Relaxation Properties of Human Umbilical Cord Blood at 1.5 Tesla

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Abstract

Purpose—To characterize the MRI relaxation properties of human umbilical cord blood at 1.5 Tesla.

Methods—Relaxometry measurements were performed on cord blood specimens ($N=88$, derived from 6 caesarean deliveries) spanning a broad range of hematocrits ($Hct=0.19–0.76$) and oxygen saturations ($sO_2=4–100\%$), to characterize the dependence of T_1 and T_2 on these blood properties. Adult blood data ($N=31$ specimens, derived from two volunteers) were similarly studied to validate our experimental methods by comparison with existing literature. Using biophysical models previously developed for adult blood, new model parameters were estimated, which relate Hct and sO_2 to the observed cord blood relaxation times.

Results—Fitted biophysical models explained more than 90% of the variation in T_1 and T_2 . In general, T_2 relaxation times of cord blood were longer (by up to 35%) than those of adult blood, while T_1 relaxation times were slightly shorter (by up to 10%).

Conclusion—The models and fitted parameters presented here can be used for calibration of future MRI investigations of fetal and neonatal blood physiology. This study is an important step in facilitating accurate, non-invasive assessments of fetal blood oxygen content, a valuable diagnostic parameter in the identification and treatment of fetal hypoxia.

Keywords

umbilical cord blood; longitudinal relaxation time; transverse relaxation time; oxygenation; hematocrit; fetus

Introduction

Accurate, non-invasive measurement of fetal blood oxygen content would enable better diagnosis and management of fetal hypoxia, a primary cause of perinatal mortality and morbidity. Blood oxygen content, cO_2 , which is proportional to oxygen saturation and hematocrit, can be combined with blood flow measurements to calculate O_2 transport parameters such as oxygen delivery and consumption, facilitating evaluation of placental function and fetal metabolism. Ultrasound, the clinical standard for the assessment of fetal well-being, detects hypoxia through the observation of collateral blood flow redistribution, which can be transient and difficult to interpret [1]. In contrast, due to a magnetic susceptibility difference between oxy- and deoxyhemoglobin, T_1 and T_2 relaxation times of blood are directly sensitive to oxygen saturation and hematocrit. With the help of appropriate calibration data, vascular relaxometry measurements can therefore be used for non-invasive cO_2 measurement.

The feasibility of relaxometry measurements in human fetal vessels using magnetization prepared, motion corrected, fast imaging techniques has recently been demonstrated [2, 3]. As such, the potential for accurate, non-invasive estimation of fetal blood cO_2 is now within reach. However, existing calibration data, which can be used to estimate oxygen saturation and hematocrit from blood relaxation times, has been derived using adult blood specimens. These relationships may not be applicable to fetal blood, which differs from its adult counterpart in several respects. In particular, fetal blood is rich in the fetal form of hemoglobin (HbF), which has a greater oxygen affinity to facilitate the transfer of oxygen from maternal to fetal blood within the placenta [4]. In addition, fetal erythrocytes are 20% larger and 50% less permeable to water, and fetal plasma is less viscous (by 20% at late gestation), due to a lower protein concentration [4–6].

Anticipating that these differences would affect MRI relaxation properties, the objective of this study was to characterize the relationships between fetal blood relaxation times and the oxygen saturation and hematocrit. Relaxometry measurements were performed at 1.5T on umbilical cord blood samples harvested from human caesarean deliveries. To our knowledge, this is the first characterization of the relaxation properties of fetal blood at this field strength. The relationships derived from this investigation are intended to serve as a calibration and reference for future MRI investigations of fetal and neonatal blood physiology.

Theory

Variations in T_1 and T_2 relaxation times of blood as a function of blood oxygen saturation, sO_2 , and hematocrit, Hct , can be described using simple biophysical models, which represent blood as a two-compartment system (plasma and erythrocytes).

Blood T_1 Model

The rate of water exchange between plasma and erythrocytes is much higher than the difference between their individual longitudinal relaxation rates. The condition of fast exchange is therefore fulfilled and the blood longitudinal relaxation rate, $R_1 = T_1^{-1}$, is given by the compartment-weighted sum [7]:

$$R_1 = Hct \cdot R_{1,ery} + (1 - Hct) \cdot R_{1,plas}, \quad (\text{Equation 1})$$

where $R_{1,ery}$ and $R_{1,plas}$ are the relaxation rates of erythrocytes and plasma, respectively. Since deoxyhemoglobin (dHb) behaves as a weak paramagnetic contrast agent [8], $R_{1,ery}$ exhibits a dependence on oxygen saturation, given by:

$$R_{1,ery} = R_{1,ery,0} + r'_{1,dHb} \cdot \left(1 - \frac{sO_2}{100\%}\right). \quad (\text{Equation 2})$$

$R_{1,ery,0}$ is the relaxation rate of erythrocytes containing only oxygenated hemoglobin (i.e. $sO_2 = 100\%$) and $r'_{1,dHb}$ is related to the relaxivity of dHb.

Blood T_2 Model

The transverse relaxation rate, $R_2 = T_2^{-1}$, of blood is described by the Luz-Meiboom (L-M) model, which similarly includes a weighted sum of erythrocyte ($R_{2,ery}$) and plasma ($R_{2,plas}$) relaxation rates. To account for the T_2 -shortening effect of spin exchange between the two frequency-shifted compartments, an exchange term, $R_{2,ex}$, is also included:

$$R_2 = Hct \cdot R_{2,ery} + (1 - Hct) \cdot R_{2,plas} + R_{2,ex}. \quad (\text{Equation 3})$$

The erythrocyte relaxation rate, $R_{2,ery}$, is a sum of plasma ($R_{2,plas}$), diamagnetic ($R_{2,dia}$), and oxygenated- ($R_{2,oxy}$) and deoxygenated ($R_{2,deoxy}$) hemoglobin contributions:

$$\begin{aligned} R_{2,ery} &= R_{2,plas} + R_{2,dia} + sO_2 \cdot R_{2,oxy} + (1 - sO_2) \cdot R_{2,deoxy} \\ &= R_{2,plas} + R_{2,dia} + R_{2,oxy} + (1 - sO_2) \cdot (R_{2,deoxy} - R_{2,oxy}). \end{aligned} \quad (\text{Equation 4})$$

The exchange component of the observed relaxation rate, $R_{2,ex}$, is given by:

$$R_{2,ex} = Hct \cdot (1 - Hct) \cdot (\Delta\omega)^2 \cdot \tau \left[1 - \frac{2\tau}{\tau_{180}} \cdot \tanh\left(\frac{\tau_{180}}{2\tau}\right) \right], \quad (\text{Equation 5})$$

where τ_{180} is the refocusing interval and τ is the exchange correlation time (i.e. $\tau^{-1} = \tau_{ery}^{-1} + \tau_{plas}^{-1}$; τ_{ery} and τ_{plas} are the mean residence times for spins within erythrocytes and plasma). ω , the frequency difference between the two sites is given by:

$$\begin{aligned}\Delta\omega &= \gamma B_0 [\omega_{dia} + sO_2 \cdot \omega_{oxy} + (1-sO_2) \cdot \omega_{deo}] \text{ (rads/s)} \\ &= \gamma B_0 [(\omega_{dia} + \omega_{oxy}) + (1-sO_2) \cdot (\omega_{deo} - \omega_{oxy})] \text{ (rads/s)}, \quad \text{(Equation 6)}\end{aligned}$$

where γ is the gyromagnetic ratio for hydrogen, B_0 is the field strength (1.5 T for this study), and ω_{oxy} , ω_{deo} , and ω_{dia} denote the frequency shifts for oxygenated and deoxygenated Hb and residual diamagnetic sites, respectively [9].

Methods

Sample Collection

Umbilical cord blood was harvested from six placentas following caesarean delivery. Inclusion criteria for the study were women with no maternal medical conditions or fetal abnormalities who had reached at least 37 weeks' gestation at time of delivery. The study was approved by the local research ethics board (REB). Informed written consent was obtained from each patient prior to delivery and the collection procedure and subsequent handling of data followed institutional ethical guidelines.

Each placenta was obtained immediately after delivery with the distal end of the umbilical cord clamped to prevent blood loss. The placenta was positioned with the fetal surface and umbilical cord facing up. An 18 gauge needle attached to a 10 cc syringe was inserted into the umbilical vein (proximal to the clamp site) and 7 mL of blood was drawn and transferred into vacutainer tubes for standard blood laboratory testing, which included hemoglobin chromatography (to determine *FHbF*, the fraction of fetal hemoglobin), and a complete blood count (CBC).

A second clamp was placed proximal to the previous needle insertion site and a second 18 gauge needle attached to a 150 mL blood collection bag was inserted into the umbilical vein (again proximal to the most recent clamp). To prevent coagulation, 1 mL of sodium heparin was added to the blood bag prior to collection. To promote flow, the collection bag was positioned below the placenta and 30–50 mL of blood was collected (depending on how much blood was available).

In addition to the fetal blood collections, adult blood was also collected from two volunteers (30 mL samples from each). The additional adult data was collected for the purpose of comparing the MRI properties of fetal and adult blood under equivalent conditions. Although the properties of adult blood at 1.5T are well described in the literature, the reported experimental conditions vary (e.g. bovine *vs.* human blood samples, temperature, sample age, etc.) [10].

Adult blood samples were drawn by routine antecubital venipuncture and collected into 3–4 pre-heparinized vacutainer tubes (Becton-Dickinson, Rutherford, NJ) with 10 mL capacity. Adult blood specimens did not undergo laboratory analysis, but were otherwise processed and scanned in the same manner as the fetal samples (see below).

Specimen Preparation & Handling

Prior to any processing, room air was introduced into the cord blood collection bag, followed by warming and agitation to allow the blood to fully oxygenate. For adult blood specimens, oxygenation was achieved by opening (and then resealing) the vacutainer tubes (and similarly warming and agitating). The initial oxygenation step ensured that all samples started with an equivalent oxygen saturation ($sO_2=100\%$) which was independent of the conditions during collection and transport.

The collected blood was then divided into 15 specimens of 1–2 mL volume, which were processed to provide a range of hematocrits and oxygen saturations (described below) and stored in additive free vacutainer tubes of 4 mL capacity (Becton-Dickinson, catalog number 366704). For a subset of specimens from each collection (at least 5), the hematocrit was unaltered. However, to investigate a broader range of *Hct* values, specimens with different hematocrits were also prepared. This was achieved by centrifugation of blood at 2800 RPM for 15 minutes (to separate plasma and erythrocytes) followed by the removal/addition of appropriate volumes of separated plasma. Specifically, *Hct* values evenly spanning a range of 0.2–0.8 were targeted. Given blood samples with a typical *Hct* of 0.45, this meant removing up to 80% of the separated plasma to increase *Hct*. Conversely, decreasing *Hct* meant adding up to 220% more separated plasma.

Specimens with different oxygen saturations were prepared by exposure to nitrogen gas. Specifically, nitrogen gas was delivered through a 20-gauge needle inserted through the vacutainer tube rubber stopper. A second 20-gauge needle was inserted into the stopper for venting. Blood was deoxygenated through multiple cycles which included gas flow followed by a five-minute period of warming and gentle agitation (tubes were placed on a warming pad on top of a rocking platform). Specimens with a range of oxygen saturations were created by varying the number of cycles (from 1–7), the duration of the gas flow period (from 30–50 seconds) and the gas flow rate (from 6–50 mL/min). Immediately after deoxygenation, needles were removed and the tube stopper was covered with a layer of paraffin film (Parafilm, Sigma-Aldrich, Missouri, USA) to prevent further gas contamination. To ensure stability, specimens were kept in the parafilm sealed vacutainer tubes for the remainder of the experiment (i.e. transport and scanning) until extraction for blood gas analysis.

All samples were processed and MRI scanned within 36 hours of collection. While not being processed/scanned, blood was stored in a 4°C fridge. Immediately following MRI, all specimens underwent blood gas analysis to determine the hemoglobin concentration, oxygen saturation, sO_2 , and methemoglobin fraction, f_{met} . Specimens with non-physiological values for the methemoglobin fraction ($f_{met}>2\%$) [11] were excluded from further analysis (this arose in 2 out of 90 specimens). Analyzer measurements of hemoglobin concentration were converted to hematocrit values by dividing by the mean corpuscular hemoglobin concentration (MCHC). For fetal blood specimens, MCHC values were obtained from the results of the complete blood count (CBC) analysis. For adult specimens, the typical value of 33 g/dL was assumed [12].

MRI Acquisition

MRI data were acquired on a 1.5T Avanto Syngo system (Siemens Healthcare, Erlangen, Germany). All 15 of the prepared specimens from each collection were imaged simultaneously. Prior to scanning, specimens were warmed to 37°C in a heated water bath for one hour with regular manual agitation (every 3 minutes). A heated bath was also used to maintain the temperature at $37\pm0.5^\circ\text{C}$ during scanning. The tubes were held in the bath by a purpose built holder and the entire unit (bath plus specimens) was placed in a 4 channel head coil for imaging. Within the bore, specimen tubes were oriented perpendicular to the main magnetic field, B_0 . To prevent erythrocyte sedimentation, specimens were manually agitated prior to each scan and scan durations did not exceed 3 minutes. Specifically, the loaded specimen holder was removed from the bath and gently inverted three times. No effect from this manipulation was apparent in the subsequent scans – there were no visible air bubbles and no ghosting due to residual fluid motion (i.e. sloshing) was observed.

All acquisitions were of a single 5 mm thick slice which cut through the center of all the specimen tubes. T_1 was measured using a Modified Look Locker Inversion Recovery (MOLLI) pulse sequence. The MOLLI sequence is a fast T_1 mapping technique developed for cardiac applications, which uses a balanced steady state free precession acquisition to obtain images at multiple inversion times, TI , following an adiabatic inversion pulse [13]. The MOLLI acquisition consisted of 3 inversions with 5 TI images acquired per inversion. This provided 15 inversion times ranging from 50–5300 ms. There was a 5 second free relaxation period between subsequent inversions.

T_2 was measured using a multi-echo spin echo sequence with 32 echoes, $TR=1000$ ms, and a scan duration of 2.2 minutes. To mitigate the effects of imperfect refocusing due to the slice profile of the 180° pulses, the refocusing slice thickness was set to 10 times the excitation thickness (this solution was possible because of the single slice acquisitions). T_2 was measured at 6 different echo spacings, $\tau_{180} = 6.7, 12, 18, 24, 36, 48$ ms. Both T_1 and T_2 acquisitions were performed with a 120×120 mm² field-of-view and a 128×128 matrix size.

Sequence Validation—For validation purposes, in a subset of blood specimens, MOLLI-based T_1 estimates were compared to standard inversion recovery (IR) T_1 measurements. IR data was acquired with a turbo spin echo pulse sequence with an echo train length of 20, $TR/TE=9000/8.3$ ms and a scan duration of 1.1 minutes. Data was acquired at 12 inversion times, TI , ranging from 24–6000 msec. Specimens were agitated prior to every second TI acquisition.

To rule out T_2 misestimation due to imperfect refocusing pulses and incomplete spoiling of stimulated echo pathways [14], T_2 measurements obtained from the multi-echo spin echo sequence were compared to those obtained from a single-echo acquisition. Measurements were performed on 5 doped water samples with Gadolinium concentrations of 1, 2, 4, 6, and 8 mM. Multi-echo data was collected with the parameters stated above and single-echo data was acquired with $TR=10$ seconds and $TE=10, 20, 40, 60, 80$ and 100 ms.

Data Analysis

T_1 , T_2 Estimation—Blood relaxation times were estimated from fits performed on the mean signal magnitude within manually drawn regions-of-interest (ROIs) inside each specimen tube. ROIs contained an average of 35 pixels. To calculate T_1 the signal, S , in the inversion recovery measurements was fit to the following equation:

$$S = \left| A + B \cdot e^{-\frac{TI}{T_1^*}} \right|. \quad (\text{Equation 7})$$

As is typical for Look-Locker data, the apparent relaxation time T_1^* and the constants A and B were then used to estimate the true longitudinal relaxation time, T_1 [15]:

$$T_1 = T_1^* \left(\frac{B}{A} - 1 \right). \quad (\text{Equation 8})$$

T_2 was determined by a mono-exponential fit to signal intensity, S , as a function of echo time, TE :

$$S = S_0 \cdot e^{-\frac{TE}{T_2}}. \quad (\text{Equation 9})$$

Although the multi-echo spin echo pulse sequence included 32 echoes, not all of these were included in the fit. Only echo times with signal, $S > 2 \cdot S_{out}$, where S_{out} refers to the mean pixel intensity in a noise ROI, were included in the fit. In instances where there were fewer than two points which met this condition (which arose for low oxygen saturations where $\tau_{180} \approx 36$ ms), T_2 could not be determined and the data point was excluded from further analysis.

Uncertainties in T_1 and T_2 relaxation times were determined by bootstrap analysis, based on random sampling of the ~ 35 pixels within the blood tube ROIs [16]. The number of pixels in each random sample was equal to the number of pixels within the original ROI; however, due to random selection (with replacement), pixels within the original ROI could be included in a sample 0, 1, or multiple times. Each bootstrapping iteration (there were 5000 in total) included calculation of the mean signal intensity of the selected pixels at each TI or TE and re-performance of the T_1 or T_2 fit. This method was chosen as a simple means of establishing how signal intensity variations within each ROI (which arise due to random noise and as a result of field inhomogeneities) propagate into uncertainties in the estimated relaxation time.

Model Fitting— $R_1 = T_1^{-1}$ data was fit to equations 1 and 2, while $R_2 = T_2^{-1}$ was fit using equations 3 to 6. Weighted non-linear least squares fits were performed using all cord blood data combined, and then using all adult data combined (making for 4 fits in total). Fits to R_1 data provided estimates of 3 parameters: $R_{1,ery,0}$, $R_{1,plas}$, and $r_{1'}^{dHb}$. Fits to R_2 data provided

estimates of the following 6 parameters: $R_{2,plas}$ ($R_{2,dia} + R_{2,oxy}$), ($R_{2,deoxy} - R_{2,oxy}$), τ , ($\omega_{dia} + \omega_{oxy}$), and ($\omega_{deo} - \omega_{oxy}$).

The quality of the fits was assessed using the root-mean-square (RMS) percent deviation:

$$RMSD = 100\% \sqrt{\frac{1}{N} \sum_{i=1}^N \frac{(fit_i - data_i)^2}{data_i^2}}, \quad (\text{Equation 10})$$

where N refers to the number of data points included in the fit. In addition, the coefficient of determination, R^2 , was used to determine the proportion of observed variance in the relaxometry data that was explained by the fitted models [17]:

$$R^2 = 1 - \frac{\sum_{i=1}^N (data_i - fit_i)^2}{\sum_{i=1}^N (data_i - \langle data \rangle)^2}. \quad (\text{Equation 11})$$

Similar to the uncertainties in the relaxation time estimates (see above), uncertainties in fitted model parameters were determined by bootstrapping. In this case, bootstrap iterations (5000 in total) consisted of re-fitting the models to a random sample (with replacement) of all the relaxometry data. Reported parameter uncertainties refer to the standard deviation (i.e. 68% confidence) of parameter estimates across bootstrap iterations.

Results

Sequence Validation

A comparison of T_1 measurements obtained from MOLLI and IR pulse sequences is shown in Figure 1a. The two sequences produced consistent results, with the percent difference between MOLLI and IR T_1 measurements never exceeding 10% (and averaging 0.6%). Similarly, deviation between single-echo and multi-echo T_2 estimates was small (see Figure 1b), not exceeding 10% (and averaging 3.6%). As expected for the doped water samples, no dependence of T_2 on the refocussing interval, τ_{180} , was observed.

Blood Specimen Properties

Relaxometry measurements were performed on a total of 88 cord blood specimens (6 caesarean deliveries $\times$ 15 specimens/delivery, minus 2 specimens with $f_{met} > 2\%$). Blood processing (see Methods section) provided specimens with hematocrits ranging from 0.19–0.76 and oxygen saturations ranging from 4–100%. Two plasma specimens (i.e. $Hct=0$) were also included. The average methemoglobin fraction, f_{met} , of cord blood specimens was $1.1 \pm 0.3\%$. The mean fetal hemoglobin fraction, $FHbF$, was $80 \pm 4\%$.

Measurements were also performed on 31 adult blood specimens (2 collections $\times$ 15 specimens/collection, plus one plasma specimen). Hct and sO_2 of these specimens ranged

from 0.17–0.72 and 16–100%, respectively. The average f_{met} was $0.9 \pm 0.2\%$. Properties of fetal and adult blood samples are summarized in Table 1.

Blood Relaxometry

T_1 data for all adult and cord blood specimens are plotted in Figures 2a and 2b, respectively. The effect of hematocrit and oxygen saturation on blood T_1 is evident in these plots. In particular, there was a strong decrease in T_1 with increasing hematocrit. Slight shortening of T_1 with decreased oxygen saturation was also apparent. T_1 data along with the fit of the model (see Eqs. 1 and 2) are shown in Figure 3.

Adult and cord blood T_2 data acquired at $\tau_{180}=6.7$ are shown in Figure 4. The trends with hematocrit and oxygenation are similarly visible: T_2 increased with sO_2 and decreased with Hct . The effect of Hct on T_2 was strongest as sO_2 approached 100%. T_2 data along with the Luz-Meiboom model fit are shown for adult and cord blood in Figures 5 and 6, respectively. Due to the dependence of blood T_2 on the pulse sequence echo spacing, individual plots are provided for each value of τ_{180} .

Parameters obtained from fits to adult and cord blood relaxation data are summarized in Table 2. $RMSD$ (Eq. 10) and R^2 (Eq. 11) values for each fit are also indicated. Model parameters obtained from previously published adult blood measurements at 1.5T and 37°C ([18, 19]) are also shown for comparison. Overall, variations in T_1 and T_2 with blood properties were effectively explained by the biophysical models. Based on the coefficient of determination, R^2 , the proportion of relaxation time variance accounted for by the fitted models was consistently over 90%. The $RMSD$ of Luz-Meiboom model fits to T_2 data increased with τ_{180} . Specifically, fits to cord blood T_2 data had an $RMSD$ of 7.1%, 9.4%, 10.1%, 12.7%, 14.9%, and 17.6% for $\tau_{180}=6.7, 12, 18, 24, 36$, and 48 ms, respectively.

Figure 7 shows plots (for each τ_{180}) of cord blood T_2 data as a function of sO_2 , which include only those specimens with unaltered Hct . This is therefore a characterization of the effect of sO_2 on the T_2 of normal cord blood. A calibration equation of the form:

$$\frac{1}{T_2} = \frac{1}{T_{2,0}} + K_0 \left(1 - \frac{sO_2}{100\%} \right) + K_1 \left(100 - \frac{sO_2}{100\%} \right)^2 \quad (\text{Equation 12})$$

was computed for each τ_{180} , where coefficients $T_{2,0}^{-1}$, K_0 , and K_1 were calculated based on the fitted L-M model parameters and the average Hct of the collected cord blood specimens ($\langle Hct \rangle = 0.47$). The calibration equation is plotted for each τ_{180} and the calculated coefficients and $RMSD$ are indicated.

Discussion

Using cord blood specimens harvested from human caesarean deliveries, we have performed a comprehensive characterization of the relaxation properties of fetal blood at 1.5T.

Cord Blood Specimen Properties

Measured properties of the cord blood specimens obtained from complete blood count and blood gas analysis ($\langle Hct \rangle = 0.47 \pm 0.05$, $\langle FhbF \rangle = 80 \pm 4\%$, and $\langle f_{met} \rangle = 1.1 \pm 0.3\%$) agree with values reported in the literature. In a study of 438 full term newborns, the reported average cord blood hematocrit was 0.47 ± 0.04 [20]. In an investigation of 68 healthy newborns, $FhbF$ values ranging from 74.3–94.3% (with an average of 85.5%) were measured by chromatography [21]. Across 42 healthy pregnancies, an average cord blood methemoglobin fraction of $1.27 \pm 0.03\%$ was reported in a recent study [22].

Adult Blood

Along with the cord blood measurements, we also acquired data from a small number of adult blood specimens. The primary focus of this study was to characterize the MRI properties of cord blood; however, adult blood measurements were included to validate the measurement methodology. *In-vitro* blood measurements incorporate a variety of methodological choices, such as the duration of sample storage prior to scanning, the technique for manipulating oxygenation (e.g. physical vs. chemical), and the frequency and method of agitation during scanning (to prevent erythrocyte sedimentation). Consistency of the adult blood measurements with the existing literature confirms that the data were not affected by some unique bias associated with our specific combination of experimental conditions and lends credence to the cord blood measurements that we report.

The T_2 model parameters obtained by fitting our adult blood data largely agree with those reported in the literature, with the exception of $(R_{2,deoxy} - R_{2,oxy})$ and $(\omega_{deo} - \omega_{oxy})$ (see Table 2). The value of $(R_{2,deoxy} - R_{2,oxy})$ in [19] is 8.1 s^{-1} , whereas our estimate was -2.0 s^{-1} . The parameter $(R_{2,deoxy} - R_{2,oxy})$ describes the component of T_2 oxygen dependence which arises purely from the relaxation rate difference between oxy- and deoxyhemoglobin, rather than from the effect of spin exchange between two sites (erythrocytes and plasma) with an oxygen-dependent frequency difference. Accurate determination of $(R_{2,deoxy} - R_{2,oxy})$ requires separation of the rate-difference effect from the much stronger effect of spin exchange, which can only be achieved by minimizing exchange with a very short echo spacing, τ_{180} . Due to gradient limitations, the minimum τ_{180} in this study was 6.7 ms, compared to 2 ms in [19] (using a spectroscopic acquisition). This may explain the disagreement and the high degree of uncertainty in our estimates of this parameter.

The value of $(\omega_{deo} - \omega_{oxy})$ in [19] is -0.31 ppm , which is smaller than our estimate of -0.69 ppm . Due to the lack of parameter uncertainties reported in [19], the extent of disagreement is difficult to assess. The parameter $(\omega_{deo} - \omega_{oxy})$ describes the oxygen-dependent frequency shift between erythrocytes and plasma. A smaller value for $(\omega_{deo} - \omega_{oxy})$ results in a longer T_2 relaxation time (due to reduced exchange-mediated dephasing) given equivalent τ_{180} , Hct and sO_2 . This difference in T_2 will be largest at low sO_2 . For example, with $\tau_{180} = 12 \text{ ms}$, $Hct = 0.45$, and $sO_2 = 0$, the fitted parameters in [19] predict a T_2 relaxation time of 63 ms, while our parameters predict $T_2 = 28 \text{ ms}$. The shorter T_2 value is more consistent with the work of Wright *et al* [23], which, under equivalent conditions, provides a T_2 of 35 ms. Although the value of $(\omega_{deo} - \omega_{oxy})$ is not reported explicitly in [23], this comparison suggests a value much closer to our own.

The adult T_I data is consistent with the T_I measurements reported in [24]. T_I values of 1531 ± 29 ms and 1429 ± 21 ms were measured in the sagittal sinus of 3 females and 3 males, respectively. Assuming an oxygen saturation of 70% and a hematocrit of 0.4 for females and 0.45 for males, our fitted T_I model parameters predict relaxation times of 1554 ms and 1486 ms, respectively. Our estimate of $R_{I,plas} = 0.41 \pm 0.01 \text{ s}^{-1}$ is smaller, but similar to the value of $0.49 \pm 0.04 \text{ s}^{-1}$ reported by [18].

Biophysical Models

The models used to fit the blood data explained the majority (>90%) of the observed variance in T_I and T_2 relaxation times. Unaccounted sources of variance include measurement noise and biological variation between subjects. A potential source of biological variability not incorporated into the models is differences in the fraction of fetal hemoglobin, $FHbF$ ($\langle FHbF \rangle = 80 \pm 4\%$, range = [72%–87%]). However, no trend was detected between $FHbF$ and the measured relaxometry values. For example, collections #2 and #4 had 72% and 87% $FHbF$, respectively, but similar relaxometry values (square and diamond data symbols in Figs. 2b, 4b and 7).

The biophysical models used in this study are well established in the literature. However, unlike [7, 8], the T_I model used here did not account for differences in water content between erythrocytes and plasma. Under physiological conditions, the erythrocyte water content is approximately 70%, while the water volume fraction in plasma is roughly 95% [8]. Accordingly, the Hct terms in Eq. [1] are replaced with f_{ery} , the fractional water content in erythrocytes:

$$f_{ery} = \frac{0.7 \cdot Hct}{0.7 \cdot Hct + 0.95 \cdot (1 - Hct)} \quad (\text{Equation 13})$$

Although this modification is commonly included in blood T_I models, it does not appear in the Luz-Meiboom T_2 model, where, given the assumption of fast exchange, it should apply equally well (see Equation 3). While replacing Hct with f_{ery} may be more accurate from a theoretical perspective, since it is the water signal that we measure, it did not improve the description of the data: the RMSD of the T_I fits decreased by only 0.3% and 0.04% for the adult and fetal data, respectively. For the T_2 fits the RMSD increased by 1.5% and 2.2%, respectively. Furthermore, the use of Hct in place of f_{ery} in Equations [1] and [3] results in simpler, more direct relationships between the relaxation rate and the hematocrit - the parameter of clinical relevance. Based on these observations, Hct , rather than f_{ery} , is used throughout this work.

The calibration coefficients (T_{20}^{-1} , K_0 and K_I) provided in Figure 7, which describe the relationship between blood T_2 and sO_2 at $Hct = 0.47$, can be used to compute sO_2 from vascular T_2 measurements performed in normal fetuses/neonates. Calibration coefficients for non-normal Hct or echo spacings which are not indicated in Figure 7 can be computed from the fitted L-M model parameters provided in Table 2. Figure 7 is similar to Figure 3 of Wright *et al.* [23], which displays calibration data for adult blood oximetry. It is worth noting, however, that the calibration equations in [23] do not include a linear term (i.e.

$K_0=0$), which is equivalent to the assumption that $(R_{2,deoxy}-R_{2,oxy}) = (\omega_{dia}+\omega_{oxy}) = 0$. The absence of the linear term in the data fit is likely to affect the remaining coefficients (T_{20}^{-1} and K_1), complicating the comparison of the values indicated in Figure 7 to those in [23].

As is evident in Figure 7, disagreement between the L-M model fit and the T_2 data increased with τ_{180} . This trend, which is expected given the assumption of a short τ_{180} in the derivation of the L-M model [25], could reduce the accuracy of oximetry measurements derived from T_2 data acquired at large echo spacing ($\tau_{180}>25$ ms). This source of error can, however, be easily avoided by performing T_2 measurements with $\tau_{180}<25$ ms. This condition, which is already a requirement for accurate measurement of short T_2 values (which arise at lower sO_2), is easily fulfilled by the vast majority of clinical scanners.

Several publications have reported better characterization of T_2 data with the Jensen-Chandra (J-C) model, which, unlike the L-M exchange model, attributes changes in T_2 as a function of τ_{180} to the diffusion of water molecules through field inhomogeneities induced by deoxyhemoglobin-containing erythrocytes [10, 26, 27]. Nevertheless, the performance of the J-C model in fitting our cord blood data was significantly poorer (RMSD=24%). This discrepancy may be a result of the comprehensive nature of the fit in this study, which included more than 450 data points, as it was performed simultaneously on all the cord blood T_2 data. In [10], the comparison of L-M and J-C models was based on the quality of fits to T_2 vs. τ_{180} , which were performed individually on each specimen. In [27], while some model parameters, such as the L-M model exchange correlation time, τ , were constrained to be equivalent for different specimens, the number of specimens was small ($N=7$), only normal Hct values were included ($Hct=0.52\pm0.04$) and the sO_2 range was narrower (40–100%).

As was demonstrated in [10], testing the performance of the J-C model involved replacing the expression for the exchange term, $R_{2,ex}$ (Eq. [5]), with the diffusion-based expression derived in [26]. The remaining terms in Eq. [3], which describe the transverse relaxation rate of blood in the absence of deoxyhemoglobin induced field inhomogeneities were unchanged. Application of the J-C model to blood data requires adopting a model of uniformly magnetized, randomly distributed spheres for blood [10, 26, 27]. The reduced quality of the global J-C model fit may therefore reflect the limitations of a random spheres model for blood.

In terms of the number of points and the range of Hct and sO_2 values, our work provides the most comprehensive fit of the Luz-Meiboom model ever to be performed. The effectiveness of the model in describing this breadth of data is a confirmation of its robustness. This quantity of data was achieved by dividing each blood collection into multiple (~15) specimens and combining all specimens from all collections in a single, global fit. Handling the data in this manner was effective in fulfilling this study's purpose - to obtain normative values of the relaxometry model parameters for a fetal population. However, as a result of the amalgamation of data, the parameter uncertainties reported in Table 2 include a within-subject contribution associated with variability/error in the measurement process, as well as a between-subject contribution from biological variability amongst the different samples. The high quality of the data fits and high coefficient of determination (>90%) suggests that

adding model parameters to account for physiological factors that can contribute to biological variability (e.g. plasma protein content, fetal hemoglobin fraction) is unwarranted and would render the fits unstable. In principle, however, a larger study with more subjects could improve upon the precision of the parameter estimates by decomposing the uncertainty into within-subject and between-subject components through a non-linear mixed effects analysis.

Cord vs. Adult Blood Relaxometry

While the relaxation characteristics of fetal cord blood and adult blood were similar, the unique physiological properties of cord blood did lead to some differences. Importantly, the ~80% difference in the fraction of fetal hemoglobin between cord and adult blood (i.e. $FHbF_{cord} \sim 80\%$, while $FHbF_{adult} \sim 0\%$) may not be the only source of variation. There may also be a contribution from the size and permeability differences between adult and fetal erythrocytes and the reduced protein content in fetal plasma. Many of the fitted model parameters (see Table 2) exhibited small but significant (i.e. $p < 0.05$) differences between cord and adult blood. These include $R_{2,plas}$, $r_{1'}^{dHb}$, $\omega_{dia} + \omega_{oxy}$, $\omega_{deo} - \omega_{oxy}$, and τ . In the paragraphs that follow, we reflect on how the unique physiological properties of cord and adult blood may manifest through these parameter variations.

- $R_{2,plas,cord} = 1.60 \pm 0.03 \text{ s}^{-1}$ vs. $R_{2,plas,adult} = 1.95 \pm 0.08 \text{ s}^{-1}$: The difference in the transverse plasma relaxation rate may be explained by viscosity and protein content. The reduced viscosity of fetal plasma is largely attributed to a reduction in high molecular weight proteins (e.g. fibrinogen) [28]. Large proteins with slow rotational correlation times (i.e. $\tau_c > 10^{-5}$ seconds) primarily contribute to transverse relaxation through static dipole-dipole interactions [29]. Their absence can therefore reduce the transverse relaxation rate, while leaving the longitudinal relaxation rate, $R_{1,plas}$ unaffected.
- $r_{1'}^{dHb,cord} = 0.29 \pm 0.03 \text{ s}^{-1}$ vs. $r_{1'}^{dHb,adult} = 0.17 \pm 0.03 \text{ s}^{-1}$: The difference in $r_{1'}^{dHb}$, the parameter governing the dependence of blood T_1 on oxygen saturation, may be attributable to the known structural variations between adult and fetal hemoglobin. If the structure of HbF allows water protons greater access to the paramagnetic heme iron than in HbA, this will strengthen the dipole-dipole interaction, leading to an increase in the relaxivity of fetal deoxyhemoglobin.
- $(\omega_{dia} + \omega_{oxy})_{cord} = -0.071 \pm 0.006 \text{ ppm}$ vs. $(\omega_{dia} + \omega_{oxy})_{adult} = -0.044 \pm 0.009 \text{ ppm}$ & $(\omega_{deo} - \omega_{oxy})_{cord} = -0.62 \pm 0.01 \text{ ppm}$ vs. $(\omega_{deo} - \omega_{oxy})_{adult} = -0.69 \pm 0.01 \text{ ppm}$: Frequency shifts $\omega_{dia} + \omega_{oxy}$ and $\omega_{deo} - \omega_{oxy}$ relate to the susceptibility difference between erythrocytes and plasma as well as the shape and packing of erythrocytes [30]. Variations between adult and fetal blood may therefore reflect a susceptibility difference between the two types of hemoglobin (i.e. HbA and HbF), but may also result from differences in erythrocyte geometry – fetal erythrocytes are 21% larger in volume, 11% larger in diameter, and have 13% more surface area [31].
- $\tau_{cord} = 2.27 \pm 0.09 \text{ ms}$ vs. $\tau_{adult} = 2.9 \pm 0.2 \text{ ms}$: The shortened exchange correlation time, τ , for cord blood is difficult to interpret, as it runs contrary to the reduced

surface area-to-volume-ratio (see above) and permeability of fetal erythrocytes. It is, however, well known that interpretation of τ as an exchange lifetime is complicated by the effects of diffusion and potential violation of the L-M model's assumption of fast exchange on the NMR time scale (i.e. that the exchange rate, τ^{-1} , is much higher than the erythrocyte-plasma frequency difference) [10, 19].

Parameter differences between cord and adult blood are such that throughout the Hct , sO_2 and τ_{180} parameter space, the cord blood transverse relaxation rate, $R_{2,cord}$ is consistently lower (by up to 25%) than that of adult blood ($R_{2,adult}$). In general, R_2 differences between cord and adult blood increase with τ_{180} and decrease with sO_2 . Conversely, the cord blood longitudinal relaxation rate, $R_{1,cord}$ is consistently higher than that of adult blood ($R_{1,adult}$), by as much as 11%. Differences between $R_{1,cord}$ and $R_{1,adult}$ increase as sO_2 approaches zero. Restating the above in terms of relaxation times, given equivalent Hct and sO_2 , $T_{2,adult} < T_{2,cord} < 1.35 \cdot T_{2,adult}$ and $0.9 \cdot T_{1,adult} < T_{1,cord} < T_{1,adult}$. Although these small but significant differences were detectable in this comprehensive, *in-vitro* investigation, given the 5–10% errors typically associated with *in-vivo* relaxometry, differences between cord and adult blood may be too small to detect. Plots of T_1 vs. sO_2 and T_2 vs. sO_2 ($\tau_{180}=12$ ms) derived from the fitted model parameters for cord and adult blood at $Hct=0.47$ are shown in Figure 8.

As a result of the slightly longer T_2 for cord blood, use of an adult-derived calibration for fetal/neonatal blood oximetry will lead to overestimation of sO_2 . For example, with $Hct=0.47$, $\tau_{180}=12$ ms and a true fetal blood sO_2 of 20% (which would correspond to a T_2 of 48 ms), use of the adult blood relationship yields an sO_2 estimate of 29%. The degree of overestimation decreases with sO_2 : with $Hct=0.47$ (Figure 8b), it is less than 5% when $sO_2=50\%$ and less than 1% when $sO_2=90\%$. However, as mentioned above, with typical T_2 measurement errors, these relatively small differences in oxygen saturation may not be significant compared to the overall uncertainty in sO_2 estimation.

Strictly speaking, the MRI properties of cord blood presented here are appropriate to describe late-gestation fetal (>35 weeks) and neonatal (<1 month old) blood. This age range corresponds to a relatively stable period in the normal time course for fetal hemoglobin levels during gestation and after birth. *FHbF* drops roughly linearly from ~100% at 24 weeks gestation to ~80% at birth [32]. After birth, the decline of fetal hemoglobin follows three stages: 1) a plateau lasting 2–3 weeks; 2) a linear *FHbF* decrease down to 5% until approximately 100 days of age; and 3) a second, much slower linear decrease to normal adult *FHbF* levels (<1%) up to ~200 days of age [33]. Given our observations, which suggest a relatively subtle difference in magnetic properties between adult and fetal hemoglobin, the applicable age range may be even broader.

The relaxation properties of cord blood at 3 Tesla have been described in a recent publication by Liu *et al.* [34]. The findings differ from the results of this study in several respects. Unlike our observations of a slightly shortened T_1 in cord blood, Liu *et al.* report significantly longer T_1 relaxation times for cord blood compared to adult blood, due to a longer plasma T_1 (cord $T_{1,plas}=1000/0.30 \text{ s}^{-1}=3333$ ms compared to adult $T_{1,plas}=1000/0.38$

$s^{-1}=2631$ ms). Although, like us, they report longer T_2 relaxation times in cord blood, the T_2 differences are larger than what we observed and tend to increase, rather than decrease, with sO_2 .

The origin of these discrepancies is difficult to determine. The discordance is too large for the effect of field strength (3T vs. 1.5 T) to be a likely explanation. Although there is a difference in specimen age between the two studies (Liu *et al* performed MRI scans within 6 hours of blood collection, compared to 36 hours in this study), this is not likely to be the source of the disagreement. In theory, prolonged storage of blood samples could lead to increased methemoglobin (mHb) content, which causes shortening of relaxation times, particularly T_1 [35]. However, the mHb fractions (f_{met}) of the cord blood specimens in this study, which were measured after each MRI session, were well within the expected *in-vivo* range (0.6–2.5% [11]). Furthermore, the hematologic parameters (e.g. white blood cell count, corpuscular volume, hemoglobin concentration, etc.) of heparinized cord blood stored at 4°C have been shown to be very stable for up to 72 hours [36]. Finally, the possibility that our relaxation measurements were biased by some biochemical confound introduced by our specimen handling/processing procedure was ruled out by the consistency of the adult blood measurements with literature values.

Conclusion

We have characterized the relaxation properties of cord blood at 1.5 Tesla over a broad range of hematocrits and oxygen saturations using specimens obtained from human caesarean deliveries. Variations in cord blood T_1 and T_2 as a function of Hct and sO_2 were well described by biophysical models previously developed in adult blood. The data and fitted model parameters we report can be used for the interpretation of future MRI investigations of fetal and neonatal blood physiology.

Results showed that, in general, relaxation properties of cord blood and adult blood are similar. T_1 relaxation times of cord blood were slightly shorter (by up to 10%) than those of adult blood, while T_2 relaxation times were longer (by up to 35%). The similarity in relaxation properties is encouraging, as it supports the findings of studies which used adult-derived calibrations to perform fetal/neonatal oximetry (e.g. [2, 3]). As the first characterization of the MRI properties of fetal blood at 1.5 Tesla, this work represents a key step in the adaptation of non-invasive vascular oximetry techniques towards a fetal population.

Acknowledgments

The authors thank the donors, the RCWIB BioBank, the Lunenfeld-Tanenbaum Research Institute, and the Mount Sinai Hospital/University Health Network Department of Obstetrics and Gynaecology for the human specimens used in this study (<http://biobank.lunenfeld.ca>). Thanks also to Natasha Milligan, Clinical Research Associate, for facilitating REB approval and performing patient recruitment. Finally, thanks to Martin Durant, PhD, for useful discussions and for providing python code for data fitting and ROI analysis (available at <https://github.com/martindurant>). This work was supported by the Canadian Institutes of Health Research (funding reference number MOP130403).

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Figure 1.

Validation of pulse sequences for relaxometry measurements. a) Comparison of T_1 estimates derived from standard inversion recovery and MOLLI (Modified Look-Locker Inversion Recovery) pulse sequences in a subset of blood specimens. b) Comparison of T_2 estimates in Gd-doped water samples obtained from single- and multi-echo pulse sequences. The consistency between the techniques is evident from the proximity of data points to the identity line shown on each plot. Note that data symbols in (b), which represent multi-echo T_2 measurements at different echo-spacings (τ_{180}), are virtually coincident.

Figure 2.

T_1 of all (a) adult and (b) cord blood specimens. Different data markers are used for each subject. Data points are colored according to oxygen saturation, sO_2 (see colorbar), as measured by blood gas analysis. Plasma data points were assigned no color (since sO_2 is undefined). The effect of hematocrit and oxygen saturation on blood T_1 is immediately apparent: T_1 decreases with hematocrit and increases (slightly) with oxygen saturation.

Figure 3.

T_1 of (a) adult and (b) cord blood specimens along with the model fit. Contour lines represent the T_1 predicted by the model for different values of the hematocrit (see contour labels). Data points are colored according to hematocrit (see colorbar). Fit quality can be judged by the similar color of data points and nearby contour lines.

Figure 4.

T_2 of all (a) adult and (b) cord blood specimens at an echo spacing of 6.7 ms. Different data markers are used for each subject. Data points are colored according to hematocrit (see colorbar). The effect of hematocrit and oxygen saturation on blood T_2 is apparent: T_2 increases with sO_2 and decreases (slightly) with hematocrit.

Figure 5.

Luz-Meiboom model fit to adult blood T_2 data. Individual plots are provided for different values of the echo-spacing, τ_{180} (indicated above). Contour lines represent the fitted model T_2 for different values of sO_2 (see contour labels). Data points are colored according to sO_2 (see colorbar). Similar to Figure 3, fit quality can be judged by the proximity of similarly colored data points and contour lines.

Figure 6.

Luz-Meiboom model fit to cord blood T_2 data. For further explanation see caption of Figure 5.

Figure 7.

Cord blood T_2 vs. sO_2 for normal Hct ($Hct=0.47 \pm 0.05$). Each plot corresponds to a different echo spacing (as indicated). Different data symbols represent different subjects. Solid lines are the prediction of the global Luz-Meiboom model fit (see Methods). Calculated calibration coefficients (see Eq. 12 in the text) are indicated on each plot, along with the corresponding RMSD.

Figure 8.

Plots of (a) T_1 and (b) T_2 as a function of oxygen saturation for cord and adult blood.

Graphs were generated at $Hct=0.47$ and $\tau_{180}=12$ ms from fitted model parameters provided in Table 2. Shaded regions correspond to the 95% confidence interval.

Table 1

Summary of blood sample properties

Collection	Type	MCHC (g/dL)	FHbF (%)	Initial Hct, Hct ₀	Hct range	sO ₂ range (%)
1	Cord	33.3	79.9	0.50	0.28–0.72	11–100
2	Cord	33.9	87.4	0.46	0.32–0.71	4.1–100
3	Cord	33.1	80.0	0.56	0.0, 0.37–0.76	12–100
4	Cord	32.9	72.0	0.44	0.33–0.61	14–100
5	Cord	33.2	80.5	0.44	0.25–0.68	7.5–100
6	Cord	34.4	80.1	0.42	0.0, 0.19–0.65	12–100
7	Adult*	(33.2)	(0)	0.37	0.0, 0.29–0.72	18–100
8	Adult*	(33.2)	(0)	0.40	0.17–0.61	15–100

* Entries in brackets indicate values for adult blood based on population averages, rather than measured. The two right-most columns indicate the *Hct* and *sO₂* ranges spanned by the specimens derived from each collection after blood processing. An entry of 0.0 in the *Hct* range corresponds to a plasma sample.

MCHC=mean corpuscular hemoglobin concentration, FHbF=fetal hemoglobin (HbF) fraction, *Hct*₀=natural, unprocessed blood hematocrit, *Hct*=hematocrit, *sO₂*= oxygen saturation.

Table 2Summary of model parameters from T_1 and T_2 data fits

	parameters	$R_{1,plus}(s^{-1})$	$R_{1,oxy,0}(S^{-1})$	r_1' (ms)	$\omega_{dia} + \omega_{oxy}$ (ppm)	$\omega_{deo} - \omega_{oxy}$ (ppm)	τ (ms)	$RMSD$ (%)	R^2 (%)
T_1	Measured								
	cord	0.44 ± 0.01	0.95 ± 0.01	0.29 ± 0.03	-0.071 ± 0.006	-0.62 ± 0.01	2.27 ± 0.09	4.5	92
	adult	0.41 ± 0.01	0.94 ± 0.02	0.17 ± 0.05				3.5	96
Literature	adult	0.49 ± 0.04^a	--	--				--	--
T_2	Measured								
	cord	1.60 ± 0.03	5.1 ± 0.1	-3.1 ± 0.7	-0.044 ± 0.009	-0.69 ± 0.01	2.9 ± 0.2	12.5	92
	adult	1.95 ± 0.08	4.9 ± 0.2	-2.0 ± 1.1				8.9	96
Literature	adult	$1.6^b, 2.0 \pm 0.2^a$	5.5^b	8.1^b	-0.063^b	-0.310^b	2.5^b	--	--

* Previously published model parameters for adult blood scanned at 1.5T and 37°C are shown for comparison (fourth and eighth rows).

^a obtained from [18].^b obtained from [19].