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MRI Quantification of Human Fetal O₂ Delivery Rate in the Second and Third Trimesters of Pregnancy

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Abstract

Purpose—The purpose of this study was to estimate fetal O₂ delivery rate *in vivo* across a range of gestational ages (GA). Toward this, a calibration equation for T₂-based oximetry was derived.

Methods—Umbilical cord blood of varying hematocrit (Hct) and oxygen saturation (HbO₂) levels was prepared and T₂ measured using a T₂-prepared balanced steady-state free precession (T₂-bSSFP) sequence at 1.5T. The relationship between blood R₂=1/T₂, HbO₂ and Hct was established based on the model $R_2 = (1 - \text{Hct})R_{2,\text{plasma}} + \text{Hct} R_{2,\text{RBC}} + k \cdot \text{Hct} \cdot (1 - \text{Hct}) \cdot (1 - \text{HbO}_2)^2$. Experimental R₂, HbO₂ and Hct levels were fit to the model yielding values of k, R_{2,plasma} and R_{2,RBC} (R₂ of plasma and erythrocytes). Umbilical vein (UV) T₂ measured *in vivo* was then converted to HbO₂ yielding, together with blood flow rate (BFR), fetal O₂ delivery rate in 22 pregnancies (GA 30±3 weeks).

Results—Constants derived from the fit ($R^2 = 0.94$) were: $k=83.1\text{s}^{-1}$, $R_{2,\text{plasma}} = 1.1\text{s}^{-1}$, $R_{2,\text{RBC}} = 12.9\text{s}^{-1}$. R_{2,RBC} and k were found to be larger than those obtained for adult blood, likely due to differences in dominant hemoglobin type. Data suggest that the use of adult blood calibration could entail errors up to 10% in fetal blood HbO₂. Average UV BFR (89.5±17.2 mL/min/kg), HbO₂ (84±7%), and fetal O₂ delivery rate (15.1±3.8 mL O₂/min/kg) were independent of GA. Fetal O₂ delivery rate agreed well with results obtained with invasive methods at term.

Conclusion—The present work describes strategies for measuring UV BFR and HbO₂ *in vivo* and estimates fetal O₂ delivery rate noninvasively with quantitative MRI during the second and third trimesters of pregnancy.

Keywords

fetal oxygen delivery rate; T₂-based oximetry; fetal blood; oxygen saturation

Introduction

The placenta is a transient organ present during pregnancy. The main function of the placenta is to provide a nurturing environment for fetal development and sustainability. One of the chief functions of the placenta is to transport O₂ between the maternal and fetal circulations (1), which is established at the end of the first trimester (2). However, in this process, maternal and fetal blood never mix; fetal capillaries are embedded inside chorionic villi, a structure analogous to the alveoli in the lung. Highly oxygenated maternal blood enters the intervillous space in the placenta through the spiral arteries. Oxygen is then transferred to the fetal circulation by diffusion and delivered to the fetus via the umbilical vein (UV). Hemoglobin O₂ saturation (HbO₂) at the UV is only on the order of 70–80% (3,4), low HbO₂ is compensated for by high fetal cardiac output. After fetal O₂ extraction, deoxygenated blood (HbO₂ 40–60%) (5,6) returns to the placenta through the umbilical arteries, where carbon dioxide diffuses into the maternal compartment of the placenta and are then removed through the maternal venous system (Figure 1). Hence, the proper establishment of the utero-placental and placental-fetal circulations is critical to the viability of the fetus.

Placental dysfunction is widely accepted as the cause of common adverse pregnancy outcomes. However, the etiology of abnormal placental development remains unknown. Importantly, clinically available tools are inadequate for evaluating placental function. Therefore, methods that allow quantification of O₂ delivery rate to the fetus throughout pregnancy *in vivo* would be of great interest to maternal-fetal medicine as they could provide new insight into placental and fetal development. Fetal O₂ delivery rate in physiological units normalized to fetal mass (mL O₂/min/kg), is defined as:

$$\text{Fetal O}_2 \text{ Delivery Rate} = C_a \cdot \text{BFR} \cdot \text{HbO}_2 / \text{fetal mass} \quad [1]$$

where C_a is the oxygen carrying capacity of fetal hemoglobin in units of mL O₂/mL ($C_a = 1.33 \text{ mL O}_2/\text{g Hb} \cdot [\text{Hb}]$, where $[\text{Hb}]$ is the blood hemoglobin concentration in g/mL blood), BFR is the umbilical vein (UV) blood flow rate (in mL/min) and HbO₂ is oxygen saturation measured at the UV. Quantitative MRI allows for the estimation of BFR and fetal mass from widely used MR techniques such as phase-contrast MRI (PC-MRI) and structural MR images (7,8). Further, HbO₂ can be estimated via T₂-based oximetry, which requires a calibration equation to convert blood T₂ values to HbO₂ (9).

The present authors have previously derived a calibration equation for human adult blood using a T₂-prepared bSSFP (T₂-bSSFP) sequence (10). However, due to differences in the biophysical (11), rheological (12) and biochemical properties between fetal and adult hemoglobin, the calibration equation derived from adult blood may not be accurate for fetal blood. Therefore, the purpose of this study was twofold: first to measure T₂ of human fetal blood samples of varying Hct for a wide range of HbO₂ levels using a T₂-bSSFP sequence to derive a calibration equation, and second, to estimate fetal O₂ delivery rate *in vivo* across a range of gestational ages (GA) with quantitative MRI.

Methods

Ex Vivo Blood Sample Preparation

Fetal whole blood (average 30 ± 18 mL, range 10–70 mL) was collected from the umbilical cord of 13 fetuses at term (GA > 37 weeks), yielding a total of 77 3 mL fetal blood samples. Upon delivery, the umbilical cord was clamped on one end and dissected from the newborn. Subsequently, the placenta was immediately taken to a laboratory and placed on a container that was raised approximately 50 cm. The container had a perforation at the base through which the umbilical cord was inserted. Removal of the clamp then allowed blood to flow and be collected into open 7 mL Vacutainer glass tubes, each containing 12 mg of K_3 EDTA (Becton, Dickinson and Company, New Jersey, USA). Tubes were capped and inverted to mix with the EDTA agent to prevent clotting. Lastly, the specimens were stored at 4°C before being transported to the hematology laboratory for preparation (to achieve the desired HbO_2 and Hct levels). The Institutional Review Board of the University of Pennsylvania approved the study, and all subjects gave oral and written informed consent.

Both, Hct and HbO_2 levels of the blood samples, were varied as described in (10). Briefly, an extended range of Hct values was achieved by separating plasma and RBC via centrifugation. Both components were then redistributed, resulting in samples of various Hct. Subsequently, HbO_2 was varied by exposing samples to N_2 gas at 37°C for different durations. Samples were taken every 10 minutes with a 3 mL syringe, HbO_2 and hemoglobin (Hb) concentration were measured with a clinical blood gas analyzer (ABL 700 series; Radiometer, Copenhagen, Denmark). Syringes were sealed using a vinyl plastic putty (Critoseal; Fisher Scientific, Pennsylvania, USA) and parafilm. Complete blood count was performed (HEMAVET HV950FS, Drew Scientific, Florida, USA) to calculate the Hct to Hb ratio for each Hct preparation. Sealed syringes were kept at 37°C and placed in a cylindrical container filled with distilled water at the same temperature for the MRI. All samples were prepared and scanned within 24 hrs of delivery.

Hemoglobin Composition of Fetal Blood

Clarified hemolysates were prepared by hypotonic lysis followed by centrifugation using standard methods. Approximately 20 μ L of lysates from experimental samples were analyzed for identification of fetal and adult hemoglobin levels by cation-exchange high-performance liquid chromatography (HPLC) using previously described methods, with slight modifications (13–16). A Hitachi D-7000 Series (Hitachi Instruments, Inc., San Jose, CA), and a weak cation-exchange column (Poly CAT A: 35 mm $\times$ 4.6 mm, Poly LC, Inc., Columbia, MD) were used. Hemoglobin isotype peaks were eluted with a linear gradient of phase B from 0% to 80% at A_{410nm} (Mobile Phase A: 20 mM Bis-Tris, 2 mM KCN, pH 6.95; Phase B: 20 mM Bis-Tris, 2 mM KCN, 0.2 M sodium chloride, pH 6.55). Clarified lysates from normal human red blood cells (RBCs, exclusively Hb A), as well as a commercial standard containing fetal and adult hemoglobin, together with hemoglobin types S and C (FASC reference material, Trinity Biotech USA Inc., Jamestown, NY, USA) were utilized as reference isotypes (Supporting Figure S1). Fetal hemoglobin fraction was estimated as the ratio of the amplitude of the fetal hemoglobin peak to the total hemoglobin,

defined at the sum of fetal and adult hemoglobin as all samples were of typical development fetuses and hemoglobin types S and C were not present.

Ex vivo MRI Experiments

Experiments were conducted at 1.5T (Siemens Avanto, Erlangen, Germany) with a 10-channel head coil. The T₂-bSSFP sequence and data reconstruction have been previously described in (10). Briefly, this sequence consists of three parts: spin saturation, T₂-preparation and bSSFP readout (Figure 2). The magnetization is nulled by a saturation pulse and allowed to partially recover during the T_{sat} period prior to each T₂-preparation, which consist of non-selective 90° RECT excitation pulse, followed by *n* MLEV-4 type refocusing pulses (*n* = 0, 1, 2, 3, 4) with inter-refocusing pulse interval $\tau_{180}=12\text{ms}$, each followed by a composite tip-up (270_x360_{-x}) pulse. Lastly, bSSFP signal encoding is preceded by 10 linear ramp-up pulses for signal stabilization and 14 reference lines are collected for phase correction during partial-Fourier reconstruction in linear order.

These methods were used to derive a calibration equation to relate fetal blood T₂ to HbO₂ and Hct. Imaging parameters: TR = 4400 ms, T_{sat} = 4000 ms, bSSFP sequence parameters: TE/TR = 1.9/3.8 ms, field of view (FOV) = 160×160 mm², acquisition matrix 128×94, voxel size = 1.25×1.25×5 mm³, T₂-preparations TEs of 0, 48, 96, 144 and 192ms, flip angle (FA) = 60°, partial-Fourier acquisition and total scan duration = 22 s. T₂ of the blood samples was extracted using a three-parameter fit to the equation $S(t) = S_0 e^{-t/T_2} + C$, with C representing the steady-state amplitude of the bSSFP signal.

Relaxation Model and Analysis

The relationship between blood relaxation rate R₂ (1/T₂) and HbO₂ was first established by Wright et al (9) who resorted to a semi-empirical rendition of the Luz-Meiboom equation for two-site chemical exchange (17):

$$R_2 = R_{2o} + K(1 - \text{HbO}_2)^2 \quad [2]$$

Here, R_{2o} is the relaxation rate of fully oxygenated blood and K represents the relaxivity. This model was subsequently expanded by van Zijl et al to incorporate the effect of Hct on blood R_{2o} and K (18):

$$\begin{aligned} R_2 &= (1 - \text{Hct})R_{2,\text{plasma}} + \text{Hct} R_{2,\text{RBC}} + k \cdot \text{Hct} \cdot (1 - \text{Hct}) \cdot (1 - \text{HbO}_2)^2 \quad [3] \\ R_2 &= R_{2o}(\text{Hct}) + K(\text{Hct}, k)(1 - \text{HbO}_2)^2 \end{aligned}$$

It can then be observed that R_{2o} scales linearly with Hct (19,20), while the relaxivity K depends on both Hct and the empirical parameter *k*. The relaxation rates of blood R₂ were plotted versus (1 - HbO₂)² and Hct, and the data fit to the model described by Equations [3] and [4] yielding constants *k*, R_{2,plasma}, and R_{2,RBC}.

***In vivo* MRI Studies**

The purpose of these studies was to quantify fetal O₂ delivery rate in the second and third trimester *in vivo*. Exclusion criteria included: known uterine fibroids, pre-gestational diabetes, connective tissue disorders affecting vascular development (e.g. lupus or vasculitis), body mass index greater than 35 before or at the start of pregnancy, known major structural fetal anomaly or genetic syndrome, and any history of oophorectomy. Toward this goal 30 pregnant women (GA: 30±3 weeks, range 23–35 weeks) were scanned using two 4-element body matrix coils at 1.5T (Siemens Avanto, Erlangen, Germany) in supine position. Fetal O₂ delivery rate in physiological units (mL O₂/min/kg) was obtained as the product of C_a of fetal hemoglobin, BFR and HbO₂ with the latter two quantities measured at the UV, divided by fetal mass (Figure 1). Half-Fourier single-shot turbo spin-echo (HASTE) imaging with the following parameters was used to identify the umbilical cord: TR/TE = 405/60 ms, FOV 430×430 mm², and voxel size = 1.7×1.7×4 mm³, bandwidth = 475 Hz/pixel.

Blood flow velocity was measured using PC-MRI during a single breath hold with the following imaging parameters: VENC = 20cm/s, TR/TE = 10.6/6.5 ms, TE = 6.5 ms, FOV 200×200 mm², acquisition matrix = 320×320, voxel size = 0.625×0.625×5 mm³, FA = 20°, bandwidth = 260 Hz/pixel, resulting in a total scan time of 9 s. In some instances, fetal motion during PC-MRI acquisition resulted in displacement of the umbilical cord in the scanning plane causing motion artifacts that could be observed on the magnitude and complex difference intensity images. In such case, PC-MRI was repeated multiple times to obtain two motion-artifact free datasets. Blood flow rate was then estimated as the product of flow velocity (averaged from two phase-contrast MRI datasets) and UV cross-sectional area. The flow velocity and cross-sectional area, used were calculated as the average from two artifact free datasets. The regions of interest (ROIs) of the UV were manually traced on the magnitude PC-image using Osirix (Pixmeo SARL, Bernex, Switzerland).

Umbilical vein HbO₂ was estimated as described above for the *in vitro* experiments (10) using the following imaging parameters: TR = 3900 ms, T_{Sat} = 3000 ms, bSSFP TE/TR = 1.9/3.8 ms, FOV = 307×307 mm², matrix size = 384×206 (partial Fourier), voxel size = 0.8×0.8×5 mm³, FA = 60°, number of acquisitions = 5, resulting in a total scan time of 1:40 mins. Therefore, T₂-bSSFP data were acquired three times: at the prescribed plane and 8mm above and below. Imaging planes were chosen where the umbilical cord was abutting the fetus or where it could be visualized in at least three sequential localizer images. No motion correction was implemented. To account for displacement of the UV between T₂-bSSFP images corresponding to specific echo times, the ROI used to estimate the average signal at each TE and repetition was manually repositioned. An elliptical ROI of the UV was initially traced on the first echo image, but was appropriately repositioned in subsequent images if deemed necessary (without modifying the shape of the initial ROI). Typical ROI placement correction was 2–3 pixels. Care was taken to avoid inclusion of Wharton's jelly (tissue embedding the umbilical vessels) in the ROI.

Fetal volume was estimated by manually segmenting the HASTE coronal images using Osirix (Pixmeo SARL, Geneva, Switzerland). Total fetal volume was estimated as the product of the total number of pixels that belonged to the fetus segmentation and the volume

of a voxel element in dm^3 . Fetal volume (in dm^3) was then converted to mass using the previously determined relationship: $\text{mass (kg)} = 1.03 \cdot \text{volume (dm}^3) + 0.12 \text{ (kg)}$ (7).

Results

Ex-vivo Umbilical Cord blood T_2 vs HbO_2 Calibration

Fetal hemoglobin fraction could be estimated in samples from 8 of the 12 pregnancies (all >36 weeks) included in the study, yielding an average of $80 \pm 7\%$ (range 68–90%). No correlation was observed between fetal hemoglobin fraction, and either blood T_2 or HbO_2 . The experimental transverse relaxation rates R_2 from all 77 samples plotted versus $(1 - \text{HbO}_2)^2$ (with HbO_2 having been obtained by blood gas analysis), and Hct, are depicted in Figure 3a. The Hct to Hb ratio was found to be 3.62 ± 0.12 for these samples. The surface obtained by 3-parameter fitting the data to Equations [2] and [3] was then used to estimate constants k , $R_{2,\text{plasma}}$ and $R_{2,\text{RBC}}$. Decay rates increase with decreasing HbO_2 (depicted as increasing $(1 - \text{HbO}_2)^2$ in Figure 3c), as expected. The coefficient of determination of the fit was $R^2 = 0.94$ and the root mean square error between data points and predicted surface was 1.6 s^{-1} . The fitted value of k was 83.1 s^{-1} (95% CI 77.2–89.0) yielding the following relationship between R_{2o} and Hct: $R_{2o} = 1.1(1 - \text{Hct}) + 12.9\text{Hct} = 11.8\text{Hct} + 1.1 [\text{s}^{-1}]$ for Hct values in the range of 0.24 to 0.71, corresponding to $R_{2,\text{plasma}} = 1.1$ (95% CI –0.85–3.06) and $R_{2,\text{RBC}} = 12.9 \text{ s}^{-1}$ (95% CI 9.30–16.50). Figure 3c depicts profile lines through the surface in Figure 3a for a range of Hct values. Note the increase in slope (relaxivity K) with increasing Hct up to a value of 0.5, beyond which K decreases due to the quadratic Hct term in Equation [3]. The intercept R_{2o} however, continues to increase as a function of Hct (Equation [3]) (19,20) as a result of the well-known increase in relaxation rate with protein concentration.

Table 1 shows the fitted values of k , $R_{2,\text{plasma}}$, and $R_{2,\text{RBC}}$ for previously reported adult (10) and fetal calibration equations. $R_{2,\text{plasma}}$ was found to be lower in fetal blood (1.1 s^{-1} vs 1.5 s^{-1}), consistent with the lower viscosity of fetal plasma (see Table 1). While $R_{2,\text{RBC}}$ and k , which are a function of the intra- to extracellular frequency shift and exchange lifetime (9), were found to be 11% larger for fetal blood than that obtained previously for adult blood, potentially due to increased RBC volume, decreased deformability and differences in hemoglobin types (12,21,22). The average bias in HbO_2 estimates introduced by using the adult blood calibration and T_2 -prep bSSFP sequence (as opposed to that for fetal blood) is 4 – 7% depending on the Hct, in the range of expected values at the umbilical vessels (Figure 3d). This bias varies across the range of HbO_2 due to the quadratic relationship between HbO_2 and blood R_2 .

Fetal O_2 Delivery Rate

Fetal mass in the subjects studied ranged from 0.7 to 2.7 kg and could be fit to an exponential $y = 0.05e^{0.11x}$ with y = mass in kg and x = GA in weeks ($R^2 = 0.93$, p -value < 0.001, Figure 4). This empirically-derived relationship and is in good agreement with population median values (23). Umbilical vein BFR data were successfully acquired in 26 of the 30 participants enrolled. Representative magnitude and velocity images used for the estimation of BFR are shown in Figure 5. Flow velocity at the UV was not found to be

significantly correlated with GA (average 8.1 ± 1.5 cm/s, Figure 6a). In agreement with previous reports (24), the cross-sectional area was found to increase with GA ($R^2=0.45$, p -value <0.05 , Figure 6b), resulting in a significant increase in BFR as pregnancy progresses ($R^2=0.68$, p -value <0.05 , Figure 6c). However, after normalization with respect to fetal mass, this relationship became non-significant (average BFR 88.2 ± 18.1 mL/min/kg, Figure 6d). Umbilical vein oximetry data were successfully acquired in 26 of the 30 enrolled participants (average ROI diameter 7 ± 4 pixels, range 5–9 pixels). Representative images of the five echoes acquired using T_2 -bSSFP and corresponding fitted T_2 decay are shown in Figures 7a and 7b. Oxygen saturation at the UV was quantified based on the values of k , $R_{2,plasma}$, and $R_{2,RBC}$ determined above. The value of Hct used for each participant was the population average for GA (25). Oxygen saturation at the UV was found to be independent of GA (Figure 7c), average HbO_2 being $84 \pm 7\%$ ($T_2=138 \pm 8$ ms), which is in good agreement with previously reported values measured using invasive methods (26).

Fetal O_2 delivery rate was estimated as the product of C_a of fetal hemoglobin, BFR and HbO_2 at the UV. The value of C_a (11) was scaled to the concentration of Hb based on the GA population average (25). Both BFR and HbO_2 data were successfully acquired in 22 of the 30 participants enrolled.

In Figure 8a fetal O_2 delivery rate (mL O_2 /min) is plotted against MRI-based fetal weight; indicating a significant relationship between these two measurements ($R^2=0.65$, p -value <0.01) with a slope of 16.3mL O_2 /min/kg. Oxygen delivery in units of mL O_2 /min significantly increased as pregnancy progressed as a result of increasing BFR ($R^2=0.76$, p -value <0.001 , Figure 8b). However, after normalization to fetal mass O_2 delivery rate was found to be invariant throughout GA (average 15.1 ± 3.8 mL O_2 /min/kg; Figure 8c).

Discussion

The purpose of this study was to demonstrate the feasibility of estimating fetal O_2 delivery rate *in vivo* across a range of gestational ages with quantitative MRI at 1.5T. Phase-contrast MRI and T_2 -based oximetry were used to estimate UV BFR and HbO_2 , respectively. In order to attain this objective, a calibration equation had to be derived using fetal blood from umbilical cord blood samples to convert blood T_2 to HbO_2 values based on T_2 measurements with a T_2 -prepared balanced SSFP sequence since the biophysical, chemical, and rheological properties of fetal and adult blood differ from each other. For instance, a large fraction of total Hb present in the fetal circulation is fetal hemoglobin (22), which has a higher oxygen affinity than adult hemoglobin. In addition, fetal hematocrit in the third trimester of pregnancy is considerably higher than the normal physiological range in adults (25). This property is counterbalanced by reduced fetal plasma viscosity (12). These differences between adult and fetal blood prompted the derivation of a calibration equation for converting fetal blood T_2 to HbO_2 as a means to estimate fetal O_2 transport. As shown in Figure 3c the error incurred, if instead the adult calibration curve were used, could be as large as 10%. If average HbO_2 and fetal O_2 delivery were estimated utilizing the calibration curve generated for adult blood would result in an underestimation of $4 \pm 3\%$ (HbO_2 units, range 1–10%) and 1 ± 1 mL O_2 /min (range 0–4 mL O_2 /min), respectively. Although these values are similar to each other, in 6 cases it was not possible to convert fetal blood T_2 to

HbO₂ values using the adult blood calibration equation as the measured blood T₂ was larger than T₂₀, resulting in invalid HbO₂ estimations.

The relationship between T₂ and HbO₂ depends on magnetic field strength, inter-refocusing interval of the T₂-preparation and readout strategy. In some previously reported T₂-HbO₂ calibration pulse sequences the center of k-space is scanned immediately after T₂ preparation (9,27). In that situation, the spatial encoding is not relevant and the established calibration equation can be used as long as the T₂-preparation parameters are identical to those used to determine that particular calibration. This would also be the case for centric encoding with bSSFP readout, which, however, is not recommended since it causes artifacts due to undesirable k-space modulation. Sequential encoding is therefore preferable as implemented in the present work. For this reason, the center of k-space is not acquired immediately after T₂-preparation, and the sequence employed to determine the calibration equation for conversion of blood T₂ to HbO₂ *ex vivo* needs to match the one used to conduct the *in vivo* studies. In the present work, bSSFP spatial encoding was based on a standard Siemens sequence consisting of a 10-pulse cycle ramp-up catalyzation and linear ordering that included the acquisition of 14 reference lines before traversing the center of k-space in the 25th pulse cycle. Between the end of the T₂-preparation and the acquisition of the center of k-space the MRI T₂-prepared signal decays as a function of T₂/T₁. Therefore, in order to utilize the calibration equation described above both the spatial encoding and T₂-preparation parameters must be closely matched to those presented here which, however, should be straightforward to achieve.

Fetal blood samples used to establish the aforementioned calibration equation were obtained after delivery at term (GA>37 weeks). In the present study we have used this equation to extract HbO₂ information from fetuses of average GA of 30±3 weeks (range 23–35 weeks). Changes in fetal blood composition throughout gestation may influence the accuracy of the HbO₂ estimation. These changes include variations in the fetal hemoglobin fraction (22), which in the blood from eight of the umbilical cords used averaged 87%. However, since at earlier GA this fraction is even larger, any contribution from adult hemoglobin can be ignored. Further, total Hb concentration, Hct (25) and total plasma protein content (28) increase throughout pregnancy. These changes lead to progressively higher fetal whole-blood viscosity, which would affect R_{2,plasma} and thus whole-blood R₂ (as well as R₁). However, raised whole-blood viscosity throughout gestation has been shown to be mainly attributable to hematocrit (29). Therefore, the effect of the variations in fetal blood composition, except those resulting from changes in hematocrit and hemoglobin concentration throughout gestation can be assumed to be negligible.

In the present study, population average Hct values were used as means to estimate subject-specific hematocrit *in vivo* (25) since Hct measurements on fetal blood is only possible upon delivery and therefore would not account for variations during pregnancy. However, it is known that the longitudinal relaxation rate, 1/T₁, scales linearly with Hct (19,30). This relationship has recently been used to convert fetal whole-blood T₁ to Hct in the context of calibrating spin-echo T₂ measurements of fetal blood to HbO₂ (27). The empirically determined association between blood water T₁ and Hct may improve the accuracy of future T₂-based oximetry studies.

Fetal O₂ delivery has been previously measured in humans at late gestation (36 weeks of GA) with quantitative MRI (8) and at term (38–42 weeks of GA) using a combination of ultrasound and invasive methods (26). Sun et al utilized PC-MRI with metric-optimized gating (31) to measure BFR and T₂-based oximetry to estimate HbO₂ at the fetal abdominal insertion of the UV (8). In contrast, the BFR data reported by Acharya et al, were based on Doppler ultrasound, obtained within 24 hours prior to delivery (26). In the latter study oxygen saturation of the umbilical vessels was measured immediately after delivery with blood gas analysis, along with neonatal mass (26). However, to the best of the present authors' knowledge, fetal O₂ delivery in humans *in vivo* had not previously been reported across a range of GAs.

The average UV BFR values reported by the above two studies somewhat differ from those in the present work: 89.5±17.2 mL/min/kg, versus 129±28 mL/min/kg (8), 67±30 mL/min/kg (26). The differences in GAs among these three studies may to some extent account for the discrepancies in BFR. Although in the present study no changes in BFR were found across GAs, this result cannot be extrapolated to the full-term case due to the limited number of study subjects. In fact, fetal mass-normalized BFR has been previously reported to decrease towards term (23,32). Differences in BFR may then be related to the different methods used to measure flow velocity.

Overall, fetal mass estimates are in excellent agreement with those expected in the second trimester of pregnancy (33), but smaller by approximately 0.4kg in the third trimester (Supporting Figure S2a). Before normalizing to fetal mass, reported BFR for a GA of 35 weeks was approximately 400 mL/min (8) and 247 mL/min (26), compared to 230 mL/min in the present study (33). Our BFR results thus are comparable to those reported by Acharya et al measured with Doppler ultrasound, which has been shown to accurately quantify blood flow measurements when performed appropriately (34). Similarly, BFR data across GAs are in excellent agreement with those measured by Bellotti et al (23), also acquired by Doppler ultrasound (Supporting Figure S2b). Based on the population average values of UV BFR (mL/min) and fetal mass (kg), fetal mass-normalized BFR (mL/min/kg) across GAs can be estimated. Supporting Figure S2c shows that BFR results are in fair agreement with expected values. The difference in shape between the plots depicted in Supporting Figure S2c (open circles) are due to the fact that fetal growth rate is larger than the rate of BFR increase rate towards term (1). However, in the present study the estimated growth rates for both parameters were the same (0.11, Figure 4b and Figure 6c) potentially due to the limited number of subjects in late GAs. Finally, the bias in BFR values between the present study and those reported by Sun et al may be due to differences in the methods. Future work is necessary to determine the accuracy of these three different methodologies in measuring BFR at the umbilical vessels.

The values for HbO₂ reported by Acharya et al (26) are ~18% lower than those estimated in the present study, which conform to other observations suggesting a decrease in HbO₂ toward the end of pregnancy (35). Therefore, this apparent disparity may be caused by the lower GAs of the participants in the present study and differences in measurement methods. Nevertheless, our HbO₂ estimates (84±7%) are in good agreement with those reported previously (80–82%) from invasive (1,3,4) and, more recently, MRI-based oximetry (8) for

second and third trimesters of pregnancies. Despite differences in BFR and HbO₂, the estimated values of fetal O₂ delivery calculated here (15.3±3.7 mL O₂/min/kg) are in good agreement with those measured with invasive methods by Acharya et al (13.4±6.9 mL O₂/min/kg) (13), but lower than those by Sun et al (20.4±4.2 mL O₂/min/kg) (8).

The present calibration equation and methods may, in theory, also be applied to quantifying fetal O₂ consumption rate to study fetal O₂ metabolism, which can be estimated via Fick's principle:

$$\text{Fetal O}_2 \text{ Consumption Rate} = C_a \cdot \text{BFR} \cdot \text{AvO}_2 / \text{fetal mass} \quad [4]$$

Here, AvO₂ is the arteriovenous difference in HbO₂ between umbilical vein and arteries. As presented herein, it is feasible to estimate UV HbO₂, however, the measurement of blood T₂ in the umbilical arteries is difficult due to the small vessel size. For example, the typical diameter of the umbilical arteries is 4mm at 36 weeks of gestation (36), therefore limiting the number of pixels across the vessel diameter and possibly compromising the accuracy of the measured T₂. An alternative approach is to measure T₂ in the fetal abdominal aorta from which the umbilical arteries branch off, thereby yielding a surrogate of umbilical artery HbO₂ as shown in a recent study involving third trimester pregnancies (8). Future work will focus on assessing the feasibility of T₂-based oximetry to estimate fetal O₂ consumption in the second and third trimesters of pregnancy.

In conclusion, the derived calibration curve for fetal blood should ensure improved accuracy in fetal-blood oximetry. Finally, this work demonstrates the feasibility of noninvasively measuring fetal O₂ delivery *in vivo* with a combination of T₂-based oximetry and phase-contrast flow imaging in the umbilical circulation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Creasy, RK., Resnik, R., Greene, MF., Iams, JD., Lockwood, CJ. Creasy and Resnik's maternal-fetal medicine : principles and practice. Vol. xxiv. Philadelphia, PA: Elsevier/Saunders; 2014. p. 1294
2. Wang, Y., Zhao, S. Vascular Biology of the Placenta, Integrated Systems Physiology: from Molecules to Function to Disease. San Rafael (CA): 2010.
3. Brinkman CR 3rd. Umbilical blood flow and fetal oxygen consumption. Clin Obstet Gynecol. 1970; 13(3):565–78. [PubMed: 5493912]
4. Arikan GM, Scholz HS, Petru E, Haeusler MC, Haas J, Weiss PA. Cord blood oxygen saturation in vigorous infants at birth: what is normal? BJOG. 2000; 107(8):987–94. [PubMed: 10955430]

5. Weiner CP, Williamson RA. Evaluation of severe growth retardation using cordocentesis--hematologic and metabolic alterations by etiology. *Obstet Gynecol.* 1989; 73(2):225–9. [PubMed: 2536145]
6. Siggaard-Andersen O, Huch R. The oxygen status of fetal blood. *Acta Anaesthesiol Scand Suppl.* 1995; 107:129–35. [PubMed: 8599265]
7. Baker PN, Johnson IR, Gowland PA, Hykin J, Harvey PR, Freeman A, Adams V, Worthington BS, Mansfield P. Fetal weight estimation by echo-planar magnetic resonance imaging. *Lancet.* 1994; 343(8898):644–5. [PubMed: 7906814]
8. Sun L, Macgowan CK, Sled JG, Yoo SJ, Manlhiot C, Porayette P, Grosse-Wortmann L, Jaeggi E, McCrindle BW, Kingdom J, et al. Reduced fetal cerebral oxygen consumption is associated with smaller brain size in fetuses with congenital heart disease. *Circulation.* 2015; 131(15):1313–23. [PubMed: 25762062]
9. Wright GA, Hu BS, Macovski A. 1991 I.I. Rabi Award. Estimating oxygen saturation of blood in vivo with MR imaging at 1.5 T. *J Magn Reson Imaging.* 1991; 1(3):275–83. [PubMed: 1802140]
10. Rodríguez-Soto AE, Abdulmalik O, Langham MC, Schwartz N, Lee H, Wehrli FW. T2-prepared balanced steady-state free precession (bSSFP) for quantifying whole-blood oxygen saturation at 1.5T. *Magn Reson Med.* 2017
11. Gregory IC. The oxygen and carbon monoxide capacities of fetal and adult blood. *J Physiol.* 1974; 236(3):625–34. [PubMed: 4822578]
12. Rampling MW, Whittingstall P, Martin G, Bignall S, Rivers RP, Lissauer TJ, Bailey PC. A comparison of the rheologic properties of neonatal and adult blood. *Pediatr Res.* 1989; 25(5):457–60. [PubMed: 2717261]
13. Abdulmalik O, Safo MK, Lerner NB, Ochotorena J, Daikhin E, Lakka V, Santacroce R, Abraham DJ, Asakura T. Characterization of hemoglobin bassett (alpha94Asp-->Ala), a variant with very low oxygen affinity. *Am J Hematol.* 2004; 77(3):268–76. [PubMed: 15495251]
14. Abdulmalik O, Safo MK, Chen Q, Yang J, Brugnara C, Ohene-Frempong K, Abraham DJ, Asakura T. 5-hydroxymethyl-2-furfural modifies intracellular sickle haemoglobin and inhibits sickling of red blood cells. *Br J Haematol.* 2005; 128(4):552–61. [PubMed: 15686467]
15. Abdulmalik O, Safo MK, Seeholzer SH, Asakura T, Hasbrouck NC, Russell JE. Hb Baden: structural and functional characterization. *Am J Hematol.* 2010; 85(11):848–52. [PubMed: 20872549]
16. Abdulmalik O, Ghatge MS, Musayev FN, Parikh A, Chen Q, Yang J, Nnamani I, Danso-Danquah R, Eseonu DN, Asakura T, et al. Crystallographic analysis of human hemoglobin elucidates the structural basis of the potent and dual antisickling activity of pyridyl derivatives of vanillin. *Acta Crystallogr D Biol Crystallogr.* 2011; 67(Pt 11):920–8. [PubMed: 22101818]
17. Luz Z, Meiboom S. Nuclear Magnetic Resonance study of the protolysis of trimethylammonium ion in aqueous solution. *The Journal of Chemical Physics.* 1963; 39:366–370.
18. van Zijl PC, Eleff SM, Ulatowski JA, Oja JM, Ulug AM, Traystman RJ, Kauppinen RA. Quantitative assessment of blood flow, blood volume and blood oxygenation effects in functional magnetic resonance imaging. *Nat Med.* 1998; 4(2):159–67. [PubMed: 9461188]
19. Bryant RG, Marill K, Blackmore C, Francis C. Magnetic-Relaxation in Blood and Blood-Clots. *Magnetic Resonance in Medicine.* 1990; 13(1):133–144. [PubMed: 2319929]
20. Thulborn KR, Waterton JC, Matthews PM, Radda GK. Oxygenation dependence of the transverse relaxation time of water protons in whole blood at high field. *Biochimica et Biophysica Acta (BBA) - General Subjects.* 1982; 714(2):265–270. [PubMed: 6275909]
21. Riopel L, Fouron JC, Bard H. Blood viscosity during the neonatal period: the role of plasma and red blood cell type. *J Pediatr.* 1982; 100(3):449–53. [PubMed: 7062182]
22. Galacteros F, Guillaud-Bataille M, Feingold J. Sex, gestational age, and weight dependency of adult hemoglobin concentration in normal newborns. *Blood.* 1991; 78(4):1121–4. [PubMed: 1868243]
23. Bellotti M, Pennati G, De Gasperi C, Battaglia FC, Ferrazzi E. Role of ductus venosus in distribution of umbilical blood flow in human fetuses during second half of pregnancy. *Am J Physiol Heart Circ Physiol.* 2000; 279(3):H1256–63. [PubMed: 10993792]

24. Boito S, Struijk PC, Ursem NT, Stijnen T, Wladimiroff JW. Umbilical venous volume flow in the normally developing and growth-restricted human fetus. *Ultrasound Obstet Gynecol.* 2002; 19(4): 344–9. [PubMed: 11952962]
25. Jopling J, Henry E, Wiedmeier SE, Christensen RD. Reference ranges for hematocrit and blood hemoglobin concentration during the neonatal period: data from a multihospital health care system. *Pediatrics.* 2009; 123(2):e333–7. [PubMed: 19171584]
26. Acharya G, Sitras V. Oxygen uptake of the human fetus at term. *Acta Obstet Gynecol Scand.* 2009; 88(1):104–9. [PubMed: 18991146]
27. Portnoy S, Osmond M, Zhu MY, Seed M, Sled JG, Macgowan CK. Relaxation properties of human umbilical cord blood at 1.5 Tesla. *Magn Reson Med.* 2017; 77(4):1678–1690. [PubMed: 27059881]
28. Linderkamp O, Versmold HT, Riegel KP, Betke K. Contributions of red cells and plasma to blood viscosity in preterm and full-term infants and adults. *Pediatrics.* 1984; 74(1):45–51. [PubMed: 6204271]
29. Christensen RD, Baer VL, Gerday E, Sheffield MJ, Richards DS, Shepherd JG, Snow GL, Bennett ST, Frank EL, Oh W. Whole-blood viscosity in the neonate: effects of gestational age, hematocrit, mean corpuscular volume and umbilical cord milking. *J Perinatol.* 2014; 34(1):16–21. [PubMed: 24030677]
30. Lu H, Clingman C, Golay X, van Zijl PC. Determining the longitudinal relaxation time (T1) of blood at 3.0 Tesla. *Magn Reson Med.* 2004; 52(3):679–82. [PubMed: 15334591]
31. Jansz MS, Seed M, van Amerom JF, Wong D, Grosse-Wortmann L, Yoo SJ, Macgowan CK. Metric optimized gating for fetal cardiac MRI. *Magn Reson Med.* 2010; 64(5):1304–14. [PubMed: 20632406]
32. Acharya G, Wilsaard T, Rosvold Berntsen GK, Maltau JM, Kiserud T. Reference ranges for umbilical vein blood flow in the second half of pregnancy based on longitudinal data. *Prenat Diagn.* 2005; 25(2):99–111. [PubMed: 15712315]
33. Alexander GR, Kogan MD, Himes JH. 1994–1996. U.S. singleton birth weight percentiles for gestational age by race, Hispanic origin, and gender. *Matern Child Health J.* 1999; 3(4):225–31. [PubMed: 10791363]
34. Hoyt K, Hester FA, Bell RL, Lockhart ME, Robbin ML. Accuracy of volumetric flow rate measurements: an in vitro study using modern ultrasound scanners. *J Ultrasound Med.* 2009; 28(11):1511–8. [PubMed: 19854966]
35. Soothill PW, Nicolaides KH, Rodeck CH, Campbell S. Effect of gestational age on fetal and intervillous blood gas and acid-base values in human pregnancy. *Fetal Ther.* 1986; 1(4):168–75. [PubMed: 3454532]
36. Persutte WH, Lenke RR. Transverse umbilical arterial diameter: technique for the prenatal diagnosis of single umbilical artery. *J Ultrasound Med.* 1994; 13(10):763–6. [PubMed: 7823338]

Figure 1.

(a) Schematic of oxygen transport between maternal and fetal circulation. Maternal arterial circulation delivers highly oxygenated blood to the placenta, however, maternal and fetal blood are never in direct contact. O₂ diffuses from the maternal to the fetal compartment and is delivered to the fetus via the umbilical vein (UV). After O₂ extraction deoxygenated blood returns to the placenta via umbilical arteries, where carbon dioxide and waste products diffuse to the maternal compartment and are removed by the maternal venous system. Fetal O₂ delivery rate may be used as means to evaluate placental function and (b) is estimated as the product of the oxygen carrying capacity of hemoglobin (C_a), hemoglobin (Hb)

concentration, blood flow rate (BFR) and oxygen saturation (HbO₂) at the UV, normalized by fetal mass. All of the above parameters can be determined via quantitative MRI methods.

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Figure 2.

T₂-prepared bSSFP sequence consisting of saturation pulses to null the magnetization after each T₂-preparation, 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 ($\tau_{180}=12\text{ms}$), and bSSFP encoding with linear ramp-up signal stabilization and partial Fourier sampling.

Figure 3.

(a) Blood R_2 as a function of the square of deoxyhemoglobin fraction $(1 - HbO_2)^2$ and Hct from 77 fetal blood samples, measured with a T_2 -prepared bSSFP sequence along with fitted surface $R_2 = R_{20}(Hct) + k \cdot Hct \cdot (1 - Hct) \cdot (1 - HbO_2)^2$ in gray; $R^2=0.94$. Red and blue markers represent data points above and below the fitted surface, respectively. (b) Plot of the residuals of R_2 measurements w.r.t the fitted surface of panel (a). (c) Profiles of R_2 versus $(1 - HbO_2)^2$ derived from data of panel A. Thin lines correspond to profiles across the fitted surface in panel A for Hct values in the range of 0.25–0.65. Thick lines correspond to Hct values of 0.3, 0.4, 0.5 and 0.6. The corresponding K and R_{20} values are 17.4, 19.9, 20.8, and 19.9 s $^{-1}$ and 215, 171, 142, 122 ms, respectively. (d) Plot of bias in fetal blood HbO_2 estimation introduced by using the adult blood calibration equation to convert fetal blood T_2 to HbO_2 values, for the same range of Hct values.

Figure 4.

(a) Representative coronal half-Fourier single-shot turbo spin-echo (HASTE) image of a gravid uterus. Red contour depicts the segmentation of the fetus on a single image. Segmentations of the entire fetal body were used to estimate fetal mass from volume information. Fetal volume was then converted to mass using the relationship determined by Baker et al: $\text{mass (kg)} = 1.03 \cdot \text{volume (dm}^3\text{)} + 0.12 \text{ (kg)}$. (b) Plot of fetal mass in kg versus gestational age (GA).

Figure 5.

Examples of phase-contrast (PC) MRI (a–c) magnitude images and (d–f) velocity maps of the umbilical vein (UV) of three different participants. Data were acquired during a single breath hold and flow velocity measurements were the average of two datasets without fetal motion, as observed in the complex difference intensity images.

Figure 6.

Plots of the parameters used to extract fetal mass-normalized blood flow rate (BFR, mL/min/kg). The regions of interest of the umbilical vein used to estimate (A) flow velocity (cm/s) and (b) cross-sectional area (mm²) were manually traced on phase-contrast (PC) MRI images. (c) Blood flow rate (mL/min) was estimated as the product of flow velocity and cross-sectional area and (d) was normalized to fetal mass.

Figure 7.

(a) Example of images used to estimate umbilical vein blood T_2 . TE: echo time. (b) Plot of a T_2 decay of the umbilical vein (UV) blood signal *in vivo*. T_2 was estimated with a three-parameter fit $S(t) = S_0 e^{-t/T_2} + C$, including TE correction. (c) Oxygen saturation (HbO_2) at the UV was measured across gestational ages (GAs) in 26 participants *in vivo*. Open points indicate cases in which UV regions of interest (ROIs) are 6 or more pixels in diameter. Solid points indicate cases when ROIs are only 5 pixels in diameter.

Figure 8.

Plot of (a) fetal O₂ delivery rate versus MRI based fetal mass, (b) estimated fetal O₂ delivery rate and (c) normalized with fetal mass across gestational ages (GAs) measured *in vivo* with quantitative MRI methods.

Table 1

Fitted parameters of the calibration equations to convert fetal and blood T₂ to HbO₂ values.

Parameter	Adult ⁽¹⁸⁾	Fetal
k (s ⁻¹)	74.2	83.1
R _{2,plasma} (s ⁻¹)	1.5	1.1
R _{2,RBC} (s ⁻¹)	11.6	12.9
Hematocrit range (%)	0.23–0.53	0.24–0.71
R ²	0.95	0.94
RMSE (s ⁻¹)	1.1	1.6
RMSD (%)	5.4%	6.9%