, we used a pulsed ASL PICORE QUIPSS II sequence [Wong et al., 1998] to simultaneously acquire CBF and BOLD weighted images to examine CBF and the BOLD stimulus response. Since the ASL signal to noise ratio is lower than BOLD FMRI [Luh et al., 2000] this study targeted a primary sensory area, the auditory cortex, where the site of the BOLD response to auditory stimulation is predictable and the hemodynamic response is robust.

MATERIALS AND METHODS

Subjects

Thirty-three healthy individuals participated in the study; eleven 8 year olds ($M = 9.09$ years, range = 7.98–10, 7 males), ten 12 year olds ($M = 12.43$ years, range = 11.52–13.22, 3 males) and 12 adults ($M = 24.27$ years, range = 22.55–28.37, 6 males). These age groups were anticipated to represent different phases of developmental change in CBF [Barthel et al., 1997; Biagi et al., 2007; Chiron et al., 1992; Takahashi et al., 1999, Wang et al., 2003]. Children 8 years of age represent a period when CBF is likely to be elevated and 8 year olds can remain still voluntarily during image acquisition. The 12-year-old group represents a phase when the peak of CBF begins to decrease. Participants were recruited from local science fairs and through advertisements in a local parent magazine. All participants were screened for sources of metal in their bodies prior to scanning. In addition, individuals with psychological or neurological conditions, learning disabilities, and those born more than 3 weeks premature were excluded from participation. All participants were right-handed, based on parent report. Adults and parents of child participants gave informed consent and children gave informed assent in accordance with the Institutional Review Boards at San Diego State University and the University of California, San Diego. In addition to the final sample size above, data from additional children were excluded due to gross motion detected upon visual inspection (three 8 year olds and one 12 year old) or an incomplete scan session (three 8 year olds).

Auditory Stimulus

Participants listened to an auditory stimulus through headphones while in the MRI scanner. Auditory stimuli consisted of instrumental music [Andersson and Ulvaeus, 1975] presented in a block design. A functional run began with 40 s of rest, after which 20 s of task and 40 s of rest were alternated for a total of 4 cycles and a total duration of 4 min 40 s per run.

Image Acquisition

Images were acquired on a 3 Tesla General Electric Signa whole body system, with an 8-channel receive only head coil and a body transmit coil. An ASL PICORE QUIPSS II sequence with a dual-echo spiral readout [Wong et al., 1998] was used to acquire CBF and BOLD data simultaneously. Five 6 mm axial slices were positioned through primary auditory cortex. The tagging band was a 100 mm thick slab positioned 10 mm inferior to the edge of the lowest imaging slice ($TR = 2$ s, $TI_1 = 600$ ms, $TI_2 = 1,500$ ms, $TE_1 = 9.5$ ms, $TE_2 = 30$ ms, $\theta = 90^\circ$, $FOV = 24 \times 24$ cm, matrix size = 64×64). The transit time and temporal width of the bolus were selected based on

our previous measurement of these parameters in children 8 and 12 years of age. With this sequence, the acquisition of an image in which the blood has been exposed to an inversion pulse (tag image), is alternated with the acquisition of an image without inverted magnetization of the blood (control image) throughout the time series. The difference between the control and tag images from the first echo yields a CBF time series, and the average between the control and tag images from the second echo yields a BOLD time series. A 3-min resting state scan was acquired first and followed by four functional runs. In addition, a cerebrospinal fluid (CSF) reference scan ($TR = 4$ s, $TI_1 = 700$ ms, $TI_2 = 1,400$ ms, $\theta = 90^\circ$, $FOV = 24 \times 24$ cm, matrix size = 64×64 , $TE = 9.5$ ms) and a minimum contrast scan ($TR = 2$ s, $TI_1 = 700$ ms, $TI_2 = 1,400$ ms, $\theta = 90^\circ$, $FOV = 24 \times 24$ cm, matrix size = 64×64 , $TE = 11$ ms) were collected and used to quantify resting state CBF in absolute units. A high-resolution structural image was acquired using a 3D fast spoiled gradient (FSPGR) pulse sequence (1.0 mm slice thickness; $TR = 7.6$ ms, $TE = 2.9$ ms, $TI = 450$ ms, $\theta = 12^\circ$; $FOV = 25 \times 25$ cm; matrix = 256×256 ; $NEX = 1$) for an anatomical reference. Subjects were carefully positioned in the scanner to ensure that their anterior and posterior commissures were aligned within the same plane at the time of scanning.

Physiological Data

Cardiac pulse and respiratory effort were recorded throughout the functional runs to reduce physiological noise and increase the signal to noise ratio in the ASL data off-line [Restom et al., 2006]. A pulse oximeter (InVivo, Orlando, FL) was placed on the participant's left index finger to record the pulse rate. A respiration belt with an attached respiratory effort transducer (BIOPAC Systems, Goleta, CA) was placed around the participant's chest to record respiratory effort. For five subjects (two participants in each the 8-year-old and adult age groups and one in the 12-year-old group), physiological data were not usable due to errors in collection or transfer. The ASL data from these subjects were retained and included in the group analyses. Additionally, since caffeine has been found to influence CBF change [Perthen et al., 2008] participants were asked to refrain from drinking caffeinated beverages the day of their appointment.

Preprocessing

Data analysis was performed using Analysis of Functional NeuroImages (AFNI) software suite [Cox, 1996]. For each ASL time series, the first four time points acquired before the magnetic signal reached steady state were removed from analysis. From each ASL functional run a CBF and a BOLD time series were computed. A CBF time series was generated from the images from the first echo with a running subtraction of each tag image from the

average of its surrounding nearest neighbor control images. A BOLD time series was generated from the second echo images with a running average of each tag image with the mean of its nearest neighbor control images [Liu and Wong, 2005]. Physiological noise was removed as described by Restom et al. [2006] and 3D volume registration within and between runs was performed for motion correction. Two runs for each subject were analyzed and the runs were selected based on the order of acquisition and on motion screening criteria. For the majority of subjects runs 1 and 2 were used. In some cases, movement in one or the other of those scans exceeded our criterion for motion, in which case we used the next of the additional runs that met criterion. All subjects' time series were visually inspected for movement. In addition, the data were screened quantitatively using the output parameters from the 3D volume registration. From these parameters the maximum displacement between images in the time series was derived. Datasets were retained for further analysis if fewer than 10% of the time points in a series had a maximum displacement of 2 mm or more [Shih et al., 2011]. No participants were excluded based on this criterion.

On an individual subject basis, for the CBF and BOLD time series separately, the first and second runs were concatenated and analyzed with a multiple regression model using 3dDeconvolve. The auditory stimulus function was the regressor of interest and nuisance regressors included six motion parameters (derived from the image registration process), a baseline and linear trend. To correct for multiple comparisons within the ROI, AlphaSim was used to generate a Monte-Carlo simulation that estimated a minimum cluster volume of $337.5 \mu\text{l}$ with a voxel-wise probability of 0.05, which corresponded to a cluster-wise activation probability of $P \leq 0.05$. Suprathreshold clusters in the CBF activation maps were spatially contiguous with or overlapped clusters in the BOLD maps. Clusters meeting these criteria were retained for further analysis.

Region of Interest Analysis

The structural images were aligned with the functional images to localize the task-related CBF and BOLD changes. ROIs were manually drawn on the high-resolution anatomical images in the axial plane in their native matrix size. The ROI encompassed the left primary auditory cortex and consisted of the anterior and posterior transverse gyri. The anterior boundary was the fundus of the first transverse sulcus and the posterior boundary was the fundus of the second transverse sulcus [Penhune et al., 1996; Steinmetz and Galaburda, 1991]. The left hemisphere was selected based on known asymmetries (left greater than right) in the posterior perisylvian region including Heschl's gyrus [Chance et al., 2006; Morosan et al., 2001; Penhune et al., 1996; Warrier et al., 2009] and the planum temporale [Geschwind and Levitsky, 1968; Preis et al.,

Figure 1.

ROI in auditory cortex. A single slice of the ROI tracing is shown in both the axial and sagittal planes.

1999; Steinmetz et al., 1989] in order to promote greater uniformity (between subjects and in interrater reliability) in manually delimiting the ROI. Anatomical tracings were resampled to the resolution of functional images to serve as a mask for those time series. Figure 1 shows an axial view of the ROI and a sagittal view of the same ROI tracing in a single slice through the transverse gyri. Two raters who were blind to the identity and the age group of the brains independently traced the ROI for all participants. In addition, one of the raters traced the full set of ROIs twice, at two separate times. For the interrater reliability the intraclass correlation was 0.99, and for the intrarater reliability the correlation was 0.98.

For suprathreshold clusters within the ROI, a mean CBF and a mean BOLD time series were derived. In each of the mean time series, the four cycles of task and rest were averaged to create a single mean response cycle for each participant. From this average cycle, multiple indices were derived for each subject. The absolute CBF change between rest and stimulation was calculated as the difference between the average signal value during the initial rest period and the average signal value during the time of peak response, defined as 7–13 TRs after the onset of the stimulus to allow for the hemodynamic rise time. The percent of CBF change and the percent of BOLD signal change were calculated as the percent change in signal between initial rest and peak. Quantification of resting state CBF was derived from the 3-min resting state series, and the average resting CBF of voxels within the ROI was calculated.

Statistical Analysis

Separate one-way analysis of variance (ANOVA) tests were used for age group comparisons (8 year olds, 12 year olds, and adults) of mean resting CBF, absolute CBF change, percent change in CBF, and percent change in the BOLD signal during auditory stimulation versus rest. A one-way ANOVA also compared the number of activated voxels in the age groups. Least significant difference tests

(LSD) between age groups were conducted for each of these measures.

RESULTS

The analysis of resting CBF showed a main effect of age group, $F(2,30) = 10.21$, $P < 0.001$. Comparisons between age groups revealed that 8-year-old children ($M = 73.09$, $SE = 3.50$) had significantly greater resting CBF levels than 12 year olds ($M = 57.99$, $SE = 3.67$), $P = 0.006$ and adults ($M = 51.59$, $SE = 3.35$), $P < 0.001$. No significant differences were observed between 12 year olds and adults. Figure 2 shows the mean resting CBF levels for each age group.

Analysis of absolute change in CBF between rest and listening to music revealed a main effect of age group, $F(2,30) = 3.83$, $P = 0.03$. In addition to increased resting CBF, children had higher activity-driven CBF as well. Eight year olds ($M = 56.18$, $SE = 6.08$) had significantly greater absolute CBF change between rest and auditory stimulation than adults ($M = 37.29$, $SE = 4.05$), $P = 0.01$. No significant differences were found between 8 year olds and 12 year olds ($M = 46.16$, $SE = 4.45$) or between 12 year olds and adults. The average time series of absolute change CBF for each age group are shown in Figure 3. The black bar indicates the time points defined as the peak (7–13 TRs after the onset of the stimulus).

Despite age-related differences in resting and absolute CBF change, no significant differences were observed between age groups for the percent of CBF change between rest and auditory stimulation, $F(2,30) = 0.40$, $P = 0.67$, ($M_s = 29.92$, 35.01 , 31.73 for 8 year olds, 12 year olds, and adults, respectively). That is, when the baseline differences were taken into account by expressing the signal at each time point as a percentage of the signal during the first 40 s of rest, there were no age group differences. The average time series of the percent of CBF change for each age group are shown in Figure 4.

Figure 2.

Resting CBF (mean $\pm$ SE) in each age group. The 8-year-old group shows a greater rate of CBF than the 12-year-olds and adults.

Figure 3.

Functional CBF response to auditory stimulation. Mean timeseries of the absolute CBF change from baseline and standard error bars at each time point for each group. The single "on/off" cycle represents the mean of the 4 cycles within a run. In the younger children, the absolute increase in CBF in response to auditory stimulation is larger than for older children and adults.

Figure 4.

Functional percent CBF change during stimulation. The mean timeseries for each age group show the percent of CBF change during stimulation relative to baseline, with standard error displayed. There are no significant differences between the groups when the relative increases above baseline are compared. The single "on/off" cycle represents the mean of the 4 cycles within a run.

While young children had significantly greater resting and absolute change in activity-driven CBF, no significant differences were found between age groups for the percent of BOLD signal change between rest and stimulation, $F(2,30) = 0.07$, $P = 0.93$, (M_s for 8 year olds, 12 year olds, and adult groups are as follows, 0.49, 0.51, 0.45). The average time series of percent BOLD change for each age group age is shown in Figure 5.

Age group analysis of estimated baseline CBF, average signal during the first 40 s of rest, in the functional runs

shows the same age-related differences as CBF estimated from the resting state scan $F(2,30) = 7.04$, $P = 0.003$. In the 8-year-old group, baseline CBF was greater than that of the 12-year-old group ($P = 0.053$), and the adults ($P = 0.001$). However, baseline CBF of 12 year olds did not differ from adults ($P = 0.12$).

Additional group comparisons that excluded the five subjects whose physiological data were not usable displayed the same findings. Comparisons of resting CBF showed group differences, $F(2,25) = 14.20$, $P < 0.001$. Specifically, 8-year-old children ($M = 76.13$, $SE = 2.90$) had significantly greater resting CBF levels than 12 year olds ($M = 60.63$, $SE = 3.56$), $P = 0.002$, and adults ($M = 52.71$, $SE = 3.02$), $P < 0.001$. There was also a main effect of age group for the absolute increase in CBF during stimulation $F(2,25) = 3.63$, $P = 0.04$, with 8 year olds showing ($M = 56.74$, $SE = 6.79$) a higher increase than adults ($M = 38.67$, $SE = 4.63$), $P = .01$. Neither group significantly differed from 12 year olds ($M = 42.46$, $SE = 2.78$), $P = 0.058$. Again, the percent of CBF increase was not different between age groups $F(2, 25) = 0.774$, $P = 0.472$ ($M_s = 28.44$, 28.79, 32.48, $SEs = 2.45$, 2.43, 2.79 for 8 year olds, 12 year olds, and adults, respectively). Comparisons of the BOLD response were also nonsignificant between groups, $F(2, 25) = 0.628$, $P = 0.54$, (M_s for 8 year olds, 12 year olds, and adults are 0.56, 0.57, 0.42, with corresponding $SEs = 0.137$, 0.103, 0.076).

To control for possible sex differences in the relationship between age and CBF or the BOLD response, separate partial correlation analyses examined the association between subject age and dependent measures of flow and BOLD, with sex as a control variable. Results resembled the findings from the one-way ANOVA analyses. Resting CBF showed a significant negative correlation with age,

Figure 5.

BOLD percent signal change during stimulation. The mean timeseries of the BOLD responses (and standard error) for each age group show comparability in the magnitude of response despite age-related differences in resting CBF and absolute CBF increase during stimulation.

$r(30) = -0.53$, $P = 0.001$, as did absolute change in CBF, $r(30) = -0.42$, $P = 0.02$. However, neither the stimulus-driven percent change in CBF nor in BOLD correlated significantly with age while controlling for sex, $r(30) = 0.01$, $P = 0.96$, and $r(30) = -0.03$, $P = 0.86$, respectively.

Age group comparisons of the number of voxels within suprathreshold clusters of activation showed no group differences for CBF, $F(2, 30) = 2.98$, $P = 0.07$, or for BOLD, $F(2, 30) = 2.52$, $P = 0.10$. The mean number of voxels in CBF clusters and the standard error for 8 year olds, 12 year olds, and adults are as follows: 12.09 (2.74), 17.8 (2.72), 22.67 (3.64). For BOLD clusters, the mean voxel count and standard error for the same age groups, respectively, are 23.54 (3.20), 30.10 (4.54), and 36.08 (4.32).

DISCUSSION

Noninvasive arterial spin labeling reveals age-related differences in CBF in typically developing volunteer children. Greater CBF in children than in adults is evident during resting state. The absolute difference between CBF during resting baseline and stimulus-related activity is also significantly different between the age groups. However, when we take the baseline differences into account by expressing the CBF stimulus-response in terms of percent change above baseline, then the age groups are no longer significantly different. Use of the QUIPSS II technique provided an opportunity to examine the BOLD hemodynamic response in direct correspondence to CBF. The percent of BOLD signal increase above resting baseline does not show a significant change with age. Thus although the rate of flow is greater at rest and during neural activity in children, the normalized amplitude of the BOLD response is stable across age.

This profile of resting state CBF and its changes with age coincides with reports based on PET or SPECT. In our study, 8-year-old children have greater CBF than 12 year olds and adults. The elevated rates in the younger child group compared to young teens and adults suggest that a decline to adult levels occurs in preteen and adolescent years. Previous studies of development from the first years of life to adulthood show CBF elevation during childhood and decline during adolescence [Barthel et al., 1997, Chiron et al., 1992, Ogawa et al., 1989, Takahashi et al., 1999]. The timing of the peak and the point of decline vary between studies. These variations likely reflect differences in parsing the age range and the resultant composition of the age groups. Barthel et al. examined children 4 to 15 years of age and showed a decrease in local CBF across the age range. By splitting the range into two groups, they revealed a trend in higher CBF in the younger, 4- to 10-year-old group than in the 11- to 15-year-old group, similar to our findings. Also consistent with our findings, Ogawa and colleagues show that 5- to 9-year-olds have greater regional CBF than 10- to 15-year-olds. Further they find that the values for the 10- to 15-year-old group are

comparable to adults. Takahashi et al. who investigated regional CBF in children from the first year of life to 16 years of age noted peak values at 7 years of age, earlier than our data suggest. It is possible that their earlier peak reflects the fact that the 8 to 16 year old range was analyzed as a single group. Similarly, in another broad study of CBF from 2 days to 19 years of age, Chiron et al. showed increased cortical CBF through 5–6 years compared with the older group of 6 to 19 year olds and adults. Our study concurs with the overall contour of developmental change in CBF in previous reports. Instances where the timing of peak CBF indicated in our study appears to differ from other studies may be attributable to differences in age groupings and the occurrence of our targeted age bands falling within a single group of another study. These differences in grouping present a challenge in articulating the profile of developmental change more precisely. Clearly ongoing research would benefit from larger sample sizes with an even distribution of age bands across the first years of life to adulthood.

To date, few studies have used ASL to study resting state CBF during childhood to delineate the pattern of normal development. These reports support the current findings. A large age span study conducted by Biagi et al. [2007] measured CBF with ASL in 44 patients 4 to 78 years of age. They examined three groups, children (4–12 years old), teens (13–19 years old), and adults (20–78 years old). Their ASL analysis revealed declining CBF values between groups as age increased. In a qualitative assessment of CBF values between 4 and 12 years of age, they described CBF levels as fairly stable until 10 and 12 years of age when a sharp decline was evident. Wang et al.'s [2003] focus on early development dovetailed with Biagi et al.'s study. Wang et al. examined seven infants and children between 1 month and 10 years of age (mean = 3.7 years) and reported the measurements of their CBF were 30% higher than adults. Recently, a large-scale study of typically developing children mapped changes in resting CBF from 5 to 18 years of age and documented regional differences in timing. They found that CBF corrected for gray matter density reaches a peak around 5 years in the occipital lobe and between 9 and 12 years of age across most other brain regions [Taki et al., 2011].

In addition to measurements of resting state CBF, this study uses QUIPSS II ASL to examine the CBF response to stimulation of the auditory cortex and its relationship to the BOLD response in typically developing children. The study shows that the higher CBF seen in children relative to adults in resting state is also evident in the stimulus-driven response. Comparison of the absolute difference in CBF between rest and response during stimulation shows a difference between the youngest group and adults. However, the difference between the two child groups, as seen in resting state, is not significant. It is possible that the peak of resting and activity-driven CBF occur earlier than we estimated based on the literature. We might have detected differences with a sample younger than 8 years

of age, although both Biagi et al. [2007] and Taki et al. [2011] report resting CBF declines after age 9. Interestingly, the BOLD response reveals no difference in percent change between any of the groups. Thus, despite the elevation in resting CBF displayed in the younger age group relative to adolescents and adults, the BOLD response remains consistent.

An important consideration in the analyses of the functional response to the auditory stimulation is the baseline state. In this study a period of rest before the blocks of auditory stimulus constituted the baseline. With this design, we cannot rule out the possibility that a participant may have actively listened for the onset of the music and altered their baseline due to their attentive state or that the sound of the scanner throughout the run provided auditory input during the baseline period that elevated the signal. Were that the case, we might expect to see limited CBF and BOLD signal increases during stimulation and perhaps an absence of group differences. However, we see that there are age group differences in the CBF signal during baseline that are consistent with those seen in the resting state scan and we observe corresponding group differences in absolute CBF increase in response to the stimulus, which demonstrates a dynamic range of response.

The finding of consistency in the amplitude of the BOLD response across the age groups complements the few studies that have expressly examined the BOLD signal or the physiological underpinnings of the BOLD effect in children. The studies that have investigated characteristics of the BOLD signal time course in children have used tasks designed to drive sensory (visual) and motor cortices while minimizing the engagement of cognitive processes that might introduce performance-based differences in neural activity, as in the current study. The time course of the BOLD hemodynamic response showed no difference in the timing of the rise or peak response, or magnitude of response in children relative to adults [Richter and Richter, 2003, Wang et al., 2003]. Yet children did show a shorter latency in onset of the post-stimulus decline of the signal following the stimulus offset [Richter and Richter, 2003]. The comparability in BOLD response in children and adults was also seen during both brief and prolonged stimulation [Wenger et al., 2004]. In a converging approach, Thomason and colleagues studied the BOLD signal in a breath holding condition that generates a BOLD signal by creating a state of hypercapnia and associated vascular changes, rather than prompting neural activity in response to a stimulus [Thomason et al., 2005]. They found regional differences in the BOLD signal produced by the vascular response. Across the whole brain, some regions showed a greater percent of BOLD signal change in children than in adults. However, in the lateral temporal lobes, which encompassed our ROI, the BOLD response showed no age-related differences, consistent with the current finding.

One possible account for the stability of the BOLD response despite high resting state flow in the immature

brain is that additional physiological mechanisms, such as metabolic processes, may also be elevated and offset the high CBF in the BOLD signal. It is recognized that cerebral glucose metabolism is elevated in the developing brain. Regional cerebral metabolic rates for glucose (rCMRglu) measured with 2-deoxy-[^{18}F]-fluoro-D-glucose (FDG) PET show an increase from the first days of life until 3 to 4 years of age when levels are approximately twice as high as adults. The level of rCMRglu is sustained in younger children (3 to 8 years of age) relative to older children (9 to 15 years of age) and adults [Chugani et al., 1987]. Additionally, regional cerebral metabolic rate for oxygen (rCMRO₂) also shows a profile of an early overshoot above adult levels. rCMRO₂ measured with PET demonstrates higher rates in early childhood (between 3 and 8 years of age) than in adulthood in many brain regions, while some sites attained adult levels between 8 and 16 years of age or later. rCMRO₂ levels were higher in primary sensory areas than association regions [Takahashi et al., 1999]. Thus the relative balances of CBF with rCMRglu and with CMRO₂ may be comparable across age groups. Another contributing factor could be increased CBV during early childhood in response to stimulation. An increase of CBV with increased CBF and a moderate increase in CMRO₂ would have an effect of decreasing the concentration of oxyhemoglobin and reducing the BOLD response.

The pattern of rapid CBF, rCMRglu, and rCMRO₂ increase in the first years, followed by a peak in the school age period, and later decline during adolescence resembles and coincides with multiple indices of structural brain development, such as synaptic density [Huttenlocher, 1990], dendritic density [Purpura, 1975], and gray matter volume [e.g., Courchesne et al., 2000; Giedd et al., 1999]. In addition, myelination is ongoing throughout childhood. Thus, a greater rate of blood flow and upregulated metabolism may be necessary to generate and maintain this characteristic early overproduction in the immature brain prior to the later refinement and elimination of processes and connections that shape the mature system.

At the same time, it is important to acknowledge that the relationship between CBF, metabolism of oxygen and glucose, and their relative contributions to the BOLD signal are not fully understood. Historically it was thought that neural activity and energy (adenosine triphosphate, ATP) expenditure triggered metabolic feedback signals to increase CBF to resupply oxygen and glucose. However, early PET studies demonstrated that stimulus-driven CBF increases exceeded and uncoupled from CMRO₂ to produce hyperemia [Fox and Raichle, 1986], which is the basis of the BOLD effect [Ogawa et al., 1990, 1992]. Ongoing research has revealed greater complexity in the mechanisms that affect CBF and that contribute to hyperemia. For example, CBF can be affected by multiple feed forward signals arising from synaptic activity, both from neurons (e.g., glutamate and nitric oxide release) and astrocytes (e.g., potassium, arachidonic acid metabolites) [Attwell et al., 2010]. In regard to flow-metabolism

coupling, initially glucose was seen as the main metabolic substrate, which was converted to ATP via the serial combination of nonoxidative glycolysis and oxidative phosphorylation. More recently, the roles of astrocyte glycolysis and lactate as a metabolic substrate have become a central focus. For instance, the astrocyte-neuron lactate shuttle (ANLS) hypothesis [Pellerin and Magistretti, 1994], asserts that the majority of glucose is taken up by astrocytes and metabolized through nonoxidative glycolysis to fuel neurotransmitter recycling and the restoration of extracellular potassium homeostasis. Lactate produced in this process is taken up by neurons and serves as their main metabolic substrate for neurotransmission via oxidative phosphorylation. In addition, some lactate is released into blood, which triggers CBF increase and contributes to hyperemia [Mintun et al., 2004]. Thus, in this model, factors other than oxidative metabolism govern CBF and are reflected in the BOLD signal. While neurophysiological research is based largely on animal and *in vitro* studies, Lin et al. [2010] provided support for this model in an examination of the relationship between CBF and oxidative metabolism (measured by CMRO₂) and nonoxidative metabolism (measured by lactate production) with MRI and 1H MRS in adults. Although ANLS is an attractive hypothesis, it is also challenged by alternative interpretations and contrasting models [see Mangia et al., 2009]. Consequently, there is uncertainty in the current specifications of the neurobiology and physiology underpinning the BOLD signal.

In the developing brain, the question of how these different factors involved in neurovascular coupling and the BOLD signal may change has yet to be fully considered. To date, knowledge about the timing and nature of developmental change in components of neurovascular pathways that affect CBF, such as vascular development, neuronal expression of nitric oxide synthase and nitric oxide release, and astrocyte morphology and expression of their glutamate receptors and potassium channels relies on animal models. Whether patterns of postnatal maturation seen in animals are evident in the human brain and at what ages potential changes occur remain to be determined [Harris et al., 2011].

As this is the first study to our knowledge to examine functional ASL and its relationship to the BOLD response in children, it will be important to confirm the current findings in future studies. We recognize that our results are based on a modest sample and that it remains possible that our sample size may have limited our ability to detect age-related differences. It will be particularly valuable to test the findings of comparability between age groups in the percent of signal change in the CBF and BOLD responses in a larger group of subjects. Another limitation in this study was the unequal number of males and females in the child groups. Auxiliary analyses that controlled for the variable of sex, however, yielded results consistent with our original findings. Even so, it would be advantageous to have a large balanced sample designed to

compare the two sexes directly. In addition, Jain et al. [2012] recently demonstrated that the accuracy of resting CBF quantification with pseudo-continuous ASL, compared with an estimate of global blood flow derived from phase-contrast MRI angiography as a standard, can be improved by the inclusion of blood T1 measurements. We did not acquire blood T1 data in the current study; yet, future studies would benefit from the inclusion of a phase-contrast scan and *in vivo* measurement of blood T1.

In the future, the investigation of CMRO₂ in children with a calibrated BOLD technique would provide valuable information about the relationship between CBF, oxygen consumption, and the BOLD signal. The interpretation that the observed stability in the BOLD response across age groups may be attributable to higher flow in combination with greater oxygen metabolism at younger ages to produce a BOLD response comparable to that of adults who have lower flow and lower oxygen consumption could be tested directly using a calibrated BOLD technique. Traditional calibrated BOLD induces an experimental condition of hypercapnia by having a subject breathe a gas mixture of 5% carbon dioxide, which has the effect of increasing CBF and the BOLD signal presumably without affecting CMRO₂. Comparison of hypercapnia and normocapnia conditions provides a basis for calibration of the BOLD signal, which can be used for the calculation of CMRO₂ in ASL and BOLD fMRI [Davis et al., 1998]. Future directions also include the examination of other cortical regions and vascular territories to see whether the findings in the primary auditory cortex generalize to other sensory cortices and to association regions. The purpose of targeting a sensory region was to generate reliable and robust CBF and BOLD responses in participants. However, there is heterochrony in the maturation of the cerebrum, with the sensory regions preceding association cortices [e.g., Gogtay et al., 2004, Huttenlocher and Dabholkar, 1997], so examination of possible regional differences in cerebrovascular dynamics will be essential.

CONCLUSIONS

The goals of this study were to determine whether or not elevated resting state CBF would be evident in two middle school age groups as previously reported and further, whether the anticipated elevation would be seen in CBF and the BOLD signal during stimulation as well. This study reveals that CBF differences between the youngest children and adults are present both at rest and in reaction to sensory stimulation. The higher rate of CBF seen in the 8-year-old children would predict a greater increase in percent CBF and BOLD signal during stimulation. Yet, the percent of stimulus-driven CBF increase and the magnitude of the BOLD response are comparable between children and adults. This set of results suggests that in young children, additional mechanisms involved in the cerebrovascular changes that occur with neural activity and result

in a local decrease in deoxyhemoglobin concentration to produce the BOLD effect, such as metabolic processes, are also elevated. An upregulation of CMRO₂ or an increase in cerebral blood volume, for example, could offset the increased CBF and in result in a BOLD response that resembles adults. Together the complementary measures of resting CBF, stimulus-driven CBF, and the BOLD response reveal age-related differences in the hemodynamics that give rise to the BOLD effect, but are not evident from the measurement of the BOLD signal alone.

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