Noninvasive monitoring of red blood cell transfusion in very low birthweight infants using diffuse optical spectroscopy

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Abstract. Red blood cell (RBC) transfusion guidelines are designed to maintain adequate tissue oxygenation by increasing blood oxygen-carrying capacity. However, since tissue oxygenation is not measured, RBC transfusion guidelines are mostly subjective. Clinical evidence of oxygen transport/consumption mismatches in infants is often unclear and confounded by multiple factors. Invasive hemoglobin measurements can contribute further to anemia if performed too frequently. Diffuse optical spectroscopy (DOS) is a noninvasive quantitative method to measure the tissue oxy, deoxy, and total hemoglobin concentrations (ctO_2Hb, ctHb, ctTHb), as well as mixed arterial-venous tissue hemoglobin saturation (stO_2). Our objective is to determine if DOS can assess changes in tissue oxygenation in very low birth weight (VLBW) infants undergoing RBC transfusions. DOS measurements of ctO_2Hb and ctHb are performed on 10 VLBW infants before and within 24 h after RBC transfusion. Seven nontransfused infants are studied to evaluate hemodynamic variations independent of RBC transfusion. Tissue near-infrared absorption and scattering values are measured using a four-wavelength (690, 750, 810, and 830 nm) frequency-domain tissue oximeter (OxiplexTS, ISS, Champaign, Illinois). In transfused subjects, DOS demonstrates significant increases in ctO_2Hb (48 ± 13 versus 74 ± 20 μM, $p<0.002$), ctTHb (87 ± 17 versus 107 ± 24 μM, $p=0.004$), and stO_2 (54 ± 8 versus $68\pm6\%$, $p<0.004$) post-transfusion. DOS measurements correlate with mean hemoglobin increases for all infants ($r=0.83$, $p<0.0001$). No significant DOS changes occurred in the nontransfused group. Calculations of the differential path length for these transfused subjects show high variability ($\sim$20%). DOS may serve as a noninvasive bedside tool to assess tissue oxygenation in infants and provide a functionally based transfusion trigger.
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1 Introduction

1.1 *Neonatal Anemia*

Neonatal anemia is a frequent occurrence in neonatal intensive care units (NICUs) and is a common cause of tissue hypoxia (i.e., low tissue oxygenation) in very low birthweight (VLBW) infants (401 to 1500 g). (We define anemia as low hemoglobin concentration, and tissue hypoxia as a general oxygen debt.) It is typical for blood hemoglobin concentrations in term and preterm infants to decrease over the first 10 weeks after birth, due to both physiologic and iatrogenic reasons. For example, Ohls reports that infants between 1001 to 1500 g at birth experience a drop in blood hemoglobin concentration from 15.1 g dL^{-1} 1 day after birth down to 9.1 g dL^{-1} 9 weeks later.[1] Routine hemoglobin sampling further depletes the oxygen-carrying capacity of the blood.

However, blood hemoglobin concentration is only one factor that contributes to tissue oxygenation. Red blood cell (RBC) transfusion guidelines are designed to maintain adequate tissue oxygenation by increasing blood oxygen-carrying capacity. Unfortunately, tissue oxygenation is not measured and is not easily assessed in clinical situations. For this reason, RBC transfusion criteria for infants are largely subjective, based on collective experience and consensus within groups. Guidelines are generally compiled to standardize transfusion practices while studying the effects of other treatments or protocols rather than the result of research aim-

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ing to identify the best criteria or guidelines for transfusing infants.[2]

RBC transfusions are given to 60 to 80% of all VLBW infants during their initial hospital course.[3] Because the symptoms of tissue hypoxia are nonspecific, objectively demonstrating the benefit from correction of anemia is difficult. Recent studies comparing infants receiving transfusions show that in large populations with varying transfusion practices, there are no measurable differences in outcomes (e.g., days on ventilator, O_2 requirement at 28 days, rate of growth, incidence of chronic lung disease, or length of stay).[4,5] The ability to assess reliably and noninvasively the degree of tissue hypoxia, as well as the response to intervention, would improve our understanding of the clinical significance of anemia in VLBW infants.

1.2 *Optical Detection of Neonatal Anemia*

Near-infrared spectroscopy (NIRS) has a strong sensitivity to hemoglobin, and can also penetrate deeply enough into tissues to sample muscle and brain. Considering the success of NIRS in detecting tissue hemoglobin, investigators have sought to monitor tissue hemoglobin to detect and treat neonatal anemia. Previous investigators have studied changes in tissue oxygenation in response to transfusions and/or blood loss in both neural[6–9] and peripheral tissues.[9,10] These NIRS studies have generally measured reflectance intensity differences and have shown promise in evaluating relative changes in tissue oxygenation.

Tissue-level measurements that reflect inadequate systemic oxygen transport (SOT) to meet oxygen demands are preferable to measurements of absolute hemoglobin concentration or level of hematocrit.[11] Along these lines, Wardle et al.[12] compared transfusion decisions for VLBW infants using traditional clinical practices (blood hemoglobin measurement and clinical judgment) versus fractional oxygen extraction (FOE) using NIRS with venous occlusion. Infants in the NIRS group were transfused for FOE >0.47, but could also be transfused at lower FOE if deemed necessary by the clinicians. No difference was found in the number of transfusions given to infants in either group. 59% of infants transfused using hemoglobin criteria and clinical judgment had FOE <0.47. The investigators concluded that either the FOE criteria was not sufficiently sensitive, or that clinicians may have relied on vague and nonspecific indicators for transfusion.[12]

1.3 *Aims of this Study*

Our long-term goal is to determine if an optical assessment of tissue oxygenation could assist in the neonatal transfusion decision-making process. The study hypothesis is that diffuse optical spectroscopy (DOS) can quantify improvements in tissue oxygenation resulting from RBC transfusions. This preliminary study was designed to explore DOS as a means to quantify tissue concentrations of oxy- (ctO_2Hb) and deoxy- (ctHb) hemoglobin in response to VLBW infant RBC transfusion.[13] (Our notation is designed to comply with the suggestions of Zander and Mertzlufft: the c denotes a concentration (μM), the t denotes the tissue, and the T denotes total.) We do not consider at this stage the direct clinical benefit of DOS, but rather we offer an assessment of the sensitivity and capabilities of DOS.

Our DOS approach differs in general from previous NIRS approaches, because we have measured tissue scattering by using a frequency domain photon migration (FDPM) approach, as opposed to using *a priori* photon path length estimates. We have assessed the pretransfusion state as well as the post-transfusion responses in muscle tissue by measuring *absolute* concentrations of tissue hemoglobin in both oxygen-bound and oxygen-free forms. We also investigated the dependence of our results on the choice of wavelengths (two versus four). Finally, we calculated the differential path length factor (DPF) for each wavelength using the frequency-domain measured absorption (μ_a) and reduced scattering (μ_s') coefficients. Though others have observed changes in tissue hemoglobin concentrations, and thus changes in tissue oxygenation, our analysis is important because the majority of NIRS measurements do not measure scattering, and as such, the influence of scattering on the results is unknown.

2 Methods

2.1 *Study Population*

This study was approved by the University of California, Irvine Institutional Review Board (protocol 2001-2011). Ten VLBW receiving RBC transfusion based on clinical indications by their respective neonatologists were enrolled following parental written informed consent. The decision to transfuse infants at our institution, as at most NICUs, is made on review of an infant's blood hemoglobin level and overall severity of clinical condition, using criteria quite similar to (and partially derived from) that used by Ohls et al. in their recent erythropoietin study.[2] Seven LBW infants not receiving transfusions were also enrolled to evaluate temporal changes in measurements in the absence of RBC transfusion.

2.2 *Procedure*

All transfused infants underwent DOS measurements of ctHb and ctO_2Hb immediately prior to transfusion. The transfused infants were measured again 6 to 24 h post-transfusion. The seven nontransfused infants were measured approximately 24 h apart. Standard invasive hemoglobin and blood gas measurements were taken before and after transfusions as per standard NICU protocol. No additional blood sampling was obtained for this study, thus invasive blood data were not available for the nontransfused infants on the second day.

2.3 *Diffuse Optical Spectroscopy Measurements*

These DOS measurements used FDPM technology in the NIR to measure tissue absorption and scattering properties.[14] The principal NIR tissue absorbers include oxygenated and deoxygenated hemoglobin (O_2Hb and Hb, respectively), particularly between 600 to 850 nm.[15] Because diffusive light samples a large volume of tissue, DOS reports on tissue at the mixed arterial-venous (capillary) level.[16] Pulse oximetry, a more familiar application of NIR light, measures *arterial* oxygen saturation (saO_2), and does not quantify tissue hemoglobin concentrations. Most NIRS systems reported in the literature make assumptions about the path length of light in tissue or report tHb to tO_2Hb ratios.[17] Photon path lengths are directly measured in DOS, thus allowing the separation of ab-

sorption from scattering and quantitation of absolute tissue hemoglobin concentrations.

The NIR DOS instrument (OxiplexTS, ISS, Champaign, Illinois) used for this study is a dual-channel tissue oximeter, featuring two wavelengths and two independent channels.[18,19] A custom system, featuring 830/690 nm for one channel and 810/750 nm for the other, was provided by ISS. The instrument was configured so that a single channel could be used with all four wavelengths. Four distances ranging from 10 to 20 mm were used for a single measurement. Tissue absorption spectra measured at four wavelengths (690, 750, 810, and 830 nm) were translated into hemoglobin concentrations using previously measured extinction coefficients.[20] Tissue water concentration was assumed to be 75% of pure water.

All optical signals were directed via optical fibers contained in a custom designed plastic handheld probe applied with gentle pressure on the skin surface. Measurements were performed in the interscapular region while the infant lay prone in a quiet state. The interscapular site was chosen because 1. it was easily accessible, 2. it has a large amount of muscle, and 3. it has a relatively thin fat layer. The acquisition time for an individual measurement of ctHb and ctO_2Hb was less than 1 s, although faster acquisition times may be achieved. Each of these concentration measurements was averaged for approximately 30 s to minimize physiologic and motion variations. These measurements were then repeated several times by removing and replacing the probe on the skin surface to determine probe-to-tissue coupling variations. The total acquisition time was less than 5 min per infant.

Theoretical calculations estimate that the *average* penetration of the light into the tissue in our experimental configuration is approximately 5 mm below the skin, and roughly 90% of the signal is derived from a region ranging in depth from 2 to 8 mm.[21] Total tissue hemoglobin concentration (ctTHb) and tissue hemoglobin saturation (stO_2) were calculated directly from the measurement of ctHb and ct O_2Hb according to the formulae: $ctTHb = ctO_2Hb + ctHb$ and $stO_2 = ctO_2Hb/ctTHb^*100$. Details of these calculations are described elsewhere.[22] The tissue was assumed to be macroscopically homogeneous in accordance with diffusion theory. Due to reasons outlined in the discussion section, we assume that the myoglobin concentration is zero and has no effect on these measurements. Additionally, we assumed that the contributions of other NIR-absorbing chromophores (such as cytochrome and lipids) to be negligible.

2.4 *Statistical Analysis*

Comparisons between the 10 pre- and post-transfusion DOS measurements were performed using the nonparametric two-tailed paired Wilcoxon ranked sum test, assuming significance within a confidence interval of 95% ($\alpha = 0.05$). Analysis of variance (ANOVA) was also performed with a 95% confidence interval to discriminate between pretransfusion, post-transfusion, and nontransfused population stO_2 measurements. These stO_2 values were transformed using a simple logarithm to preserve normalcy for the ANOVA analysis (Shapiro-Wilk W test, $W < 0.019$). Correlations between DOS measurements and invasive blood hemoglobin sampling were performed using a weighted regression analysis using all 17 infants. We report the correlation coefficient (r) for all linear fits. Error bars for individual measurements represent the standard deviation from successive repeated measurements. Error bars for population data are the standard deviation of the population (i.e., not the standard error of the mean).

Table 1 Demographic characteristics of transfused and nontransfused infants (mean ± SD of the mean).

	Transfusion group	Nontransfused group
Number of infants	10	7
Birthweight (g)	796 ± 46	1530 ± 420
Study weight (g)	1370 ± 710	1890 ± 560
Gestational age at birth (wk)	26.4 ± 1.5	31.1 ± 2.6
Postnatal age (days)	35 ± 24	27 ± 18
Number of prior transfusions	2 ± 2	1 ± 1
Baseline blood hemoglobin (g/dl)	8.67 ± 1.0	12.3 ± 3.1

2.5 *Differential Pathlength Calculations*

The DPF is a correction to the Beer-Lambert law to account for multiple scattering.[23] This correction modifies the photon path length L as $L = \rho \times DPF$, where ρ is the source-detector separation. The DPF then is a scale factor between the known geometric photon path (i.e., ρ), and the actual photon path L. We have adopted the following formula for the calculation of the DPF from frequency-domain data in a semi-infinite medium geometry as shown by Fantini et al.:[17]

$$\mathrm{DPF} = \frac{\sqrt{3}}{2}\sqrt{\frac{\mu_s'}{\mu_a}}\frac{\rho\sqrt{3\mu_a\mu_s'}}{\rho\sqrt{3\mu_a\mu_s'}+1}. \quad (1)$$

Since our measurements required ρ values ranging from 10 to 20 mm, all DPF values are reported using the average distance of 15 mm.

3 Results

Table 1 demonstrates the infant characteristics for each population, where the error bars represent the standard deviation (SD) of the population. Infants served as their own controls for the measurements of transfusion response.

3.1 *Time Course for a Single Transfusion*

Figure 1 presents a measurement of ctO_2Hb in a single subject over the entire transfusion period (started at $t = 0$ and finished at $t = 240$ min). Over time, DOS measurements demonstrated clear changes in the tissue concentrations of hemoglobin resulting from this transfusion. Error bars are the result of repeated measurements at each time point. The instrument was recalibrated at each time point to remove any drift effects. Similar results were observed for the ctTHb. Prior to transfu-

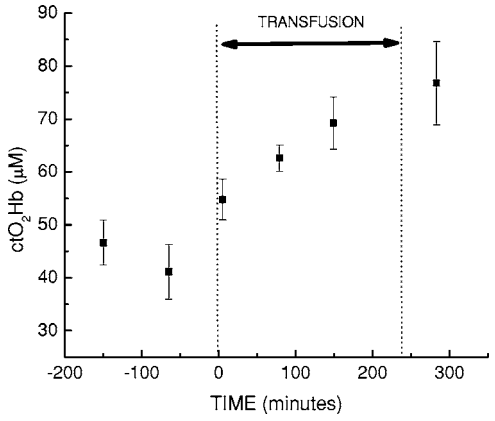

Fig. 1 Plot of noninvasively measured ctO_2Hb in a single infant during a transfusion. Time $t=0$ represents immediately prior to the transfusion. Error bars represent the SD of the results of repeated measurements at each time point. The system was calibrated at each measurement to ensure that the changes are not due to instrumental drift. The transfusion starts at the second data point. The same general trend exists for ctTHb (not shown).

sion, the two values overlap within the errors, thus establishing a baseline value. During the course of the transfusion (i.e., between the dotted lines), the increase of ctO_2Hb was linear with a slope of 0.101 ± 0.003 μM/min.

3.2 *Population Measurements*

Figure 2 presents comparisons between the initial and final measurements for each subject in terms of ctO_2Hb, ctHb, and stO_2. Figures 2(a), 2(c), and 2(e) represent the ten transfused subjects, whereas Figs. 2(b), 2(d), and 2(f) represent the seven nontransfused subjects. Each pair of graphs are plotted on the same scale. It is clear from the figure that both ctO_2Hb and stO_2 always increased in the transfused sample population. The nontransfused sample population displays both increases and decreases in ctO_2Hb and stO_2, and are generally of a much smaller magnitude. ctHb generally decreases in the transfused sample population.

Table 2 lists DOS-measured and calculated parameters for all ten transfused infants before and after transfusion. Error bars represent the SD of the sample population average. DOS measurements demonstrated statistically significant increases (as evidenced from the paired Wilcoxon ranked sum test) in ctO_2Hb, ctTHb, and stO_2 post-transfusion. ctO_2Hb increased for every subject ($p=0.0001$). Although ctHb changes after transfusion were not statistically significant ($p=0.086$), there was a downward trend after transfusion for nine of the ten infants. There was also a statistically significant increase in the ctTHb ($p=0.0007$). Post-transfusion blood sampling confirmed mean hemoglobin increases; these values are provided in Table 2 for comparison with DOS results. No changes in μ_s' were observed before and after transfusion at any wavelength. Additionally, there were no changes in saO_2 as reported by standard pulse oximetry (95.5 ± 3.7 to 95.4 ± 2.8). Similar results have been observed elsewhere.[8,9] There were no statistically significant changes in DOS over time for the seven nontransfused infants.

Table 2 Summary of pre- and post-transfusion measurements. * is a significant result.

	Pre ($N=10$)	Post ($N=10$)	$p<$
ctO_2Hb(μM)	48±13	74±20	0.002*
ctHb (μM)	40±10	33±6	0.11
ctTHb (μM)	87±17	107±24	0.004*
stO_2(%)	54±8	68±6	0.004*
Blood hemoglobin (g/dl)	8.7±1	12±1	0.002*
saO_2(%)	95.5±3.7	95.4±2.8	1.0

3.3 *Comparison with Invasive Sampling*

Figure 3 demonstrates the high degree of correlation between invasive blood sampling and DOS measurements of ctO_2Hb for all 17 infants. The straight line represents a fit of the independent initial measurements for all 17 subjects (squares). The extra points (triangles) represent the post-transfusion values for the ten transfused subjects. Repeated invasive hemoglobin values were not obtained for the nontransfused group.

The line in Fig. 3 is the result of an error-weighted linear fit through all points ($r=0.83, p<0.0001$, slope $=5.15 \pm 0.28$ $\mu Mg^{-1}dL$, intercept$=0.46 \pm 2.7$ μM). Error bars represent the SD of each individual measurement. The quality and results of the fit were not affected by forcing the intercept to zero (same r value).

3.4 *DOS as a Predictor of Anemia*

Figure 4 presents a scatter plot of the stO_2 values for each of the three categories of measurements, pretransfusion (PRE, squares), post-transfusion (POST, circles), and nontransfusion (NON, up triangles). The sample population averages for each category are plotted immediately to the right of the scatter plot along with the category SD (down triangles). These results are similar to Fig. 3, since infants with lower amounts of hemoglobin tended to have a lower stO_2 (not shown here). The range of DOS stO_2 values approximates that of the nontransfused group after the transfusion. A standard ANOVA performed on these three groups yielded $F=0.0003$, with a power of 0.99 at a 95% confidence interval. Analysis by the Tukey-Kramer HSD method, a more conservative multiple comparison test, concluded a significant difference between the PRE and POST values, but no significant difference between the POST and NON groups (also at 95% confidence).

3.5 *Spectral Considerations*

To determine the differences between two and four wavelength spectroscopy, we recalculated our four wavelength data using only two wavelengths. One set was comprised from each Oxiplex TS channel. Thus, we compared the results that would have been acquired using 1. 830 and 690 nm, 2. 810 and 750 nm, and 3. all four wavelengths.

ANOVA revealed no significant differences between the measurements of the ten pretransfusion measurements in any DOS parameter for these three sets of wavelengths. In general, the ctTHb was unchanged ($F=0.926$), and the ctO_2Hb was unchanged ($F=0.817$), but the ctHb was slightly higher,

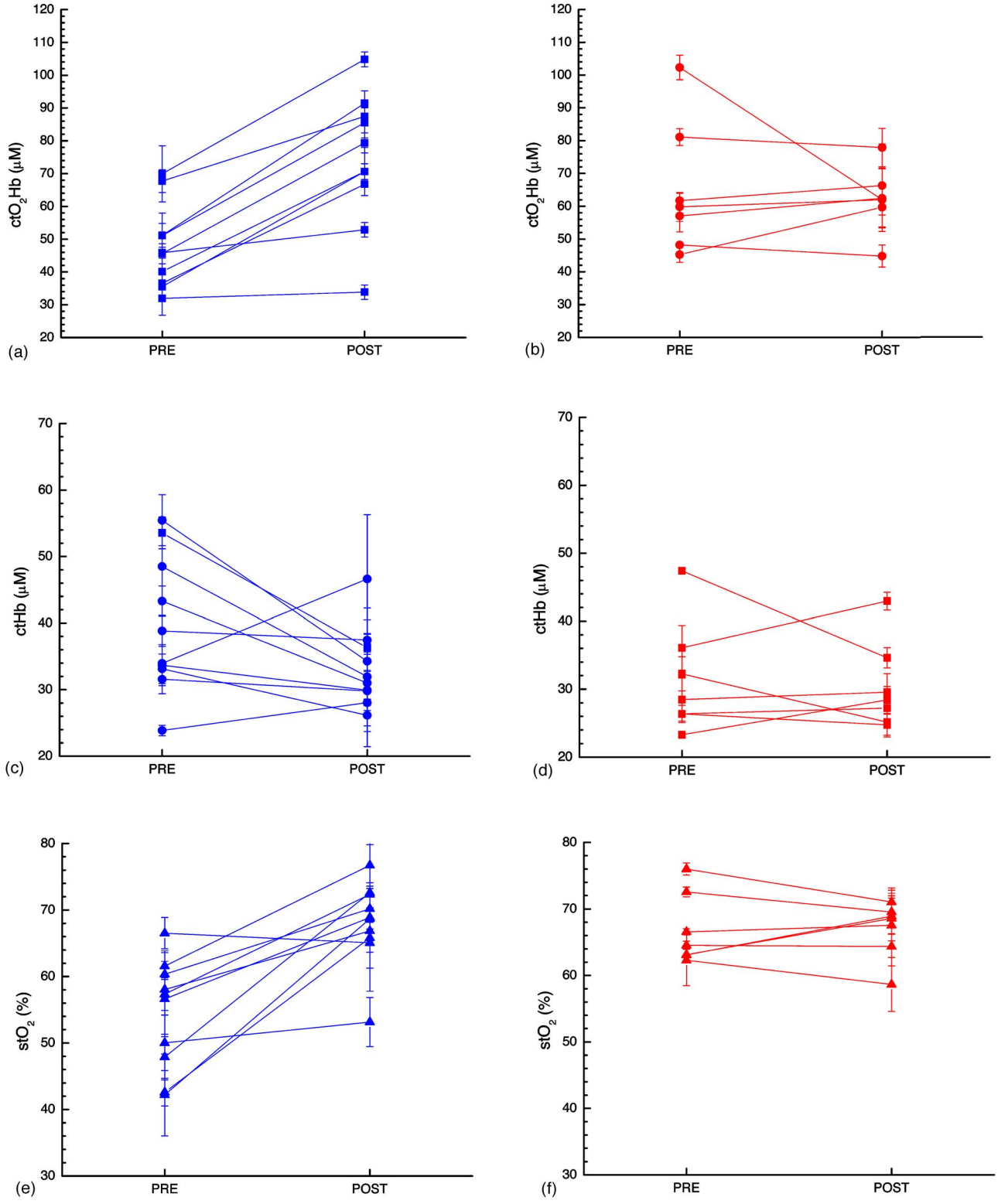

Fig. 2 Before/after comparisons for each of the measured subjects for ctO_2Hb, (a) and (b), ctHb (c) and (d), and stO_2 (e) and (f). Note that each pair of graphs are plotted on the same scale. For each parameter, (a), (c), and (e) represent the ten transfused subjects, while (b), (d), and (f) represent the seven nontransfused subjects. Lines connect the measurements for each subject. This graphical representation of Table 2 demonstrates that ctO_2Hb and stO_2 changes for each transfusion subject were positive and large, but on average, the nontransfused subjects did not change.

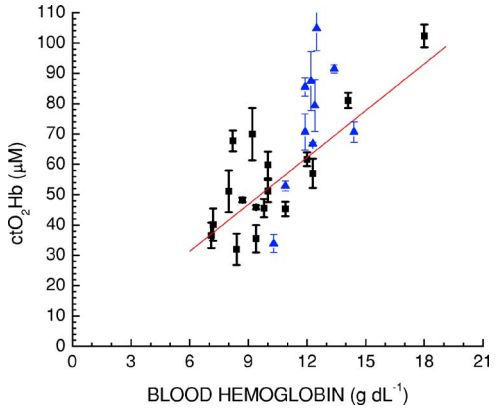

Fig. 3 Absolute comparison between invasively measured hemoglobin and DOS measured ctO_2Hb. Nontransfused as well as transfusion infants are shown (squares). Error bars represent the SD of the results from repeated individual measurements (17 subjects, 34 measurements). The solid line is an error-weighted ($r=0.83, p<0.0001$) fit involving the initial measurements on all 17 subjects. Squares represent the post-transfusion measurements (but were not included in the fit).

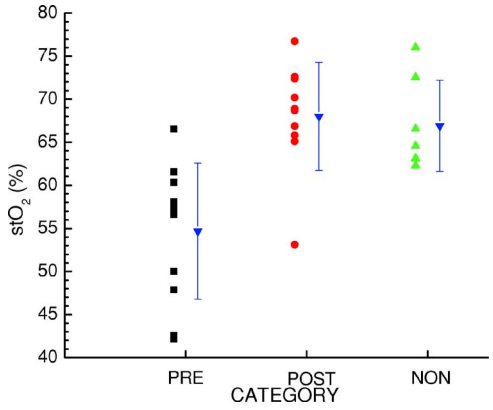

Fig. 4 Plot of DOS tissue hemoglobin saturations by category for all infants. Ten pretransfusion infants (squares) have a lower average stO_2 (55±8%) compared to ten post-transfusion infants (circles, 68±6%) and seven nontransfused infants (up triangles, 67±5%). The averages for each group (down triangles) are plotted immediately next to each group. Error bars represent the SD of the sample population. Note that the nontransfused population does not represent a matched control group for the transfused infants.

though not significantly, for the 810/750 nm combination ($F=0.218$). In all, we do not expect to see any significant trend differences between using the 830/690, 810/750, or 830/690/810/750 for this dataset. In addition, the magnitude and significance of differences observed by DOS was essentially the same for all three wavelength combinations.

3.6 *Differential Path Length*

Using Eq. (1), we calculated the DPF for each of the ten transfused infants at all four wavelengths. The average values, complete with population errors, are provided in Fig. 5. Each of the symbols represents one of the ten subjects at a particular wavelength. The star symbols to the right are the population average (and error) for a given wavelength. The variance in the DPF over the transfused population was 21.4% for all wavelengths, but only 14.5% for all wavelengths over the normal population.

Statistically significant changes were not observed in tissue scattering or DPF between pre- and post-transfusion subjects for any wavelength. Statistically significant changes were also not observed in tissue scattering or DPF between the nontransfused and transfused groups. In general, both DPF and tissue scattering were higher in the nontransfused group. Additionally, the standard deviation over the population was higher in the nontransfused group relative to the transfused group for both tissue scattering (16 to 29%) and DPF (21 to 14%).

4 Discussion

4.1 *Absolute DOS Results*

The present study shows that DOS measurements consistently document absolute, quantifiable increases in ctO_2Hb, ctTHb, and stO_2 in muscle tissue following RBC transfusions in VLBW infants. The magnitude of our changes in muscle corresponds well with those measured in the brain by Dani et al.[8] Using NIRS technology, Torella, Haynes, and McCollum[9] demonstrated a modest relative increase in stO_2 in peripheral tissue (1.6%). The difference in the magnitude of change is likely due to differences in NIR instrumentation and modeling.

In contrast to conventional NIR measurements, the DOS technique reports absolute values of ctHb and ctO_2Hb, and thus also absolute stO_2, insofar as the conditions of the diffusion model are met. Our measured absolute ctO_2Hb correlated well with routinely sampled hemoglobin concentrations (Fig. 3); no calibration curve of any kind was required. We stress that we do not expect a perfect correlation between blood hemoglobin and tissue hemoglobin (for example, during local tissue ischemia). However, we do expect that the two measurements will be similar under normal conditions.

Absolute values of stO_2 may also be used to detect tissue hypoxia, and the need for transfusion, as shown in Fig. 4. Some infants showed stO_2 values that are consistent with healthy, metabolically normal tissue, while others showed lower stO_2 values consistent with increased metabolic demand (as seen in tumors and exercising muscle in previous studies)[24–27] or reduced delivery. Additionally, the post-transfusion average stO_2 was comparable to that of the nontransfused group's average stO_2 (69±7% and 67±5%, respectively, Table 2). These data together suggest that infants with stO_2 values in the lower range may have been in a greater state of SOT/tissue demand mismatch, and derived greater benefit from the transfusions, than those with higher stO_2 values. We also observe that the dynamics of the changes in ctO_2Hb during transfusion (i.e., Fig. 1) may also play an important role in assessing therapeutic efficacy. For example, rapid increases are likely to have a different significance than slower increases. This observation will be the subject of future studies.

Nontransfused infants were also measured to ensure that machine drift and probe placement errors were not responsible for the DOS-measured changes observed in the transfused population. Although the nontransfused infants are not

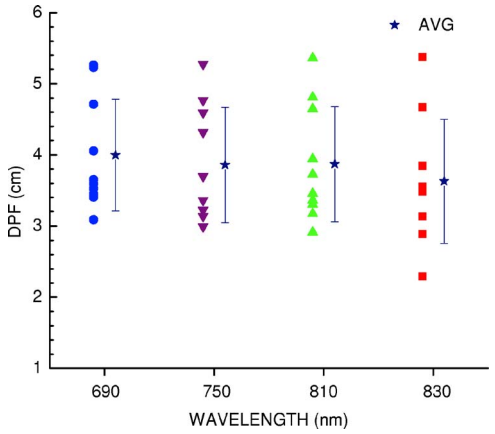

Fig. 5 Plot of the distribution of calculated DPF values from the ten transfused subjects for each wavelength. The stars and error bars next to each column represent the population average for that particular wavelength. Note the variation is approximately 20% for each wavelength. The source-detector separation was taken to be the mean of the four distances (15 mm).

matched to the transfused population, we can regardless be confident that the measured physiological variations in the transfused population are not the result of measurement errors.

The changes we have observed were not due to changes in heart rate or blood pressure. No significant changes in heart rate occurred after transfusion (161±10 to 159±10). In this study, blood pressure changes were not monitored; however, others have shown no changes in mean arterial pressure in response to transfusion.[8,9]

4.2 *Effects of Myoglobin*

Myoglobin and hemoglobin have similarly shaped extinction spectra within the NIR,[28] making it difficult, if not impossible, to distinguish between them *in vivo* with only four wavelengths. However, skeletal muscle myoglobin content varies with age.[29] Kagen and Christian reported that the concentration of myoglobin found in neonatal pectoral and psoas muscles is approximately 0.22 mg/g (wet weight of muscle), which is only about 5% of the adult value.[30] Thus, it is highly improbable that myoglobin affected our results. The linearity of Fig. 3 additionally suggests the effect of myoglobin did not distort our results.

4.3 *Discussion of Outliers*

Two points in Fig. 2(e), where the stO_2 did not appear to change after transfusion, warrant comment. For one subject with an initial point near 66%, stO_2 may represent a case where the effect of the transfusion was minimal, since the initial stO_2 value was very close to the nontransfused group. The outlier infant, with an stO_2 value near 50% in Fig. 2(e), differed from the other transfused subjects in two ways: 1. this infant had received seven prior transfusions, and 2. this infant had the greatest post-natal age. The reason why this infant did not show a significant increase in tissue oxygenation following transfusion is unclear, and suggests further study. Due to both the large number of prior transfusions and the low stO_2 improvement, we conjecture the benefit from transfusion for this neonate is uncertain. Although the neonate met traditional transfusion criteria, the benefit from transfusion was either nonexistent or short lived due to ongoing blood loss.

4.4 *Spectral Bandwidth*

The results of this study did not depend on the choice of four available wavelength combinations: 1. 830 and 690 nm, 2. 810 and 750 nm, and 3. 830, 690, 810, and 750 nm. It is impossible to say from this study which is the "best" combination of wavelengths, because there is no comparison that could be made against a gold standard. It is important to remember that limited, discrete wavelength sampling of tissue hemoglobin is potentially subject to large errors, even in stO_2.[31]

Comparing two to four wavelengths, it is not surprising to find similar results. Only after acquiring broadband measurements of the tissue will the effects of limited discrete wavelengths be understood.[32] Using custom-built broadband DOS technology, we plan to investigate this issue.[33,34]

The use of broadband DOS would not only allow for more accurate hemoglobin quantitation, but would also provide the concentrations of other chromophores such as water and lipids.[32] These additional chromophores might be used to qualify the measured tissue hemoglobin concentrations in terms of body composition at the measured tissue site. For example, the influence of lipid layers or the fraction of sampled muscle tissue could be factored into the optical assessment of tissue hypoxia.

4.5 *Effects of Fetal Versus Adult Hemoglobin*

All infants were transfused with blood from adult donors. In this study, the fraction of fetal hemoglobin present in each infant was not taken into account. Two issues are of concern here: 1. differences between fetal and adult hemoglobin absorption spectra, and 2. differences between fetal and adult hemoglobin oxygen binding properties. Since the relative amounts of fetal and adult concentrations were unknown, our calculations of ctHb and ctO_2Hb are subsequently affected.

Differences between fetal and adult hemoglobin spectra cannot account for the large transfusion-induced ctO_2Hb increases we have observed. On average, adult O_2Hb extinction coefficients are about 4% higher than fetal extinction coefficients at the four wavelengths we have used.[35,36] The Hb extinction coefficients are about 3% lower at 690 (where the absorption of Hb is much higher than the other wavelengths), but about 10% higher at 810 and 830. By changing extinction coefficients from fetal to adult hemoglobin, we expect to see approximately the same ctHb but a reduced ctO_2Hb, as opposed to the increase we observed. Thus, the increases we have observed cannot be due to differences between the extinction coefficients of adult and fetal hemoglobins.

It is also important to note that the hemoglobin-oxygen binding curve differs between adult and fetal hemoglobins. Fetal hemoglobin has a higher affinity for oxygen (i.e., a lower P_{50} value) than adult hemoglobin does.[37] Knowledge of the P_{50}, or equivalently the balance of fetal and adult hemoglobins, is important for determining the range of adequate saturation post-transfusion.[38–40] However, this lower oxygen affinity will not affect the direct measurements of the tissue hemoglobin concentration *per se*.

A lower P_{50} can affect the balance between measurements of ctO_2Hb and ctHb. For example, it is possible that the outlier discussed in Sec. 4.3 could be due to the fact that the subject had predominately only adult hemoglobin due to the large number of transfusions (seven). We are currently investigating if there are enough spectral differences between adult and fetal hemoglobin such that broadband DOS could quantify the fractions of adult and fetal hemoglobin.

4.6 *Variation in Tissue Scattering*

The DPF values we have measured in muscle are consistent with DPF values measured by others in the infant brain.[41,42] We observed about a 20% variation in the DPF over the transfused population at each wavelength. The large degree of variation in this population could be due to the variance in coupling between probe and infant. However, similarly scaled variations were observed by Duncan et al., who measured over 280 DPFs in the brains of neonates, children, and adults.[42] In their age-corrected data, Duncan et al. observed a "wide scatter of values" and suggested that the DPF be measured at the time of each investigation. Assuming a constant value of the DPF for our population will therefore result in similar variations in the recovered hemoglobin concentrations. This problem was avoided by measuring the tissue scattering for each subject.

We expected to see increases in the reduced scattering as a result of the transfusion, but we did not observe these increases. Some evidence has suggested a link between changes in hemoglobin and changes in reduced scattering during a venous occlusion protocol.[43] We have previously observed a drop in reduced scattering and hemoglobin concentration in hemorrhage animal models using a broadband frequency-domain instrument.[44]

4.7 *Study Limitations*

Although the results of this pilot study are promising, there are limitations. The ctO_2Hb is a function of many variables, including cardiac output, mean arterial pressure, and F_iO_2.[45] Blood pressure and assessment of cardiac function were not specifically evaluated in these infants (none of the infants were on vasopressor medications during this study, and none were on mechanical ventilation). However, as described earlier, we did not detect changes in heart rate, and other groups have not detected changes in mean arterial pressure in response to transfusion. Markers of impaired tissue perfusion such as lactate levels and base deficits were also not measured. In future studies, correlations between low stO_2 values, decreased perfusion, and lactic acidosis would help to support our findings.

The biggest limitation of this study lies in the establishment of a control population properly matched with the transfused VLBW infants. Thus, the nontransfused group of Fig. 4 cannot be taken to be a normal population average consistent with the VLBW transfused population. However, we note that the stO_2 values of the healthy subjects and the post-transfusion subjects agree significantly. Further study using carefully matched control populations will be required to make certain that this comparison could be used as an indicator of tissue hypoxia. We also plan, as has Wardle et al., to include the effects of symptomatic and asymptomatic infants.[40]

5 Conclusions

This pilot study demonstrates consistent, quantifiable, and absolute increases in the tissue ctO_2Hb, ctTHb, and stO_2 following RBC transfusion in VLBW infants. Using the OptiplexTS, we demonstrated that: 1. the choice of wavelength combinations (2 or 4) did not affect our results, and 2. the high variation in DPF implies that the tissue reduced scattering should be measured. We have shown that the absolute level of ctO_2Hb as well as the stO_2 could be reliable indicators of the degree of tissue hypoxia in muscle tissue. With further validation of this technique, stO_2, together with absolute ctO_2Hb values, may be able to predict those infants with challenged O_2 perfusion at the tissue level, thereby aiding clinical decisions regarding the therapeutic need for RBC transfusions.

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