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Investigating the Magnetic Susceptibility Properties of Fresh Human Blood for Non Invasive Oxygen Saturation Quantification

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Abstract

Quantification of blood oxygen saturation on the basis of a measurement of its magnetic susceptibility demands knowledge of the difference in volume susceptibility between fully oxygenated and fully deoxygenated blood ($\Delta\chi_{do}$). However, two very different values of $\Delta\chi_{do}$ are currently in use. In this work we measured $\Delta\chi_{do}$ as well as the susceptibility of oxygenated blood relative to water, $\Delta\chi_{oxy}$, by MR susceptometry in samples of freshly drawn human blood oxygenated to various levels, from 6 to 98% as determined by blood gas analysis. Regression analysis yielded 0.273 ± 0.006 and -0.008 ± 0.003 ppm (cgs) respectively, for $\Delta\chi_{do}$ and $\Delta\chi_{oxy}$, in excellent agreement with previous work by Spees et al (MRM 2001;45:533–542).

Keywords

MR oximetry; MR susceptometry; Susceptibility imaging; Blood oxygen saturation

Introduction

Hemoglobin is derived from the words 'heme' meaning iron and 'globin' referring to its globular shape. The heme iron transitions from a low-spin state in oxyhemoglobin to a high-spin state in deoxyhemoglobin accounting for the former being diamagnetic and the latter paramagnetic. This oxidation state dependence of hemoglobin's magnetic properties is the basis of contrast for various MRI methods such as blood oxygenation level dependent (BOLD) MRI (1), susceptibility weighted imaging (SWI) (2), detection of acute hemorrhage (3), etc. While the above methods exploit qualitative differences in bulk magnetic susceptibility between oxy and deoxy-hemoglobin, their quantification enables determination of intravascular oxygen saturation.

In recent years several articles have appeared in which venous oxygen saturation was quantified by magnetic resonance techniques (2,4–8). These approaches can be broadly classified as T_2 (7–8) and T_2' (6) based intravascular and extravascular methods, and susceptibility-based phase methods (4,9). The former rely on an empirical relationship to derive oxygen saturation from relaxation rates while the latter are essentially calibration-free. Phase methods are based on an exact solution of Laplace's equation for the scalar

magnetic potential, yielding a simple equation of the fractional induced field inside, relative to the field outside the vessel. The induced field can be translated to oxygen saturation values by approximating the vessel as a long cylinder combined with the knowledge of the volume susceptibility difference between fully oxygenated and deoxygenated blood ($\Delta\chi_{do}$) and hematocrit (Hct). Haacke et al used the above approach to estimate oxygen saturation in cerebral pial veins (4). Since then, several groups have applied the method for various *in vivo* applications (5,10–12). However, much of the prior work has relied on two very different values of $\Delta\chi_{do}$, 0.18 ppm (13) and 0.27 ppm (14) (CGS units), yielding substantially different oxygen saturations. Although several prior studies have evaluated this constant in various ways, no consensus appears to exist on its actual value (13–16).

Phase based oxygen saturation measurements have recently been used to evaluate global and regional cerebral oxygen extraction fractions (*OEF*) and cerebral metabolic rate of oxygen consumption (*CMRO₂*) at rest as well as during metabolic and motor tasks (10,12,17–18). The soundness and reliability of the results of these experiments is incumbent on the accuracy of the derived venous oxygen saturation. Thus, knowledge of the correct value of $\Delta\chi_{do}$ is of paramount importance.

In this work we aimed to measure $\Delta\chi_{do}$ using a framework similar to that utilized by Weisskoff et al (13). The experiments were planned to simulate *in vivo* conditions and to overcome some of the limitations of previous studies. The methodology used in the present work has previously been shown to yield accurate and reliable susceptibility values(19). Additionally, we examined potential sources of error in oxygen saturation derived by MR susceptometry and compared these to values obtained by blood gas analysis, widely regarded as the clinical gold standard.

Methods

Blood Preparation

Fresh whole-blood was collected from the antecubital vein of seven healthy human volunteers via venipuncture under an Institutional Review Board (IRB) approved protocol. The samples were drawn in 7mL K₂EDTA (1.7mg per mL of blood) Vacutainer tubes (Becton, Dickinson and Company, NJ, USA). Following collection the blood was stored over ice and immediately transferred to the hematology lab for preparation. All blood specimens were used within 6 hours after collection.

The samples were pipetted into 2mL large-surface-area cylindrical tubes (diameter = 20mm, height = 6mm) to ensure maximal exposure to the diffusing gases and placed into an Eppendorf Thermomixer (New Brunswick, NJ, USA) via a custom-designed mount. This mount had an air-tight seal with inlet/outlet nozzles for adding gases. Subsequently, the blood was oxygenated to varying levels (range: 6–98 %) by either exposure to room air or a continuous stream of N₂ gas, while being maintained at 37°C. To prevent the RBCs from settling or foaming and to ensure maximal surface area for gas diffusion, the samples were continuously agitated. The samples had variable incubation periods to achieve their desired level of oxygenation (verified by blood gas analysis).

Blood samples from multiple large surface-area 2mL tubes were transferred into 6mL glass cylindrical tubes (diameter: 10 mm; height: 75mm) and sealed with a rubber stopper in preparation for MR scanning. Subsequently, the blood was injected into the MR sample tube via an inflow needle and another needle was placed for air outflow. The above procedure ensured that the tubes were filled completely with no remaining air spaces. The sample tubes were gently tumbled to prevent the blood from settling and maintained at a temperature of 37°C.

Four to five air-tight blood samples of different oxygenation were prepared per subject in the above manner (total of 30 samples with variable oxygenation levels for the entire study). The samples for each subject were placed in a cylindrical container filled with distilled water at 37°C and brought to the MRI suite for scanning. The setup in MR scanner included a heating pad to ensure a constant temperature of 37°C throughout the experiment. Care was taken to scan the samples quickly (less than 1.5min after they were placed in the scanner) to prevent red blood cell (RBC) settling. Prior work had shown that settling of RBCs can substantially affect the NMR line shape; with settling occurring within 2–3 minutes (14). After MRI, oxygen saturation level and hematocrit in each tube were re-measured in the hematology lab using the Radiometer Blood Gas Analyzer (Model: ABL 725, Radiometer Medical ApS, and Denmark). To examine the precision of the phase-based MRI method, samples from the last three subjects were scanned five times each. Between measurements, the sample tube assembly was removed from the scanner and gently tumbled to prevent RBC settling.

MR Protocol

All MRI measurements were performed on a 3T Siemens Tim Trio system (Siemens Medical Solutions, Erlangen, Germany) using a 12-channel head coil. The cylindrical container was placed in the scanner with its long axis parallel to the B_0 field. A 2D gradient-recalled Echo (GRE) sequence was used to obtain axial phase maps with the following imaging parameters, voxel size: (voxel size = $1 \times 1 \times 5 \text{ mm}^3$, FOV = $76 \text{ mm} \times 76 \text{ mm}$, flip angle = 25° , TE = 7.2ms, TR = 70ms, number of echoes = 2, echo spacing (ΔTE) = 2.5ms, total scan time ~11s). Phase difference ($\Delta\phi$) was computed by taking the difference of the phase images from the two echoes.

The susceptibility differences between various compartments (such as air, tissue etc.) cause field inhomogeneities and result in low spatial-frequency modulations of the phase signal. To minimize this interfering effect a retrospective correction method was implemented, which approximates the field inhomogeneity by a second-order polynomial. The compartments where oxygen saturation was to be determined were masked out and the phase difference image was weighted with the corresponding masked magnitude image. The robustness and accuracy of the method for quantifying susceptibility has been previously validated by some of the present authors (19). The experimental set-up, duplicated in the current work, consisted of an array of sample tubes filled with Gd-doped water of various concentrations and known volume susceptibilities.

The susceptibility difference ($\Delta\chi$) between blood and water was computed from the phase difference ($\Delta\phi$) between an ROI placed inside the cross sectional area of each tube and the surrounding water as

$$\Delta\chi = \frac{3\Delta\phi}{\gamma B_0 \Delta TE} \quad (1)$$

$\Delta\chi_{do}$ was obtained from the slope of $\Delta\chi/Hct$, where Hct are the individual hematocrit levels, and the concentration of deoxy-Hb ($1-HbO_2$) obtained from blood gas analysis (Eq 2):

$$\frac{\Delta\chi}{Hct} = \Delta\chi_{do} (1 - HbO_2) + \Delta\chi_{oxy} \quad (2)$$

where $\Delta\chi_{oxy}$ is the susceptibility difference between fully oxygenated blood and water. In this model it is assumed that the $\chi_{plasma} \sim \chi_{water}$ (14).

Statistical Analysis

Regression lines were calculated using standard linear regression. Bland-Altman method was used to evaluate agreement between blood gas and phase based oxygen saturation measurements. The Bland-Altman test is a statistical method to compare two different methods of measurement and test if one can be replaced by another. The mean difference between the two methods for measuring oxygen saturation ('bias') and 95% limits of agreement ($1.96 \times$ Standard deviation) are then calculated. In order to be equivalent, 95% limits must include 95% of differences between the two measurement methods (20). All analyses were performed using JMP (Version 7, SAS Institute Inc., Cary, NC).

Results

Representative phase difference maps for calculating blood susceptibility are shown in Figure 1. The slope of the regression line of $\Delta\chi/Hct$ versus deoxygenation yielded a value of 0.273 ± 0.006 ppm (CGS units) for $\Delta\chi_{do}$ and a y-intercept of -0.008 ± 0.003 ppm corresponding to $\Delta\chi_{oxy}$, the susceptibility of fully oxygenated blood relative to water (Figure 2). Hematocrit and deoxygenation ($1-HbO_2$) ranges for the measurements were 36 to 49% and 2 to 94%, respectively. Repeated measurements of blood susceptibility at a given oxygen saturation and hematocrit level yielded a coefficient of variation of $<5\%$. Figure 3 shows Bland-Altman plots for assessing the agreement between phase and blood gas analyzer derived oxygen saturation measurements. The mean bias and limits of agreement were -0.7% and $\pm 6.4\%$ HbO_2 , respectively. A 95% confidence interval for the mean yielded a lower and upper limit of -2.0 and 0.6% HbO_2 , respectively, suggesting that the two measurements are not significantly different from one another. Figure 4 illustrates the sensitivity of the derived oxygen saturation to hematocrit, $\Delta\chi_{do}$ and $\Delta\phi$. A fractional error of σ_{Hct} , $\sigma_{\Delta\chi_{do}}$ and $\sigma_{\Delta\phi}$ in Hct, $\Delta\chi_{do}$ and phase ($\Delta\phi$), respectively, introduces an absolute error ε_{HbO_2}

$$\varepsilon_{HbO_2} = (1 - HbO_2) \times \sigma \quad (3)$$

in the derived oxygen saturation estimates; where $\sigma = \sqrt{\sigma_{Hct}^2 + \sigma_{\Delta\chi_{do}}^2 + \sigma_{\Delta\phi}^2}$ (see Appendix for derivation of Eq. 3). Thus, errors in the above parameters affect lower oxygen saturation values more severely. Additionally, for a given phase value the sensitivity of HbO_2 measurements to uncertainties in Hct and $\Delta\chi_{do}$ is greatest at low values of Hct and $\Delta\chi_{do}$ as is evident from the increased slope of the surface plot in Figure 4a at low values of these parameters. Figure 4b illustrates that phase errors lead to greater absolute errors in HbO_2 at low Hct values.

Discussion and Conclusions

In this work we determined the bulk volume susceptibility difference between fully oxygenated and deoxygenated human blood by varying blood oxygen saturation ex-vivo in a controlled environment. The ability of the proposed phase difference method and processing technique to determine $\Delta\chi$ with high precision and accuracy has been verified previously (5,19). Further, we evaluated the agreement of MR susceptometry derived oxygen saturation measurements with the clinical gold standard, blood gas measurements.

The value of $\Delta\chi_{do}$ obtained in the present study (0.273 ± 0.006 ppm) is in excellent agreement with the work of Spees et al based on both NMR and SQUID magnetometry (14). They obtained $\Delta\chi_{do} = 0.27$ ppm using both methods. Additional support for this value comes from calculations based on Cerdonio's model using the magnetic moment of deoxyhemoglobin as initially determined by Pauling et al (14–15).

Our measurement of $\Delta\chi_{do}$ is also in good agreement with earlier magnetic cell capture experiments by Kondroskii et al who studied the cellular trajectories of single RBC's in response to a known magnetic field, from which they deduced a $\Delta\chi_{do}$ value of 0.29ppm (21). More recent and technologically improved experiments by Zborowski et al on RBC magnetophoretic motilities yielded a value of 0.26ppm for $\Delta\chi_{do}$ (22).

However, our measured value of $\Delta\chi_{do}$ differs significantly from Weisskoff et al's (13) who examined the phase difference between whole blood oxygenated to various levels and distilled water. Based on oxygen saturation determined by blood gas analysis, they obtained a $\Delta\chi_{do}$ value of 0.18ppm. This value is of the same order as an early rough estimate by Thulborn et al (16).

Although our experimental methodology somewhat resembles that used by Weisskoff, who performed field mapping with an asymmetric spin-echo sequence using an echoplanar readout, there are notable issues with that study that could have caused systematic errors. First, they used expired human blood. Hemorheological and hematological parameters are known to alter significantly with storage age. For example, erythrocytes swell, deform, lyse, become rigid and form micro-aggregates (23). Additionally, blood chemistry can change substantially, with pH and 2, 3-DPG levels dropping with longer durations ex-vivo (23–24). These changes significantly alter hemoglobin's oxygen binding affinity. Second, there was no mention of any precautions taken to prevent blood from settling, which is known to substantially affect the NMR line shape within 2–3 minutes (14). Thus, the conditions in Weisskoff and Kiihne's experiments do differ substantially from those of freshly drawn or *in vivo* blood.

In addition to the value for $\Delta\chi_{do}$, the intercept of the graph in Figure 2 provides an estimate for the value $\Delta\chi_{oxy}$. The observed value for $\Delta\chi_{oxy}$ corroborates that fully oxygenated blood is slightly more diamagnetic than water. This finding was first reported by Pauling et al (25). However, experiments by Cerdonio et al in the late 1970s put this into question. Their measured magnetic moment for oxyhemoglobin suggested a possible paramagnetic contribution (26–27). In line with our measurements, recent work by Savicki et al and Spees et al (14,28) has not provided evidence for such paramagnetism.

Most prior studies in which whole-blood oxygen saturation via the long-cylinder approximation and phase difference method was measured have ignored the contribution of $\Delta\chi_{oxy}$ in equation 2; assuming its effect to be negligible (4–5,11,29). Our results indicate that neglecting this contribution can introduce a slight bias (on the order of 2–3% HbO_2) towards overestimating the oxygen saturation values. The effects of ignoring the diamagnetic contribution from fully oxygenated blood can be significant in some hematologic disorders. A case in point is sickle-cell disease (*SCD*). Sakhnini et al (30), who examined the magnetic properties of hemoglobin in *SCD* patients, found that fully oxygenated *HbS* is more diamagnetic than normal hemoglobin.

Our results indicate that the mean bias $\pm$ limits of agreement between MR susceptometry derived and blood gas analyzer measured HbO_2 values were $-0.7 \pm 6.4\%$. The data thus suggest that HbO_2 can be quantified by MR susceptometry with clinically acceptable levels of agreement. The model used to derive whole-blood oxygen saturation from susceptibility measurements relies on the accuracy of *Hct*, $\Delta\chi_{do}$ and phase as errors in their values are propagated into the derived oxygen saturation values. As predicted by Equation 3 and illustrated in Figure 4, uncertainties in these parameters cause the greatest errors at low oxygen saturation values as the errors scale according to the level of deoxygenation ($1-HbO_2$). Thus, a population based estimate of *Hct* might suffice when using MR susceptometry to determine oxygen saturation in arterial vessels. However, an accurate

measurement of Hct is critical when applying this method in vessels where low oxygen saturations are expected. In general, uncertainties in HbO_2 estimates are dominated by errors in Hct and $\Delta\chi_{do}$ at low oxygen saturations and by low phase SNR at high oxygenation levels.

It should be noted that our measured value $\Delta\chi_{do}$ might not be applicable to all clinical scenarios. Our experimental $\Delta\chi_{do}$ was calculated with normal adult hemoglobin. Caution must be exercised when using this constant for oxygen saturation determination in individuals with variable hemoglobin types (e.g. HbF , HbS etc.). There is some disagreement in literature on the possible contribution of steric heme-heme interactions on the net dipole moment of hemoglobin (calculated per heme). Traditionally myoglobin has been used to study such interactions as it consists of a single heme group conjugated to a globin chain. Thus it is representative of true magnetic moment of heme attached to globin in the absence of any heme-heme interactions. While early experiments on the magnetic moment of myoglobin found values similar to those obtained for hemoglobin (per heme) (31), later experiments contradicted this finding (32). Such interactions, if present, could have implications on the magnetic moment of deoxyHb and hence $\Delta\chi_{do}$. It is conceivable that in the event of variations in globin chains (e.g. individuals with hemoglobinopathies), the aforementioned steric heme-heme interactions could be affected, amongst other variables, thereby altering $\Delta\chi_{do}$.

In conclusion, our results strongly support a $\Delta\chi_{do}$ value of 0.27ppm for normal adult human blood. Deviations from the present value in prior work are likely due to experimental inadequacies.

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Appendix

From Equations 1 and 2, HbO_2 can be calculated as,

$$HbO_2 = 1 - \frac{3\Delta\phi}{\gamma Hct \Delta\chi_{do} B_0 \Delta TE} \quad (A1)$$

Constants other than Hct , $\Delta\chi_{do}$ and $\Delta\phi$ are user specified or empirical and can be replaced by constant k for simplicity, yielding.

$$HbO_2 = 1 - \frac{k\Delta\phi}{Hct \Delta\chi_{do}} \quad (A2)$$

Using basic rules of error propagation, the absolute error (ϵ) in HbO_2 can be estimated as

$$\epsilon = \sqrt{\left(\frac{\partial HbO_2}{\partial \Delta\phi}\right)^2 (d\Delta\phi)^2 + \left(\frac{\partial HbO_2}{\partial Hct}\right)^2 (dHct)^2 + \left(\frac{\partial HbO_2}{\partial \Delta\chi_{do}}\right)^2 (d\Delta\chi_{do})^2} \quad (A3)$$

Computing partial derivatives and substituting $d\Delta\phi = \Delta\phi \cdot \sigma_{\Delta\phi}$, $dHct = Hct \cdot \sigma_{Hct}$ and $d\Delta\chi_{do} = \Delta\chi_{do} \cdot \sigma_{\Delta\chi_{do}}$ with σ representing the fractional error, yields

$$\varepsilon = \frac{k\Delta\phi}{Hct\Delta\chi_{do}} \sqrt{(\sigma_{\Delta\phi}^2 + \sigma_{Hct}^2 + \sigma_{\Delta\chi_{do}}^2)} \quad (A4)$$

Rearranging and substituting Eq. A2, gives

$$\varepsilon = (1 - HbO_2) \sqrt{(\sigma_{\Delta\phi}^2 + \sigma_{Hct}^2 + \sigma_{\Delta\chi_{do}}^2)} \quad (A5)$$

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Figure 1.

Representative phase difference images of cylindrical sample tubes filled with blood oxygenated to various HbO_2 levels, oriented parallel to the B_0 field and immersed in distilled water. Note the change in contrast at different oxygenation levels.

Figure 2.

Susceptibility difference ($\Delta\chi$) between blood and surrounding distilled water normalized to individual hematocrit (Hct) level in each subject plotted against the level of deoxygenation. The slope of the regression line represents the susceptibility difference between fully oxygenated and deoxygenated blood ($\Delta\chi_{do}$) and intercept the susceptibility difference between fully oxygenated blood and water ($\Delta\chi_{oxy}$).

Figure 3.
Bland-Altman plots showing the agreement between MR susceptometry and Blood Gas Analyzer derived oxygen saturation values based on $\Delta\chi_{do} = 0.27\text{ppm}$ and $\Delta\chi_{oxy} = 0.008\text{ppm}$. The red and green lines indicate the mean bias and the limits of agreement ($1.96 \times$ standard deviation), respectively.

Figure 4.

Surface plot of HbO_2 as a function of (a) susceptibility difference between fully deoxygenated and fully oxygenated blood, $\Delta\chi_{do}$, and hematocrit, Hct , for a phase value corresponding to 65% HbO_2 for $Hct = 0.42$ and $\Delta\chi_{do} = 0.27$ ppm (b) phase difference ($\Delta\phi$) and Hct for $\Delta\chi_{do} = 0.27$ ppm. The plots illustrate that errors in HbO_2 measurement due to uncertainties in $\Delta\chi_{do}$, Hct and $\Delta\phi$ scale with the level of deoxygenation.