Tissue oxygenation monitoring using resonance Raman spectroscopy during hemorrhage

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BACKGROUND: The ability to monitor the patient of hemorrhage noninvasively remains a challenge. We examined the ability of resonance Raman spectroscopy to monitor tissue hemoglobin oxygenation (RRS-StO_2) during hemorrhage and compared its performance with conventional invasive mixed venous ($SmvO_2$) and central venous ($ScvO_2$) hemoglobin oxygen saturation as well as with near-infrared spectroscopy tissue hemoglobin oxygenation (NIRS-StO_2).

METHODS: Five male swine were anesthetized and instrumented followed by hemorrhage at a rate of 30 mL/min for 60 minutes. RRS-StO_2 was continuously measured from the buccal mucosa, and NIRS-StO_2 was continuously measured from the forelimb. Paired interval measures of $SmvO_2$, $ScvO_2$, and lactate were made. Pearson correlation was used to quantify the degree to which any two variables are related. Receiver operating characteristic (ROC) area under the curve values were used for pooled data for RRS-StO_2, NIRS-StO_2, $SmvO_2$, and $ScvO_2$ to compare performance in the ability of tissue oxygenation methods to predict the presence of an elevated arterial blood lactate level.

RESULTS: Sequential RRS-StO_2 changes tracked changes in $SmvO_2$ (r 0.917; 95% confidence interval [CI], 0.867 0.949) and $ScvO_2$ (r 0.901; 95% CI, 0.828 0.944) during hemorrhage, while NIRS-StO_2 failed to do so for $SmvO_2$ (r 0.283; 95% CI, 0.04919 0.4984) and $ScvO_2$ (r 0.142; 95% CI, 0.151 to 0.412). ROC curve performance of oxygenation measured to indicate lactate less than or greater than 3 mM yielded the following ROC area under the curve values: $SmvO_2$ (1.0), $ScvO_2$ (0.994), RRS-StO_2 (0.972), and NIRS-StO_2 (0.611).

CONCLUSION: RRS-StO_2 seems to have significantly better ability to track central oxygenation measures during hemorrhage as well as to predict shock based on elevated lactate levels when compared with NIRS-StO_2. (*J Trauma Acute Care Surg*. 2014;76: 402 408. Copyright © 2014 by Lippincott Williams & Wilkins)

KEY WORDS: Hemorrhagic shock; tissue hemoglobin oxygen saturation; resonance Raman spectroscopy; near-infrared spectroscopy; noninvasive monitoring; swine.

Detecting the presence of shock, its severity, and the adequacy of resuscitations continue to be high priorities in the development of technologies for the care of trauma patients. Because of the rapid echelons of care that a trauma patient passes through including the prehospital setting, noninvasive monitoring technologies are appealing. It has been argued that technologies designed to monitor for evidence of hypoxia at the tissue level would provide the most value to help prevent underresuscitation and overresuscitation.[1] To this end, technologies such as gastric tonometry, sublingual tonometry, transcutaneous gas measurement, near-infrared spectroscopy, and others have been studied.[2–7] Each of these relies on principles of microcirculatory oxygen transport and the fact that the postextraction compartment of tissue is dominated by venous blood. While each has advantages, none has been uniformly accepted, and each has the potential to be confounded.

We are exploring the use of resonance Raman spectroscopy (RRS) as a method to monitor tissue oxygenation. RRS has been valuable in the structural and ligand binding assessment of heme proteins.[8] The vibrational bands of heme are well-known but have only recently been explored to provide medically relevant information.[9,10] The spectroscopic basis for the application of RRS of hemoglobin lies in the resonance vibrational enhancement of hemoglobin in the deep violet region, allowing simultaneous identification and monitoring of the proportion of oxy and deoxy species of hemoglobin in a concentration-dependent manner with a single wavelength of light.[11] This produces a chemical fingerprint of the species with little or no interference from other compounds in the tissue being interrogated (Fig. 1).[11–13]

In this pilot study, we hypothesized that RRS would detect changes in buccal mucosal hemoglobin oxygen saturation (RRS-StO_2) in response to hemorrhage and that it would track changes in mixed and central venous hemoglobin oxygen saturation. Furthermore, we hypothesized that it would perform as well or better in this regard as compared with the use of near-infrared spectroscopy–derived tissue hemoglobin oxygen saturation (NIRS-StO_2).

MATERIALS AND METHODS

The Virginia Commonwealth University Institutional Animal Care and Use Committee approved this study, which

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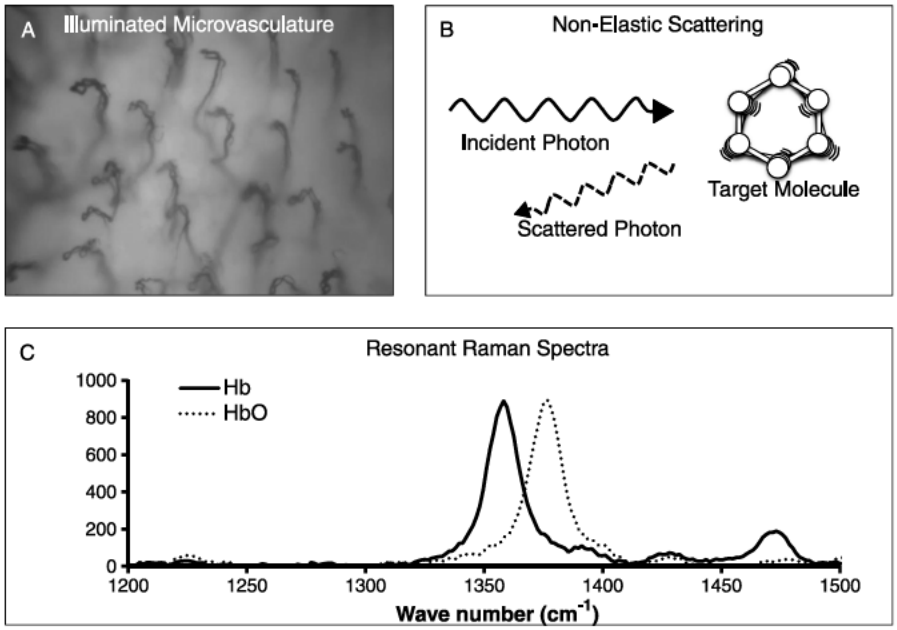

Figure 1. *A,* Example of oral microvasculature being interrogated by RRS (see text for details). *B,* Depiction of Raman vibrational spectroscopy phenomena. *C,* Example of resonant Raman spectra of oxyhemoglobin and deoxyhemoglobin using excitation at 405 nm.

adhered to the Guide for the Care and Use of Laboratory Animals (National Research Council, revised 2011).

Five male Yorkshire swine with a mean (SD) weight of 38 (1.5) kg were used in the study. Animals were fed a standard swine diet and were given one half of their morning feed ration on the morning of the experiment. Animals were sedated with intramuscular mixture of ketamine (20 mg/kg) and xylazine (2 mg/kg) before anesthesia induction. Anesthesia was induced with 4-mg/kg to 8-mg/kg intravenously administered propofol and maintained with intravenous α-chloralose (40–50-mg/kg intravenous bolus followed by 10-mg/kg/h infusion). Animals were intubated and ventilated (Fabius GS, Draeger Medical Inc., Telford, PA) with a tidal volume of 8 mL/kg to 10 mL/kg and 0.3 FIO_2. Ventilation was adjusted to produce an end-tidal CO_2 value of 38 mmHg to 44 mmHg (Biopac Systems Inc., Goleta, CA). Ventilation was unchanged after baseline was established and for the duration of the experiment. Core temperature of 38°C to 38.5°C was maintained through feedback via a rectal temperature probe and heating blanket (Blanketrol II, Cincinnati Sub-Zero Products, Cincinnati, OH).

Continuous electrocardiogram monitoring was performed using a standard 3-lead configuration (Biopac Systems Inc.). Mean arterial pressure (MAP) and arterial blood sampling was performed via an arterial catheter surgically placed in the carotid artery. A pulmonary artery catheter (Swan-Ganz Catheter, 746F8, Edwards Lifesciences, Irvine, CA) was inserted into the pulmonary artery via the right external jugular vein for monitoring of mixed venous hemoglobin oxygen saturation ($SmvO_2$) and central venous hemoglobin oxygen saturation ($ScvO_2$). The left external jugular vein was cannulated for fluid administration. The left femoral artery was exposed and cannulated for subsequent controlled arterial hemorrhage using a 9 Fr catheter (Smith Medical, Dublin, OH).

Tissue oxygen saturation measurements (StO_2) were performed using two technologies. (1) The microvascular oximeter (Pendar Medical, Cambridge, MA) uses RRS to determine relative concentrations of oxyhemoglobin and deoxyhemoglobin. A 405-nm, 4-mW laser coupled to a complex plastic fiberoptic cable illuminates an area of tissue approximately 3 mm in diameter and less than 1 mm deep. An autofluorescence imaging system using the same wavelength was used to take images of the oral mucosa from one of the authors (D.V.) to demonstrate an example of the type of oral microvasculature being interrogated with RRS (Fig. 1). The laser light excites oxyhemoglobin and deoxyhemoglobin molecules into distinct vibrational states resulting in a differential wavelength shift of the scattered light (Fig. 1). The spectrum of the scattered light is captured in the device's charged-coupled device-based spectrometer each second, with distinct sharp peaks linearly proportional to the concentration of oxyhemoglobin and deoxyhemoglobin. The spectral peaks are used to calculate the percentage of tissue oxygen saturation (StO_2) as the relative ratio of concentration of oxygenated hemoglobin to that of total hemoglobin.[11–13] To increase precision of the result, 20 to 80 spectra are averaged. The hemoglobin oxygen saturation (%) value is updated on the integrated monitor each second. The RRS-StO_2 sensor was placed on the buccal mucosa.

(2) NIRS (INVOS, Covidien, Mansfield, MA). The device uses a range of light frequencies between 650 nm and 1,100 nm. At these wavelengths, tissue is translucent. In addition, at these wavelengths oxyhemoglobin and deoxyhemoglobin differentially absorb light.[14] The technique is used in a reflectance mode in which the light source and detectors are spaced

TABLE 1. Average Baseline Characteristics of Animals

Baseline	Weight, kg	Blood Volume mL	Heart Rate, beats/min	MAP, mmHg	Hemoglobin, g/dL	Lactate, mmol/L	$SmvO_2$, %	$ScvO_2$, %	RRS-StO_2, %	NIRS-StO_2, %
Mean	38.4	2,496	109	98	8.8	0.7	60.8	64.4	62.0	53.4
SD	1.5	98.6	6.6	20.7	1.2	0.2	4.7	4.0	4.7	7.0

to allow the differential absorption spectra of oxyhemoglobin and deoxyhemoglobin to be obtained 2 cm below the surface while excluding signal from more shallow tissue. The device continuously samples spectra and updates values every 5 seconds. The NIRS sensor was placed over the left upper forelimb of the animal.

Once instrumentation was completed, animals were monitored for a 20-minute baseline period. At the end of baseline, hemodynamics, $SmvO_2$, $ScvO_2$, RRS-StO_2, and NIRS-StO_2 were collected as were arterial lactate and hemoglobin oxygen saturation (SaO_2) (ABL800, Radiometer America, Westlake, OH).

Five minutes after baseline measurement, controlled hemorrhage was started through the femoral artery at a rate of 30 mL/min (Masterflex Pump: Cole Parmer Instruments, Court Vernon Hills, IL). Baseline parameters described previously were recorded every 5 minutes for 60 minutes. At 60 minutes, animals were euthanized with potassium chloride (2 mEq/kg).

Statistical Analysis

Descriptive statistics are expressed as means and SDs. Pearson correlation was used to quantify the degree to which any two variables are related. We used unpaired *t* test to quantify differences between the variables. Where necessary, 95% confidence intervals (CIs) are included. Summary statistics using receiver operating characteristic (ROC) area under the curve (AUC) values were used for pooled data for RRS-StO_2, NIRS-StO_2, $SmvO_2$, and $ScvO_2$ to compare performance in the ability of tissue oxygenation methods to predict the presence of elevated lactate levels. Linear regression was used to quantify the relationships between variables to account for the repeated-measures structure of the data. Data analysis was performed on GraphPad Prizm6 (GraphPad Software, Inc., La Jolla, CA).

RESULTS

Five animals were studied. Tables 1 and 2 list the mean and SD of baseline and end-of-hemorrhage values for each oxygenation indicator as well as major hemodynamic variables. Sequential regional RRS-StO_2 changes tracked changes in $SmvO_2$ ($r = 0.917$; 95% CI, 0.867–0.949) and $ScvO_2$ ($r = 0.901$; 95% CI, 0.828–0.944) during hemorrhage, while NIRS-StO_2 failed to do so for $SmvO_2$ ($r = 0.283$; 95% CI, 0.04919–0.4984) and $ScvO_2$ ($r = 0.142$; 95% CI, −0.151 to 0.412). Scatter plots (Fig. 2) demonstrate the relationship between the variables. There were significant differences between the correlation coefficients when comparing RRS-StO_2 and NIRS-StO_2 with $SmvO_2$ (0.917 and 0.283, $p < 0.0001$) and when comparing with $ScvO_2$ (0.901 and 0.142, $p < 0.0001$). Unpaired *t* tests revealed no significant difference between RRS-StO_2 and both $SmvO_2$ and $ScvO_2$ ($p > 0.05$), whereas it indicated a significant difference between NIRS-StO_2 and both $SmvO_2$ and $ScvO_2$ ($p < 0.0001$).

ROC AUC (Fig. 3) was used to compare the ability of the various oxygenation monitoring methods to detect shock based on lactate levels. Lactate levels equal to or greater than 3 mM and equal to or greater than 4 mM were used to distinguish between shocked and nonshocked states. ROC yielded the following AUC values for lactates equal to or greater than 3 mM; $SmvO_2$ (1), $ScvO_2$ (0.994), RRS-StO_2 (0.972), and NIRS-StO_2 (0.611). When used to distinguish between lactate levels equal to or greater than 4 mM, AUC values were as follows: $SmvO_2$ (0.994), $ScvO_2$ (0.998), RRS-StO_2 (0.952), and NIRS-StO_2 (0.589) (Fig. 4). Nearly identical performance was noted when lactate thresholds of 5 mM and 6 mM were used.

Linear regression comparing RRS-StO_2 and $SmvO_2$ yielded a slope of 1.01 (95% CI, 0.892–1.11) with a Y intercept at 6.99 (95% CI, 3.366–10.62), meaning that when $SmvO_2$ equals zero, RRS-StO_2 will equal 6.99. A linear regression comparing NIRS-StO_2 and $SmvO_2$ yielded a slope of 0.126 (95% CI, 0.021–0.229) with a Y intercept at 44.96 (95% CI, 41.49–48.44), meaning that when $SmvO_2$ equals zero, NIRS-StO_2 will equal 44.96. There was a significant difference between the slopes ($F = 134.48$, $p < 0.0001$). Nearly identical results were obtained when the same analysis was performed comparing RRS-StO_2 and NIRS-StO_2 with $ScvO_2$.

DISCUSSION

Rapidly detecting the presence of impending shock, its degree when present, and its resolution during treatment continue to be high priority goals in the field of critical care. While invasive approaches using oxygen transport parameters such as $SmvO_2$ and $ScvO_2$ are valuable, their invasive nature makes their use in earlier echelons of care problematic.[15–18] Noninvasive technologies, which can be easily applied and

TABLE 2. Average End-of-Hemorrhage Characteristics of Animals and Average Total Blood Loss

End of Hemorrhage	Hemorrhage Volume, %	Heart Rate, beats/min	MAP mmHg	Hemoglobin, g/dL	Lactate, mmol/L	$SmvO_2$, %	$ScvO_2$, %	RRS-StO_2, %	NIRS-StO_2, %
Mean	66.3	172	22	7.5	11.2	5.6	7.6	6	48
SD	7.9	58	9.97	2.4	0.98	1.64	2	1.7	12.28

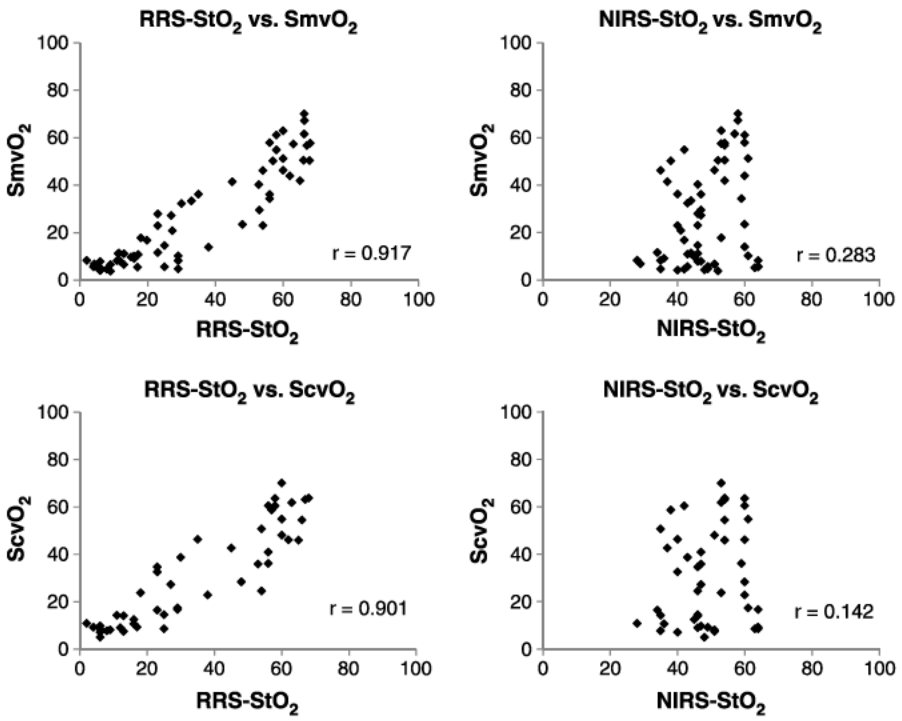

Figure 2. Scatter plots and Pearson correlations comparing RRS-StO2 and NIRS-StO2 with SmvO2 and ScvO2 (see text for details).

allow for continuous monitoring, would potentially offer significant value.

In this swine model of hemorrhagic shock, RRS-StO_2 of the oral mucosa tracked simultaneous changes in $ScvO_2$ and $SmvO_2$ statistically and clinically significantly better compared with NIRS. In addition, when compared with an elevated lactate as an independent marker of shock, the ability of RRS-StO_2 to detect a change was superior to NIRS-StO_2. Because RRS-StO_2 and NIRS-StO_2 are both local measures of tissue oxygenation versus global tissue aggregate measures ($SmvO_2$ and $ScvO_2$), we felt that comparing these values to a "shared" metabolic link would assist in evaluating their respective performance. Furthermore, because of the biphasic relationship of oxygen delivery and oxygen consumption, lactate levels are not elevated until a critical level of oxygen delivery and tissue oxygen extraction is reached.[19] Because animals have greater physiologic reserve than humans do, lactate levels are not elevated until SvO_2 levels drop to less than 40%. This study confirms our previous work in small animals and in vitro settings demonstrating the accuracy of RRS to measure hemoglobin oxygen saturation over the entire range of clinically relevant hematocrit and hemoglobin oxygen saturation levels and similar performance of RRS-StO_2 to $ScvO_2$ in signaling elevated lactate levels.[12,20]

The Raman effect is a measurable loss or gain of energy of scattered photons that correspond to the vibrational energies of a molecule in response to excitation from a light source at a precise wavelength (Fig. 1).[8] This type of vibrational spectroscopy

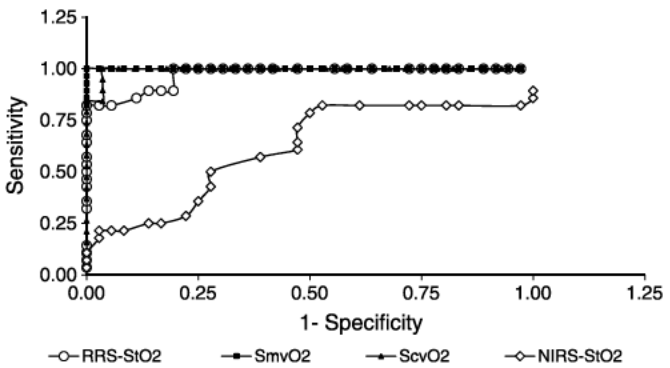

Figure 3. ROC AUC comparing the performance of SmvO2, ScvO2, RRS-StO2, and NIRS-StO2 to discriminate between shocked and nonshocked animals based on lactate levels equal to or greater than 3 mM.

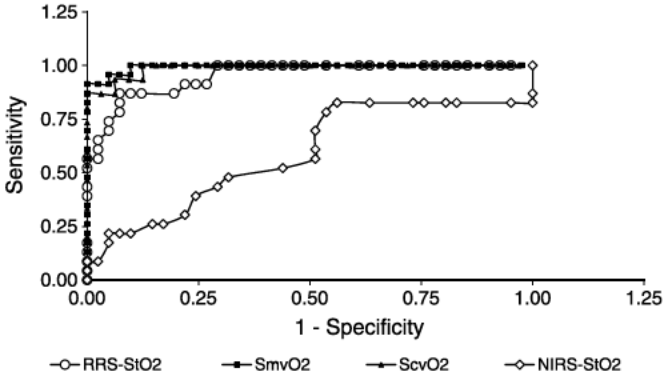

Figure 4. ROC AUC comparing the performance of SmvO2, ScvO2, RRS-StO2, and NIRS-StO2 to discriminate between shocked and nonshocked animals based on lactate levels equal to or greater than 4 mM.

is fundamentally different from the differential absorption spectroscopy (the basis for NIRS).[14] While Raman scattering is a low-intensity phenomenon requiring sensitive detectors, the signal is greatly enhanced when the excitation energy is in resonance with the electronic transition of the species being interrogated. This allows for the selective detection of very low concentrations of a molecular species in a complex mixture. In this study, the 405-nm wavelength used is near the peak for the heme Soret band, thus allowing for the production of strong resonance spectra of oxyhemoglobin and deoxyhemoglobin.[11] This resonance results in an enhanced signal-to-noise ratio.

Reasons for the improved performance of RRS-StO_2 over NIRS-StO_2 may in part be based on the contamination of the NIRS-StO_2 signal with myoglobin.[14] The NIRS spectra of oxymyoglobin and deoxymyoglobin are indistinguishable from that of oxyhemoglobin and deoxyhemoglobin. Studies have dealt with this issue by adding the species together (HbO_2 + MbO_2) and (Hb + Mb). Thus StO_2 as determined by NIRS (which include signal from muscle) would be determined as (HbO_2 + MbO_2) / (HbO_2 + MbO_2 + Hb + Mb). Awareness of this is important for several reasons including the fact that the P50 of myoglobin is 5 mmHg as opposed to 27 mmHg for hemoglobin and is thus fully saturated under most conditions.[21,22] In addition, in human skeletal muscle, myoglobin and hemoglobin will exist in approximately equal concentrations.[14,23,24] In the setting of hemorrhage, the fractional signal from myoglobin may even be increased during resuscitation, as hematocrit decreases during crystalloid resuscitation. Evidence using proton magnetic resonance (which is able to distinguish deoxy Mb from deoxy Hb) indicates that myoglobin might account for the majority of the NIRS signal.[25,26] Other confounders such as pigment and fat may explain why baseline values in volunteers can widely vary.[27] It may also explain why NIRS has not shown more sensitivity and specificity over blood pressure and lactate as an earlier warning system in either the setting of trauma or sepsis and why some are attempting to use dynamic changes in NIRS produced in response to vaso-occlusion to gain additional information.[6,27–29] Recent studies have examined the performance of commercially available NIRS devices on humans at baseline and in response to ischemic occlusion including the device used in this study. These studies demonstrate significant differences in performance between devices in baseline readings and in response to ischemia as well as significant differences in values at different sites including differences in repeatability at the same site with the same device.[30–32]

At the wavelength used for RRS-StO_2 measurement, the expected depth of penetration is less than 1 mm and is not contaminated by muscle, pigment, or fat in the buccal tissues. Previous studies using RRS on the sublingual surface of the tongue noted no contribution of tongue myoglobin from the tongue mucosa site after performing saline perfusion of the tongue.[20] The oral mucosa as shown in this study and others seems to be very sensitive to changes in perfusion.[33–35] However, because the buccal mucosa receives its blood supply from branches of the internal carotid artery and its microvascular orientation is more looped, it could be argued that it would not be an appropriate site of monitoring.[36] Furthermore, since RRS-StO_2 is only a reflection of the balance between mucosal blood flow and metabolism, it could be argued that the oral mucosa would not be metabolically active enough to reflect the global aggregates of tissue oxygenation such as $ScvO_2$ and $SmvO_2$. We have previously demonstrated that the StO_2 in the sublingual mucosa of rats completely desaturates in less than 60 seconds after cardiac arrest.[20] It was thus essential in this study to follow $ScvO_2$ and $SmvO_2$ to their lowest obtainable values and compare buccal mucosa RRS-StO_2 values with them. The very low values of RRS-StO_2 and their correlation to low $ScvO_2$ and $SmvO_2$ values supports this as an attractive tissue site for interrogation.

While it could be argued that the values of $ScvO_2$, $SmvO_2$, and RRS-StO_2 less than 40% are not relevant, clinical studies in heart failure and sepsis have demonstrated humans with values well less than 40% accompanied by elevated lactate levels.[15,16,37] Although values this low have not been reported in human traumatic hemorrhage, this may be caused by the fact that patients are rarely instrumented and monitored quickly before resuscitation. The ability to track such low values might also provide value in monitoring cardiac arrest resuscitation where values would be expected to be lower.[38]

In addition to the small number of animals, this study has several limitations. We tested only one manufacture's brand of NIRS monitoring. While there are several other manufacturers of this technology for monitoring peripheral tissue perfusion of the skeletal muscle, all are based on similar principals, with their differences being mainly where the suggested site of monitoring is and their algorithmic approach to signal analysis.[14] Therefore, the use of other devices may have yielded different NIRS performance. However, as indicated earlier, performance between devices have been noted to be significantly different and potentially not comparable with each other even in the same individual.[30–32] We monitored only at one site (proximal forelimb). This area in our animals lacked redundancy of skin and fat and was believed to be suitable for reproducibility. While others have used the hind limb, there should not be a significant difference from an anatomic standpoint since the animals are quadrupeds. However, we did desire not to confound NIRS-StO_2 with potential changes in blood flow to the hind limb caused by the placement of the 9 Fr (61 cm) femoral artery catheter used to hemorrhage since the tip of this catheter lies at the bifurcation of the abdominal aorta. Still, others have used the hind limb (with different NIRS technology), so we cannot rule out a difference in performance had we chosen another site or NIRS device.[39] We did not resuscitate animals, so we cannot report on the performance of either RRS-StO_2 or NIRS-StO_2 to track changes during resuscitation. We felt, however, that it was essential to demonstrate the ability of RRS to track StO_2 to its lowest levels during exsanguinating levels of hemorrhage as microvascular levels of hemoglobin drop and to prove that the oral mucosa would reflect central oxygenation. We note that there is a lack of such studies using NIRS.

Lastly, our baseline $ScvO_2$ and $SmvO_2$ were lower than might be expected. This may be caused by the fact that young swine are highly susceptible to iron-deficient anemia secondary to low tissue stores at birth and extremely rapid growth.[40] The swine we used did not receive iron supplementation in their diet.

CONCLUSION

In this model of hemorrhagic shock, RRS-StO_2 tracked changes in $SmvO_2$ and $ScvO_2$ well and seemed to have similar discriminatory power of $SmvO_2$ and $ScvO_2$ to detect shock based on lactate levels. Use of NIRS-StO_2, however, did not track $SmvO_2$ and $ScvO_2$ during hemorrhage and demonstrated less ability to discriminate shock states as compared with RRS-StO_2. While RRS-StO_2 may be a promising technique for the noninvasive evaluation of hemorrhage, additional testing will be required including its ability to track changes in tissue oxygenation during resuscitation.

AUTHORSHIP

M.H.T. and K.R.W. designed this study. M.H.T., G.T.D., and K.R.W. collected and analyzed the data. All authors contributed to data interpretation. M.H.T. and K.R.W. wrote the manuscript. M.H.T., R.W.B., J.T., I.T.F., P.R., D.V., and K.R.W. contributed to critical revision.

DISCLOSURE

K.R.W., R.W.B., J.T., and I.T.F. have intellectual property on the use of RRS for tissue oxygenation monitoring assigned to Virginia Commonwealth University. P.R. and D.V. are officers in Pendar Medical, which have licensed the Raman spectroscopy technology from Virginia Commonwealth University.

REFERENCES

1. Ward KR, Ivatury RR, Barbee RW. Endpoints of resuscitation for the victim of trauma. *J Intensive Care Med*. 2001;16:55 75.
2. Marik PE. Sublingual capnography: a clinical validation study. *Chest*. 2001;120:923 927.
3. Ivatury RR, Simon RJ, Islam S, Fueg A, Rohman M, Stahl WM. A prospective randomized study of end points of resuscitation after major trauma: global oxygen transport indices versus organ-specific gastric mucosal pH. *J Am Coll Surg*. 1996;183:145 154.
4. McKinley BA, Butler BD. Comparison of skeletal muscle PO2, PCO2, and pH with gastric tonometric P(CO2) and pH in hemorrhagic shock [see comments]. *Crit Care Med*. 1999;27:1869 1877.
5. Cohn SM, Crookes BA, Proctor KG. Near-infrared spectroscopy in resuscitation. *J Trauma*. 2003;54:S199 S202.
6. Cohn SM, Nathens AB, Moore FA, Rhee P, Puyana JC, Moore EE, Beilman GJ. Tissue oxygen saturation predicts the development of organ dysfunction during traumatic shock resuscitation. *J Trauma*. 2007;62: 44 54; discussion 55.
7. Gottrup F, Gellett S, Kirkegaard L, Hansen ES, Johansen G. Effect of hemorrhage and resuscitation on subcutaneous, conjunctival, and transcutaneous oxygen tension in relation to hemodynamic variables. *Crit Care Med*. 1989;17:904 907.
8. Hanlon EB, Manoharan R, Koo TW, Shafer KE, Motz JT, Fitzmaurice M, Kramer JR, Itzkan I, Dasari RR, Feld MS. Prospects for in vivo Raman spectroscopy. *Phys Med Biol*. 2000;45:R1 59.
9. Spiro TG. Resonance Raman spectroscopy: a new structure probe for biological chromophores. *Accts Chem Res*. 1974;7:339 344.
10. Spiro TG. Resonance Raman spectroscopy as a probe of heme protein structure and dynamics. *Adv Protein Chem*. 1985;37:111 159.
11. Ward KR, Barbee RW, Reynolds PS, Torres Filho IP, Tiba MH, Torres L, Pittman RN, Terner J. Oxygenation monitoring of tissue vasculature by resonance Raman spectroscopy. *Anal Chem*. 2007;79:1514 1518.
12. Torres Filho IP, Terner J, Pittman RN, Proffitt E, Ward KR. Measurement of hemoglobin oxygen saturation using Raman microspectroscopy and 532-nm excitation. *J Appl Physiol*. 2008;104:1809 1817.
13. Torres Filho IP, Terner J, Pittman RN, Somera LG, Ward KR. Hemoglobin oxygen saturation measurements using resonance Raman intravital microscopy. *Am J Physiol Heart Circ Physiol*. 2005;289:H488 H495.
14. Ward KR, Ivatury RR, Barbee RW, Terner J, Pittman R, Torres Filho IP, Spiess B. Near infrared spectroscopy for evaluation of the trauma patient: a technology review. *Resuscitation*. 2006;68:27 44.
15. Ander DS, Jaggi M, Rivers E, Rady MY, Levine TB, Levine AB, Masura J, Gryzbowski M. Undetected cardiogenic shock in patients with congestive heart failure presenting to the emergency department. *Am J Cardiol*. 1998;82:888 891.
16. Rivers E, Nguyen B, Havstad S, Ressler J, Muzzin A, Knoblich B, Peterson E, Tomlanovich M. Early goal-directed therapy in the treatment of severe sepsis and septic shock. *N Engl J Med*. 2001;345:1368 1377.
17. Scalea TM, Hartnett RW, Duncan AO, Atweh NA, Phillips TF, Sclafani SJ, Fuortes M, Shaftan GW. Central venous oxygen saturation: a useful clinical tool in trauma patients. *J Trauma*. 1990;30: 1539 1543.
18. Scalea TM, Holman M, Fuortes M, Baron BJ, Phillips TF, Goldstein AS, Sclafani SJ, Shaftan GW. Central venous blood oxygen saturation: an early, accurate measurement of volume during hemorrhage. *J Trauma*. 1988;28: 725 732.
19. Barbee RW, Reynolds PS, Ward KR. Assessing shock resuscitation strategies by oxygen debt repayment. *Shock*. 2010;33:113 122.
20. Ward KR, Torres Filho I, Barbee RW, Torres L, Tiba MH, Reynolds PS, Pittman RN, Ivatury RR, Terner J. Resonance Raman spectroscopy: a new technology for tissue oxygenation monitoring. *Crit Care Med*. 2006;34: 792 799.
21. Gayeski TE, Connett RJ, Honig CR. Minimum intracellular PO2 for maximum cytochrome turnover in red muscle in situ. *Am J Physiol*. 1987; 252:H906 H915.
22. Gayeski TE, Honig CR. Direct measurement of intracellular O2 gradients; role of convection and myoglobin. *Adv Exp Med Biol*. 1983;159:613 621.
23. Moller P, Sylven C. Myoglobin in human skeletal muscle. *Scand J Clin Lab Invest*. 1981;41:479 482.
24. Nemeth PM, Lowry OH. Myoglobin levels in individual human skeletal muscle fibers of different types. *J Histochem Cytochem*. 1984;32:1211 1216.
25. Nighswander-Rempel SP, Kupriyanov VV, Shaw RA. Relative contributions of hemoglobin and myoglobin to near-infrared spectroscopic images of cardiac tissue. *Appl Spectrosc*. 2005;59:190 193.
26. Tran TK, Sailasuta N, Kreutzer U, Hurd R, Chung Y, Mole P, Kuno S, Jue T. Comparative analysis of NMR and NIRS measurements of intracellular PO2 in human skeletal muscle. *Am J Physiol*. 1999;276:R1682 1690.
27. Crookes BA, Cohn SM, Bloch S, Amortegui J, Manning R, Li P, Proctor MS, Hallal A, Blackbourne LH, Benjamin R, et al. Can near-infrared spectroscopy identify the severity of shock in trauma patients? *J Trauma*. 2005;58:806 813; discussion 813 816.
28. Moore FA, Nelson T, McKinley BA, Moore EE, Nathens AB, Rhee P, Puyana JC, Beilman GJ, Cohn SM. Massive transfusion in trauma patients: tissue hemoglobin oxygen saturation predicts poor outcome. *J Trauma*. 2008;64:1010 1023.
29. Mayeur C, Campard S, Richard C, Teboul JL. Comparison of four different vascular occlusion tests for assessing reactive hyperemia using near-infrared spectroscopy. *Crit Care Med*. 2011;39:695 701.
30. Fellahi JL, Butin G, Fischer MO, Zamparini G, Gerard JL, Hanouz JL. Dynamic evaluation of near-infrared peripheral oximetry in healthy volunteers: a comparison between INVOS and EQUANOX. *J Crit Care*. 2013;28: 881.e1 881.e6.
31. Hyttel-Sorensen S, Hessel TW, Greisen G. Peripheral tissue oximetry: comparing three commercial near-infrared spectroscopy oximeters on the forearm. *J Clin Monit Comput*. 2013.
32. Nygren A, Rennerfelt K, Zhang Q. Detection of changes in muscle oxygen saturation in the human leg: a comparison of two near-infrared spectroscopy devices. *J Clin Monit Comput*. 2013.
33. De Backer D, Dubois MJ. Assessment of the microcirculatory flow in patients in the intensive care unit. *Curr Opin Crit Care*. 2001;7:200 203.
34. Verdant CL, De Backer D, Bruhn A, Clausi CM, Su F, Wang Z, Rodriguez H, Pries AR, Vincent JL. Evaluation of sublingual and gut mucosal microcirculation in sepsis: a quantitative analysis. *Crit Care Med*. 2009;37: 2875 2881.

35. Ward KR, Tiba MH, Ryan KL, Torres Filho IP, Rickards CA, Witten T, Soller BR, Ludwig DA, Convertino VA. Oxygen transport characterization of a human model of progressive hemorrhage. *Resuscitation*. 2010;81: 987 993.
36. Massey MJ, Larochelle E, Najarro G, Karmacharla A, Arnold R, Trzeciak S, Angus DC, Shapiro NI. The microcirculation image quality score: development and preliminary evaluation of a proposed approach to grading quality of image acquisition for bedside videomicroscopy. *J Crit Care*. 2013.
37. Rady MY, Rivers EP, Martin GB, Smithline H, Appelton T, Nowak RM. Continuous central venous oximetry and shock index in the emergency department: use in the evaluation of clinical shock. *Am J Emerg Med*. 1992;10:538 541.
38. Rivers EP, Martin GB, Smithline H, Rady MY, Schultz CH, Goetting MG, Appleton TJ, Nowak RM. The clinical implications of continuous central venous oxygen saturation during human CPR. *Ann Emerg Med*. 1992;21: 1094 1101.
39. Taylor JH, Mulier KE, Myers DE, Beilman GJ. Use of near-infrared spectroscopy in early determination of irreversible hemorrhagic shock. *J Trauma*. 2005;58:1119 1125.
40. Staton HC, Mersmann HJ, eds. *Swine in Cardiovascular Research*. Boca Raton, FL: CRC Press; 1986;II:21.