Effect of cerebrovascular changes on brain DTI quantitation: a hypercapnia study

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Abstract

Quantitative diffusion tensor imaging (DTI) offers a valuable tool to probe the microstructural changes in neural tissues in vivo, where absolute quantitation accuracy and reproducibility are essential. It has been long recognized that measurement of apparent diffusion coefficient (ADC) using DTI could be influenced by the presence of water molecules in cerebrovasculature. However, little is known about to what extent such blood signal affects DTI quantitation. In this study, we quantitatively examined the effect of cerebral hemodynamic change on DTI indices by using a standard multislice echo planar imaging (EPI) spin echo (SE) DTI acquisition protocol and a rat model of hypercapnia. In response to 5\% CO\textsubscript{2} challenge, mean, radial and axial diffusivities measured with diffusion factor ($b$-value) of $b=1.0\text{ ms}/\mu\text{m}^2$ were found to increase in whole brain (1.52\%±0.22\%, 1.66\%±0.16\% and 1.35\%±0.37\%, respectively), gray matter (1.56\%±0.23\%, 1.63\%±0.14\% and 1.47\%±0.45\%, respectively) and white matter regions (1.45\%±0.28\%, 1.88\%±0.33\% and 1.10\%±0.26\%, respectively). Fractional anisotropy (FA) was found to decrease by 1.67\%±0.38\%, 1.91\%±0.59\% and 1.46\%±0.30\% in whole brain, gray matter and white matter regions, respectively. In addition, these diffusivity increases and FA decreases became more pronounced at a lower $b$-value ($b=0.3\text{ ms}/\mu\text{m}^2$). The results indicated that in vivo DTI quantitation in brain can be contaminated by vascular factors on the order of few percentages. Consequently, alterations in cerebrovasculature and hemodynamics can affect the DTI quantitation and its efficacy in characterizing the neural tissue microstructures in normal and diseased states. Caution should be taken in designing and interpreting quantitative DTI studies as all DTI indices can be potentially confounded by physiologic conditions and by cerebrovascular and hemodynamic characteristics.

Keywords: DTI; Quantitation; Diffusivity; Hypercapnia; Hemodynamics; Cerebrovasculature

1. Introduction

Diffusion tensor imaging (DTI) offers a valuable tool to characterize water diffusion behavior in biological tissues [1–4]. It provides an in vivo and noninvasive capability to measure the tissue water diffusion properties by estimating a triaxial diffusivity ellipsoid, which is irreplaceable by other imaging modalities. As water diffusion is anisotropic in nerve fibers due to myelination and restrictions from other inherent axonal structures [5], DTI has demonstrated remarkable success in describing orientational neuroarchitecture and connectivity in the central nervous system and in characterizing white matter (WM) integrity [4,6–8]. Taking the advantages of the quantitative nature of DTI, valuable information with regard to the degree of physiological and pathophysiological associated microstructural changes can be accessed during aging and brain development [9,10], neurological and psychiatric disorders [11,12], brain injuries and tumor [13,14], and brain plasticity and cognitive functions [15,16].

It has been demonstrated extensively that measurement of apparent diffusion coefficient (ADC) using DTI could be influenced by the presence of water molecules in brain vasculature [17]. Potential sources of such interference include cerebral blood flow (CBF) response [18], cerebrovascular reactivity [19] and tissue temperature change [20]. As a result, DTI quantitation would be complicated when changes in neural microstructure are coupled with pathophysiological
alterations in vasculature such as in aging [21–25]. On the other hand, in DTI studies of brain plasticity, subtle microstructural alterations were reported on training or psychiatry models [16,26–29]. However, CBF or cerebral blood volume (CBV) alterations could also be induced by factors such as training, anxiety, neural activities and respiration changes, which would potentially introduce heterogeneities or misinterpretation of the in vivo findings [30]. Thus, alterations in cerebrovasculature and hemodynamics can potentially affect the DTI quantitation and its efficacy in characterizing the neural tissue microstructures in normal and diseased states. Therefore, it is imperative to evaluate the extent of hemodynamic effects on DTI indices quantitation. Such study would allow us to better understand the reproducibility and sensitivity of in vivo DTI measurements.

Hypercapnia can induce vessel dilation and alter blood acidity, resulting in CBF and CBV elevation in brain [31,32]. It has been widely used to study the hemodynamic change in the absence of neuronal activation [33–35]. It was also reported that hypercapnia could lead to diffusion-weighted signal increases in a wide range of $b$-values in human subjects [18]. In this study, we aimed to quantitatively examine the effect of cerebrovasculature and hemodynamic changes on DTI quantitation globally as well as in gray matter (GM) and WM regions in a well-controlled rat model of hypercapnia.

2. Methods

2.1. Hypercapnia paradigms

Normal adult Sprague–Dawley rats ($N = 6$) were anesthetized with a mixture of isoflurane/air (3% for induction and 1.5%–2% for maintenance) via a nose cone. Each rat individually underwent hypercapnic challenges by inhalation of 5% CO$_2$ and 95% air via a nose cone [36]. Each session consisted of six pairs of ON ($\sim$4 min 15 s) and OFF (100% air inhalation, $\sim$7 min 5 s) CO$_2$ periods. Three DTI trials were performed consecutively for each ON period and five DTI trials for each OFF period, yielding a total of 48 consecutive DTI trials per session. Animals were kept warm using a warming pad with circulating water. Respiration rate, heart rate, saturation of peripheral oxygen (SpO$_2$) and rectal temperature were consecutively monitored (SA-Instruments, Stony Brook, NY, USA) throughout the experiments.

2.2. Magnetic resonance imaging (MRI) protocols

All experiments were conducted using a 7-T MRI scanner with a maximum gradient of 360 mT/m (70/16 PharmaScan, Bruker Biospin GmbH, Germany). A birdcage transmit-only coil with a 72-mm inner diameter in combination with an actively decoupled receive-only quadrature surface coil was used. Diffusion-weighted images (DWIs), together with five images without diffusion sensitization ($b=0.0$ ms/$\mu$m$^2$, $b_0$ images), were acquired using spin echo (SE) echo planar imaging (EPI) sequence along six gradient directions and two $b$-values (1.0 and 0.3 ms/$\mu$m$^2$). The total scan time for each DTI trial was 85 s. Forty-eight consecutive DTI trials were performed for each animal with the hypercapnia paradigms described. The imaging parameters were: repetition time/echo time=2500/31.1 ms, $\delta/\Delta=5/17$ ms, field of view=4.5×4.5 cm$^2$, data matrix=96×96 (zero-filled to 256×256) and slice thickness=1 mm (0.2 mm gap); multislices were acquired sequentially from posterior to anterior brain.

2.3. Data analysis

For each rat, DWIs and $b_0$ images from all 48 DTI trials with varying $b$-values were first co-registered to compensate for the eddy-current-induced displacements by AIR 5.2.5 [37]. The mean of the co-registered five $b_0$ images and the mean of DWIs from six diffusion directions were computed for each DTI trial. DTI index maps from each trial were generated by a home-written MATLAB program. Mean diffusivity (MD), fractional anisotropy (FA), axial diffusivity and radial diffusivity maps were calculated with $b$-values 0 versus 1.0 ms/$\mu$m$^2$ and 0 versus 0.3 ms/$\mu$m$^2$, respectively, as previously described [38].

To obtain multislice masks of individual animals, whole brain mask in each slice was firstly defined based on the averaged FA and MD maps from the 48 trials of each animal. Based on the mean FA and MD values, the brain was segmented into GM (MD<1.6 $\mu$m$^2$/ms, 0.03<FA<0.31), WM (MD<1.6 $\mu$m$^2$/ms, FA>0.31) and cerebrospinal fluid (CSF) (MD>1.6 $\mu$m$^2$/ms) [39]. The segmented whole brain mask and regional masks were used to quantify the changes in whole brain, GM and WM in various DTI indices and for histogram analysis.

Evaluation of quantitation changes and histogram comparisons between normocapnia and hypercapnia were made by extracting two DTI trials from each of the CO$_2$ ON and OFF pairs. The trial right before CO$_2$ inhalation represented normocapnia, and the last trial of CO$_2$ inhalation represented hypercapnia. The mean percentage change of each animal was calculated by averaging $[(S_{\text{hypercapnia}}−S_{\text{normocapnia}})/S_{\text{normocapnia}}*100]$ from each of the six pairs, where $S_{\text{hypercapnia}}$ and $S_{\text{normocapnia}}$ were the DTI parametric values in the extracted hypercapnia and normocapnia index maps, respectively. Data were presented as mean±standard deviation unless otherwise specified.

3. Results

Fig. 1 summarizes the consecutive physiological recordings of all animals throughout the experiments. During CO$_2$ inhalation, respiration rate increased from about 50 to 65 respirations per minute, SpO$_2$ increased from around 81% to 87% Sat, and heart rate slightly decreased from around 378 to 365 beats per minute. Respiration rate, SpO$_2$ and heart rate returned to baseline levels and reached a steady state before the next CO$_2$ inhalation session. The
rectal temperature was maintained at 37.15°C±0.15°C throughout the experiment without apparent changes between normocapnia and hypercapnia.

Fig. 2 illustrates representative masks of whole brain, GM and WM overlaid on a mean FA map. Note that GM regions (such as cerebral cortex, hippocampus, thalamus and caudate putamen) and WM regions (such as corpus callosum, fimbria, external capsule, internal capsule and optical tract) were well segmented in individual separate masks for each animal.

Quantitation changes of $b_0$ and DWIs between normocapnia and hypercapnia in whole brain, GM and WM are summarized in Table 1. Both $b_0$ and diffusion-weighted signals increased during hypercapnia. The signal increases in the mean DWIs were significantly smaller than those in $b_0$ at both $b=1.0$ ms/$\mu$m$^2$ ($P=4.8 \times 10^{-4}$ in whole brain, $P=6.0 \times 10^{-4}$ in GM and $P=1.9 \times 10^{-4}$ in WM) and $b=0.3$ ms/$\mu$m$^2$ ($P=7.5 \times 10^{-4}$ in whole brain, $P=9.1 \times 10^{-4}$ in GM and $P=5.2 \times 10^{-4}$ in WM) using a two-tailed paired Student’s $t$ test across animals.

Fig. 3 illustrates the time courses of DTI indices computed with $b$-values of 0.0 ms/$\mu$m$^2$ versus 1.0 and 0.3 ms/$\mu$m$^2$ in whole brain. MD and radial and axial diffusivities were found to increase during CO$_2$ inhalation. The time courses corresponded well with the CO$_2$ manipulation paradigms, clearly indicating the effect of hypercapnia on diffusivity quantitation. In addition, FA gradually decreased during hypercapnia, suggesting that the anisotropy measurement in whole brain region was also affected by CO$_2$ manipulation.
Fig. 4 demonstrates the time courses of DTI indices computed with 
\( b \)-values of 0.0 ms/\( \mu \)m\(^2\) versus 1.0 and 0.3 
ms/\( \mu \)m\(^2\) in GM (left column) and WM (right column). The 
patterns of time courses were similar to those in whole brain 
quantitation. In Table 2, radial diffusivity was found to 
increase more than axial diffusivity, and FA decreased in 
both regions during hypercapnia. Statistical analyses showed 
that compared to GM, WM increased significantly less in 
axial diffusivity (\( P=3.9\times10^{-6} \) and \( P=4.9\times10^{-8} \) for \( b=1.0 \)
and \( b=0.3 \) ms/\( \mu \)m\(^2\), respectively) and MD (\( P=1.9\times10^{-7} \) and
\( P=4.2\times10^{-5} \) for \( b=1.0 \) and \( b=0.3 \) ms/\( \mu \)m\(^2\), respectively),
decreased significantly less in FA (\( P=1.7\times10^{-2} \) and
\( P=1.8\times10^{-2} \) for \( b=1.0 \) and \( b=0.3 \) ms/\( \mu \)m\(^2\), respectively),
but increased significantly more in radial diffusivity
(\( P=6.6\times10^{-4} \) for \( b=1.0 \) ms/\( \mu \)m\(^2\)) using two-tailed paired 
Student’s \( t \) tests. 

Comparing between \( b=1.0 \) and 0.3 ms/\( \mu \)m\(^2\), DTI 
quantitation followed a similar trend in correspondence 
with CO\(_2\) manipulation in whole brain, GM and WM (Figs. 3 
and 4). However, the percentage changes between normo-
capnia and hypercapnia computed with \( b=0.3 \) ms/\( \mu \)m\(^2\) were
more than twice than with \( b=1.0 \) ms/\( \mu \)m\(^2\) in all DTI index 
maps except axial diffusivity in WM (Table 2). 

Fig. 5 illustrates the distributions of DTI quantitation in 
different brain regions of a representative animal during 
normocapnia and hypercapnia. Distribution profiles shifted 
distinctly to higher values in MD and radial and axial 
diffusivities during hypercapnia in all animals, indicative of 
the hypercapnic effect on diffusivity quantitation. A larger 
extent of shifts in MD and radial and axial diffusivities was 
found in GM compared to WM, but the shifts in FA were less 
evident compared to diffusivity shifts in all regions.

4. Discussion

This study demonstrated quantitatively that the cerebro-
vasculature and hemodynamic changes induced by hyper-
capnia substantially altered MD, radial diffusivity, axial 
diffusivity and FA quantitation. Accompanied with CBF

<table>
<thead>
<tr>
<th>( b )-value</th>
<th>Mean DWIs at ( b=0.3 ) ms/( \mu )m(^2)</th>
<th>Mean DWIs at ( b=1.0 ) ms/( \mu )m(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0 ms/( \mu )m(^2)</td>
<td>1.59%±0.22% 0.94%±0.11%**</td>
<td>0.84%±0.09%**</td>
</tr>
<tr>
<td>0.3 ms/( \mu )m(^2)</td>
<td>1.62%±0.24% 0.97%±0.11%**</td>
<td>0.86%±0.10%**</td>
</tr>
<tr>
<td>1.0 ms/( \mu )m(^2)</td>
<td>1.46%±0.19% 0.88%±0.18%**</td>
<td>0.81%±0.12%**</td>
</tr>
</tbody>
</table>

Whole brain, GM and WM quantitation changes (mean±standard deviation, \( n=6 \)). The increase in the mean DWIs at \( b=1.0 \) or 0.3 ms/\( \mu \)m\(^2\) was significantly smaller than that in \( b_0 \), respectively, using two-tailed paired 
Student’s \( t \) tests (**\( P<.01 \)).

Fig. 3. Time courses of MD (in \( \mu \)m\(^2\)/ms), radial diffusivity (radial, in \( \mu \)m\(^2\)/ms), axial diffusivity (axial, in \( \mu \)m\(^2\)/ms) and FA in the whole brain computed with 
\( b \)-value 0.0 ms/\( \mu \)m\(^2\) versus \( b=1.0 \) or 0.3 ms/\( \mu \)m\(^2\), respectively. Error bars represent the standard error of the mean. Gray shades indicate 5% CO\(_2\) inhalation.
and CBV elevations, diffusivity measurements were elevated and FA decreased. The extent of changes was observed to be tissue dependent, whereby GM had greater changes in MD, axial diffusivity and FA, and a smaller change in radial diffusivity compared to WM. Such differences might be ascribed to the varying distributions of cerebrovasculature...
and distinct hemodynamics in different brain components. The decrease in FA during CO2 inhalation in all regions might also be attributed to the pronounced pseudodiffusion effect in association with the relatively random capillary vasculature.

It is believed that conventional diffusion imaging could include perfusion effect [17], and the sensitivity to such incoherent perfusion motion would be varied with $b$-value [40,41]. In this study, the hypercapnia-induced quantitation changes were more pronounced in the indices computed with $b$-value of 0.3 ms/μm² compared to 1.0 ms/μm², suggesting more pronounced pseudodiffusion effect from blood perfusion with lower $b$-value. This further confirmed that perfusion intervention was a major source of the alterations in DTI quantitation. In this study, the increases of diffusion weighted signal in the whole brain, WM and GM were less

<table>
<thead>
<tr>
<th>Whole brain</th>
<th>MD</th>
<th>axial</th>
<th>radial</th>
<th>FA</th>
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<tbody>
<tr>
<td>$b=1.0$ ms/μm²</td>
<td>1.52±0.22%</td>
<td>1.35±0.37%</td>
<td>1.66±0.16%</td>
<td>−1.67±0.38%</td>
</tr>
<tr>
<td>$b=0.3$ ms/μm²</td>
<td>3.59±0.28%</td>
<td>2.79±0.69%</td>
<td>3.81±0.96%</td>
<td>−3.78±0.32%</td>
</tr>
<tr>
<td>GM</td>
<td>1.56±0.23%</td>
<td>1.47±0.45%</td>
<td>1.63±0.14%</td>
<td>−1.91±0.59%</td>
</tr>
<tr>
<td>$b=1.0$ ms/μm²</td>
<td>3.71±0.28%</td>
<td>3.07±0.81%</td>
<td>3.77±1.01%</td>
<td>−4.14±0.54%</td>
</tr>
<tr>
<td>$b=0.3$ ms/μm²</td>
<td>1.45±0.28%</td>
<td>1.10±0.26%</td>
<td>1.88±0.33%</td>
<td>−1.46±0.30%</td>
</tr>
<tr>
<td>WM</td>
<td>3.25±0.34%</td>
<td>2.14±0.50%</td>
<td>4.06±0.91%</td>
<td>−3.44±0.56%</td>
</tr>
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</table>

Whole brain, GM and WM quantitation changes (mean±standard deviation, $n=6$) in various DTI index maps computed with $b$-value 0.0 ms/μm² versus $b=1.0$ or 0.3 ms/μm², respectively. Compared to GM, WM quantitation increased significantly less in MD ($b=1.0$ and $b=0.3$ ms/μm²) and axial diffusivity ($b=1.0$ and $b=0.3$ ms/μm²), decreased significantly less in FA ($b=1.0$ and $b=0.3$ ms/μm²) and increased significantly more in radial diffusivity ($b=1.0$ ms/μm²), respectively, using two-tailed paired Student’s $t$ tests ($*P<.05; **P<.01$).

Fig. 5. Histogram comparisons between hypercapnia and normocapnia. MD (in μm²/ms), radial diffusivity (radial, in μm²/ms), axial diffusivity (axial, in μm²/ms) and FA were evaluated in the masked whole brain (A), GM (B) and WM (C) regions in a representative animal.
than 1% during hypercapnia, and no clear difference in such increase was observed between b-value of 1.0 and 0.3 ms/μm². However, quantitation changes found in DTI indices were significantly b-value dependent, suggesting that DTI quantitation could be more sensitive to hypercapnia at different b-values.

It is well known that cerebrovasculature distribution is regional dependent. It was reported that the capillary density in GM was about 2 to 4 times that in WM using fluorescence microscopy [42,43]. It was also demonstrated that the baseline regional CBV was around double in GM relative to WM using various MRI techniques [44,45]. In addition, Lu et al. reported greater CBV increases in cortex (∼1.24%) than in subcortical area (∼1.19%) during 5% CO₂ hypercapnia using gradient echo EPI sequences in rat and attributed such differences to the spatially varying regulation of hemodynamics [36]. In the current study, hypercapnia-induced CBF and CBV elevation in brain is expected to contribute to the observed diffusivity increases; therefore, it is not surprising that diffusivities (except radial diffusivity) increased more in GM than in WM during hypercapnia. Histogram analysis showed more apparent global shifts to higher diffusivity in GM and whole brain regions during hypercapnic state.

The choice of sequence setting and interslice distance could affect the in-flow effect of blood signals and thus influence the extent of blood perfusion contribution to the apparent diffusion measurement [46]. It may also affect the captured hypercapnia-induced DTI quantitation changes. Hence, the diffusivity percentage change was examined using different slice gaps while keeping other imaging parameters unchanged. The same slices with acquisition gap of 2.3 mm and 0.1 mm were acquired in a single hypercapnia session. It was found that MD increase in acquisition gap=2.3 mm (∼2.28%) was larger than that in acquisition gap=0.1 mm (∼1.53%) during hypercapnia responses. This suggested that the absolute diffusivity increases could also depend on the blood inflow saturation effect among slices. A smaller interslice gap might lead to greater flow-saturation effect, and the extent of perfusion alterations to DTI quantitation might be smaller.

The overall time courses in DTI parametric quantitation and physiology recordings in this study were reproducible across animals. As expected, respiration rate increased, and heart rate decreased in response to hypercapnia [33,47]. Spo₂ increased during hypercapnia likely due to the elevated hemoglobin saturation when respiration rate increased. These data were consistent with previous physiology studies by other groups [33,36,48]. Temperature is known to affect molecular Brownian motion and diffusion coefficient [20]. Zhu et al. reported that a 4.5-min hypercapnia at 5% CO₂ could change the body and deep brain temperature difference from −0.2°C to around −0.18°C due to metabolic rate alteration [49]. In our study, rectal temperature variation was maintained within ±0.15°C throughout the experiment. Given that the temperature sensitivity of diffusion coefficient was estimated to be around 2%/°C [20], any brain temperature change during hypercapnia likely plays a negligible role in the diffusivity increase as observed in the current study. It was noted that the time courses of DTI quantitation did not always reach equilibrium within each CO₂ inhalation period and might change further using longer inhalation duration. However, since it was reported that prolonged hypercapnia might depress respiratory activity in rat [50], we intended to use a short CO₂ inhalation period of around 4.5 min each to minimize abnormal physiological responses while maintaining adequate sensitivity for DTI quantitation.

In addition to GM and WM, CSF has been reported to possess functional changes during hypercapnia [51]. A preliminary analysis was performed to examine whether DTI quantitation in CSF could also be affected by CO₂ challenge. The cerebral aqueduct, which possesses no choroid plexus, was selected for DTI measurements. A single voxel which had a maximum MD value in the cerebral aqueduct was chosen for quantitation analysis. It was found that MD and axial and radial diffusivity dropped 5.6%, 6.5% and 6.2%, respectively, at b=1.0 ms/μm² during hypercapnia (N=5). Although Piechnik et al. presented a CSF volume shift during hypercapnia [52] and Cohen-Adad et al. proposed the blood-oxygenation level dependent (BOLD) responses in the CSF region around spinal cord upon hypercapnia [51], the sources of the DTI alterations in the CSF are uncertain and are beyond the scope of this paper.

It is noteworthy that isoflurane, which was used for anesthesia in the current study, is a potent cerebrovasodilator [47,53]. It has been reported that isoflurane depressed cerebrovascular reactivity to CO₂ relative to awake conditions [54,55] and potentially reduces hypercapnia-evoked BOLD increases [35,36,48]. Therefore, it is possible that the use of isoflurane would reduce the hypercapnia-evoked DTI parametric changes.

5. Conclusions

Our data demonstrated that MD and radial and axial diffusivities increased in whole brain, GM and WM regions in response to hypercapnia. Given that such effect was more pronounced at a lower b-value, perfusion intervention might be the main source of the alterations in DTI quantitation. The FA decrease during CO₂ inhalation could be due to an enhanced pseudodiffusion effect associated with relatively random capillary vasculature. These results indicated that in vivo DTI quantitation in brain can be interfered by vascular factors on the order of few percentages. Consequently, alterations in cerebrovasculature and hemodynamics can affect the DTI quantitation and its efficacy in characterizing the neural tissue microstructures in normal and diseased states. Caution should be taken in designing and interpreting quantitative DTI studies as all DTI indices can be potentially

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