Breathing manoeuvre-dependent changes in myocardial oxygenation in healthy humans

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Received 19 June 2013; revised 24 July 2013; accepted after revision 29 August 2013; online publish-ahead-of-print 27 September 2013

Aims
CO₂ is an intrinsic vasodilator for cerebral and myocardial blood vessels. Myocardial vasodilation without a parallel increase of the oxygen demand leads to changes in myocardial oxygenation. Because apnoea and hyperventilation modify blood CO₂, we hypothesized that voluntary breathing manoeuvres induce changes in myocardial oxygenation that can be measured by oxygenation-sensitive cardiovascular magnetic resonance (CMR).

Methods and results
Fourteen healthy volunteers were studied. Eight performed free long breath-hold as well as a 1- and 2-min hyperventilation, whereas six aquatic athletes were studied during a 60-s breath-hold and a free long breath-hold. Signal intensity (SI) changes in T₂*-weighted, steady-state free precession, gradient echo images at 1.5 T were monitored during breathing manoeuvres and compared with changes in capillary blood gases. Breath-holds lasted for 35, 58 and 117 s, and hyperventilation for 60 and 120 s. As expected, capillary pCO₂ decreased significantly during hyperventilation. Capillary pO₂ decreased significantly during the 117-s breath-hold. The breath-holds led to a SI decrease (deoxygenation) in the left ventricular blood pool, while the SI of the myocardium increased by 8.2% (P = 0.04), consistent with an increase in myocardial oxygenation. In contrast, hyperventilation for 120 s, however, resulted in a significant 7.5% decrease in myocardial SI/oxygenation (P = 0.02). Change in capillary pCO₂ was the only independently correlated variable predicting myocardial oxygenation changes during breathing manoeuvres (r = 0.58, P < 0.01).

Conclusion
In healthy individuals, breathing manoeuvres lead to changes in myocardial oxygenation, which appear to be mediated by CO₂. These changes can be monitored in vivo by oxygenation-sensitive CMR and thus, may have value as a diagnostic tool.

Keywords
BOLD-sensitive MRI • carbon dioxide • vasodilation • T₂*-weighted imaging • apnoea • hyperventilation

Introduction
Oxygenation-sensitive cardiovascular magnetic resonance (CMR) can be used to assess myocardial tissue oxygenation by taking advantage of the blood oxygen level-dependent (BOLD) effect in T₂*-weighted imaging sequences. These sequences exploit the magnetic properties of haemoglobin as an endogenous contrast: while oxygenated haemoglobin (oxyHb) is diamagnetic (i.e. exhibits a weak stabilization of the magnetic field surrounding the molecule), deoxygenated haemoglobin (deoxyHb) is paramagnetic, destabilizing the surrounding field and thereby leading to a loss of magnetic field homogeneity. Therefore, increasing deoxyHb levels will result in a signal intensity (SI) decrease.¹,² This corresponding decrease in SI was demonstrated in myocardial ischaemia studies.³

In previous studies using oxygenation-sensitive CMR, vasodilators were utilized in order to decouple myocardial blood flow from its demand and to induce excess perfusion.⁴,⁵ Using adenosine-induced coronary vasodilation, we could recently show that this vasodilatory effect leads to a measureable SI increase, which was linearly related to coronary sinus blood oxygenation.⁶

Vascular tone and tissue blood flow are tightly regulated by its local metabolic demand. Several metabolites are known to affect vascular autoregulation, with O₂ and CO₂ being the two agents with the most direct feedback.⁷,⁸
As we and others have shown, a systemic increase in CO₂ tension leads to an increase in cerebral and myocardial blood flow. The exact pathways of CO₂ controlled vasodilation remain unclear, but different theories have been outlined by Deussen. Importantly, an increase in systemic PaCO₂ modulates tissue blood flow independently from local metabolic demands and will consequently lead to an excess perfusion similar to pharmacologic vasodilators. In a swine model, we recently observed that breath-holds lead to CO₂-dependent changes in myocardial oxygenation. Yet, there are no human data. Thus, the utility of manipulating systemic CO₂ levels by voluntary breathing manoeuvres to induce changes in myocardial oxygenation and eventually as a vasodilator for diagnostic testing is unknown.

We aimed to monitor myocardial oxygenation during voluntary breathing manoeuvres in humans, including testing the feasibility of this protocol in combination with oxygenation-sensitive CMR to monitor coronary vascular function in healthy volunteers. We hypothesized that, in healthy volunteers, there are consistent changes in myocardial oxygenation during long breath-holds (increased CO₂ with a subsequent increase in blood flow, resulting in an increased oxygenation) and hyperventilation (decreased CO₂ with a subsequent decrease in blood flow, resulting in a decreased oxygenation) that can be detected by oxygenation-sensitive CMR.

### Methods

#### Experimental protocol

Eight healthy volunteers and six aquatic athletes were recruited to perform breathing manoeuvres in a 1.5-T clinical magnetic resonance imaging (MRI) scanner (Siemens Avanto, Siemens, Erlangen, Germany). Volunteers were required to have a minimum age of 18 years and provide informed consent. Aquatic athletes had to be able to comfortably hold their breath for at least 60 s. Exclusion criteria consisted of any conditions of previous or known cardiovascular disease, respiratory disease, vasoactive medication, pregnancy, consumption of coffee, tea or cigarettes 12 h prior to the scan, as well as general exclusion criteria for MRI exams.

The healthy normal volunteers performed three breathing manoeuvres; a free maximal breath-hold and two sets of hyperventilation of 1 and 2 min each. Aquatic athletes were asked to perform a timed 60-s breath-hold as well as a free maximal breath-hold. Using a 32-channel cardiac phased array coil, BOLD-sensitive, steady-state free precession, gradient echo cine images were continuously acquired during the breath-holds in mid-left ventricular short-axis views (slice thickness 10 mm, echo time 2.78 ms, repetition time 65 ms, flip angle 90°, field of view 280 × 157.5, and matrix 128 × 72). For the acquisition of hyperventilation images, two baseline cines and two post-hyperventilation cines were acquired during a short breath-hold. Each cine loop was composed of 20 phases covering the entire cardiac cycle, obtained by retrospective ECG gating. Prior to and immediately after a breathing manoeuvre, capillary pO₂ and pCO₂ were acquired. The hands were warmed to arterialize the blood.

#### Image analysis

The images were analysed with the certified software for CMR images (crm®, Circle Cardiovascular Imaging, Inc., Calgary, AB, Canada). Image quality was graded prior to SI measurement using visual assessment based on a 1–4 scale: 1 = good image quality, 2 = mildly impaired image quality resulting in <10% of the total myocardial area excluded, 3 = limited image quality resulting in >10% of the myocardium or >1 phases from the cine to be excluded, 4 = image with insufficient quality for analysis. The mean myocardial SI in the images was automatically calculated after manual tracing of endocardial and epicardial contours of all images. Additionally, left ventricular blood pool contours were traced to assess changes of SI caused by changes in arterial haemoglobin saturation (Figure 1).

For the breath-hold experiments, the first two cine series of the breath-hold were averaged for all cardiac phases and compared with the final two cines. If breath-holds were shorter resulting in four or less cine series, only the first cine was compared with the final one. For the hyperventilation experiments, the two baseline scans were averaged accordingly and compared with two post-hyperventilation images. The area under the curve (AUC) was calculated from the SI of all 20 phases and expressed as %-change SI from baseline to provide a single SI value incorporating representative data of the entire cardiac cycle.

All images were analysed by two readers, and the average change in myocardial SI from both readers was reported.

#### Statistical analysis

To determine the SI changes resulting from the breathing manoeuvres, the AUC from the beginning of the breath-hold/after hyperventilation was compared with those at the end of the breath-hold or after hyperventilation, respectively, using a paired t-test and expressed as %-change SI. A one-way analysis of variance (ANOVA) and a Tukey–Kramer post hoc test were used to compare the %-change SI between the different breathing manoeuvres. Inter-observer variability of the MR analysis was assessed with an intra-class correlation (ICC). The changes in blood gases from baseline and after a breathing manoeuvre were analysed using a paired t-test, and the correlation was calculated between the changes in BOLD-SI, blood gases and heart rate (HR). A D’Agostino’s-Pearson normality test was performed to assess normal distribution within the data points. Multiple regression analysis was performed with these variables using blood gases and HR as the independent variables.
and BOLD-SI as the response variable to determine which factor was primarily responsible for the variation in BOLD-SI. Additionally, analyses were also performed with normalization for HR (SI/HR). A P-value of <0.05 was regarded statistically significant. Statistical analysis was completed with SPSS version 19 (SPSS, Chicago, IL, USA) and Graph Pad Prism (GraphPad Software, San Diego, CA, USA).

Results

There were five breathing manoeuvre groups available for analysis: 1-min hyperventilation (HV60 s, n = 7), 2-min hyperventilation (HV120 s, n = 5), short free breath-hold (BH35 s, n = 6), timed 60-s breath-hold (BHS8 s, n = 6), and long free breath-hold (BH117 s, n = 5). In the HV60 s experiment, one volunteer had to be excluded due to insufficient image quality. In the HV120 s group, two studies had to be excluded due to poor image quality, whereas one volunteer had to abort the experiment due to hyperventilation side effects. One volunteer was excluded from the timed 60-s breath-hold due to bad image quality. Subjects whose breathing manoeuvres did not meet predefined criteria were reallocated to the appropriate breathing manoeuvre group prior to image analysis.

Blood gas analysis

Capillary pO2 dropped significantly by 16.3 mmHg after the longest breath-hold, BH117 s (P = 0.02). In contrast, myocardial SI increased by 8.2% in the HV120 s group, whereas one volunteer had to abort the experiment due to hyperventilation side effects. One volunteer was excluded from the timed 60-s breath-hold due to bad image quality. Subjects whose breathing manoeuvres did not meet predefined criteria were reallocated to the appropriate breathing manoeuvre group prior to image analysis.

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CMR results

In the HV120 s group, myocardial SI decreased by −7.5 ± 1.8% (P = 0.02). In contrast, myocardial SI increased by 8.2 ± 2.8% (P = 0.04) after the longest breath-hold (BH117 s) (Table 1 and Figure 2). Although not significant, the HV60 s group shows a trend for a SI decrease, while an increase was observed in both the BH35 s and BHS8 s cohorts. A change in arterial blood SI in the left ventricle was only observed in the BH117 s group (−6.8%, P = 0.02). ANOVA showed a difference between at least two breathing manoeuvre groups when comparing the %-change in SI (F_{2,24} = 4.7, P < 0.01).

The D’Agostino’s-Pearson normality test showed a normal distribution within the data points. Both the CO2 values and the myocardial SI changes passed the normality test. When variables were assessed individually, a correlation was found between myocardial SI and pCO2 (Table 2 and Figure 3). A negative correlation was found between HR and both CO2 (r = −0.62, P < 0.01) and myocardial BOLD-SI (r = −0.43, P = 0.02), but regression analysis showed the variance inflation factor between pCO2 and HR was <10, so the variables were assessed together in one model with multiple regression (Table 2). While pCO2, pO2 and HR can explain changes seen in myocardial SI (r^2 = 0.23, F_{2.24} = 4.4, P = 0.01), CO2 was the only variable which was independently correlated with myocardial SI changes. Moreover, there was no direct relationship found between the absolute myocardial SI and HR. When a correction for HR was performed, the relative SI change was more pronounced for both breath-hold and hyperventilation experiments (Table 1). With these values corrected for HR, the increases in SI after each breath-hold were significantly different from the changes caused by hyperventilation (F_{4,24} = 8.8, P < 0.05). ANOVA showed a stronger correlation with pCO2 changes, when SI was corrected for HR (r = 0.68, P < 0.01).

Image quality

Of 33 scans, 13 (39%) were graded as good, with the highest score of 1. Images with minor artefacts were graded medium image quality (n = 13, 39%) and three images were graded as poor (n = 3, 9%), resulting in 29 of the 33 scans being analyzable (88%). Four (12%) studies were non-analyzable due to breathing artefacts and thus excluded from the analysis. ICC between the two observers was excellent (ICC = 0.90, [0.80; 0.95]).

Discussion

Our data indicate that BOLD-CMR can monitor CO2-dependent coronary function with consecutive changes in myocardial oxygenation using simple breathing manoeuvres in healthy volunteers.

BOLD-sensitive CMR of myocardial oxygenation

Various factors determine myocardial tissue oxygenation. During constant blood flow (and thus constant O2 supply), an increased O2 demand would result in deoxygenation and thus a SI decrease, a decreased O2 demand would have an opposite effect. In the setting of constant O2 demand, an increase in blood flow leads to a

<table>
<thead>
<tr>
<th>Breathing manoeuvre</th>
<th>n</th>
<th>ΔpO2</th>
<th>ΔpCO2</th>
<th>ΔSI</th>
<th>ΔSI HR-corrected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperventilation (120 s)</td>
<td>5</td>
<td>1.5 ± 4.2</td>
<td>−8.7 ± 0.6*</td>
<td>−7.5 ± 1.8*</td>
<td>−25.6 ± 3.3*</td>
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<tr>
<td>Hyperventilation (60 s)</td>
<td>7</td>
<td>−1.4 ± 3.5</td>
<td>−5.9 ± 1.1*</td>
<td>−5.8 ± 4.3</td>
<td>−24.3 ± 7.1*</td>
</tr>
<tr>
<td>Breath-hold (35 s)</td>
<td>6</td>
<td>+7.3 ± 3.6</td>
<td>+0.1 ± 1.0</td>
<td>+3.6 ± 2.0</td>
<td>+19.8 ± 10.9</td>
</tr>
<tr>
<td>Breath-hold (58 s)</td>
<td>6</td>
<td>+5.8 ± 6.0</td>
<td>+0.8 ± 2.0</td>
<td>+6.9 ± 3.7</td>
<td>+9.0 ± 7.4</td>
</tr>
<tr>
<td>Breath-hold (117 s)</td>
<td>5</td>
<td>−16.3 ± 5.5*</td>
<td>+2.4 ± 1.2</td>
<td>+8.2 ± 2.8*</td>
<td>+21.3 ± 7.3*</td>
</tr>
</tbody>
</table>

Mean (± SEM) change of capillary gas pressure (mmHg) obtained from a finger prick prior to and after the breathing manoeuvres and SI change in oxygenation-sensitive CMR (mean % change ± SEM) without and with HR correction (*P < 0.05). The results indicate a decrease of myocardial oxygenation in hyperventilation and an increase during breath-holds, which is dependent on the duration of the manoeuvre.
decreased fraction of deoxyHb, exhibiting the BOLD-effect used for oxygenation-sensitive CMR imaging.

The observed BOLD-effect is predominantly due to changes of inhomogeneities in the magnetic field occurring in and around the myocardial capillaries. In the presence of a vasodilator without an increase in myocardial oxygen consumption in the healthy myocardium, the opening of arterioles increases the total oxygen supply without a matching change of the demand and thereby reduces the deoxyHb content in the capillary beds. Accordingly, this leads to an increase in BOLD-SI or $T^*_2$, of the tissue in the affected perfusion bed. Importantly, SI changes in BOLD-sensitive images acquired during steady conditions have been shown to exclusively reflect changes in Hb oxygenation.

CO$_2$-mediated changes in coronary perfusion

Myocardial CO$_2$ has been known to control coronary flow, but has recently been assessed with CMR imaging. Using oxygenation-sensitive CMR in an anaesthetized swine model, it was recently demonstrated that breath-holds of 1-min increased myocardial tissue oxygenation. This increase in myocardial BOLD-SI was correlated with that in paCO$_2$. Although arterial pO$_2$ dropped significantly in the animal study, the increase in CO$_2$-mediated coronary blood flow compensated the drop in haemoglobin saturation in the heart, thus myocardial oxygen supply was not compromised during a limited period of apnoea. The anaesthetics required in an animal model are known to affect vascular tone as well as myocardial contractility and could therefore be a confounder of oxygenation-sensitive CMR by reducing myocardial oxygen demand. Therefore, this study addressed these concepts in a human model with controlled breathing manoeuvres. In the present volunteer study, there was a significant increase in the 117-s breath-hold and a significant decrease in BOLD-SI in the 120-s hyperventilation experiment. However, not significant, we could see a trend for a consistent increase in BOLD-SI in all breath-hold experiments. Longer breath-holds yielded higher increases in BOLD-SI. The significant drop in capillary pO$_2$ and left ventricular blood pool in the longest breath-hold supports our findings from our previous study that CO$_2$-mediated increase in blood flow can compensate for desaturation and prevent compromised myocardial oxygenation during long effective breath-holds. In fact, mild hypercapnic hypoxia may have further increased myocardial blood flow. Broten et al. and Beaudin et al. demonstrated that hypercapnia and hypoxia have
synergistic effects and result in even greater blood supply to the myocardium.

Interestingly, hyperventilation resulted in a significant drop in BOLD-SI, indicating a drop in myocardial tissue oxygenation. Reduced myocardial blood flow is a result of the coronary vasoconstriction caused by hyperventilation. Consequently, deoxygenation of the myocardium occurs as the oxygen extraction outweighs the coronary supply, presupposing a constant myocardial workload. Since there was a negative correlation between HR and change in pCO2, an increasing myocardial workload due to increased HRs may be another factor leading to a drop in BOLD-SI during hypocapnia. Yet, in a study of healthy elderly men, hypocapnia induced through hyperventilation resulted in a decrease in myocardial blood flow independent of cardiac workload.

We could demonstrate that the change in myocardial oxygenation is strongly associated with CO2. The correlation exhibits how the known coronary flow controlling effects of both hypocapnia and hypercapnia can be visualized with non-invasive imaging. In fact, long effective breath-holds with a systemic rise of CO2 resemble the effects of adenosine-induced hyperperfusion in oxygenation-sensitive CMR in healthy volunteers.

Capillary blood gas changes
The drops in pCO2 prove the effectiveness of the hyperventilation manoeuvres. The 117-s breath-hold yielded a significant drop in pO2, while we were unable to detect a significant change in pCO2. A limited oxygen supply.26

Changes in pCO2 was the only depending factor for that in BOLD-SI (Table 2). Blood pressure values were not available and are not factored into the cardiac workload calculations.

While long effective breath-holds may replace the use of pharmacologic vasodilators, hypocapnic vasoconstriction in relation with an increase in HR may, in fact, prove to be an endogenous coronary stress test in cardiovascular oxygen-sensitive imaging in the future.

Image quality
Although image quality remains a challenge with BOLD-CMR in a 1.5-T system, there was a good agreement found between the two readers. Slightly higher discrepancies were only seen in studies with reduced image quality.

Potential future clinical application
In a review article by Friedrich and Karamitsos, the authors highlight the advantages of oxygenation-sensitive CMR. Oxygenation-sensitive CMR can provide functional information of myocardial oxygenation in addition to existing CMR protocols and has already shown its potential as a non-invasive and radiation-free tool in patients with ischaemia. It does not require contrast agents, which is an advantage in patients with severely impaired renal function. However, these protocols use adenosine or other pharmacologic vasodilators, which can have severe side effects (e.g. chest pain, dyspnoea, arrhythmia). The use of endogenous vasodilators (e.g. breath-hold) for patients is a very exciting prospect as it is safe and presumably free of side effects. Future studies need to show if breathing manoeuvres can distinguish between myocardium subtended to healthy from that subtended to diseased vessels when using oxygenation-sensitive CMR.

Limitations
Our results are limited by the small sample size (eight healthy normals and six aquatic athletes). Image quality is a known issue with
oxygenation-sensitive CMR studies, especially at 1.5 T, but was found to be acceptable. A hyperventilation was not performed in the aquatic athletes; therefore, there is no comparison between groups for hypocapnic challenges. The expected changes in blood gases were not reflected by the capillary gas measurements as volunteers resumed breathing already while the samples were obtained. With an arterial line in place, these samples could have been acquired closer to the scan. However, this was not an option for a study with healthy volunteers. We used a cardiac phase array coil for our study. As for all studies using SI as the measured parameter, the inhomogeneous distribution of the sensitivity within the reception field may have had an impact on our results. We, however, think that this impact was negligible, since we compared the SI change over the course of an intervention (breath-hold) in the same region and thus, can expect a proportional change of SI.

Conclusion

Long effective breath-holds and hyperventilation lead to changes in myocardial oxygenation that are likely mediated by pCO2-dependent vasodilation or vasoconstriction. Therefore, BOLD-CMR is a useful technique for monitoring CO2-dependent coronary function during controlled breathing manoeuvres in healthy volunteers.

Acknowledgements

D.P.G. and M.G.F. had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Conflict of interest: M.G.F., D.P.G., K.F. and J.A.F. is a advisor and a shareholder of Circle Cardiovascular Imaging, Inc., Calgary, AB, Canada. Patent [WIPO (PCT) Patent Appl No. CA2013/050608; BLG Ref: PAT 7197W-90], which protects the use of breathing manoeuvres to induce changes in myocardial oxygenation for diagnostic purposes. Declaration of Helsinki: The study was performed in accordance with the ethical principles stated in the Declaration of Helsinki, 1964, as revised in Washington, 2002. The Conjoint Health Research Ethics Board of the University of Calgary approved the study.

Funding

This work was supported by The Husky Energy Inc., as part of the Husky Energy Program for the Early Detection of Cardiovascular Disease, yet did not have a role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

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