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T₂-prepared balanced steady-state free precession (bSSFP) for quantifying whole-blood oxygen saturation at 1.5T

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Abstract

Purpose—To establish a calibration equation to convert human blood T₂ to the full range of oxygen saturation levels (*HbO*₂) and physiologic hematocrit (*Hct*) values using a T₂-prepared bSSFP sequence (T₂-SSFP) at 1.5T.

Methods—Blood drawn from ten healthy donors (29.1±3.9 years old) was prepared into samples of varying *HbO*₂ and *Hct* (n=79), and imaged using T₂-SSFP sequence at 37°C and inter-refocusing interval τ_{180} =12ms. The relationship between blood T₂, *HbO*₂ and *Hct* was established based on the model $R_2 = R_{2,plasma} + Hct(R_{2,RBC} - R_{2,plasma}) + k \cdot Hct \cdot (1 - Hct) \cdot (1 - HbO_2)^2$. Measured *R*₂ and *HbO*₂ levels were fit by the model yielding values of *R*_{2,plasma}, *R*_{2,RBC} and *k*. T₂-SSFP and the established calibration equation were applied to extract *HbO*₂ at the superior sagittal sinus (SSS) *in vivo* and compared with susceptibility-based oximetry.

Results—Constants derived from the fit were: $k = 74.2 [s^{-1}]$, $R_{2,plasma} = 1.5[s^{-1}]$, $R_{2,RBC} = 11.6[s^{-1}]$, the *R*² of the fit was 0.95. Average *HbO*₂ at the SSS in seven healthy volunteers was 65±7% and 66±7% via T₂- and susceptibility-based oximetry, respectively. Bland-Altman analysis indicated agreement between the two oximetric methods with no significant bias.

Conclusion—The calibration constants presented here should ensure improved accuracy for whole-blood oximetry based on T₂-SSFP at 1.5T.

Keywords

T2-based oximetry; whole blood; oxygen saturation

Introduction

Knowledge of the arteriovenous difference in oxygen saturation ($\text{SaO}_2 - \text{SvO}_2$) and blood flow rate enables calculation of whole-organ metabolic rate of oxygen (MRO_2) via Fick's Principle. While bulk flow into or out of the organ of interest is relatively straightforward to measure by phase-contrast MRI, and SaO_2 is obtained by pulse oximetry, quantification of SvO_2 is more challenging. Two methods have emerged during the past two decades for estimating blood hemoglobin oxygen (HbO_2) saturation, both relying on deoxyhemoglobin's inherent relative paramagnetism (1). Fully deoxygenated blood is less diamagnetic than fully oxygenated blood; its susceptibility is greater (difference in bulk or volume susceptibility between fully deoxygenated and fully oxygenated blood at 100% hematocrit, $\chi_{\text{do}} = 0.27$ ppm, in cgs units) (2, 3). Since there is a linear relationship between fractional deoxyhemoglobin (dHb) concentration and blood magnetic susceptibility, χ , the latter yields oxygen saturation. However, χ is not directly obtainable, but instead its effect on local field is measured (typically via phase mapping) and χ extracted via dipole inversion (4). In the case of blood vessels that conform to the geometry of a straight cylinder relatively parallel to B_0 , inversion is trivial (5–7). The method, also referred to as susceptometry-based oximetry (SBO) has since been used for whole-brain oximetry in humans (8, 9). However, the method is generally less practical for SvO_2 quantification in abdominal vessels due to vessel curvature and orientation, lack of reference tissue for computing blood-tissue phase difference, and presence of often severe field inhomogeneity. An additional problem in abdominal imaging is physiologic motion from breathing and peristalsis.

An alternative technique for measuring HbO_2 exploits the modulation of T_2 of blood water protons resulting from intra- to extracellular chemical exchange (10) and diffusion of the water molecules in the inhomogeneous field surrounding red blood cells (RBCs) (11). The contribution of each mechanism to the transverse relaxation rate ($R_2 = 1/T_2$) is determined by the water exchange lifetime, τ_{ex} , (~10 ms) (10), field strength and the measurement technique. At typical imaging field strengths blood R_2 varies quadratically with dHb fraction and field strength (10).

Wright et al first developed a method for T_2 -based HbO_2 mapping in humans *in vivo*, composed by a T_2 preparation involving a train of nonselective composite 180° pulses followed by center-out spiral readout (12–14). Derivatives of this method for SvO_2 mapping of abdominal and pelvic vessels have since been practiced for studying fetal oxygen supply during pregnancy (15). However, unlike susceptometry, T_2 -based oximetry requires a calibration curve to convert T_2 to oxygen saturation levels. Calibration equations are typically semi-empirical versions of the Luz-Meiboom equation for two-site exchange between chemically shifted protons (16) even though this model is not quite correct since spins, as pointed out above, experience a continuum of magnetic fields as the water molecules diffuse in the local field gradients in the vicinity of RBCs, along with undergoing chemical exchange.

Virtually all approaches involve a T_2 -preparation period, typically consisting of a train of composite nonselective phase-reversal RF pulses, followed by a readout period. Calibration curves at 1.5 and 3T have been described for spiral (12), EPI (17) and multi-echo spin echo

readouts (18). For applications in the body balanced steady-state free precession (bSSFP) has advantages in terms of SNR efficiency, allowing higher in-plane resolution without excessively prolonging acquisition time (19). However, a calibration curve describing the relationship between HbO_2 and T_2 of whole blood estimated using MLEV-based T_2 -prepared bSSFP does not currently exist. Unlike with EPI or spiral readout, where the measured magnetization is only determined by the T_2 preparation, this is not the case when the readout itself involves RF excitation pulses as in bSSFP. The purpose of this work, therefore, was to measure T_2 of human blood samples of varying Hct for a wide range of HbO_2 saturation levels using a T_2 -prepared bSSFP sequence and to derive a calibration curve.

Methods

Ex Vivo Blood Samples Preparation

Fresh whole blood (30–40mL) was collected via forearm venipuncture from ten healthy adult volunteers (29.1 ± 3.9 years old, three male), yielding a total of 79 samples. Blood was placed into 7mL Vacutainer glass blood collection tubes containing 12mg of K_3 EDTA (Becton, Dickinson and Company, NJ, USA). Specimens were then immediately transported to the hematology lab for sample preparation. The Institutional Review Board of the University of Pennsylvania approved this study, and all volunteers gave oral and written informed consent.

In order to extend the range of examined Hct values, blood from five volunteers was centrifuged to separate plasma from RBCs. Care was taken to avoid cross-contamination of blood components from different donors as incompatible blood types may cause sample agglutination. Plasma was then extracted and redistributed across samples to vary Hct and blood samples were mixed using a vortex mixer to incorporate RBCs and plasma. Thereafter, blood was transferred into a 6-well polystyrene culture plate (well dimensions 35.4mm in diameter and 17.4mm in height, Corning Inc., NY, USA), mounted on a temperature-controlled shaker platform (Thermomixer R, Eppendorf). The purpose of this setup was to continuously agitate blood samples to avoid RBC sedimentation and to maintain blood temperature at 37°C during sample preparation. Once in wells and on the incubation platform, blood was exposed to room air for 5 minutes to reach full oxygenation. An air-tight lid was then placed around the 6-well plate creating a chamber. The device had inlet and outlet nozzles used to feed N_2 gas into the chamber as a means to vary oxygen saturation. The top surface of the device had silicone regions (3mm in diameter) that allowed drawing blood with a syringe and needle, while restricting room air from flowing into the chamber. Every 10 minutes a blood sample was withdrawn and HbO_2 and Hb concentration measured with a clinical blood gas analyzer (Radiometer Copenhagen, ABL 700 series), which suctioned 0.25mL of blood directly from a 3mL (10mm in diameter and 68 mm in length) syringe. Once a desired oxygenation level was achieved the syringe was filled with prepared blood and sealed using a vinyl plastic putty (Critoseal, Fisher Scientific, PA, USA) and parafilm. This process was repeated to achieve the entire range of HbO_2 saturation levels (5–100%). Hemoglobin concentration was converted to Hct values using the standard factor of 3.

Prepared samples were initially kept in a dry bath at body temperature and subsequently transferred to a cylindrical container filled with distilled water at 37°C for MRI, and scanned within 10 minutes. This assembly was then placed into the bore of the scanner with the cylinder axis of container and test tubes parallel to the static field, and scanned with the protocol described below. All samples were scanned within 12hrs of blood draw.

MRI Experiments

Experiments were conducted at 1.5T (Siemens Avanto) with a 10-channel head coil. A single slice 5-echo T₂-prepared bSSFP sequence was used to extract the T₂ of prepared blood samples. Prior to each T₂-preparation the magnetization was reset via saturation and allowed to recover for a period of T_{Sat} seconds (Fig. 1A). Non-selective T₂-preparation was initiated with a 90° RECT excitation pulse, followed by *n* MLEV-4 type refocusing pulses (*n* = 0, 1, 2, 3, 4) with inter-refocusing pulse interval $\tau_{180}=12\text{ms}$, corresponding to T₂-preparation TEs of 0, 48, 96, 144 and 192ms, each followed by a composite tip-up (270_x360_{-x}) pulse (20) (Fig. 1B). After T₂-preparation, the magnetization was read out with bSSFP with partial-Fourier acquisition. Balanced SSFP signal encoding was preceded by 10 linear ramp-up pulses for signal stabilization (21) and 14 reference lines were collected for phase correction during partial-Fourier reconstruction using projection onto convex set (22) (Fig. 1C), i.e. the k-space center traversed on 25th pulse cycle. Imaging parameters for this sequence were: TR=4400ms, T_{Sat}=4000ms, bSSFP sequence parameters: bSSFP TE/TR=1.9/3.8ms, FOV=128×128mm², voxel size=1.25×1.25×5mm³, FA=60°, total scan duration 22 seconds. The data was fit to the equation $S_A(t) = S_0 e^{-t/T_2} + C$ to extract S₀, T₂ and C, the latter representing the (non-zero) steady-state bSSFP signal amplitude (23). Image reconstruction and analysis were performed with an in-house written Matlab (Mathworks, Natick, MA) script. The values of S_i at each TE were estimated as the average signal inside a region of interest (ROI) defined by thresholding (24) the first echo (TE=0) image. Additionally, T₂-preparation TEs were corrected for the period the magnetization is temporarily stored along the longitudinal axis throughout the execution of composite refocusing pulses (14). Values of T₁ and T₂ chosen for this correction were 1400 (25) and 140ms (12), respectively; yielding the corrected echo times 0, 43.3, 86.5, 129.8, and 173.1ms.

Relaxation Model and Analysis

The relationship between blood T₂ and *HbO₂* for the T₂-prepared bSSFP sequence was established based on the model initially described by Wright et al (12) derived from the Luz-Meiboom equation for two-site chemical exchange (16):

$$R_2 = R_{2o} + K(1 - HbO_2)^2 \quad [1]$$

where R₂ is the transverse relaxation rate of partially oxygenated blood and R_{2o} is the relaxation rate of fully oxygenated blood, which can be regarded as constant at given field strength and blood hematocrit. Here, *K* is the relaxivity that depends on i) field strength, ii) average exchange time of water in blood τ_{ex} , iii) refocusing pulse interval τ_{180} of the T₂-preparation, and iv) the specific readout technique. Often, variations in hematocrit that also

affect relaxivity are ignored thereby limiting application of the model to the *Hct* range of the samples used to estimate these constants. Hence, incorporating the known dependence of R_{2o} (26) and K (27) on *Hct* into Eq. [1] would be advantageous when *Hct* values are near the upper and lower limits of the physiological range. Van Zijl et al (28) expanded the equation accordingly:

$$R_2 = R_{2o}(Hct) + Hct \cdot (1 - Hct) \cdot (\Delta\omega)^2 \tau_{ex} \left[1 - \frac{2\tau_{ex}}{\tau_{180}} \tanh \frac{\tau_{180}}{2\tau_{ex}} \right] \quad [2]$$

Here, $\omega = \alpha\omega_o(1 - HbO_2)$, is the frequency shift between water protons inside the RBC and plasma, where ω_o is the resonance frequency, and α is dimensionless constant associated with the susceptibility and shape of RBCs. Furthermore, R_{2o} can be simply described by the relaxation equation for rapid mixing of a heterogeneous sample:

$$R_{2o} = (1 - Hct) R_{2,plasma} + Hct R_{2,RBC} = R_{2,plasma} + Hct(R_{2,RBC} - R_{2,plasma}) = R_{2,plasma} + Hct R_{2,RBC-plasma} \quad [3]$$

where $R_{2,plasma}$ and $R_{2,RBC}$ are the relaxation rates of plasma and fully oxygenated RBCs. Thus, it follows that R_{2o} is expected to scale linearly with *Hct* (thus $R_{2,RBC-plasma}$ is the slope of this linear relationship) as experimentally established earlier (11, 26). We note that the present model is somewhat simpler than the one described in ref. 33 in that $R_{2,RBC}$ is considered independent of HbO_2 with the modulation of T_2 solely determined by the exchange term. It is convenient to lump some of the parameters in Eq. [2] into a semi-empirical constant $k = (\alpha\omega_o)^2 \tau_{ex} \left[1 - \frac{2\tau_{ex}}{\tau_{180}} \tanh \frac{\tau_{180}}{2\tau_{ex}} \right]$. We can then rewrite Eq. [2] as:

$$R_2 = R_{2o}(Hct) + k \cdot Hct \cdot (1 - Hct) \cdot (1 - HbO_2)^2 = R_{2o}(Hct) + K(Hct, k)(1 - HbO_2)^2 \quad [4]$$

Here, $K(Hct, k)$ is the relaxivity as defined in Eq. [1]. We therefore plotted the transverse relaxation rates of blood R_2 versus $(1 - HbO_2)^2$ and *Hct*, and the surface described by Eqs. [3,4] was fitted to determine the constants k , $R_{2,plasma}$ and $R_{2,RBC}$, thereby yielding $R_{2o}(Hct)$ and $K(Hct, k)$.

In vivo Cross-Validation of T_2 -Prepared bSSFP Oximetry

Venous HbO_2 at the superior sagittal sinus (SSS) was estimated via susceptibility- and T_2 -based oximetry, and compared against each other. The SSS was selected because its geometry allows for the implementation of SBO as a means to study cerebral O_2 metabolism. Susceptometry-based oximetry is a well-established, robust method that exploits the intrinsic susceptibility of deoxyhemoglobin for quantification of whole-blood HbO_2 (see, for example, (28)). HbO_2 is estimated as:

$$HbO_2 = \left[1 - \frac{2|\Delta\phi|}{\gamma \cdot \chi_{do} \cdot \Delta TE \cdot B_0 \cdot \left(\cos^2\theta - \frac{1}{3} \right) \cdot Hct} \right] \quad [5]$$

Here, ϕ is the average inter-echo phase difference between intravascular blood and surrounding tissue, $\chi_{do} = 0.27 \text{ ppm}$ (3) is the susceptibility difference (in cgs units) between fully deoxygenated and fully oxygenated erythrocytes, TE is the inter-echo spacing of the gradient-echo acquisition, and θ is the angle of the SSS with respect to B_0 (7). Imaging parameters used for single slice SBO at the SSS were: $TR=40\text{ms}$, $TE=7.84\text{ms}$, $FOV=176 \times 176\text{mm}^2$, voxel size $=1 \times 1 \times 5\text{mm}^3$, $FA=15^\circ$. The ROI used to measure the phase of intravascular blood was determined by manually placing a rectangular box around the SSS in the magnitude image and using a threshold as defined in (24) to confine selection to the vessel. Further, all edge pixels were removed to avoid partial volume effects on SBO phase measurements, creating the final SSS mask. The reference tissue ROI was selected as described previously (29). Further, in order to examine the robustness of T_2 -bSSFP to blood flow velocity *in vivo*, the latter was measured in the SSS with phase-contrast MRI. Imaging parameters: $TR=21.1\text{ms}$, $TE=6.5\text{ms}$, $FOV=200 \times 200\text{mm}^2$, voxel size $=0.625 \times 0.625 \times 5\text{mm}^3$, $VENC = 30\text{cm/s}$, and $FA=15^\circ$. T_2 -bSSFP imaging parameters were: $TR=3900\text{ms}$, $T_{Sat}=3000\text{ms}$, T_2 -prep $TEs = 0, 48, 96, 144$ and 192ms , bSSFP $TE/TR=1.9/3.8\text{ms}$, $FOV=128 \times 128\text{mm}^2$, voxel size $=1.25 \times 1.25 \times 5\text{mm}^3$, $FA=60^\circ$, 5 averages and total scan duration 97.5s . Venous blood T_2 at the SSS and corresponding ROI were determined as described above and used to estimate HbO_2 values using Eq. [4]. Constants $T_{2o}(Hct)$ and $K(Hct)$ were those determined from the *ex vivo* calibration curve. Hematocrit values were determined via complete blood count performed in the outpatient lab where blood was drawn, except for one participant in whom Hct was measured via fingerprick (Hb 201+, Hemocue, Angelholm, Sweden). Intra-class correlation coefficient (ICC) and Bland-Altman tests were used to evaluate the agreement between both oximetric methods.

Results

Blood T_2 Dependence of HbO_2

Figure 2A depicts images of the five echoes acquired using T_2 -bSSFP for blood oxygenated to five HbO_2 levels. The fitted T_2 decay curves for the corresponding samples are shown in Fig. 2B. Decay rates increase with decreasing HbO_2 as expected. The experimental transverse relaxation rates R_2 from all 79 samples plotted versus $(1-HbO_2)^2$ obtained by blood gas analysis, and Hct are given in Fig. 3A. The surface obtained by fitting the data to Eqs. [3,4] was then used to estimate constants k , $R_{2,plasma}$ and $R_{2,RBC-plasma}$. The value of k obtained was 74.2s^{-1} , yielding the following relationship between R_{2o} and Hct : $R_{2o} = 10.1Hct + 1.5[\text{s}^{-1}]$ for Hct values in the range of 0.23 to 0.53 ($R^2=0.95$), corresponding to $R_{2,plasma} = 1.5$ and $R_{2,RBC-plasma} = 10.1\text{s}^{-1}$ which represent intercept and slope (i.e. the relaxivity) describing the Hct dependence of R_{2o} . The coefficient of determination of the fit was $R^2=0.95$ and the root mean squared error between data points and predicted surface was 1.1s^{-1} . Profile lines through the surface depicted in Fig. 3A are shown in Fig. 3B for a range of Hct values indicating increasing slope (K) with increasing Hct . The dependence of whole-

blood R_2 at given value of $(1-HbO_2)^2$ versus Hct was found to be non-linear. Fig. 3C shows profile lines through the fitted surface in Fig. 3A for a given HbO_2 values and varying Hct . As predicted by the model, the relationship between R_2 and Hct is approximately linear for highly oxygenated blood ($HbO_2 \geq 70\%$) and quadratic as the concentration of deoxyhemoglobin increases. Note that the profile line for $HbO_2 = 100\%$ illustrates the linear relationship between R_{20} and Hct reported previously at 4.3T (11) and subsequently also at 1.4T (26).

In vivo Cross-Validation

The purpose of these experiments was to compare T_2 - and susceptometry-derived HbO_2 in the SSS – a location accessible to both types of oximetry – to determine possible bias. Images of the five echoes acquired using T_2 -bSSFP and corresponding fitted T_2 decays are shown in Figs. 4A and B for a representative study subject. Oxygen saturation at the SSS was quantified based on the value of k determined as described above while T_{20} was estimated for each participant based on their measured Hct values using Eq. [3], and estimated values of $R_{2,plasma}$ and $R_{2,RBC-plasma}$. The magnitude and phase difference images used to measure HbO_2 via SBO for each of the seven study participants are displayed in Fig. 4C. Average HbO_2 at the SSS was $65 \pm 7\%$ (average $T_2 = 127\text{ms}$) and $66 \pm 7\%$ via T_2 - and SBO, respectively. Good agreement between both methods was found, as illustrated with the data plotted against each other, showing them distributed equally around, and deviating minimally, from the line of identity. The Bland-Altman plot (Fig. 5B) yielded a mean difference ($HbO_{2,T2} - HbO_{2,Susc}$) of $0.5 \pm 1.4\%$ indicating nonsignificant bias as the nominal difference is well within the 95% confidence interval boundaries. Further, the intra-class correlation coefficient (0.98, $p < 0.0001$) corroborates the strong agreement between the two oximetric methods.

Discussion

In this work, a calibration equation was established to convert human blood T_2 to HbO_2 based on the full range of oxygen saturation levels and physiologic hematocrit values. Whole-blood T_2 was estimated from blood samples imaged *ex vivo* using a T_2 -prepared sequence and bSSFP encoding at 1.5T and 37°C . Further, this T_2 -prepared sequence and the established calibration equation were applied to extract HbO_2 at the superior sagittal sinus *in vivo* and compared with SBO. Excellent agreement was found between the two oximetric methods at this anatomic location (Fig. 5). Even though in the central nervous system the field strength of choice is 3T we elected to carry out the present study at 1.5T given the target application of measuring O_2 saturation and organ consumption in the abdomen of pregnant women. In distinction to the original *in vivo* work conducted at 1.5T (12) the specific readout strategy used with the present method involving RF pulses for readout is expected to cause an additional modulation of the decay rate. It is therefore plausible that the derived relaxation constants could differ from those derived via EPI or spiral signal readout. With the latter readout schemes, signal is determined solely by the T_2 -preparation. However, typically, in order to achieve adequate resolution multiple interleaves are necessary with spiral k-space coverage, thus significantly prolonging scan time. Single-shot EPI is generally used in TRUST where resolution is less critical since image subtraction eliminates partial

volume blurring from static spins (albeit at the expense of a factor of two in scan time) (17, 30). However, the TRUST approach is not suited in the abdomen where vessel orientation is multi-directional. Single-shot acquisition bSSFP overcomes some of the above approaches' limitations, achieving high in-plane resolution with a single T_2 preparation per TE. During readout the signal evolves with T_2/T_1 (31) resulting in high contrast signal between surrounding tissue and blood vessels, which is further enhanced by inflowing blood to the imaging slice. For these reasons and with abdominal imaging applications in mind, bSSFP was the chosen as readout scheme for the present work in view of the target applications in the abdomen and pelvis.

Since R_2 scales quadratically with deoxyhemoglobin concentration the detection sensitivity of T_2 -based oximetry (slope of T_2 versus HbO_2 curve) varies across the full range of O_2 saturation values evident in the work of ref. (12). Sensitivity increases with τ_{180} (Eq. [2]) albeit at the expense of greater variation over the physiologic range from 40–80% HbO_2 . Uniform sensitivity throughout the entire range of physiologic HbO_2 values is clearly desirable as is high detection sensitivity. Of note is that increasing inter-echo interval τ_{180} prolongs the echo-train, thereby adversely affecting SNR. Given these somewhat conflicting requirements, a choice of $\tau_{180}=12\text{ms}$ is a reasonable compromise. At this inter-echo interval the calibration curve is reasonably linear in the physiologic range of venous HbO_2 (40–80%), while yielding a relatively large T_2 (~150ms) range (12).

In the present work, the model implicit to Eqs. [3,4] was found to fit the data very well ($R^2=0.95$). Over the physiologic range from Hct 0.3 to 0.5 (Fig. 3B) the relaxivity K increases nonlinearly from 15.6 to 18.5s^{-1} . When plotted versus Hct , R_2 varies nearly linearly at high HbO_2 but becomes gradually convex with increasing desaturation (Fig. 3C). The estimated value of $R_{2,plasma}=1.5\text{s}^{-1}$ is in excellent agreement with earlier measurements in human blood plasma at 1.5T ($R_{2,plasma}=1.5$ at 39°C) (32). Further, $R_{2,plasma}$ agrees well with values obtained in similar *ex vivo* experiments applied to blood relaxation models (33, 34). Because the present model and the one originally described by Van Zijl et al (28) differ in the dependence of $R_{2,RBC}$ on HbO_2 , the estimated value $R_{2,RBC-plasma}$ of 10.1s^{-1} cannot directly be compared to those previously reported (33). However, as described above, the variable $R_{2,RBC-plasma}$ represents the slope of the linear relationship between R_{2o} and Hct . Therefore, it is possible to compare the results presented here to others by calculating the magnitude of this slope for the full range of HbO_2 levels using reported values of $R_{2,dia} + R_{2,oxy}=5.5\text{s}^{-1}$ and $R_{2,deoxy} - R_{2,oxy}=8.1\text{s}^{-1}$ (33). The average slope was $9.5\pm 2.7\text{s}^{-1}$ (range 13.6–5.5 s^{-1}), which is in good agreement with the estimated value of $R_{2,RBC-plasma}$ 10.1s^{-1} obtained using our simplified model.

Finally, one has to bear in mind that T_2 of exchanging systems is not an absolute quantity. Even though we compare measurements at a given inter-refocusing time ($\tau = 12\text{ms}$), because of the different readout strategy used here (bSSFP with delayed scanning of $k_y(0)$, and differences in the model used), the functional dependence of T_2 on HbO_2 is expected to deviate somewhat from work by others (12, 33, 34). For comparison, calibration curves plotted as T_2 versus HbO_2 for a range of hematocrit values derived in the present work, along with the corresponding curve in (12) at $Hct=0.45$, and matching τ_{180} of 12ms, is shown in Fig. 6. Of note are the lower relaxivity (and consequently dynamic range) and T_{2o}

in the present work compared to those in (12) ($K=18.4$ versus 24.6s^{-1} , and $T_{20}=164$ versus 254ms). We attribute these disparities to the evolution of the magnetization following T_2 -preparation and the temporal location of k-space center during readout.

A number of precautions are necessary to avoid experimental errors when handling blood samples. Among these is maintenance of body temperature while varying O_2 saturation as any deviation causes a shift in the dissociation curve. Another potential source of error noted during sample preparation (2–3 hours) is plasma water evaporation, thereby affecting hematocrit. Maintaining low oxygen saturation was found to be difficult given hemoglobin's high oxygen affinity. It is therefore imperative to minimize the exposure to room air during sealing of the samples. This was achieved by removing all room air from the syringe before filling it with prepared blood and after measuring HbO_2 with the blood gas analyzer. In order to test the effectiveness of the sealing method several samples were rescanned 24 hrs after the initial experiments and oxygen saturation remeasured. No significant differences were found in either O_2 saturation or T_2 (data not shown). Nevertheless, as a precaution, all samples were measured within 12 hrs of blood draw as storage can affect oxygen affinity (35). Another potential source of error are temperature variations even though deviations from body temperature may not greatly affect T_2 . Thulborn et al found T_2 to vary by only 3% between 20 and 37°C (11).

Finally, settling of RBCs is a phenomenon that occurs in the absence of flow as in *ex vivo* experiments resulting in a broadening of the NMR spectrum as early as one minute after agitation of the sample stops (2). In the present study, all T_2 -prepared data were acquired within one minute of agitating the sample construct to avoid settling (10-second localizer followed by T_2 -bSSFP taking 22 seconds).

The comparison of *in vivo* measurements of venous HbO_2 in the SSS based on both T_2 -bSSFP sequence in conjunction with the new calibration equation with values obtained by SBO lend further credence to the accuracy of the method. The SSS was selected because it is relatively straight and runs roughly parallel to B_0 with participants in supine position in the MRI scanner. Further, the SBO model has previously been extensively evaluated, both experimentally (8) and numerically (36). However, cerebral oxygen metabolism is tightly regulated, thereby limiting the range of baseline HbO_2 values. Nonetheless, results suggest that T_2 -prepared bSSFP is a reliable and accurate technique to extract HbO_2 *in vivo*.

In conclusion, the calibration equation presented here should ensure improved accuracy for whole-blood oximetry based on T_2 -prepared SSFP. The method as described is attractive given its speed and SNR efficiency and should be applicable widely throughout the body.

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Figure 1.

T₂-prepared bSSFP sequence consisting of: **A)** saturation pulses to reset the magnetization to zero after each T₂-preparation, **B)** T₂-preparation with composite refocusing pulses in MLEV-4 pattern, repeated to achieve TE values of 0, 48, 96, 144 and 192ms at constant interpulse interval (τ₁₈₀); MLEV-4 pulses labeled blue are 90_{+x}180_{+y}90_{+x}, while those labeled red have opposite phase, i.e. 90_{-x}180_{-y}90_{-x}. **C)** bSSFP encoding with linear ramp-up signal stabilization and partial Fourier sampling.

Figure 2.

Measurement of blood T_2 for various oxygen saturation levels with a T_2 -prepared bSSFP sequence: **A)** Magnitude images of blood samples at hematocrit of 0.41, immersed in distilled water at 37°C, at five O_2 saturation levels; **B)** T_2 decay for the five experiments of panel A. T_2 was extracted using a three-parameter fit to the equation $S_A(t) = S_0 e^{-t/T_2} + C$, with C representing the steady-state amplitude of the bSSFP signal.

Figure 3.

A) Blood R_2 as a function of the square of deoxyhemoglobin fraction, $(1 - HbO_2)^2$, and Hct from 79 samples, measured with a T_2 -prepared bSSFP sequence along with fitted surface $R_2 = R_{2,plasma} + Hct R_{2,RBC-plasma} + k \cdot Hct \cdot (1 - Hct) \cdot (1 - HbO_2)^2$ in grey; $R^2=0.95$. Red and blue markers represent data points above and below the fitted surface, respectively. **B)** Profiles of R_2 versus $(1 - HbO_2)^2$ derived from data of panel A. Dotted lines correspond to profiles across the fitted surface in panel A for Hct values in the range of 0.25–0.55. Thick lines correspond to Hct values of 0.3, 0.4 and 0.5. Corresponding K and R_{20} values are 15.6, 17.8, 18.5 s⁻¹ and 219, 179, and 152 ms, respectively. **C)** Profiles of R_2 versus Hct , for different values of HbO_2 .

Figure 4.

A) Representative images of the superior sagittal sinus (SSS, white circle) acquired with T_2 -bSSFP. **B)** T_2 decay of the blood signal, along with 3-parameter fit to $S_A(t) = S_0 e^{-t/T_2} + C$, including TE correction. The parameter C represents the steady-state amplitude of the bSSFP signal. **C)** Magnitude image (left) and phase difference image (right) from susceptibility-based oximetry used to compute HbO_2 in the SSS (white circle). Black contours encompass the regions of interest used to measure the phase in intravascular blood and reference tissue.

Figure 5.

A) Plot of susceptometry versus T₂-based *HbO₂* in seven subjects (mean age $\pm$ SD 39.8 $\pm$ 3.1 years, four male, average *Hct*=0.42), with black dashed line indicating line of identity, solid red dotted line being fit to the data. **B)** Bland-Altman plot showing relative agreement between the two methods. Solid and dotted black lines indicate mean bias (0.5% *HbO₂*) and 95% confidence interval boundaries ($\pm$ 2.7%). The data suggest statistically insignificant systematic bias between the two methods.

Figure 6.

Plot of blood T_2 versus HbO_2 . Colored lines represent different hematocrit values (range 0.25–0.55) representing measurements obtained with the present T_2 -bSSFP sequence. Black line represents the calibration curve generated by Wright et al based on a similar T_2 -prepared sequence with spiral readout and samples of average hematocrit of 0.45 (12). Interpulse interval, τ_{180} , was 12ms in both sequences.