Functional Magnetic Resonance Imaging of Regional Cerebral Blood Oxygenation Changes During Breath Holding

Andreas Kastrup, MD; Tie-Qiang Li, PhD; Atsuchi Takahashi, PhD; Gary H. Glover, PhD; Michael E. Moseley, PhD

Background and Purpose—Recently, noninvasive MRI methods have been developed that are now capable of detecting and mapping regional hemodynamic responses to various stress tests, which involve the use of vasoactive substances such as acetazolamide or inhalation of carbon dioxide. The aim of this study was to assess regional cerebral blood oxygenation changes during breath holding at 1.5 T.

Methods—In 6 healthy volunteers, T2*-weighted gradient echo images were acquired for a total dynamic scanning time of 10 minutes during alternating periods of breath holding and normal breathing at 40-second intervals after inspiration, at 30-second intervals after expiration, and at 18 seconds after expiration. To quantify the relative signal changes, 2.5-minute baseline image sampling with normal breathing was carried out.

Results—Repeated challenges of breath holding of various durations induced an overall rise in blood oxygen level–dependent (BOLD) signal intensities. In general, BOLD signal intensity increases were greatest in gray matter and nonsignificant in white matter. Depending on the breath-holding duration and techniques, BOLD signal intensity increases of all activated pixels varied from 0.8% to 3.5%.

Conclusions—The present study demonstrates that cerebral blood oxygenation changes during breath holding can be detected by means of fMRI at 1.5 T. The breath-holding test, a short and noninvasive method to study cerebral hemodynamics with fMRI, could become a useful alternative to the acetazolamide or CO2 test. (Stroke. 1998;29:2641-2645.)

Key Words: cerebral blood flow ■ magnetic resonance imaging ■ vasomotor reactivity

In the normal brain, the constancy of cerebral blood flow (CBF) and volume relies on the intrinsic ability of the cerebral arteries to alter their caliber in response to variations in blood pressure (autoregulation) and changes in regional metabolic demands. As one of the major products of cerebral metabolism, CO2 can alter cerebrovascular resistance and ultimately affect CBF. Therefore, vasomotor responses of the cerebral vasculature to altered carbon dioxide tensions can easily be used for the study of CBF regulation and to determine the cerebrovascular reserve capacity.

The response of cerebral perfusion to vasodilatory stress is not merely of scientific interest but also of potential clinical interest in patients with hemodynamically relevant atherosclerotic disease. Recently, there has been renewed interest in the role of hemodynamic factors in the pathogenesis of stroke, fueled by the improved prognosis found after carotid endarterectomy for high-grade carotid artery stenoses. For insufficiently collateralized occlusions of the internal carotid artery, it has been found that hemodynamic compromise as evidenced by exhausted vasomotor reserve capacity presents a considerable risk for subsequent ischemic stroke.

In the past, cerebral hemodynamics and cerebrovascular reserve capacity have mainly been measured with positron emission tomography, single-photon emission CT, transcranial Doppler sonography, and dynamic or xenon CT. Recently, advanced, noninvasive, MRI methods have been developed that are now capable of detecting and mapping regional hemodynamic responses to various stress tests, complementing the arsenal of functional brain investigations feasible with MRI.

The majority of these techniques exploit the fact that task-related increases in flow exceed the demands of oxidative metabolism. This uncoupling between CBF and cerebral metabolism causes a similar change in venous oxygen concentration, as seen during brain stimulation. Thus, the application of a reversible vasoactive stress can be visualized noninvasively with deoxyhemoglobin-sensitive (blood oxygen level–dependent [BOLD]) as well as flow-sensitive (such as flow-sensitive alternating inversion recovery [FAIR]) MR techniques.

Several reports have successfully described investigation of cerebral hemodynamics with the BOLD technique in...
healthy volunteers after the injection of acetazolamide during hyperventilation and during CO₂ inhalation.

Besides inhalation of carbon dioxide or injection of acetazolamide, measurement of cerebrovascular reactivity (CVR) can simply and reliably be performed using the breath-holding test (BHT). This test, which uses the increase of arterial PCO₂ induced by breath holding as a vasodilative stimulus, was introduced in 1990 by Ratnatunga and Adisesiah. Preliminary experience suggests that it can be useful for obtaining functional information in patients with carotid occlusive disease.

The aim of the present study was to assess regional cerebral blood oxygenation changes during breath holding at 1.5 T with use of a blood oxygenation level–dependent (BOLD) sensitive fMRI technique. The use of different breath-holding techniques and breath-holding durations are discussed.

Subjects and Methods

The study comprised 6 healthy volunteers (4 men, 2 women), ranging in age from 27 to 33 years. All volunteers were examined after they gave their informed consent. The protocol was approved by the Institutional Review Board of the Stanford University School of Medicine.

All images were obtained with a GE Signa Horizon 1.5-T scanner (General Electric Medical Systems) equipped with an “echo-speed” gradient system. The maximum achievable gradient amplitude and slew rate were 22 mT/m and 120 Ts/m, respectively. To reduce motion artifacts, subjects were secured with pillows and padding in the head coil. T₂*-weighted images were acquired using a gradient echo version of a single-shot spiral sequence with a TE/TR of 50/3000 ms.

The design of the spiral readout gradients was based on the variable rate method proposed by Hardy and Cline with use of the Ridder search algorithm to improve execution time. For this study, we used a matrix size of 128×128 over a field view of 240×240 mm², which corresponds to a one-shot spiral readout window of 65 ms using a 100-kHz receiver bandwidth.

Four slices with an 8-mm slice thickness and 2-mm interslice spacing were imaged. Image reconstruction was performed offline on a SPARC workstation (Sun Microsystems), with use of the gridding algorithm described by Meyer et al.

The reconstruction routine also used an acquired B₀ field map to correct for linear shim terms and carrier frequency offset in each slice.

The image procedure for each volunteer scanning was as follows: sagittal localizer images were first obtained with a conventional gradient echo sequence. A midsagittal image was used to prescribe axial slices through the frontoparietal lobes of the brain. Slices above ventricles were selected to avoid possible artifacts. Fast spin-echo images were acquired at the same locations to serve as anatomic references.

In each subject, 3 studies were carried out: T₂*-weighted gradient echo images were acquired for a total dynamic scanning time of 10 minutes during alternating periods of breath holding and normal breathing at 40-second intervals after inspiration, 30-second intervals after expiration, and 18-second intervals after expiration. The time to start and to stop breathing was indicated by projecting the instruction into the magnet bore in front of the subject.

To quantify the relative signal changes, 2.5-minute baseline image sampling with normal breathing was carried out. Using a TR of 3.0 seconds, 150 frames images usable for analysis were collected for each sequence after excluding the baseline images.

The T₂*-weighted images were analyzed with a time series correlation method to produce conventional correlation maps. Statistical significance of activation was assessed by setting a significance criteria of P<0.01 for the intensity of activation and a minimum cluster size of 5 pixels. Because of a global activation feature of the breath-holding stimulus, overlay to the corresponding anatomic images was not performed. The mean values of the baseline images collected before breath-holding activation were used as reference to calculate the maximal relative BOLD signal intensity changes.

Results

Repeated challenges of breath holding of varying duration induced an overall rise in BOLD signal intensities. Figure 1 shows the topographic pattern of the breath-holding effect in a typical subject for the different breath-holding paradigms. In general, BOLD signal intensity changes were greatest in gray matter and nonsignificant in white matter. The corresponding time courses of the mean BOLD signal intensity changes of all activated pixels are presented in Figure 2. Notably, breath holding after expiration yielded an immediate T₂*-weighted SI increase (Figures 2b and 2c), whereas BOLD signal intensity declined initially during breath holding after inspiration (Figure 2a).

Using the mean values of the baseline images collected before breath-holding activation as reference to calculate the maximal relative BOLD signal intensity changes, quantitative analysis yielded a maximal BOLD signal intensity increase of 0.8% to 3.5%. Table 1 summarizes the mean percentage signal changes of all slices for repeated challenges of breath holding for each volunteer. The BOLD signal intensity changes clearly depended on the breath-holding duration and...
functions such as vision, motor skills, or language. These techniques are now capable of detecting and mapping regional hemodynamic responses to various stress tests, which involve the use of vasoactive substances such as acetazolamide or inhalation of carbon dioxide. Due to the high spatial and temporal resolution, noninvasive fMRI techniques are increasingly used to study cerebral hemodynamics and cerebrovascular reserve.14–20 Kleinschmidt et al26 demonstrated exhaustion of the autoregulatory reserve capacity in 4 patients with unilateral occlusion of the internal carotid artery, while monitoring cerebral blood oxygenation changes during vasodilatory stress.

Recently, the BHT has been introduced as an alternative, simple method for assessing cerebral hemodynamics.21–26 It has the advantages of not requiring a source of CO₂ or acetazolamide injection, and it can therefore easily be performed during a routine MRI. Previous studies have reported an excellent patient tolerance.21,24,25 In the present study, all subjects were able to hold their breath as prescribed by the study protocol, and none found it uncomfortable. The only adverse effect was a compelling urge to breathe toward the end of the apneic phase.

This is a main advantage over methods that use increased inspired CO₂ concentrations or acetazolamide, which many subjects find uncomfortable. In addition, cardiovascular side effects have been reported.29 Using the BHT, Silvestrini et al23 demonstrated a significant improvement of cerebrovascular reserve after carotid endarterectomy. Preliminary experience suggests that functional information obtained with the BHT correlates well with CO₂24 and acetazolamide tests.22

During breath holding, the increase in PacO₂ gives rise to increased CBF due to vasmotor reactivity, and this flow increase will enrich the oxyhemoglobin in the venous blood, resulting in increased signal intensity in BOLD imaging. On the other hand, the depletion of oxygen stores in the body during breath holding will increase the overall concentration of deoxyhemoglobin in the blood. Therefore, the observed signal intensity change in T2*-weighted images depends on the relative contributions of these 2 competing factors.

In the present study we have demonstrated regional cerebral blood oxygenation increases during repeated challenges of breath holding for various durations. These findings are in good accordance with the results of Stillman et al20 and Moritz et al21 who observed a 3% to 10% BOLD signal intensity increase in the gray matter during a single breath-holding paradigm in normal volunteers. In contrast to these and our findings, a BOLD signal intensity decrease during apnea in cats52–54 and humans55 has been reported. In these animal studies, however, intubation, ventilation, and anesthesia with either isoflurane or halothane pose major limitations, so that the results cannot be compared with the present human study without major restrictions. Halothane, and to a lesser extent isoflurane, influence cerebral metabolic rate of oxygen, CBF, neuronal coupling and the reactivity of the cerebral vasculature to altered carbon dioxide tensions.56 Possibly, an impaired response of the cerebrovasculature to increased PacO₂ during apnea under halothane or isoflurane anesthesia can account for the overall BOLD signal intensity decrease reported in cats, as this will enhance the effect of the

### Discussion

Functional MRI (fMRI) techniques are rapidly moving away from the perceived role of solely mapping human cognitive

#### Mean BOLD Signal Intensity Changes (% of Baseline Signal) of Significantly Activated Pixels (P<0.01) for Six Subjects During Periodic Breath-Holding Challenges

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>18 s</th>
<th>30 s</th>
<th>Initial Dip</th>
<th>Peak Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expiration</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak Value</td>
<td></td>
<td></td>
<td>Initial Dip</td>
<td>Peak Value</td>
</tr>
<tr>
<td>1</td>
<td>1.19±0.3</td>
<td>2.76±0.3</td>
<td>−0.8±0.2</td>
<td>2.51±0.2</td>
</tr>
<tr>
<td>2</td>
<td>1.38±0.4</td>
<td>2.41±0.3</td>
<td>−0.8±0.1</td>
<td>2.03±0.2</td>
</tr>
<tr>
<td>3</td>
<td>1.87±0.3</td>
<td>2.08±0.3</td>
<td>−0.7±0.5</td>
<td>3.48±0.6</td>
</tr>
<tr>
<td>4</td>
<td>0.80±0.2</td>
<td>2.76±0.2</td>
<td>−2.2±0.2</td>
<td>2.53±0.2</td>
</tr>
<tr>
<td>5</td>
<td>1.98±0.4</td>
<td>3.44±0.3</td>
<td>−1.0±0.2</td>
<td>2.75±0.4</td>
</tr>
<tr>
<td>6*</td>
<td>1.81±0.2</td>
<td>3.01±0.1</td>
<td>−1.2±0.4</td>
<td>3.04±0.2</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>1.5±0.4</td>
<td>2.74±0.4</td>
<td>−1.1±0.6</td>
<td>2.72±0.4</td>
</tr>
</tbody>
</table>

*Subject presented in Figures 1 and 2.

![Figure 2. Time courses of the mean BOLD signal intensity changes in the same subject as Figure 1 during repeated challenges of breath holding for 40 seconds after inspiration (a), 30 seconds after expiration (b), and 18 seconds after expiration (c). The mean signal-intensity changes of the T2*-weighted images of all activated pixels are given as a function of the experimental time. Arrows indicate the beginning of the first breath-holding period.](http://stroke.ahajournals.org/)

[Table showing relative signal intensity change (% of Baseline Signal) of Significantly Activated Pixels (P<0.01) for Six Subjects During Periodic Breath-Holding Challenges]

During breath holding, the increase in PaCO₂ gives rise to increased CBF due to vasmotor reactivity, and this flow increase will enrich the oxyhemoglobin in the venous blood, resulting in increased signal intensity in BOLD imaging. On the other hand, the depletion of oxygen stores in the body during breath holding will increase the overall concentration of deoxyhemoglobin in the blood. Therefore, the observed signal intensity change in T2*-weighted images depends on the relative contributions of these 2 competing factors.

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depletion of oxygen stores, with subsequent increase of the overall deoxyhemoglobin concentration.

While these speculations and differences between animal and human data await clarification, further insight into human arterial and jugular blood gas values during breath holding may be gained from the literature with data given in Reference 37. Despite a reduction in arterial oxygen saturation (SaO₂) and oxygen tension (PaO₂) during breath holding, jugular oxygen tensions (PJO₂) and oxygen saturation (SJPO₂) increase. Therefore, due to vasomotor reactivity from the rising PaCO₂, an increase in both CBF and T2*-weighted signal intensity is expected.

The time course and the magnitude of the increase in T2*-weighted signal intensity clearly depends on the breath-holding technique. Breath holding after expiration leads to an instantaneous reduction in PaO₂ and increase in PaCO₂ as well as in PJO₂/SJPO₂. In breath holding after inspiration, however, PaCO₂, PaO₂, arterial pH, and jugular Po₂ show a biphasic change. PaCO₂ is reduced at the onset, followed by a marked increase with an accompanying decrease of arterial pH. Similarly, jugular Po₂ increases after an initial small decrease. Therefore, breath holding after expiration will lead to a rapid increase in CBF and BOLD signal, thus allowing shorter breath-holding observation periods. Against the background of these reflections it was possible to detect BOLD signal intensity changes during a repeated challenge of expiration breath holding of as short as 18 seconds. Despite a 10-second-shorter observation period, 30-second breath holding after expiration yielded results comparable to those obtained with 40-second breath holding after inspiration (Table 1).

The physiological response of vasomotor tone to vasodilatory stress from either functional activation or pharmacological manipulation is one of the prime features of intact cerebral perfusion. CBF in normal individuals ensures that a fall in perfusion pressure is counterbalanced by vasodilation of cerebral arteries, which under normal conditions maintains adequate CBF. This cerebrovascular reserve can be estimated by measuring the response to a vasodilatory stimulus. Recent studies have documented a significantly increased risk in stroke or transient ischemic attack ipsilateral to a stenosis or occluded internal carotid artery in patients with impaired CVR. Therefore, assessment of CVR is recognized as an important parameter in the management of cerebrovascular diseases, and a less invasive and more readily available method to investigate CVR would be useful for clinical application. Positron emission tomography is considered the "gold standard" for assessing cerebral perfusion because of its potential to separately quantify CBF, cerebral blood volume, and fractional oxygen extraction. However, fMRI is noninvasive and does not involve exposure to radiation; therefore, it confers the advantage of repeatability. Repeated testing of a single patient will allow clinicians to study changes of cerebrovascular hemodynamics during the course of a disease. The BHT offers potential as a simple method for assessing cerebrovascular reserve, although its use may be limited in patients who have impaired respiratory function. Furthermore, several sources of error may arise, most prominently the fact that PaCO₂ may rise at different rates during breath holding in different subjects. Estimations of the arterial PaCO₂ are not possible, because a steady state does not exist.

The present study demonstrates that fMRI techniques are capable of mapping cerebral blood oxygenation responses to apnea; however, functional MRI is still in its infancy, and some precautions should be exercised when interpreting fMRI data. BOLD MR signal changes induced by pharmacological as well as functional activation encompass signals originating from both brain microvasculature and large vessels. Moreover, BOLD contrast images obtained under rapid radiofrequency pulsing conditions can have an inflow component from the large vessels that is dominated by fast-flowing macrovascular blood in arteries and veins. In the present study, however, this confounding effect was minimized through use of a long TR (3 seconds). Because BOLD effects are related to multiple physiological parameters (such as CBF and volume) and oxygen consumption, it is difficult to extract a single physiological parameter from the observed signal changes.

In conclusion, our study suggests that cerebral blood oxygen changes during breath holding can be detected by means of fMRI at 1.5 T. Although functional studies using deoxyhemoglobin contrast are possible at 1.5 T, they will benefit from higher field strength in terms of signal-to-noise ratio. The BHT is a rapid and noninvasive method for the study of cerebral hemodynamics with fMRI, and it could become a useful alternative to the acetazolamide or CO₂ test. The measures of hemodynamic reserve presented in this study may represent ways to identify individuals with impaired cerebrovascular reserve who will benefit most from interventional procedures; however, before these MRI methods are applied to clinical practice, further validation is required by comparison against more established techniques.

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