Hemoglobin measurement patterns during noninvasive diffuse optical spectroscopy monitoring of hypovolemic shock and fluid replacement

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Abstract. The purpose of this study is to demonstrate the feasibility of broadband diffuse optical spectroscopy (DOS) for noninvasive optical monitoring of differentiating patterns of total tissue hemoglobin (THC), oxy- (OxyHb), and deoxyhemoglobin (DeOxyHb) concentrations during hypovolemic shock and subsequent fluid replacement with saline and whole blood. The goal of this DOS application is to determine the efficacy of resuscitation efforts at the tissue level rather than currently available indirect and invasive measurements of hemodynamic parameters. 16 New Zealand white rabbits are hemorrhaged 20% of their total blood volume. In resuscitated animals, shed blood volume is replaced with equal volume of crystalloid or whole blood (five animals each). Physiological variables (cardiac output, mean arterial pressure, systemic vascular resistance, hematocrit) are measured invasively, while (OxyHb) and (DeOxyHb) are measured during the interventions using broadband DOS. During the pure hypovolemic hemorrhages, the decrease in THC is mainly due to the decrease in (OxyHb), since the decrease in THC due to blood loss results in decreased tissue perfusion, with a resultant increased tissue extraction of oxygen. The hemorrhage with the whole blood resuscitation model shows significant changes in (OxyHb) during resuscitation phases due to the higher oxygen carrying capacity of whole blood, as opposed to the limited volume replacement effects and the decreased tissue oxygen content from the euvolemic anemia of the saline resuscitation. Broadband DOS noninvasive optical monitoring reveals distinct patterns of total tissue hemoglobin, oxy-, and deoxyhemoglobin during hemorrhage. Further studies are needed to confirm potential clinical utility and accuracy under more complex clinical conditions in animal models and patients. © *2007 Society of Photo-Optical Instrumentation Engineers.*
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1 Introduction

The goals of fluid resuscitation from traumatic hemorrhagic shock include restoration of hemodynamic stability and tissue oxygen delivery. There are many questions regarding conventional intravenous fluid resuscitation and the optimal end points for resuscitation efforts.[1–3] Regardless of the resuscitation strategy, it is imperative to develop effective feedback monitoring systems to reduce the morbidity and mortality of hemorrhagic shock patients. Global indicators of oxygen delivery and oxygen uptake such as hematocrit, serum hemoglobin concentration, and cardiac output may be insensitive to regional hypoperfusion because of distributive changes in blood flow to protect specific organs. However, there is increasing evidence that peripheral tissue hypoxia is a major clinical event that precedes the onset of multiple organ failure.[4] The ideal device for monitoring the adequacy of resuscitation in trauma patients would have two basic characteristics. First, it would be noninvasive, not only allowing ease of placement but also permitting potential use in field settings. Second, it would provide the clinician with objective parameters that measure *in-vivo* oxygenation at the tissue level noninvasively.

Address all correspondence to Matthew Brenner, Pulmonary and Critical Care Division, Univ. of Califonia/Irvine, 101 City Dr. South—Building 53, Rm. 119, Orange, CA 92868, United States of America; Tel: 714 456 5150; Fax: 714 456 8349; E-mail: mbrenner@uci.edu

Near-infrared spectroscopy (NIRS) has been proposed to fulfill these roles for almost 40 years and has often been applied in critical care settings.[5–7] Crookes et al.[8] reported that noninvasive muscle tissue oxygen saturation (S_TO_2) determined by NIRS was more reliable than invasive oxygenation variables as an index of shock. However, these conventional NIRS devices do not allow quantitative measures of absolute concentrations of the chromophores due to their inability to accurately account for photon path lengths for each wavelength traveling through tissue volume and losses due to scattering. Instead, they provide estimates of concentration changes deviating from baseline values during variations in oxygen availability and utilization. To have wider applicability in critical care settings, further technical developments to NIRS systems are needed to allow for accurate and relatively simple measurements of light scattering and absorption by bulk tissue, which, in turn, lead to the quantitative measurements of tissue hemoglobin concentrations and oxygen saturation. Recent advances in NIRS enable accurate simultaneous tissue scattering and absorption measurements that can be used for monitoring concentrations of tissue absorbers (such as hemoglobin) in near real time in clinical settings.[9–11] Specifically, broadband diffuse optical spectroscopy (DOS) combines time-resolved frequency domain photon migration measurements with conventional cw spectroscopy. With the combination of these two principles, it is possible to overcome the critical limitation of NIRS regarding the treatment of tissue scattering properties while maintaining the rich spectral contents of broadband cw NIRS measurements.

The application of frequency domain photon migration and broadband DOS in critical care settings, especially in the setting of the acute hemorrhage, has been detailed previously.[12] However, the restoration of tissue perfusion and oxygen utilization during the resuscitation efforts are more complex than acute hypovolemic hemorrhage. Therefore, it is crucial to assess and understand the hemodynamic responses measured by broadband DOS. Since broadband DOS measures over bulk tissue volume, the measured tissue hemoglobin concentration by DOS will be related to both blood hemoglobin concentration and vascular tissue volume within the measured region. Thus, vasoconstriction or vasodilation in the arterial, capillary, or venous beds will affect average tissue hemoglobin concentration as well as changes in intravascular hemoglobin concentrations.[13] Consequently, broadband DOS total tissue hemoglobin concentrations would be expected to differ in pure hemorrhage (with associated vasoconstriction) compared with resuscitated hemorrhage (approximating acute euvolemic anemia), or with blood resuscitation.

The purpose of this study was to demonstrate the feasibility of broadband DOS noninvasive optical monitoring of hemorrhagic shock and to describe the distinct patterns of bulk tissue hemoglobin concentrations [total hemoglobin concentration (THC), OxyHb, and DeOxyHb] observed during hemorrhage, resuscitation with saline, and resuscitation with whole blood.

2 Material and Methods

2.1 Animal Model

Pathogen-free white New Zealand rabbits (Myrtle Rabbitry Incorporated, Thompson Station, Tennessee), weighing
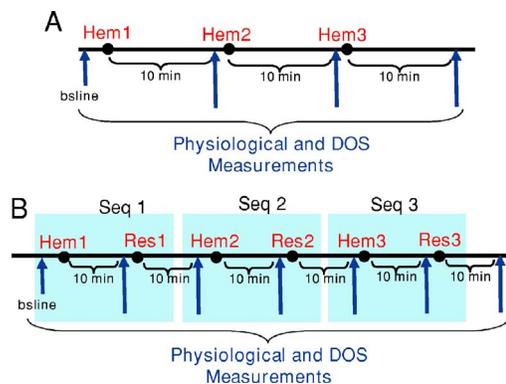
Fig. 1 Time line of measurement sequences. (A) Hypovolemic hemorrhage and (B) hemorrhages with saline and whole blood resuscitation. Blood withdrawal and resuscitation was carried out in three steps and physiological parameter monitoring and DOS measurements were conducted 10 min after each hemorrhage (and resuscitation) step. Blood withdrawal and subsequent resuscitation are grouped together as single sequence [shaded area in Fig. 1(B)].

3.5 to 4.5 kg, were used. Animals were housed in a pathogen-free animal facility and were given a commercial basal diet and water *ad libitum*. The study was approved by the Institutional Laboratory Animal Care and Use Committee, University of California, Irvine (ARC protocol 2000-2218-2).

2.2 Hemorrhage/Resuscitation Model

Six animals were used for the pure hemorrhage model. 20% blood loss (15 cc/kg) was achieved to attain hypovolemia. Blood withdrawal was carried out in three steps and physiological parameter monitoring and DOS measurements were conducted 10 min after each hemorrhage step [Fig. 1(A)]. For resuscitation models, blood volumes removed from circulation were replaced with equivalent resuscitatory volumes of 0.9% NaCl saline and whole blood [Fig. 1(B)]. Five animals were used for saline and whole resuscitation models, respectively. As with the pure hemorrhage model, blood withdrawal and blood volume replacements were carried out in three steps [Fig. 1(B)]. Physiological parameter monitoring and DOS measurements were conducted after each withdrawal and resuscitation step.

2.3 Measurement Procedures

The animals were initially sedated with an intramuscular injection of 2:1 ratio of Ketamine HC1 (100 mg/mL) (Ketaject, Phoenix Pharmaceutical Incorporated, Saint Hoseph, Michigan):Xylazine (20 mg/mL) (Anased, Lloyed Laboratories, Shenandoa, Iowa) at a dose of 0.75 mL/kg. The animal's body weight and temperature were assessed after sedation. A 22-gauge catheter was then placed and secured into the animal's marginal ear vein and maintenance anesthetic was dosed at 0.3 mL of 1:1 mixture of Ketamine:Xylazine IV (Ketamine 100 mg/mL:Xylazine 20 mg/mL) as needed. Depth of anesthesia was monitored according to established guidelines. The animals were then immediately intubated with a 3.0 cuffed endotracheal tube and placed on mechanical ventilation (dual phase control respirator, model 32A4BEPM-5R, Harvard Apparatus, Chicago, Illinois) with the following settings: tidal volume, 50 cc, respiratory rate 25 breaths/min,

and 100% oxygen. Blunt dissection was performed to isolate the femoral artery on the left thigh slightly distal to the inguinal ligament. A 20-gauge catheter was secured within the femoral artery for systemic blood pressure measurements and blood withdrawal and insertion (hemorrhage, resuscitation, and arterial sampling). The animals were shaved at the thorax and thighs. The chest wall was opened to expose the thoracic cavity, allowing direct access to the heart and aorta. A plastic probe composed of source and detector fibers at a fixed separation distance was placed on the medial surface of the right hind thigh for the broadband DOS measurements. A source and detector separation of 10 mm was used for optical measurements. Animals were euthanized by standard procedures at the end of the studies (pentobarbital sodium, Eutha-6, intravenous injection).

2.4 *Measurement Variables*

Pertinent physiological variables of animals and broadband DOS measurements were taken concurrently throughout the experiment to establish the relationship between conventional hemodynamic indices during hemorrhage, resuscitation, and peripheral tissue hemoglobin contents from broadband DOS.

Pulmonary and systemic arterial blood pressure measurements were obtained with a calibrated pressure transducer (TSD104A Transducer and MP100 WSW System, Biopac Systems, Incorporated, Santa Barbara, California). For the pulmonary arterial pressure, the transducer was connected to a fluid-filled line terminating in a 24-gauge catheter. The catheter was directly inserted into the right ventricular chamber and advanced into the pulmonary artery for the measurement duration. Systemic arterial pressures were measured similarly to pulmonary blood pressures, with the transducer-connected catheter inserted into the femoral artery.

For cardiac output (CO) measurements, a calibrated flow transducer (T106 Small Animal Flow Meter, Transonic System, Incorporated, Ithaca, New York) was placed around the ascending aorta to measure CO. Time trace of the cardiac flow was recorded for $\sim$10 sec, and the time average of the flow profile was calculated to yield the mean CO.

2.5 *Broadband Diffuse Optical Spectroscopy*

The broadband diffuse optical spectroscopy (DOS) instrument we have constructed has been previously described in the literature.[9,14] Briefly, the prototype DOS device we constructed employs both steady-state (SS) broadband measurements and frequency domain (FD) photon migration techniques. This combination is necessary because: 1. the FD technique directly measures the tissue scattering and absorption at discrete wavelengths (661, 686, 783, 814, 826, and 859 nm), and 2. the SS broadband technique provides high spectral content in a continuum across the entire spectral range measured. Thus our method makes no *a priori* assumptions about the photon path length in tissue, and provides more optimal spectral characterization. No patient calibrations are required; only a standard instrument calibration is necessary. It takes 20 sec to complete a single broadband DOS (FD and SS) measurement.

The DOS instrument recovers the entire NIR tissue absorption spectrum from 650 to 1000 nm, in essence, averaging the tissue optical properties in the sampled tissue volume for a given wavelength. Because absorption is related to absorber concentration via the Beer-Lambert relationship, we translate tissue absorption into absorber concentrations of the dominant NIR chromophores: OxyHb, DeOxyHb, water, and lipids. Tissue absorber concentrations are obtained via standard positive-constrained linear least square fits of the wavelength-dependent extinction coefficient spectra.[15]

2.6 *Statistical Analysis*

All data are presented as mean$\pm$standard error of the mean (SEM). Pairwise related changes for data points of two or more time points were assessed by a paired nonparametic test (Wilcoxon rank sum test) using Systat version 10 (Systat Software, Incorporated, Point Richmond, California). Two-sided p-values using normal approximation were reported. The differences were considered significant at $p < 0.05$.

3 Results

For each of the hemorrhage/resuscitation models, both the absolute and the changes from the baseline concentrations of OxyHb, DeOxyHb, and total hemoglobin concentration $[\text{THC}=(\text{OxyHb})+(\text{DeOxyHb})]$ are shown.

The baseline values of standard hemodynamic parameters (HCT, hematocrit; mAP, mean arterial pressure; CO, cardiac output; SVR, systemic vascular resistance) and broadband DOS parameters are listed in Tables 1 and 2, respectively. The variations of tissue water and lipid fraction of individual animals were minimal (less than 3 and 2%, respectively)

Table 1 Physiological variables at baseline. Measured values of hematocrit (HCT), cardiac output (CO), and mean arterial pressure (mAP). Data were collected from all 16 animals.

Physiological parameters	Mean±SEM*
HCT	34±4 g/dL
CO	318±77 mL/min
mAP	77±8 mmHg

Table 2 Broadband DOS variables at baseline. Calculated physiological properties, Hb concentration (μM, OxyHb, DeOxyHb, THC). The water fraction is the water concentration in tissue relative to the concentration of pure water (55.6 M), and the lipid fraction is the lipid mass density (g mL^{-1}) in tissue relative to a lipid mass density of 0.9 g mL^{-1}. Data were collected from all 16 animals.

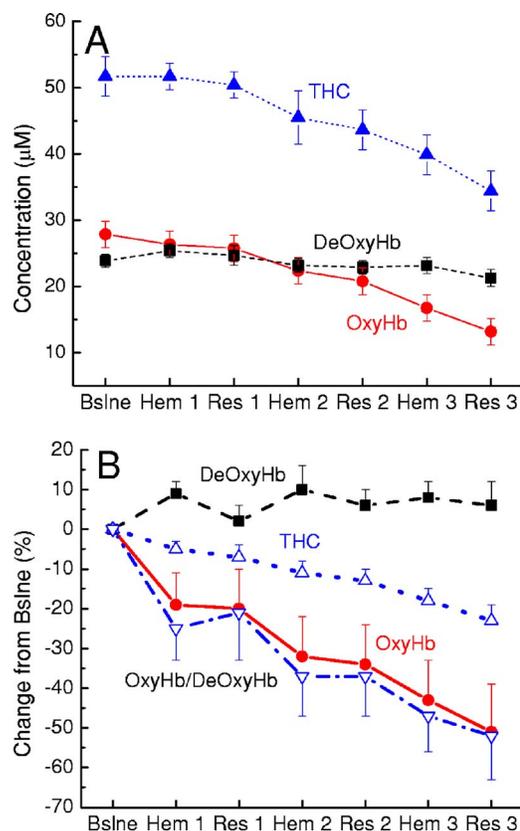| Broadband DOS parameters	Mean±SEM*
(OxyHb)	34.6±3.6 μM
(DeOxyHb)	20.7±1.6 μM
(THC)	55.3±4.3 μM
(OxyHb)/(DeOxyHb)	1.72±0.194
Water	76.5±2.8%
Lipid	9.7±0.7%
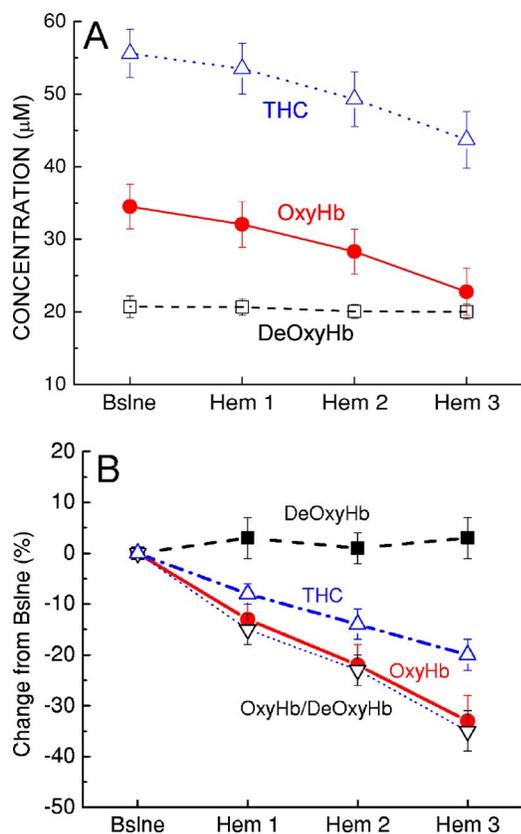
Fig. 2 Broadband DOS parameters during hypovolemic hemorrhage. (A) Progression of absolute (OxyHb), (DeOxyHb), and THC in all six animals. (B) Percent changes from the baseline values of OxyHb, DeOxyHb, THC, and OxyHb/DeOxyHb during stepwise hemorrhage in all six animals.

Fig. 3 Broadband DOS parameters during hemorrhage with saline resuscitation. (A) Progression of absolute (OxyHb), (DeOxyHb), and THC in all five animals. (B) Percent changes from the baseline values of OxyHb, DeOxyHb, THC, and OxyHb/DeOxyHb ratios compared to baseline during hemorrhage and saline resuscitation in all five animals.

throughout measurements. Since broadband DOS quantifies the absolute concentrations of OxyHb and DeOxyHb, the measurement values at each hemorrhage and resuscitation points were normalized by the baseline values, and the changes from the individual baseline were reported. As a result, the intersubject variations were effectively reduced and the clear patterns of response to hemorrhage and resuscitation can be seen. For the hemorrhage and resuscitation groups, only the measurements taken at the end of each resuscitation phase are shown and termed as sequences 1, 2, and 3 [Fig. 1(B)] to compare the different physiological responses and tissue perfusion due to two different types of fluid resuscitation strategies.

3.1 Hypovolemic Hemorrhage

Figure 2 shows the broadband DOS measured hemodynamic variables during the hypovolemic hemorrhage without the fluid resuscitation. Figure 2(A) illustrates the absolute concentrations of OxyHb, DeOxyHb, and THC after successive blood draws. These data show that the decrease in (THC) resulted from decreases in (OxyHb) because changes in (DeOxyHb) from their baseline values were insignificant during subsequent hemorrhage steps ($p \geqslant 0.6$). This trend is also apparent in Fig. 2(B), where ratios to baseline of respective parameters of all six animals are shown. The OxyHb/

DeOxyHb ratio decreases significantly with the decreasing (OxyHb) contribution ($p \leqslant 0.028$) when compared in successive hemorrhage steps.

3.2 Hemorrhage with Saline Resuscitation

The overall trends of broadband-DOS-measured hemodynamic parameters during hemorrhage with saline resuscitation were similar to those of the hypovolemic hemorrhage (Fig. 3). (OxyHb) declined significantly during the first two hemorrhage steps ($p \leqslant 0.042$), while the last hemorrhage step resulted in decrease in (OxyHb), but not significantly ($p \leqslant 0.08$). Saline resuscitation did not change (OxyHb) ($p \geqslant 0.225$). The euvolemic anemia resulted in the decreased overall oxygen content in tissue and did not restore the normal tissue oxygen profile. However, the decline in OxyHb and OxyHb/DeOxyHb ratio were delayed during the saline resuscitation phase.

3.3 Hemorrhage with Whole Blood Resuscitation

Figure 4 shows the distinct patterns of (OxyHb), (DeOxyHb), and (THC) during hemorrhage with whole blood resuscitation. Notably, (OxyHb) and (THC) were restored after reintroduction of hemorrhaged whole blood volume. However, there were still overall declines of (OxyHb) and increases in (DeOxyHb) throughout the experiments ($\sim$−34±5% and
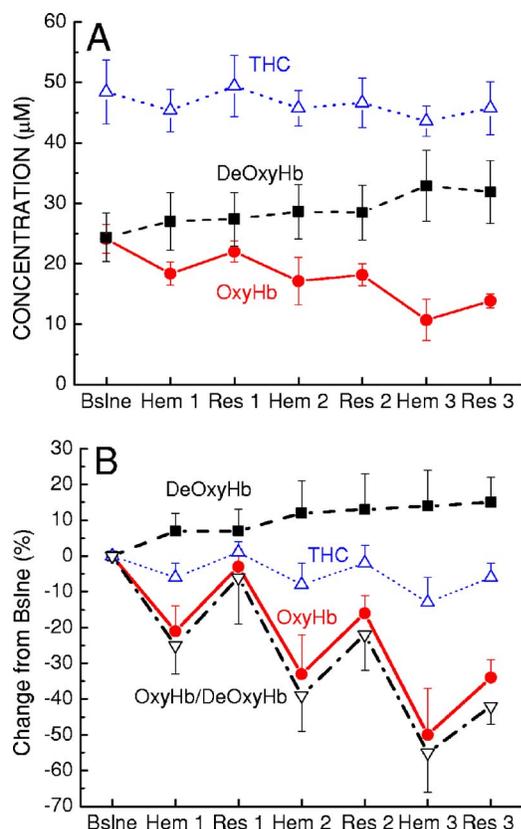
Fig. 4 Broadband DOS parameters during hemorrhage with the whole blood resuscitation. (a) Progression of absolute (OxyHb), (DeOxyHb), and THC in all five animals. (b) Changes in ratios to baseline values of OxyHb, DeOxyHb, THC, and OxyHb/DeOxyHb during hemorrhage with the whole blood resuscitation in all five animals.

+15±7% at the end, respectively). The percent change from the baseline plot of all five animals in this group [Fig. 4(B)] shows decrease in (OxyHb) during hemorrhage ($\sim -24\%$) and increase in (OxyHb) during resuscitation phases ($\sim +17\%$), which was expected due to the higher oxygen carrying capacity of the whole blood as opposed to the limited volume replacement effects of the saline resuscitation. Even though THC was restored to near baseline levels when whole blood volume was replaced (within 6%), the overall OxyHb/DeOxyHb ratio continued to decrease after the successive whole blood replacements due to the decreased (OxyHb) and increased (DeOxyHb).

3.4 Comparison between Saline and Whole Blood Resuscitation

Figure 5 summarizes the comparison of all physiological variables between saline and the whole blood resuscitation under investigation. During the hemorrhage with the saline resuscitation, there were statistically significant changes in Hct ($p \leq 0.043$) from the baseline and between each sequence. In contrast, only sequence 3 was statistically different from the baseline Hct in the whole blood resuscitation group. The mean arterial pressure (MAP) declined in both resuscitation groups, but the saline resuscitation group showed a greater decline ($\sim$ two-fold) than the whole blood group. While CO in the whole blood group continued to decline, there was an increase in CO for the saline group. These opposite changes in CO account for the different patterns in calculated systemtic vascular resistance (SVR).

Figure 6 summarizes the results of broadband DOS variable measurements in two resuscitation model groups. At the end of experiments, both groups show some similarities: decrease in (OxyHb), (THC), OxyHb/DeOxyHb ratio, and static or slight increase in (DeOxyHb). (THC) was preserved best during the hemorrhage with the whole blood resuscitation as expected, while the decline in (OxyHb) was greater with the saline resuscitation, as expected as well.

4 Discussion

This study demonstrates the feasibility of broadband DOS for noninvasive optical monitoring of hemorrhagic shock and differentiating patterns of THC, OxyHb, and DeOxyHb in tissue based on hemorrhage and resuscitation methods. Since (OxyHb) and (DeOxyHb) are quantified, it is now possible to compare tissue perfusion status during fluid resuscitation regardless of the baseline condition of subjects. In addition, broadband DOS enables us to evaluate the baseline condition of subjects in contrast to previously described NIRS methods.

Bulk broadband DOS OxyHb and DeOxyHb levels in tissues of subjects undergoing hemorrhage and resuscitation are complex. Total tissue OxyHb and DeOxyHb levels are governed by tissue blood volume, arteriolar and venular capillary volume, hemoglobin levels, and oxygen extraction ratios (as well as arterial pO_2 in the event of arterial hypoxemia).

As predicted, in the nonresuscitated hemorrhagic shock animals, total tissue hemoglobin decreased as measured noninvasively with broadband DOS (Fig. 2). With incremental blood withdrawal, reductions in tissue vascular volume lead to the reduced tissue total hemoglobin concentration measured by DOS. As peripheral tissue blood delivery decreases, oxygen extraction increases. Reductions in the capillary and tissue venous oxygen saturation in the tissue vasculature occur. This results in a relative increase in the tissue DeOxyHb level and a decrease in the tissue OxyHb level (decreased OxyHb/DeOxyHb ratio), since broadband DOS is a bulk average measurement for the regional tissue. Total tissue OxyHb decreases due to decreased blood volume, decreased hemoglobin, and increased oxygen extraction from the blood within the tissue region interrogated. Whether the DeOxyHb increases or decreases is dependent on whether the increase in DeOxyHb fraction from increased tissue oxygen extraction is outweighed by the loss in total tissue hemoglobin from hemorrhage or vasoconstriction. Bulk tissue OxyHb/DeOxyHb ratio would also theoretically be affected to some degree by any relative differences that might occur in the amount of vasodilation or vasoconstriction on the arteriolar versus venous sides of the circulation within the tissue being assessed.

In contrast, in the saline resuscitated hemorrhagic animal model (Figs. 3, 5, and 6), the animal is maintained in approximately an euvolemic state as blood is removed. As a result, tissue vascular volume is generally maintained and cardiac output increases as systemic vascular resistance decreases to compensate significant reductions in blood hemoglobin concentration and hematocrit within the vascular compartment. However, the decrease in systemic vascular resistance outweighed the increase in cardiac output in this animal model.
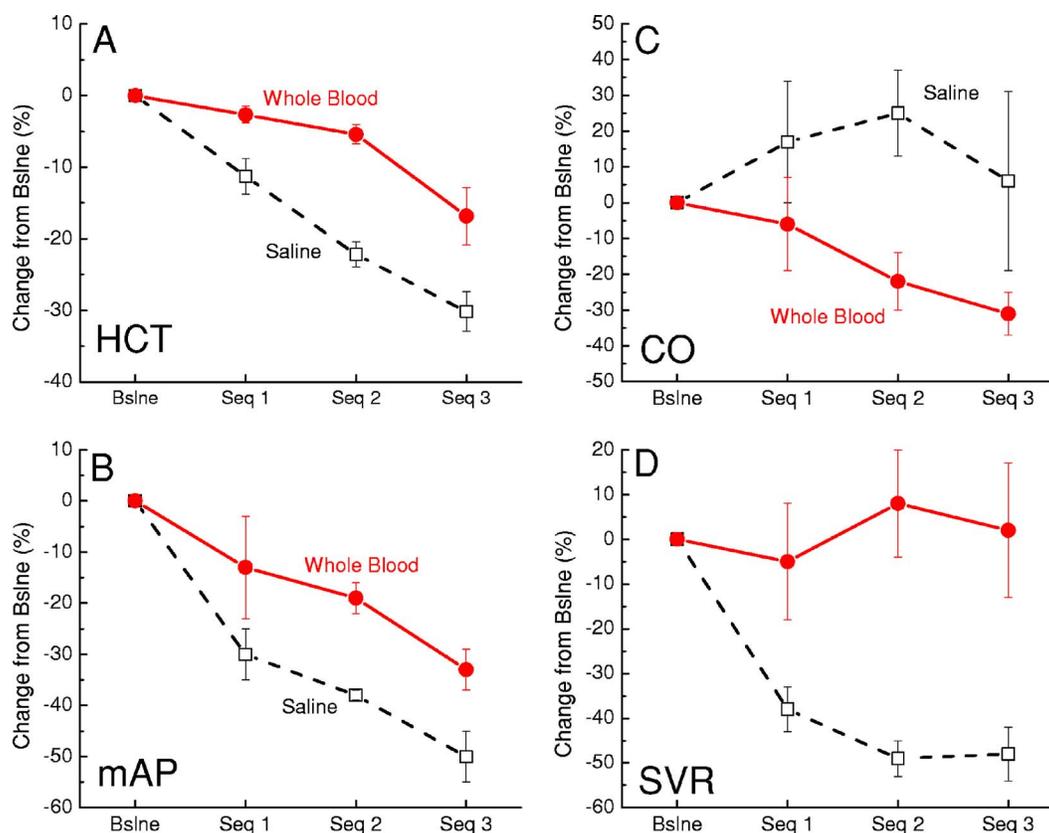
Fig. 5 Comparison of physiological parameters during three different hemorrhage models. Hematocrit (HCT), mean arterial pressure (mAP), cardiac output (CO), and systemic vascular resistance (SVR) values at the end of each sequence (hemorrhage and fluid resuscitation are grouped together as a sequence) are normalized with baseline values.

This resulted in the failure to maintain blood pressure at normal level. The bulk average tissue hemoglobin is primarily determined by decreases in blood hemoglobin in this euvolemic acute anemia hemorrhage-resuscitation model. Tissue OxyHb decreases with the fall in blood hemoglobin and with increases in oxygen extraction (Fig. 6). In ideal euvolemic fluid resuscitation, increases in cardiac output may offset some of the tissue needs for increased extraction of oxygen on the venous capillary side. Thus, the expected fall in OxyHb/DeOxyHb ratios might be less prominent compared to the nonresuscitated hemorrhage model. However, our results show that cardiac output was not able to offset the increased extraction of oxygen, and OxyHb/DeOxyHb ratio from broadband DOS measurement reflects the failure of the compensatory physiological responses.

In the whole blood resuscitated animals (Figs. 4–6), the hemorrhagic shock-induced reductions in tissue hemoglobin, OxyHb, and reduction in OxyHb/DeOxyHb levels should be essentially reversed by replacement with blood. However, the overall trend in hemodynamic patterns shows the evidence of stress from the experiment and possible compromise in tissue reperfusion capacity after hemorrhage. This may have led to incomplete restoration of the oxygen carrying capacity of the vascular system during resuscitation. Despite these potential complications, with 20% blood volume removal, change in THC was greatest within the saline resuscitation group ($-22.5\pm9.4\%$, mean$\pm$SD) and was significantly different

from the autologous blood resuscitation group ($p\leqslant0.043$, $-6.4\pm7.8\%$, mean$\pm$SD). This result shows that saline resuscitation failed to restore THC back to baseline values in tissue despite fluid volume replacement in this animal model. Also, this finding illustrates the importance of monitoring tissue THC in assessing the effectiveness of resuscitation.

The expected hemodynamic patterns were observed during the noninvasive broadband DOS monitoring of these hemorrhagic shock and resuscitation animals, demonstrating feasibility of this concept. However, there are a number of important limitations that must be addressed. Complex physiologic events occur during the hemorrhage process. Reflex responses to blood loss and hypovolemia, organ damage, mediator release, and other physiologic reactions to hemorrhagic insults may not be fully reversible with either fluid or blood resuscitation. In addition, edema, vascular permeability, pH, and other changes may affect optically measured tissue parameters. During saline resuscitation, intravascular volume changes may be occurring, since it is not possible to determine exact fluid replacement equivalents, and saline extravascular distribution. The animals are under general anesthesia, which affects blood pressure, cardiac output, vascular resistance, tissue blood volumes, some physiologic responses to hemorrhage, and necessitates some fluid replacement. In addition, despite the complex physiologic events occurring with hemorrhage, this study is an attempt at a single manipulated
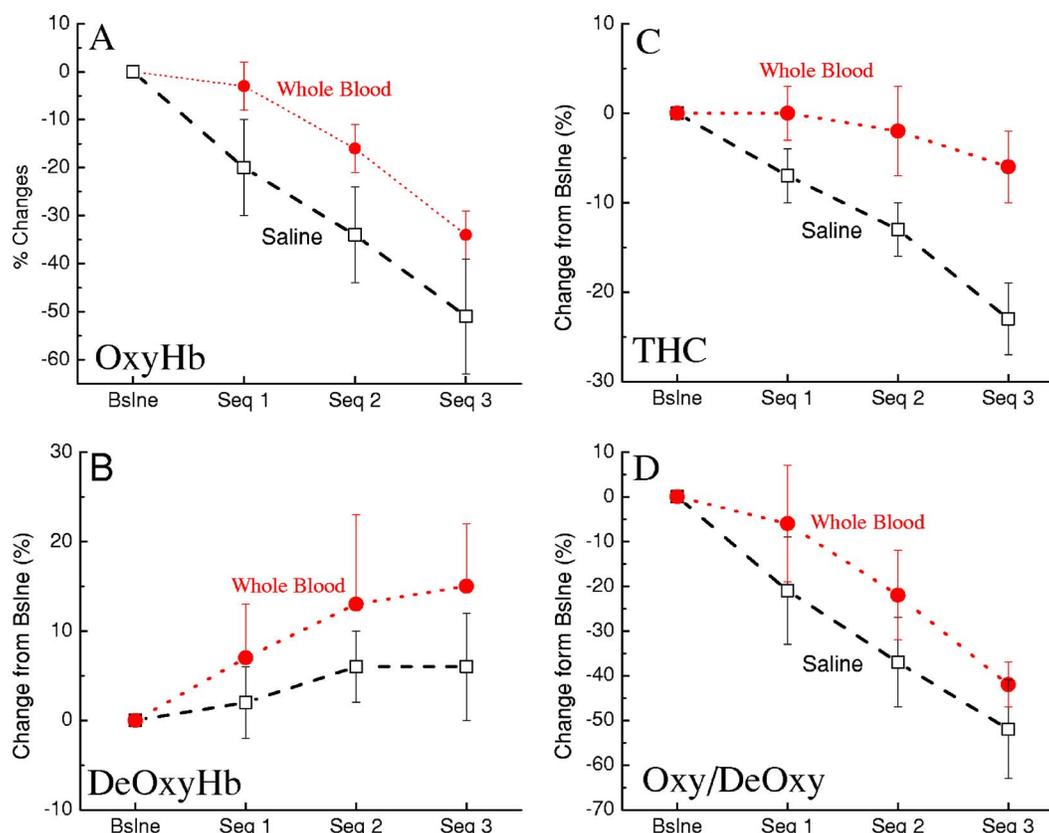
Fig. 6 Comparison of hemodynamic parameters measured by broadband DOS during two different resuscitation models. OxyHb, DeOxyHb, THC, and OxyHb/DeOxyHb values are normalized by baseline values.

variable model. The ability to monitor, track, and interpret broadband DOS tissue hemoglobin parameters may be much more complex or impossible to accurately interpret if multiple major physiologic changes are occurring (such as combined hemorrhage with sepsis, respiratory failure, hemolysis, necrosis, tissue hemorrhage, liver, or renal dysfunction). The use of pressors, vasodilators, or inotropic agents would also be expected to affect the broadband DOS hemoglobin readings.

There are technical limitations of these DOS measurements as well. The depth of penetration is determined by absorption and scattering properties of the tissues and the source detector separations. The solute concentrations reported represent a bulk average of all of the tissue components in the region of optical interrogations. Intravascular and extravascular compartments are not distinguished in this approach. Inhomogeneities within tissues, and differences in baseline and response across the different tissues, are not distinguished using the DOS methods applied in this particular study.

5 Conclusion

Recent advances in broadband DOS technology enable more accurate *in-vivo* assessment of tissue and blood component concentrations. Broadband DOS noninvasive optical monitoring reveals distinct patterns of total tissue hemoglobin, oxy-, and deoxyhemoglobin during hemorrhage. The ultimate goal of fluid resuscitation in hemorrhagic shock patients would be the improvement of tissue oxygenation, rather than simply

attempting to restore blood volume. In this sense, the ability to directly measure tissue hemoglobin concentrations and oxygenation level would be beneficial for clinicians to determine the efficacy of the resuscitation efforts rather than having to rely on currently available indirect and invasive measurements of global hemodynamic parameters. However, further studies will be needed to confirm potential clinical utility and accuracy of broadband DOS under more complex physiological conditions in animal models and patients.

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