Noninvasive Assessment of Vascular Architecture and Function during Modulated Blood Oxygenation Using Susceptibility Weighted Magnetic Resonance Imaging

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Susceptibility weighted imaging (SWI) is a BOLD-sensitive method for visualizing anatomical features such as small cerebral veins in high detail. The purpose of this study was to evaluate high-resolution SWI in combination with a modulation of blood oxygenation by breathing of air, carbogen, and oxygen and to directly visualize the effects of changing blood oxygenation on the magnetic field inside and around venous blood vessels. Signal changes associated with the response to carbogen and oxygen breathing were evaluated in different anatomic regions in healthy volunteers and in two patients with brain tumors. In the magnitude images inhalation of carbogen led to significant signal intensity changes ranging from $+4.4 \pm 1.9\%$ to $+9.5 \pm 1.4\%$ in gray matter and no significant changes in thalamus, putamen, and white matter. During oxygen breathing mean signal changes were smaller than during carbogen breathing. The method is capable of producing high-resolution functional maps of BOLD response to carbogen and oxygen breathing as well as high-resolution images of venous vasculature. Its sensitivity to changes in blood oxygenation was demonstrated by in vivo visualization of the BOLD effect via phase imaging. Magn Reson Med 54:87–95, 2005. © 2005 Wiley-Liss, Inc.

Key words: BOLD; susceptibility weighted imaging; carbogen; MRI; brain

The contrast mechanism in BOLD MR techniques for functional (1) or anatomical (2) applications is based on the fact that local magnetic field homogeneity (or inhomogeneity) determines signal intensity in a gradient echo sequence. Depending on its orientation with respect to the static magnetic field and its magnetic susceptibility, a venous vessel modifies the magnetic field in its vicinity (see, e.g., (3)). First, signal cancelation occurs within a voxel due to frequency shifts between the vessel and the surrounding parenchyma. Second, inhomogeneities of the magnetic field around the vessel cause extravascular spin dephasing (4). At higher blood oxygenation levels, the local field homogeneity around the vessel increases and spin dephasing is reduced. At the same time, the difference in the precession frequencies between spins in venous blood and spins in the parenchyma decreases; hence, cancelation between intravascular and extravascular signals is reduced. In gradient echo imaging, both effects lead to a signal increase with increasing blood oxygenation. The BOLD contrast makes use of the differences in $T_2^*$ caused by different venous blood oxygenation, which can, for example, be associated with neural activity (5). Susceptibility weighted imaging (SWI) (6) is based on the common BOLD contrast with an (optional) additional weighting by the magnetic susceptibility as provided by MR phase images. The first application of this technique was to further enhance the visibility of venous vessels in the brain using the different magnetic susceptibility of deoxygenated blood compared to surrounding tissue and provides detailed anatomic information on small venous vessels (2, 7), or venous malformations (8, 9), coining the term MR venography. The focus has been on the venous macrovessels, that is vessels that are larger than capillaries in size, these usually being the venules (with diameters of $\approx50\mu m$) and pial veins (several hundred micrometers to a millimeter in diameter). The technique is not restricted to venography, as the underlying principles of SWI apply to all susceptibility differences in general and it was subsequently used for visualizing susceptibility contrast in tumors (10, 11), hemorrhagic lesions (12), and stroke (13, 14).

More recently, gaseous modifiers of blood oxygenation such as carbogen (95% $O_2$ and 5% $CO_2$) or pure oxygen have drawn the attention of research groups (15–22). Breathing of pure oxygen leads to a decrease in cerebral blood flow (23) due to increased vasoconstriction in pial arteries (24). In order to inhibit the vasoconstrictive effect, the vasodilator carbon dioxide is added to the oxygen. Robinson et al. (15) were among the first to study signal changes in tumors (GH3 prolactinomas) due to carbogen breathing by using a gradient echo imaging sequence in a rat experiment. Using a 2D approach, they found signal increases of up to 100% in the image intensity in the tumor. Most studies mainly aimed to establish MRI methods that could help to identify tumors that show the highest response to carbogen (or oxygen) inhalation. Such tumors are susceptible to radiosensitization with carbogen because higher oxygenated cells are more radiosensitive (25). However, large intra- and intertumoral heterogeneity in response to carbogen was found (16, 26–28). The majority of previous investigations concerned animal studies,
whereas human studies conducted so far employed only 2D approaches with relatively low spatial resolution.

The purpose of this study was to quantitatively evaluate 3D high-resolution SWI as a tool for functional investigation in different cerebral regions using a modulation of blood oxygenation by breathing carbogen and oxygen and to develop techniques to directly visualize the effects of changing blood oxygenation on the magnetic field inside and around (pial) veins with diameters of a millimeter or larger. To investigate the potential of the method for visualizing changes in tumors, two patients with tumors (glioblastoma multiforme and astrocytoma) were scanned. Maps of tumor response to carbogen and air/oxygen were computed and regions of interest (ROIs) within the tumor were evaluated quantitatively.

**METHODS**

**MRI Protocol**

Fourteen healthy volunteers (20 to 36 years old, mean 26 years old) and 2 patients (patient 1, female, 49 years, with a glioblastoma multiforme; patient 2, female, 63 years, with an astrocytoma (WHO grade II)) were investigated. High-resolution, $T^*_2$-weighted, single echo images were acquired on a 1.5-T system (Magnetom Vision, Siemens, Erlangen, Germany) with a 3D, first-order velocity-compensated gradient echo sequence (29) using a quadra
ture transmit/receive birdcage head coil. (Typical parameters were TR = 67 ms, $T_E = 40$ ms, $\alpha = 25^\circ$, readout bandwidth = 78 Hz/pixel, field of view (FOV) 25.6 $\times$ 19.2 $\times$ 6.4 cm, matrix size 512 $\times$ 192 $\times$ 64, acquisition time = 14 min. Two volunteers were scanned with a slightly different acquisition matrix of 512 $\times$ 256 $\times$ 36, resulting in a scan time of 10 min).

**Breathing Protocol**

During the scans, air (first scan), carbogen (95%O$_2$, 5%CO$_2$) (second scan), and pure oxygen (third scan) were supplied through a face mask by a continuous positive airway pressure system (CF 800, Dräger Medical, Lübeck, Germany) for each subject. Gas flow rate was 25 L/min. Each volunteer and patient received a detailed explanation of the effects of carbogen to maximize their comfort. Informed consent was obtained from all individuals. The protocol was approved by the local ethics committee. Subjects lay in a supine position with the mask attached during the whole experiment. Patient 1 received all three gases; patient 2 received air and carbogen. A period of 1 min was introduced between subsequent scans to allow for a transition between the gases. During the scans measurement of oxygen saturation $S_pO_2$ and pulse rate were performed (Bruker Maglife, Bruker, Ettlingen, Germany) and the inspired and expired oxygen concentration ($F_{iO_2}$, $F_{eO_2}$) as well as the breathing rate were monitored (PM8050 MRI Dräger Medical).

**Data Analysis**

Reconstruction and all postprocessing was performed using IDL (Interactive Data Language, Research Systems, Inc., Boulder, CO, USA) except for image registration, which was done using SPM (Wellcome Department of Cognitive Neurology, London, UK). Zero filling of $k$-space data was applied to the two phase encoding directions, resulting in a matrix of 512 $\times$ 384 $\times$ 96, and complex images were reconstructed. Unwrapped and high pass filtered phase images of the data from the volunteers were created by homodyne filtering in three dimensions (30, 31), using a 3D Hamming window (width = 96 $\times$ 72 $\times$ 32 pixels) for low pass filtering in $k$-space. The phase images from the tumor patients were unwrapped using a region growing algorithm operating in image space (32, 33). This results in better phase images in the tumor, which represents a region of high field inhomogeneity where unwrapping using the homodyne detection may fail. SPM was used to realign the images in the following way (Fig. 1): The realignment parameters were obtained by realigning the magnitude images. Then the parameters were applied to the imaginary and real part of the complex images. 

**FIG. 1.** Description of the postprocessing procedure. Realignment parameters are calculated using the magnitude information. The parameters are then applied to the real and imaginary part of the image (not shown in diagram) or to the magnitude and unwrapped phase images (see text). Phase mask images are then calculated. In a final step, the phase mask and magnitude information is combined to create the susceptibility weighted image.
(volunteer data) or the magnitude and phase images (patient data). The volunteer data were Fourier transformed back into k-space where they were multiplied with the Hamming window. Complex division of the realigned complex images by the inverse FFT of the filtered data results in unwrapped and high pass filtered phase images (34). Phase masks were created by setting all positive phases to 1 and by scaling negative phases linearly between 0 and 1 (34). These were then multiplied four times (6) with the corresponding magnitude image to create a susceptibility weighted image $I_{sw}$ (29),

$$I_{sw} = M \cdot \hat{\phi}$$,

where $M$ is the magnitude image and $\hat{\phi}$ is the phase mask. For visualization purposes MR venograms were created by computing minimum intensity projections (mIPs) over several slices of $I_{sw}$ acquired during air breathing (i.e., when contrast between veins and parenchyma is largest).

The relative signal changes in the magnitude data among the three scans were evaluated in percentages according to

$$\Delta S = \frac{A - B}{B} \times 100$$,

where $A$ and $B$ stand for the mean over 3D ROIs of typically 0.5 cm$^3$ (i.e., 2000 voxels) of the data sets to be compared. The relative changes were computed for regions in cortical gray matter, frontal white matter, occipital white matter, the splenium of the corpus callosum, putamen, and thalamus in the volunteers (Fig. 2) and a t test was performed. The regions were defined manually based on anatomy. The 3D ROIs typically extended over five slices and their location was checked for each slice to ensure that they only contained the desired tissue. Visualizations of the magnetic field distribution around larger venous vessels (\~1 mm in caliber) for the three different breathing situations were calculated from the phase images by computing views of planes oriented perpendicular to the long axis of the vessel.

**RESULTS**

**Physiological Parameters**

The procedure was well tolerated by the 2 patients and by all but 1 subject. All volunteers showed the lowest pulse rate during oxygen breathing. Changes in pulse rate between air breathing and carbogen breathing showed large intersubject variability, ranging from -12 to +20%. Data of the respiration frequency $f_R$ were available from 10 subjects. Eight subjects showed an increase in $f_R$ when carbogen was administered. In 7 subjects a drop in $f_R$ occurred when the respiration protocol was switched from carbogen to oxygen. Arterial blood oxygenation $\text{SpO}_2$ was at 96.9±1.4% during air breathing, at 98.1±0.9% during carbogen breathing, and at 98.3±1.2% during oxygen breathing. Table 1 summarizes the mean and standard deviation of the parameters $\text{SpO}_2$, $f_R$, $\text{FiO}_2$, and $\text{FeO}_2$.

**Visualization**

A dramatic loss of contrast between the venous vessels and the parenchyma was observed on the venograms during carbogen breathing compared to air breathing (Fig. 3). This directly reflects the changes in venous blood oxygenation where the susceptibility difference between veins and surrounding tissue is diminished and signal loss is suppressed. Contrast due to non-heme iron (for instance in the basal ganglia) is independent of the exogenous contrast agents and appears the same in all three scans.

The ultrahigh resolution used in our approach enabled us to directly visualize the effects of changing blood oxygenation on the magnetic field inside and around larger venous blood vessels. A visualization of the local precession frequency inside a vein via phase imaging is shown in Fig. 4, where the change of the signal phase in response to changing blood oxygenation is demonstrated for two vessels perpendicular to $B_0$. At low blood oxygenation (i.e., during air breathing; Fig. 4a) the phase difference between vessels and parenchyma is larger than at high blood oxygenation (i.e., during carbogen breathing, Fig. 4b). For all angles between the vessel axis and $B_0$ larger than zero the field outside the vessel is inhomogeneous and extravascular.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Air</th>
<th>Carbogen</th>
<th>Oxygen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulse rate (min$^{-1}$)</td>
<td>72.5 ± 10.9</td>
<td>71.5 ± 10.0</td>
<td>63.9 ± 8.6</td>
</tr>
<tr>
<td>Respiration rate (min$^{-1}$)</td>
<td>12.6 ± 3.3</td>
<td>15.4 ± 3.8</td>
<td>11.8 ± 4.3</td>
</tr>
<tr>
<td>$\text{SpO}_2$ (%)</td>
<td>96.9 ± 1.4</td>
<td>98.1 ± 0.9</td>
<td>98.3 ± 1.2</td>
</tr>
<tr>
<td>$\text{FiO}_2$ (%)</td>
<td>21.5 ± 0.5</td>
<td>95.3 ± 3.0</td>
<td>100 ± 0.0</td>
</tr>
<tr>
<td>$\text{FeO}_2$ (%)</td>
<td>17.5 ± 2.1</td>
<td>93.2 ± 3.3</td>
<td>97.4 ± 2.3</td>
</tr>
</tbody>
</table>
lar spin dephasing occurs, which is shown in Fig. 5, where a visualization of the BOLD effect at work in vivo is displayed. It demonstrates the effect that leads to spin dephasing in the extravascular inhomogeneous magnetic field (4). Here, the vessel is oriented perpendicular to the static magnetic field. Figure 5 (d–f) displays the typical field pattern around a cylinder of susceptibility $\chi_{\text{int}}$ embedded in a medium of different susceptibility $\chi_{\text{ext}}$ and with the direction of the static magnetic field being perpendicular to the cylinder’s axis. Field homogeneity is modulated by blood oxygenation and is lowest (associated with strong phase variations) during breathing of air (Fig. 5d), slightly increased during breathing of pure oxygen (Fig. 5e), and highest during carbogen breathing (Fig. 5f). This is in excellent accordance with the MR signal being lowest during air breathing, slightly increased during oxygen breathing, and highest during carbogen breathing (see Fig. 3).

Quantitative Analysis

The percentage signal changes among the three breathing conditions were evaluated using the averaged magnitude data in the 3D ROIs as described under Methods. Data from four subjects were degraded by ghosting artifacts due to motion that could not be eliminated by coregistration and were discarded. In Fig. 6 a high-resolution map of the BOLD response to carbogen is displayed. It shows a maximum intensity projection over a stack of 21 slices of the 3D parametric map of relative signal change in the SWI data between air breathing and carbogen breathing (Eq. [2]). At this resolution ($0.5 \times 0.5 \times 0.67$ mm$^3$ after zero

FIG. 3. mIPs over 15 mm of the SWI data acquired during breathing of air (left), oxygen (middle), and carbogen (right). Increased blood oxygenation during oxygen and carbogen breathing diminishes field inhomogeneities around the veins as well as the phase shift between spins inside the vessel and outside the vessel. As a consequence the signal around venous vessels increases and the contrast between veins and parenchyma is reduced during oxygen breathing and dramatically reduced during carbogen breathing.

FIG. 4. Phase images of the deep brain nuclei acquired while the subject was breathing air (a) and carbogen (b) with the corresponding profiles along the lines shown below. The vessel axes are in the imaging plane. Three parallel adjacent profiles of the images were averaged for each plot. The two minima indicate the location of the two nearly parallel venous vessels (arrows) at the center of images (a) and (b). For angles between the vessel and $B_0$ larger than $54.73^\circ$ (in this case $90^\circ$) the precession frequency inside the vein is lower than in the surrounding tissue. Note also that the phase does not change in the basal ganglia where the precession frequency is determined predominantly by non-heme iron.
filling) medium-size and large venous vessels (i.e., vessels with diameters comparable to voxel size or larger) can be seen as red lines where signal increase is the largest. Quantitative signal changes in volumes of interest of typically 2000 voxels are given in Table 2.

Carbogen.

Significant changes in response to carbogen were found predominantly in cortical gray matter ($-4.5 \pm 1.8$ to $+9.5 \pm 1.4\%$). Putamen and thalamus showed small positive responses, which were not statistically significant. White matter showed a trend toward negative changes with significant signal changes in response to carbogen breathing only in one of the five ROIs.

Oxygen.

Signal changes during oxygen breathing were less pronounced in all regions and less often significant than during carbogen breathing. The largest changes were found in gray matter ($0.4 \pm 0.9$ to $3.4 \pm 0.6\%$). The signal changes $\Delta S$ among the three breathing situations are consistent for all regions, with the sum of the changes $\Delta S_{a,o}$ and $\Delta S_{a,c}$ being nearly equal to the change $\Delta S_{a,c}$,

$$\Delta S_{a,c} \approx \Delta S_{a,o} + \Delta S_{a,c},$$

where $a$, $o$, and $c$ stand for air, oxygen, and carbogen, respectively.

Tumors.

Response of the glioblastoma multiforme to both oxygen and carbogen was very heterogeneous among the six regions evaluated (Table 3 and Fig. 7), with larger signal changes for oxygen than for carbogen and relatively small differences between the scans acquired during oxygen breathing and carbogen breathing. The maps of relative signal change in Fig. 7 illustrate the strong heterogeneity in response to carbogen and oxygen. Signal increase in some regions of the tumor was much larger ($+29.20 \pm 0.54\%$ in ROI 3) than in gray matter of healthy subjects (Table 2), while in other areas of the tumor a signal decrease ($-7.74 \pm 0.95\%$) between air breathing and carbogen breathing was detected. Only in the region of the edema (ROI 5) did the response to carbogen ($+5.50 \pm 0.42\%$) exceed the response to oxygen ($+0.16 \pm 0.47\%$).

Signal change in the astrocytoma was more homogeneous with signal changes in response to carbogen of $-2.70 \pm 0.13$ to $-4.74 \pm 0.21\%$.

DISCUSSION

In this study, we evaluated the effects of breathing the gases carbogen or oxygen on MR signal intensity and phase in nine healthy subjects and two patients using high-resolution 3D BOLD imaging.

Carbogen and oxygen are known to have significant influence on BOLD signal and on blood flow. In the brain, pure oxygen leads to a vasoconstriction and to a decrease in blood flow (35). Carbogen causes vasodilation and an increase in brain blood perfusion. This leads to an increase in venous blood oxygenation and hence to an increase in BOLD signal compared to air or pure oxygen. The question of whether the changes in BOLD signal intensity are due to raised blood flow or increased blood oxygenation has been addressed in the past. Initially, signal increase was attributed to increased inflow of unsaturated spins due to vasodilation (15). In an animal study at 4.7 T Al-Hallaq et al. (16) confirmed that blood oxygenation levels measured with microelectrodes and the water resonance line width are strongly correlated, concluding that changes in BOLD signal are a reliable index of changes in tissue oxygenation. Howe et al. separated flow-related contrast from BOLD contrast using a 2D gradient echo imaging at different echo times and at different flip angles (36) and found that signal increases during carbogen breathing are dominated by the BOLD effect. In this study, we used a 3D
susceptibility weighted sequence with a slab thickness of 64 mm. Hence, inflow effects are negligible due to the long paths that fresh spins must travel. Nevertheless, we observed signal changes comparable to those found in preceding studies. This supports the hypothesis that signal changes are dominated by blood oxygenation related changes in $R_2^*$ and reductions in resonance frequency offsets and that inflow effects play only a minor role.

The mixture of 95% O$_2$ and 5% CO$_2$ was tolerated by all subjects. One subject felt claustrophobic after a few minutes in the scanner but before carbogen was administered. Claustrophobia might be aggravated by the breathing mask. Robinson et al. demonstrated in an animal study that concentrations of 1 or 2.5% CO$_2$ lead to changes in $R_2^*$ comparable to those obtained with 5% CO$_2$ (18). Powell et al. found that 98% O$_2$/2% CO$_2$ leads to similar increases in tumor oxygenation compared to conventional carbogen and that patient comfort was higher with the smaller percentage of carbon dioxide (37). Especially in patient studies, lower percentages of CO$_2$ may be favorable.

**Volunteers**

Some of the highly motivated volunteers showed so little movement that no realignment was necessary. In a clinical setting the data may be degraded by motion, making coregistration and ghost correction essential postprocessing.
steps. On the other hand, we found that motion that is not visible as a spatial mismatch of the 3D images may still lead to subtle ghosts if it occurs during sampling of low k-space frequencies. We were forced to exclude 4 of 13 data sets due to such ghosting.

Cortical and Deep Gray Matter

The mean signal increase of about 6% for cortical gray matter in volunteers between air breathing and carbogen breathing is comparable to 6.81 ± 1.96% measured at 2 T (38) and 8 ± 1.1% for healthy tissue determined at 1.9 T (17). In deep gray matter Rostrup et al. found an increase of 2.7 ± 2.6% in the thalamus and 2.0 ± 1.4% in the putamen using a 2D FLASH sequence and 2.0 ± 2.4% and 0.5 ± 0.7% using EPI (20). Again similar to our findings of 1.8 ± 1.2%/2.6 ± 0.9% (left/right thalamus) and 0.3 ± 0.9%/4.3 ± 1.9% (left/right putamen).

White Matter

In our study white matter showed a tendency toward a signal decrease, with a significant signal change during carbogen breathing in only one of the five selected white matter regions (see Table 2). During oxygen administration Losert et al. (21) reported white matter enhancement of 0.82 ± 0.08% and gray matter enhancement of 1.71 ± 0.14%, using gradient echo EPI with TE = 64 ms and a resolution of 2.2 × 2.2 × 5 mm³ at 1.5 T. Rostrup et al. (20) reported +0.8 ± 1.0% using gradient echo EPI (TE = 62 ms, resolution = 1.8 × 3.6 × 3 mm³) and +1.4 ± 0.5% using a FLASH sequence (TR/TE/α = 96 ms/62 ms/25°). White matter changes have been anomalously difficult to see. Early work with acetazolamide (39), which also increases flow by 30%, failed to demonstrate significant white matter changes, while vascular changes as reported here were well visualized. Similar results have been reported in previous studies, where white matter also showed unexpected behavior, such as a 3.5-fold increase in signal going from a 5% CO₂ gaseous mixture to a 7% CO₂ mixture in frontal WM but only an increase of about 30% in occipital WM, whereas all cortical gray matter areas showed a consistent increase of about 100% between 5% CO₂ and 7% CO₂ (20). Studies on breath hold induced hypercapnia also found no significant signal changes in white matter (40, 41). While the response of gray matter is consistent throughout different methods and studies, white matter seems difficult to assess.

Of note is also the consistency of the signal changes among the three breathing situations, where the sum of the changes between oxygen and carbogen and air and oxygen results in the change between air and carbogen for each ROI (see Table 2). This implies that the ROIs cover the same anatomic regions in all three scans. It can be also concluded from this finding that the essential parts of the k-spaces were sampled when blood oxygenation had already reached steady state, although no interscan delays of more than a minute were introduced, in order to minimize the time the subjects had to remain in the scanner.

Patients

In the data of the patient with the glioblastoma signal increases exceeded those found in gray matter and white matter of healthy volunteers. Even with image registration applied to the 3D images we found heterogeneous intratu-
Conclusions

The main advantage of the approach presented is that it allows investigation of the venous vasculature by computing venograms from the data acquired during air breathing and—at the same time—assessment of the BOLD response to the exogenous contrast agents carbogen or oxygen. The long acquisition time of about 10 to 14 min represents the main limitation of the presented method in terms of patient comfort and leads to a relatively high probability of artifacts due to motion. In the future, this problem may be overcome using segmented echo planar imaging, parallel imaging (44), and/or higher field strengths with shorter optimum echo times for BOLD contrast (10, 34). The former has already been shown to reduce scan times to 5 min with the inclusion of a fat saturation pulse, but has other well-known difficulties related to flow compensation not being as effective as the sequence we used here (45). However, parallel imaging in conjunction with either segmented EPI or high-field imaging may be the best compromise so that most flow compensation characteristics remain intact. Parallel imaging also offers new ghost correction strategies (46).

Visualization of venous vascularization and the response to changing blood oxygenation at the same time is possible using a high-resolution susceptibility-weighted imaging sequence. The effects of changing blood oxygenation on the magnetic field near venous vessels were visualized and signal changes in cortical gray matter, deep gray matter, white matter, and two tumors were evaluated. Further investigations may help to distinguish between brain areas with normal and abnormal blood supply.

References


