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14. ABSTRACT
The research project was conducted in three-parts to validate the accuracy and reliability of a specific NIRS sensor (Equanox, Nonin, Inc, Plymouth, MN) in diagnosing acute compartment syndrome following severe leg injury. Part 1 was a series of observational clinical studies, the first of which was completed at Landstuhl Regional Medical Center during year 1. The primary clinical study was originally planned to be conducted in-theater (OEF/OIF), but had to be transitioned to a FDA-regulated study conducted under abbreviated IDE requirements in the US. This study was conducted at three trauma centers in Georgia. The study was completed and the final data analysis is reported herein. Part 1 demonstrated the safety and efficacy of NIRS oximetry in diagnosing ACS, but it also identified areas for improvement. Part 2 of the project involved animal studies to address issues raised in clinical testing and to promote further understanding of NIRS response to ACS. Four animal studies were completed and they demonstrated that NIRS in a controlled in vivo setting is highly accurate in diagnosing ACS, and just as importantly, the absence of ACS in traumatized limbs. Part 3 of this project is the translation of the knowledge gained in Parts 1 and 2 into a FDA-approvable format that will be available for clinical use, to ultimately answer long unmet needs in combat casualty care.

15. SUBJECT TERMS
Near Infra-Red Spectroscopy, acute compartment syndrome, fasciotomy, wound, combat

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Table of Contents

Page

INTRODUCTION

This award covered animal and human studies to validate the accuracy and reliability of using Near Infrared Spectroscopy (NIRS) to diagnose acute compartment syndrome (ACS) in combat troops and civilians suffering high energy trauma to the legs. ACS may develop in severely traumatized limbs when swelling and bleeding increases pressure within the non-expandable muscle compartment to the extent that blood flow is cut off, resulting in ischemia and necrosis. Universally accepted treatment involves surgical release of pressure within the fascial compartments (four compartment fasciotomy) allowing the muscles to expand and re-perfuse. Missed or delayed diagnosis can have devastating consequences, increasing morbidity and mortality. Accurate diagnosis of ACS has been the greatest challenge to date, relying heavily on physician experience and preference in evaluating subjective clinical signs and symptoms. The only objective ACS diagnostic in common use is measurement of intra-compartmental pressure (ICP). However, this is an invasive procedure that can produce erroneous results, and diagnostic threshold values have been disputed. Furthermore, ICP measurements are single points in time, whereas ACS is a condition that evolves over time requiring serial monitoring over hours or even days.

The research covered under this award evaluated the direct measurement of tissue oxygenation using NIRS as a noninvasive, continuous, and responsive solution for ACS diagnosis. NIRS uses three wavelengths of near infrared (NIR) light that can penetrate skin, soft tissue, and bone, and are absorbed differentially by oxygenated and deoxygenated hemoglobin. Percent oxygen saturation (rSO_2) of hemoglobin in the muscle tissue is calculated by measuring the amount of each wavelength that is reflected back to the sensor. Previous animal and clinical studies confirmed the ability of NIRS technology to monitor perfusion in muscle compartments of the lower leg in normal, hypoxic, and traumatized conditions. In human studies rSO_2 evaluated using NIRS decreased significantly in extremities diagnosed with ACS compared to the uninjured contralateral extremity, and increased in response to fasciotomy.

BODY

The goals of the current award were to systematically evaluate the use of NIRS to diagnose ACS and to validate its diagnostic accuracy and reliability. This was achieved through a three-part program covering two clinical observational studies (Part 1; Tasks 1 and 2), four animal studies using validated porcine models of ACS (Part 2; Task 3), and the translation of study results into a proven means for detecting critical hypoperfusion of the leg compartments indicative of ACS (Part 3; Tasks 4 to 6). The final deliverable of this award will be a publically available, FDA-approved device with solid preclinical and initial clinical support for use as a diagnostic tool for ACS. Task 6 commences the next stage in the technology development which includes creation of a diagnostic algorithm and guidelines for using NIRS to diagnose ACS, and completiont of a subsequent FDA-IDE clinical trial to validate the algorithm. This research will ultimately lead to an FDA indication for using NIRS as an ACS decision-support device.

KEY RESEARCH ACCOMPLISHMENTS

- NIRS oximetry has been translated from a research tool with no routine clinical use in the setting of ACS , to a viable means for monitoring traumatized patients and diagnosing ACS. This work has unquestionably demonstrated the viability of this technological solution to the long unmet need in identifying a reliable and accurate means for diagnosing ACS.
- Key limitations to the existent and modified NIRS technology, which was initially developed to monitor cerebral tissue in anesthetized patients, were identified, sequentially addressed and most were overcome. For challenges not yet met, technological solutions with high probability of success and methods for their validation have been identified.
- Clinical guidelines in order to both obtain data as well as interpret the data in the setting of trauma were developed and validated using both animal models as well as traumatized subjects. These guidelines included identifying the need for controls, determining the optimal control sites and demonstrating a framework in order to identify well perfused subjects, subjects who are developing ischemia and ischemic compartments in ACS.

- By executing rigorous scientific studies as well as extensive presentations and publications, the the use of NIRS in traumatized patients is gaining acceptance by clinicians in the civilian and military setting.
- Animal labs were performed in highly controlled settings to confirm both the reliability of NIRS in ischemic tissue as well as the ability of NIRS to confirm adequate release of elevated pressures in the setting of ACS.
- A use specific NIRS oximeter designed as a decision-support tool for ACS has been developed based on the results collected over the grant period. The completion of this design process and validation must be completed under a subsequent award.
- The researchers designed, implemented and completed the first of two FDA requested, multi-center, FDA monitored IDE studies obtaining continual blinded NIRS oximetry data on traumatized patients over a period of 48-72 hours. The study resulted in over 30 million data points that were cleaned, analyzed and tabulated in order to define the clinical guidelines governing the use of NIRS in traumatized subjects.
- A real time, continual, noninvasive, objective, physiologic, monitor of muscle perfusion for all four compartments has been developed that can now be tested under a FDA monitored study with unblinded data for final FDA approval.

REPORTABLE OUTCOMES

TASK 1: Human Use Study – Phase 1

Task 1 covered the Phase 1 study, the purpose of which was to define the reliability and accuracy of NIRS in detecting perfusion status of the lower leg muscle compartments in injured and uninjured extremities over time. The goal of the study was to develop guidelines for using NIRS in diagnosis of ACS, monitoring patients at risk for ACS, and evaluating the adequacy of fasciotomy in patients treated for ACS. The study was conducted at the Landstuhl Regional Medical Center in Germany.

Study Design

This prospective observational study continuously monitored tissue oxygenation in the lower leg muscle compartments using NIRS and compared them to intracomparmental pressure (ICP) measurements, vital signs, and clinical examinations in three groups of study subjects. Study subjects were active duty military personnel from Operation Iraqi Freedom (OIF) and Operation Enduring Freedom (OEF) that were treated at LRMC and provided informed consent to participate. The study consisted of two control groups and one investigational group.

- Cohort 1: Normal Physiological State Control Subjects (n=45) were uninjured volunteers from the LRMC spine clinic.
- Cohort 2: Critically-Injured Physiological State Control Subjects (n=45) were severely injured military personnel that did not have injuries to the lower extremities. These were military personnel that were evacuated from theater to LRMC.
- Cohort 3: Severe Traumatic Leg Injury Investigational Subjects (n=45) were military personnel that were evacuated from theater with severe leg injuries.

Tissue oxygenation was monitored on the four compartments of each lower extremity using two Somanetics INVOS NIRS monitors. Cohort 2 and 3 subjects were monitored throughout their admission at LRMC and during their subsequent air evacuation to CONUS. Fat depth over the superficial posterior compartment was measured untrasonically (BX 200 Intelametrix), skin pigmentation was measured over the volar aspect of the arm, and vital signs were recorded on all subjects for the duration of their study participation. Additionally, ICP was measured on all compartments of the lower legs for all Cohort 3 subjects during their leg surgery.

Task 1a: Create and Submit SAMMC Human Use IRB Protocol Application, Principal Investigators Complete CITI Training; Obtain second level DoD IRB Approval.

During Y1/Q1 the Phase 1 protocol was written and approved by the SAMMC IRB, and by the second level DoD IRB. The Principal Investigators completed CITI training. This task was completed in Y1/Q1.

Task 1b: Obtain Clearance Impact Statements
This task was completed in Y1/Q1.

Task 1c: Hire 2 Research Coordinators (LRMC Site) and 1 Project Manager (J+M Shuler Site)
This task was completed in Y1/Q1.

Task 1d: Initiate Patient Enrollment in Phase 1 Study
During Y1/Q1 we trained the research team on the study procedures and prepared to begin enrollment. A number of early technical issues were encountered and overcome, such as establishing the communication between the Somanetics Vital Sync device and the Philips C70 Intellivue ICU monitors. Although this required the relatively simple technical solution of upgrading the Intellivue software, it proved to be administratively challenging, resulting in a minor delay in initiating subject enrollment. As a consequence, the study initiation date was delayed to February 2, 2010 (Y1/Q2) rather than in the previous quarter.

Task 1e: Conduct Phase 1 Prospective Observational Study
The Phase 1 study commenced early in February, 2010 (Y1/Q2) and by the end of that quarter one third (45) of the anticipated subjects had been enrolled. Throughout Y1/Q3 enrollments progressed, but at a slower rate than anticipated, such that 62% of enrollments were complete at the end of that quarter. Slower than anticipated enrollments in Y1/Q3 were the consequence of volcanic ash from Iceland preventing air evacuation flights to Germany, and resupply of NIRS sensors. Enrollment in the Phase 1 study was completed in Y1/Q4.

Task 1f: Analyze Data, Provider Feedback and Amend Phase 2 Methodology as Needed
Results from Cohort 1 were analyzed during Y1/Q4. Complete data analysis was completed by the end of Year 2. The results of the Phase 1 study are appended to this report in Jackson et al. in the form of a published manuscript.

Task 1g: Present/Publish Results of Phase 1 Study
Data from Cohort 1 was presented at the following conferences:

1. 2010 Society of Military Orthopaedic Surgeons
2. 2011 American Academy of Orthopaedic Surgeons
3. 2011 Advanced Technological Applications for Combat Casualty Care (ATACCC)

TASK 2: Human Use Study – Phase 2
The tasks stated in the grant proposal covered the Phase 2 study that was originally planned to be conducted in Combat Support Hospitals in Iraq and Afghanistan exempt from the requirement to obtain informed consent from study participants as provided for under the Common Rule for minimal risk studies using non-significant risk devices. Partway through the study review process, the USAMRMC's Human Research Protections Office (HRPO) requested that the FDA make a determination on the IDE status of the study. The FDA determined that the study should be conducted as an abbreviated IDE and therefore required study participants to provide informed consent. Subsequently JC2RT decided that informed consent could not, and should not, be requested of study participants, since consent for medical procedures is not required. As documented under Task 2a, this resulted in the Phase 2 study being transitioned to three civilian trauma centers in the State of Georgia. The tasks included in the SOWs for the Phase 2 study, the budget, and protocol were modified to reflect this change during Y2Q3.

Study Design

The Phase 2 study was a prospective, observational study evaluating the utility of NIRS to monitor tissue oxygenation in traumatized and untraumatized muscle compartments of the lower leg over time. The goal of the study was to establish diagnostic thresholds and algorithms indicative of ACS development and clinical guidelines for using NIRS as a diagnostic tool. These parameters would be validated in a subsequent interventional study.

Study subjects were patients presenting to the participating trauma centers with injuries meeting the protocol inclusion criteria. Cohort 1 subjects were critically injured patients without lower extremity trauma that were expected to stay in the ICU for at least 48 hours. These subjects served as controls for the critically injured physiological state that may induce systemic rather than regional hypoperfusion. Cohort 2 subjects were patients with injuries to the lower leg that were at high risk for developing ACS (high energy tibia fractures or gunshot wounds without tibia fracture), that could be enrolled within twelve hours of their injury. Those Cohort 2 subjects that subsequently developed ACS and required a fasciotomy transitioned into Cohort 3. Potential subjects were identified by reviewing the medical records of patients admitted to the ICU (Cohort 1) or after notification from the treating physician (Cohort 2). They were screened based on the eligibility criteria outlined in the protocol, and informed consent was sought either from the eligible patient, or from a legally authorized representative (LAR) in situation where the eligible patient was unable to consent for themselves.

Results from our earlier clinical studies revealed significant inter-subject variability in rSO_2 values measured on the lower leg compartments, but low variability in rSO_2 values between the two legs of an individual subject. Consequently, the uninjured leg is a suitable control and comparator for the injured leg. In this Phase 2 study we monitored rSO_2 in the lower leg compartments of both extremities. Additionally, rSO_2 was monitored in the arch of each foot and in the deltoid and volar compartments of one upper extremity to determine if these compartments could serve as suitable comparators in cases of bilateral lower leg trauma.

Tissue oxygenation was monitored in the twelve compartments (if possible) using the Nonin Equanox™ 7600 tissue oximeter and 8003CA regional oximeter sensors. Each 7600 oximeter has four recording channels. Three units were used to monitor each subject with the units connected and synchronized via a laptop computer. Tissue oxygenation values for each compartment were recorded every four seconds and were saved in a single spreadsheet file on the laptop. Study participation was for 48 hours (if possible) and extended up to 72 hours in Cohort 2 subjects considered at risk for developing ACS. Depth of the subcutaneous tissue overlying the superficial posterior compartment of each extremity was measured using a portable ultrasound device, and skin coloration was evaluated using a colorimeter specific for skin pigmentation. Demographic and clinical data were captured from the patient's medical records for the duration of their study participation.

Task 2a. Create and Submit Local Institutional Human Use IRB Protocol Application (Months 21 to 24).

The process for securing IRB approval for this minimal risk study of a non-significant risk device was long and difficult. **Table 1** outlines the approval processes, challenges encountered, and milestones reached. The original plan was for this study to be conducted in theater at Combat Support Hospitals (CSH) in Iraq and Afghanistan with support from the Joint Combat Casualty Research Team (JC2RT), and with waiver of informed consent as provided for under the Common Rule (46 CFR 45) for minimal risk studies and devices. The protocol was prepared in the latter part of Year 1, Quarter 1 (Y1/Q1) and was submitted to JC2RT for approval in Y1/Q2 (25 March, 2010). The expectation was that it would receive routine approval for implementation in theatre by JC2RT, scientific review from the United States Army Institute of Surgical Research (ISR), expedited ethical review by the San Antonio Military medical Center (SAMMC) Institutional Review Board (IRB), and second level ethical review by USAMRMC's Human Research Protections Office (HRPO). Enrollment was expected to commence in Y1/Q4 (August/September 2010).

However, during the second tier ethical review during Y1/Q3, HRPO raised concerns about the ability for data obtained from a waived consent protocol to be used to support an FDA regulatory submission. After lengthy discussions with relevant stakeholders, it was concluded that this Phase 2 study would not be used to support an FDA 510(k) application. The protocol and Statement of Work (SOW) were updated accordingly, the protocol being resubmitted to ISR on 07 June, 2010. This caused about a one month delay in the projected enrollment start date, which was pushed back to September 2010 to coincide with the next JC2RT deployment.

The revised protocol was resubmitted to HRPO on 08 July, was reviewed on 11 August, and all changes requested by HRPO were addressed by 08 September, 2010. However, during the review process HRPO has asked the FDA to make a determination on the Investigational Device Exemption (IDE) status of the protocol. The FDA determined that the study must be conducted under an abbreviated IDE. This changed the status of the study from one that was exempt from informed consent under the Common Rule due to its low risk to one requiring informed consent from all study participants. Discussion with JC2RT members during September indicated that it was feasible to conduct the Phase 2 study with informed consent since sufficient numbers of consentable war causalities being treated in Afghanistan, but this would extend the enrollment period by three to six months, as documented in our Year 1 Annual Report. The revised protocol including obtaining informed consent from all subjects was approved by HRPO on 06 October, 2010 with the stipulation that the study include an FDA-compliant medical monitoring plan.

The revised protocol included addition of the Camp Dwyer and Kandahar Combat Support Hospitals as study sites since the US Army was no longer supporting medical research in Iraq, and admissions to Bagram had dropped significantly due to changing missions in Afghanistan. A further setback occurred when the new commander at the Kandahar CSH stated that informed consent could not, and should not, be sought from traumatized warfighters since it was not obtained for medical procedures. Therefore, with the FDA determination that the Phase 2 study should be conducted under an abbreviated IDE requiring informed consent collided with the updated JC2RT decision that we could not seek informed consent for study participation, effectively bringing the study to a halt.

On 25 November 2010 (Y2/Q1) JC2RT updated the CENTCOM Surgeon on how HRPO's decisions had impacted this Phase 2 study, plus three other minimal risk device studies that were being conducted in theater. The issue was promoted to the Office of the Surgeon General which resulted in a petition to the FDA to waive requirement for informed consent for minimal risk studies conducted in theater. The Phase 2 study was placed on hold indefinitely until a resolution could be found. This came on 11 February, 2011 (Y2/Q2) with a decision from the FDA that informed consent could not be waived.

To complicate matters during Y2/Q2 the T.R.U.E. Research Foundation that was managing this award, suddenly and unexpectedly, filed for bankruptcy. The award was officially re-awarded to The Geneva Foundation at the end of Y2/Q3. Further, the provider of the NIRS system we were using, Somanetics Inc, was sold to Covidien, which had no interest in furthering their relationship with this study. Consequently we transferred our interest to Nonin Medical to supply NIRS equipment for the study. To accommodate these changes, the SOWs, budget, and protocol were further modified to allow the project to proceed successfully. A plan to transfer the protocol to the civilian setting, utilizing three trauma centers in the State of Georgia, was presented to CDMRP on 28 March, 2011. The plan, including a one-year increase in timeline and 9 – 10% increase in total budget to cover these unforeseen delays and increased cost of running the study in civilian hospitals was unofficially endorsed on 19 April, 2011 (Y2/Q3).

The contingency plan to transfer the Phase 2 study from the combat zone to the civilian setting was developed and implemented during Year 2, Quarters 2 and 3. Three sites were identified in Georgia with Principal Investigators capable and willing to undertake the study. Letters of support were obtained from Dr William Reisman, Dr Bruce Ziran, and Dr Chip Ogburn, the Directors of Orthopedic Trauma at Grady Memorial Hospital (GMH), Atlanta Medical Center (AMC), and Athens Regional Medical Center (ARMC) respectfully. Institutional Review Board submissions were prepared for these sites during Y2/Q3 but they could not be submitted until contracts and budgets were established with each site.

Throughout the second half of Year 2 we worked on preparing materials to conduct the Phase 2 study in the civilian environment, including preparing site-specific IRB submissions, study procedures, and data management processes. Approval to conduct the study at the three identified civilian trauma centers and to submit protocols to their IRBs was received from USAMMDA on 18 October 2011 (Y2/Q4). Initial local IRB approvals were obtained during Y3/Q1. HRPO approval for GMH was granted on 13 January 2012 (Y3/Q2), but HRPO approval for AMC and ARMC was delayed until April 11 and May 17 respectively due to issues encountered with the local IRB approvals (documented in our Y3/Q3 Quarterly Report).

Task 2b: Obtain Clearance and Impact Statements (Months 21 – 27)

This process was commenced in Y1/Q1and was completed for the Phase 2 study as initially planned for conduct in theater in Y1/Q3, with the exception of final HRPO approval which was contingent on a solution to the waived informed consent issue being determined with the FDA. The consequences of this determination being made in favor of informed consent (in Y2/Q2), the subsequent decision by JC2RT that informed consent was not possible in the combat environment, and the resulting transfer to the study to the civilian setting, required further Clearance and Impact Statements to be obtained from the three civilian trauma centers serving as study sites. These were obtained from the respective Chiefs of Orthopedic Trauma at participating sites in Y2/Q4.

Task 2c: Recruit Lead Investigators for GMH, AMC, ARMC

Recruiting lead investigators for the originally planned study to be conducted in Afghanistan and Iraq was an ongoing process since typical deployments of JC2RT team members at the participating combat support hospitals was for six months. The first on-site PI assisted in establishing the site at Bagram Air Force Base in September and October 2010 (Y1/Q4) and with identifying two additional sites at Camp Dwyer and Kandahar Air Force Base. At this stage, the Army no longer supporting research in Iraq, and, due to changing missions in Afghanistan, the case load at Bagram had decreased, requiring additional sites to be brought on in Afghanistan. In Y1/Q4 a replacement for the Bagram PI had been identified, and PIs for the two new sites had also committed to the study. The two clinical research coordinators (CRCs) at LRMC and the project manager at J+M Shuler in Athens, GA, that were hired in Y1/Q1 to manage the Phase 1 study were also going to manage the Phase 2 study.

We received the final decision from the FDA that we could not perform the Phase 2 study in theater without informed consent in February 2011 (Y2/Q2) which precluded the study be conducted in the combat zone (see section on Task 2a and **Table 1**). Our contingency plan to move the study to the civilian environment was instigated immediately. Three sites were identified in the state of Georgia that could participate in the study. Commitments were received from suitable Principal Investigators (PIs) that same quarter (Y2/Q2).

Three CRCs were hired in Q3/Y1 to conduct the study at both Atlanta sites (GMH and AMC) plus there was an existing CRC and a project manager in Athens to manage the ARMC site. All research staff underwent training on study procedures, including placement of NIRS sensors and use of the Nonin Equanox™ 7600 oximeter in Atlanta with the expectation that the study would start recruiting subjects the following quarter. During Y3/Q1 the Athens-based project manager left the area and, although still actively overseeing the study, she was based in California, not Georgia. A second project manager was hired in the last week of Y3/Q2 to manage the overall program at LRMC, since the two CRCs that had coordinated the Phase 1 study in LRMC were no longer needed once that study was completed, and the Phase 2 study had moved to Georgia.

During Y3/Q3, project manager in California and the Athens-based CRC left the study, and it became apparent that more coordinator support was needed in Atlanta. An additional CRC was hired to support the two Atlanta sites. The remaining project manager agreed to remain in the United States, being located in Atlanta until the end of Y3/Q4 and then in North Florida until the end of the program, and an additional project manager was hired to be permanently based at the Athens site. A CRC was then hired for the Athens site in Y3/Q4 to ease the demands of providing 24/7 on-call coverage.

Through to the end of Y4/Q1 the study was supported by four CRCs in Atlanta, a CRC and project manager in Athens, and a project manager in Florida. At the start of Y4/Q2 one of the full-time CRCs in Atlanta was replaced by two part-timers who could provide 24/7 coverage on weekends. The CRC at Athens left at the end of Y4/Q3 and was not replaced. When enrollment was completed at the end of Y4/Q4, the three full-time CRCs and two part-time CRCs based in Atlanta were terminated. The Florida-based project manager was employed by the study for an additional month, after which the Athens-based project manager remained on to close out the study.

Task 2d: Conduct Phase 2 Prospective Observational Study

USAMMDA and HRPO approval for the study was finalized on 18 October 2011 (Y2/Q4), and each of the three Georgia sites had local IRB approval by the end of Y3/Q1 (**Table 1**). HRPO approval of the GMH site was awarded early in Y3/Q2 (13 January, 2013). Due to issues with the local IRB approvals, discussed in our Y3/Q2 report), final HRPO approval was delayed until Y3/Q3 (AMC in April and ARMC in May). Patient screening began at GHM on 23 March and the first subject was enrolled on 26 March (Y3/Q2). AMC and ARMC followed in August (Y3/Q4 – **Table 1**).

Site	Initial IRB Approval	Initial HRPO Approval	Start of Patient Screening	First Subject Enrolled
GMH	12/20/2011	01/13/2012	03/23/2012	03/26/2012
AMC	11/10/2011	04/11/2012	08/10/2012	08/30/2012
ARMC	12/29/2011	05/17/2012	08/01/2012	08/20/2012

Table 1: Major Milestones in the establishment and initiation of clinical research sites for the NIRS study

Patient Screening

Table 2 illustrates the number of patients that were screened per month across all three sites. Screening of potential subjects commenced at GMH in March 2012 (Y3/Q2), but, due to issues with obtaining or maintaining a NIRS signal on some compartments (as discussed below) screening was suspended temporarily while the CRCs were retrained in placing sensors, and troubleshooting the NIRS equipment. Screening restarted in June, initially of potential Cohort 1 subjects, then of Cohort 2 subjects. In December 2012, screening of potential subjects was again put on hold while the contract between the site and The Geneva Foundation was renewed. Screening resumed for Cohort 2 during January and for Cohort 1 at the beginning of February. Screening of potential subjects commenced at AMC and ARMC during August 2012. It was put on hold at AMR during April and May 2013 while the change in PI was processed.

When screening started the age eligibility was 18 to 60. However, we were missing a number of otherwise eligible patients in the 60 to 65 age category, and, since there wasn't a medical reason for excluding these patients, the upper age limit was extended to 65 in protocol version 2.2 which took effect in July 2012.

Cohort 1 Screening

All patients admitted to the ICU for trauma were screened for inclusion in Cohort 1 by daily review of the medical records at GMH. The other two sites were not involved in enrolling Cohort 1 subjects. At total of 862 subjects were screened over the study resulting in 35 subjects being consented. However, 12 of these subjects were withdrawn prior to the start of NIRS monitoring leaving 23 enrolled subjects (**Figure 1**). Seven subjects were determined to be ineligible subsequent to consenting, four withdrew consent before NIRS monitoring started, and one was withdrawn.

	2012							2012
	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total

Cohort 1 - GMH	30	113	105	127	77	69		521
Cohort 2 - GMH	24	19	13	16	12	17	1	102
Cohort 2 - AMC			5	8	3	7	3	26
Cohort 2 - ARMC			7	6	8	4	2	27
Total	54	132	130	157	100	97	6	676

	2013									2013	OVERALL
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Total	TOTAL
Cohort 1 - GMH		21	40	18	58	59	84	61		341	862
Cohort 2 - GMH	11	11	11	16	18	17	12	16	8	120	222
Cohort 2 - AMC		2	1			3	7	2	3	18	44
Cohort 2 - ARMC	2	5	5	2	3	4	4			25	52
Total	13	39	57	36	79	83	107	79	11	504	1180

Table 2: Monthly screening for potential study subjects by site

Initially the time window for enrollment in Cohort 1 was the same as for Cohort 2 – 12 hours after injury. However, many potential subjects were being missed because they would not be consented within this time frame. The severely injured patient that was bound for the ICU usually had extended clinical workups, often sent directly to surgery, and didn't land in the ICU for some time after their injuries occurred. Since we were screening for Cohort 1 from the records of patients admitted to the ICU, we had no access to these patients until they were actually admitted to the ICU – often times outside the 12 hour window. Additionally, consent for most of these patients was required from an LAR, and it was often difficult to locate the appropriate LAR within the 12 hour window. Within the first two months of screening we missed seven potential subjects because

All ICU Admissions for Trauma	Number	Percentage
Expected length of stay < 48 hours	349	40.5
Bony or vascular injury to lower extremity	124	14.4
Age not in 18 to 65 range	116	13.5
Other reason	67	7.8
Declined consent	52	6.0
No LAR to provide consent	36	4.2
In police custody	35	4.1
Spinal cord injuries causing paralysis	24	2.8
ENROLLED	23	2.7
No uninjured upper extremity	9	1.0
Application of sensors would impede care	7	0.8
Outside 12 hour time window	7	0.8
Admission not trauma-related	6	0.7
Is potentially pregnant	4	0.5
Could not place sensors	3	0.3
TOTAL	862	100.0

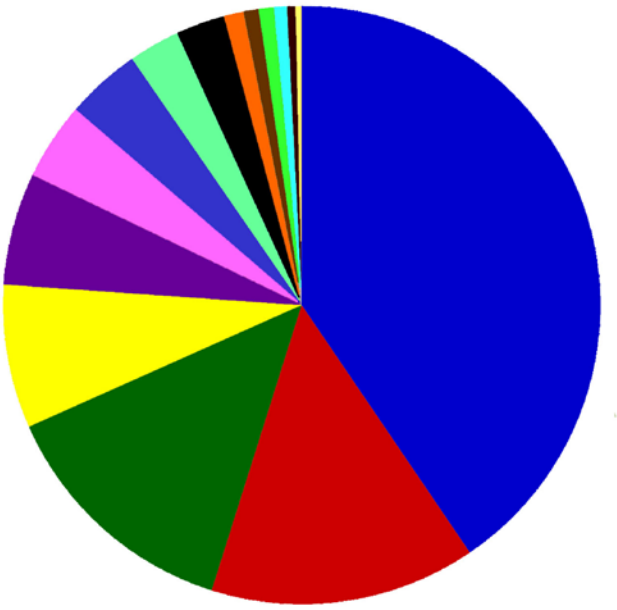


Figure 1: Analysis of screening outcomes of potential Cohort 1 subjects.

they could not be consented within the timeframe. This 12 hour window applied to Cohort 2 patients because that was the timeframe in which ACS is most likely to develop. It does not apply to Cohort 1 subjects who may develop systemic hypoperfusion at any stage in their ICU stay. Consequently, the enrollment window for Cohort 1 subjects was extended to seven days from injury. This was included in protocol version 2.2 and implemented in July 2012.

When the study started, all patients admitted to the ICU for trauma were eligible for inclusion. In protocol amendment 2.3 the provision for limiting inclusion to just patients with moderate to severe traumatic brain

injury (TBI) was implemented. The purpose for this was to obtain NIRS data on another traumatized tissue, one for which there was a wealth of control data from untraumatized tissue. This was implemented in October 2012. However, moderate to severe TBI patients proved to be few and far between, and also difficult to consent due to the need for surrogate consent from an LAR. Consequently, we reverted to including all trauma patients in May 2013.

Twenty-three (2.7%) subjects completed enrollment in Cohort 1. **Figure 1** illustrates the reasons that patients did not meet the screening criteria for inclusion in Cohort 1. The majority (40.5%) were because their expected length of stay in the ICU was less than 48 hours. Most of these were patients with mild TBI or liver lacerations that were admitted for 24 hour observations. However, some were so severely injured that they were not expected to survive. The next most common reasons for exclusion were patients with injuries to the lower extremities (14.4%) and patients outside the age range (13.5%). The need to obtain consent from an LAR for the severely injured patients also proved difficult. Thirty-six (4.2%) potential patients could not be enrolled because an appropriate LAR could not be identified and contacted within the seven day enrollment period.

Sixty-seven patients (7.8%) were excluded for reasons other than the eligibility criteria stated in the protocol. The majority of these (46.3%) were because they would not make good study candidates as they were either combative or noncompliant with their clinical care (22.4%) or they had unsuitable mental status (23.9%). Eleven (16.4%) were not enrolled because they were still in the process of being screened when the cohort closed, six had too many serious comorbidities, six were involved in an accident in which a family member had been killed, and six were already enrolled in a TBI drug study. Since TBI subjects were an important group of patients for Cohort 1, we got clearance from the PI of the drug study and the IRB to be able to co-enroll TBI patients in both studies.

Cohort 2 Screening

The study team was notified of potential Cohort 2 patients by the residents providing initial screening information on patients they were treating. If the patient met the basic eligibility criteria (severity of injury, age, at least one uninjured upper extremity) one of the CRCs would meet with the patient and do a second level screening for eligibility, and ask for consent from eligible patients. Coordinators also attended morning board rounds to catch any potential subjects that they may not have been notified of.

A total of 319 subjects were screened, 222 at GMH, 44 at AMC, and 53 at ARMC. Of these 130 (40.8%) were eligible to participate and 95 (29.8% - **Figure 2**) were enrolled and completed participation in the study with at least two hours of continuous NIRS data captured. An additional nine patients consented to participate, but did not complete the study – three were determined to be screen failures, four withdrew consent, and two were withdrawn. Thirty-five of eligible patients declined consent. The most common reasons that patients were determined to be ineligible for study participation were that their injuries did not meet severity criteria (22.9%), they could not be enrolled within 12 hours of their injury (11.9%) or they were outside the 18 to 65 year age range (7.8% - **Figure 2**).

Tibia Fracture Patients Screened	Number	Percentage
ENROLLED	95	29.8
Did not meet severity criteria	73	22.9
Could not enroll within 12 hour window	38	11.9
Declined consent	35	11.0
Outside 18 to 65 age range	25	7.8
Had vascular injury to lower extremity	11	3.4
In police custody	9	2.8
No uninjured upper extremity	8	2.5
Unable to place sensors	6	1.9
Other	5	1.6
No LAR to provide consent	4	1.3
Had fasciotomy prior to enrollment	4	1.3
Prior injury compromizing circulation in leg	3	0.9
Language barrier (not English or Spanish)	2	0.6
Had spinal cord injury causing paralysis	1	0.1
TOTAL	319	100.0

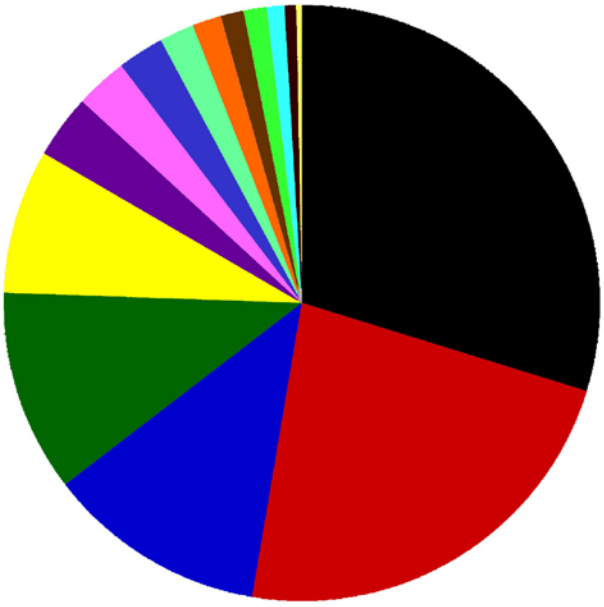


Figure 2: Analysis of screening outcomes of potential Cohort 2 subjects.

Subject Enrollments

Subjects were considered completed if at least two hours of continuous NIRS data was captured. **Table 3** shows the number of subjects across the three sites that were consented, and either completed study participation or did not. As stated earlier, a total of 21 consented subjects did not complete study participation because they were determined to be screen failures subsequent to consenting, or withdrew consent or were withdrawn from the study before two hours of NIRS data could be captured. Because of the number of subjects that did not complete study participation, the both cohort sizes were increased during the study to ensure the number of completed subjects required by the protocol were obtained. Cohort 1 was increased to 35, but we hit this limit of August 20, 2013 with only 23 completed subjects. This was considered sufficient subjects for the control cohort, so another protocol amendment to increase the sample size further was not considered. The size of Cohort 2 was also increased from 95 to 120 to ensure 95 completed subjects were accrued according to our goal in the award proposal. This commitment was achieved in September with the completion of the 95th subject.

		Consented	Did not Complete	Completed	Total Complete Subjects	Required by Protocol	Percent Complete
Cohort 1	GMH	35	12	23	23	25	92.0% *
Cohorts 2 and 3	GMH	76	8	68	95	95	100%
	AMC	16	1	15			
	ARMC	12		12			
TOTAL		139	21	118	118	120	98.3% *

Table 3: The number of subjects who were enrolled in the study and completed study participation. * No further subjects could be enrolled into Cohort 1, so enrollment was considered complete.

Transition of ACS Cases from Cohort 2 to Cohort 3

Cohort 3 subjects are Cohort 2 subjects that develop ACS and have a fasciotomy. In the original award proposal we estimated that about 25% of our high energy leg trauma subjects would develop ACS and transition into Cohort 3. We expected 25 Cohort 3 subjects to emerge from our 95 Cohort 2 subjects. However, this did not happen. Only eight cases of ACS resulting in fasciotomy were encountered (8.4% of Cohort 2 subjects), three at GMH (4.4%), three at ARMC (25.0%), and two at AMC (13.3% – **Table 4**).

We are confident that we screened all patients with tibia fractures and identified all cases of ACS at GMH and ARMC, but not at AMC. Four cases of ACS occurred at GMH that we were unable to enroll (**Figure 2**), three were diagnosed by the trauma team who performed the fasciotomies without consulting the orthopedics residents, and one was diagnosed and fasciotomized during their tibia fracture surgery. Consequently a total of twelve cases of ACS resulting in fasciotomy were encountered during the study – eight of which were enrolled subjects and four were not.

Site	Cohort 3	Cohort 2	
		Number	Percentage
GMH	3	68	4.4%
AMC	2	15	13.3%
ARMC	3	12	25.0%
TOTAL	8	95	8.4%

Table 4: The number of ACS cases that developed in the completed Cohort 2 subjects

Table 5 is an analysis of ACS cases at GMH and ARMC, the percentage of high energy fractures that resulted in ACS and the percentage of total tibia fractures that resulted in ACS. Overall, 3.6% of patients with tibia fracture developed ACS. This is consistent with other reports in the literature.

Site	Total ACS Cases[1]	High Energy Tibia Fracture Cases [2]		All Tibia Fracture Cases[3]	
		Number	Percentage	Number	Percentage
GMH	7	180	3.9%	222	3.2%
ARMC	3	31	9.7%	53	5.7%
TOTAL	10	211	4.7%	275	3.6%

Table 5: Total number of ACS cases encountered at GMH and ARMC, and the percentage of ACS cases resulting from the high energy tibia fracture cases, and all tibia fracture cases. [1] Total ACS cases at GMH include 3 that were enrolled Cohort 2 subjects and 4 cases that were identified but not enrolled. [2] High energy tibia fracture cases are all tibia fracture cases screened less those that did not meet the injury severity criteria for inclusion in the study. [3] All tibia fracture cases are all potential patients that were screened for inclusion into Cohort 2.

Task 2e: Analyze Data and Provider Feedback

The amount of data collected during this study was enormous. Delays in data monitoring and receipt of cleaned data from the CRO necessitated an 8-moth no cost extension for data analysis. The no cost extension was requested, and granted, in Y5/Q1.

End of Study Participation

Ninety-five Cohort 2 subjects remained on the study for at least two hours and were considered "completed" and analyzable subjects. The methods by which their participation was terminated is described in **Table 6**. Forty-three percent of subjects completed 48 hours of study participation. Of note, 28.4% withdrew consent for participation stating that the sensors were uncomfortable, or that being attached by sensors and cables to the monitor was too restrictive. Many of these patients were reasonably ambulatory and wanted to get out of bed at various times. It is clear that that the current set up with the 12 sensors connected to individual pods, and those pods connected to a common trunk cable via individual cables is not optimal for subject compliance. Two subjects completed participation due to adverse events that were related to their injuries, not to participation in the study. The average length of study participation for Cohort 2 and 3 subjects is presented in **Table 7**.

	GMH		AMC		ARMC		TOTAL	
	Number	Percent	Number	Percent	Number	Percent	Number	Percent
Completed 48 hours on study	34	50.0%	4	26.7%	3	25.0%	41	43.2%
Extended participation due to risk for ACS	3	4.4%	1	6.7%			4	4.2%
Ended participation due to fasciotomy	3	4.4%	2	13.3%	3	25.0%	8	8.4%
Discharge from hospital	6	8.8%	1	6.7%	6	25.0%	10	10.5%
Withdrawn from study by PI	1	1.5%	2	13.3%			3	3.2%
Withdrawn due to Adverse Event	1	1.5%	1	6.7%			2	2.1%
Subject withdrew consent	20	29.4%	4	26.7%			24	25.3%
Total	68		15		12		95	

Table 6: Status of the 95 completed Cohort 2 subjects at the end of their study participation

Length of Study Participation		
ll Cohort 2 and 3 Subjects		
Number	95	
Range	1.7 to 62.0 hours	
Mean ± Std Error	32.5 ± 1.75 hours	
1.7 to 9 hours	16	16.8%
10 to 19 hours	8	8.4%
20 to 29 hours	12	12.6%
20 to 39 hours	13	13.7%
40 to 49 hours	40	42.1%
>49 hours	6	6.3%
Cohort 3 Subjects – Fasciotomized		
Number	8	
Range	1.7 to 13.8 hours	
Mean ± Std Error	6.2 ± 1.68 hours	
Subjects that Withdrew Consent		
Number	27	
Range	2.0 to 34.6 hours	

Mean ± Std Error	17.8 ± 2.17 hours

Table 7: Length of study participation for all Cohort 2 and 3 subjects, for those subjects that underwent a fasciotomy (Cohort 3), and those that withdrew consent to participate

Of the 23 Cohort 1 subjects that completed the study, 15 (65.2%) completed 48 hours of NIRS recording, 4 (17.4%) were discharged from the ICU earlier than 48 hours, 3 (13.0%) withdrew consent and 1 (4.3%) was withdrawn by the study team.

Cohort 1 Demographics	GMH Number	GMH Percent
Gender		
Male	14	60.9
Female	9	39.1
Age		
Average	35.8	
< 20	0	0
20 to 29	7	30.4
30 to 39	9	39.1
40 to 49	5	21.7
50 to 59	0	0
60 to 65	2	8.7
Race		
Black	8	34.8
White	15	65.2
Ethnicity		
Hispanic	0	0
Not Hispanic	23	100

Table 8a: Demographic data for the 23 completed Cohort 1 subjects on the NIRS Study

Subject Demographics

Demographic data for the 23 Cohort 1 subjects and the 95 Cohort 2 and 3 subjects that completed participation in the study are presented in Tables 8a and 8b respectively. Overall the 118 subjects that completed all cohorts, 77.1% were male and 22.9% female; 53.4% were black, 44.9% were white, and 1.7% (1 Asian, 1 Native American) were of another race; and the average age was 39.4 years. The racial distribution is representative of that of the Dekalb County, GA, which is 55.6% African American and 34.9% Caucasian based on 2010 Census statistics (2010 US Census Bureau Dataset – www.globalatlantaworks.com/html/109.htm).

Cohort 2 and 3	GMH		AMC		ARMC		Total	
	Number	Percent	Number	Percent	Number	Percent	Number	Percent
Gender								
Male	59	86.8	10	66.7	8	66.7	77	81.1
Female	9	13.2	5	33.3	4	33.3	18	18.9
Age								
Average	37.8		47.3		44.8		40.2	
< 20	1	1.5					1	1.1
20 to 29	23	33.8	2	13.3	3	25.0	28	29.5
30 to 39	15	22.1	3	20.0			18	18.9
40 to 49	13	19.1	1	6.7	4	33.3	18	18.9
50 to 59	11	16.2	5	33.3	4	33.3	20	21.1
60 to 65	5	7.4	4	26.7	1	8.3	10	10.5
Race								
Black	45	66.2	8	53.3	2	16.7	55	57.9
White	22	32.4	6	40.0	10	83.3	38	40.0
Asian	1	1.5					1	1.1
American Indian			1	6.7			1	1.1
Ethnicity								
Hispanic	1	1.5			1	8.3	2	2.1
Not Hispanic	67	98.5	15	100	11	91.7	93	97.9
Injured Lower Extremity								
Left	29	42.6	5	33.3	5	41.7	39	41.1
Right	35	51.5	10	66.7	6	50.0	51	53.7
Bilateral	4	5.9			1	8.3	5	5.3

Table 8b: Demographic data for the 95 Cohort 2 and 3 subjects on the NIRS study.

Missing NIRS Data Recording Errors

The main intention of our Phase 1 study was to identify and solve all possible issues associated with NIRS recording on traumatized muscle compartments of the lower leg so that the Phase 2 study could proceed without technical difficult or hold up. Despite this, we experienced a major problem when we transitioned to continuous NIRS monitoring in the Phase 2 study. As we have been documenting in our quarterly and annual reports over the last two years, there are times when NIRS data cannot be recorded on certain traumatized compartments. Sometimes recording starts and then disappears, other times no signal can be captured right from the start. These missing data recording errors became apparent soon after the study started in March 2012, being encountered on the first three subjects enrolled at GMH. The study was placed on hold for a few weeks while the CRCs were retrained in sensor placement and on troubleshooting the NIRS device. During this time we also developed a protocol for closer monitoring of study subjects and recording of detailed information, including implementation of the NIRS Monitoring Log that documented the CRC's experience with the NIRS devices and the patient's clinical history while on the study. However, despite the additional training, these missing data recording issues persisted.

Two specific types of missing data recording errors were identified – the most common was when rSO_2 values were low and eventually stopped displaying a value, or were not able to be recorded at all, and the less common when rSO_2 values gradually increased to levels about 95% to 100% and then either stayed at those levels or the device stopped displaying a value. These oxygen saturation levels are not physiologically reasonable. We defined these as low value errors and high value errors respectfully. We have evaluated all the continuous NIRS data recordings for quality purposes to define the prevalence of the missing data recording errors. The data for each Cohort 2 and 3 subject has been graphed in blocks of about six hours to provide the ability to identify data quality and trends. We have identified instances where data is missing and

correlated these instances against entries on the NIRS Monitoring Log that document the coordinators' experience with the NIRS devices and the patient's clinical history while on the study. These logs are a meticulous record of events that happen that may cause a break in data collection, such as when the patient is moved, and information on recording quality reported by the NIRS device.

		Before Surgery – Total of 99 Injured Lower Extremities [1]				After Surgery – Total of 70 Injured Lower Extremities [2]			
		Anterior	Lateral	Deep Post	Super Post	Anterior	Lateral	Deep Post	Super Post
[3] Compartments on which a sensor was not placed	N	7	16	7	29	0	0	1	2
[4] Compartments that were monitored	N	92	83	92	70	70	70	69	68
	%	92.9	83.8	92.9	70.7	100	100	98.6	97.1
[5] Total Missing Data Recording Errors	N	30	19	31	3	21	20	30	4
	%	32.6	22.9	33.7	4.3	30.0	28.6	43.5	5.9
[6] Total Missing Data	N	37	35	38	31	21	20	31	5
	%	37.4	35.3	38.4	31.3	30.0	28.6	44.3	7.1
[7] Low Value Errors	N	24	13	20	2	15	12	10	3
	%	26.1	15.7	21.7	2.9	21.4	17.1	14.5	4.4
[8] High Value Errors	N	6	6	11	1	6	8	20	1
	%	6.5	7.2	12.0	1.4	8.6	11.4	29.0	1.5

Table 9: Prevalence of missing data errors in monitored muscle compartments of the lower extremity.

1. The total number of lower extremities that could be monitored before surgery was 99 – 91 subjects with unilateral injuries and 4 subjects with bilateral injuries.
2. The number of lower extremities that could be monitored after surgery was 70. 67 subjects had NIRS monitoring after surgery, 3 of which had bilateral injuries. 28 subjects either did not have surgery while they were on the study, or they had a fasciotomy so no further NIRS monitoring after surgery.
3. The number of compartments on which a sensor could not be placed due to location of wounds or placement of a splint.
4. Number and percentage of the total injured lower extremities that were monitored
5. Total number of each compartment on which unresolvable missing data errors were encountered, and the percentage relative to the number of compartments that were monitored.
6. Total number of missing data due to sensors not being placed and sensors not picking up a usable signal, and the percentage relative to the number of injured lower extremities available for monitoring.
7. The number of low value errors encountered and percentage relative to compartments being monitored
8. The number of high value errors encountered and percentage relative to compartments being monitored

We have defined missing data recording errors as any loss of signal that cannot be restored using standard troubleshooting of the NIRS set up. Many errors could be corrected by checking the connection between the sensor and the pod, or between the pod and the subject's skin. Often taping down the sensor or replacing the sensor resolved the issues. However, there were some errors that consistently could not be resolved. These are the ones that we are defining as "missing data recording errors" – including high value and low value errors. Every Cohort 2 and 3 subject has been evaluated for these errors, and their prevalence is documented in **Tables 9 and 10**.

Table 10 is divided into missing data recording errors that occurred before surgery and those that occurred after surgery. Prior to surgery there were 99 injured lower extremities that could be monitored – 91 subjects with unilateral injuries and 4 subjects with bilateral injuries (Table 10 [1]). Since 28 subjects either didn't have a surgery while they were participating on the study, or they had fasciotomies and terminated their participation, there were only 70 injured lower extremities after surgery – 67 subjects, 3 of which had bilateral injuries [2]. However, sensors could not be placed on every compartment of these injured extremities due to

prior placement of a splint, wounds or bandaging [3 and 4]. The superficial posterior compartment was the most difficult sensor to place under a splint, followed by the lateral with 29 and 16 unable to be placed before surgery respectfully. Table 8 [4] is the number of injured compartments on which sensors could be placed, and the percentage relative to the number of injured extremities available to be monitored.

The total number of missing data recording errors per compartment is shown in line [5] of Table 10. The majority occurred in the anterior and deep posterior compartments with 32.6% and 33.7% of sensors placed not picking up a usable signal respectively. However, if the number of sensors that were not able to be placed is added into the equation to give the total number of compartments on the injured lower extremities from which no data was obtained [6] the percentage of missing data is relatively consistent between compartments before surgery, ranging from 31.3 to 38.4%. Therefore, in our experimental model where a splint is often placed prior to placement of NIRS sensors, data is missing from over 30% of each compartment – either due to the sensor not being placed, or the sensor not picking up a usable signal. We have no way to tell whether the compartments on which sensors could not be placed would have yielded a usable signal or not. However, sensor placement is not an issue after surgery. Only three sensors could not be placed due to the position of wounds – one on the deep posterior and two on the superficial posterior. Missing data errors from compartments after surgery show that the deep posterior is the most prone to losing a signal (44.3% of the time), followed by the anterior and lateral at 30.0% and 28.6% respectively. What is interesting about the missing data from the deep posterior after surgery is that 20 of the 30 cases were the high value errors. These may be due to increased pressure on the sensor caused by the weight of the leg.

We have also analyzed the number of compartments per injured extremity that had data recording issues due to either a sensor not being placed or to missing data recording errors before and after surgery (**Table 10**). Before surgery, 33.3% of the 99 injured extremities had NIRS data recorded on all four compartments. 57.6% did not have any missing data recording errors but may have had missing sensors, while 61.6% had all four sensors placed, but may have had data recording errors. After surgery, inability to place sensors does not contribute significantly to NIRS data being lost. Of the 70 injured extremities monitored after surgery, sensors could not be placed on only three compartments. However, loss of data due to data recording errors is still significant. Only 41.4% of injured extremities had NIRS data recorded on all four compartments.

The issue of the sensors on injured extremities either not being able to pick up or maintain a signal that is usable by the Nonin 7600 oximeter causing data to be lost is significant, occurring in at least one compartment of in about 42% of injured extremities. We have been working closely with Nonin to resolve this issue. Nonin engineers suspect this issue may be due to increased amount of hemoglobin products in injured tissue soaking up the NIR light and reducing the amount of light reflected back to the sensors, thus falling outside the device's oxygenation calculation thresholds and resulting in a sensor error being generated. The 7600 oximeter uses reflected wavelength data from the sensors to generate an oxygenation valuation, but it does not capture the raw wavelength data that is needed to further investigate the missing data recording errors. As part of a resolution strategy we implemented use of the Nonin 7610 oximeter to capture raw engineering data at the beginning of the fourth period of this award. The 7610 oximeter uses the same 8003CA sensors as the 7600 oximeter. Beginning in January 2013 (Y4/Q2) we collected 7610 recordings from 39 subjects, 21 of which has missing data recording errors on at least one compartment. One minutes of baseline data was collected from all the lower extremity compartments – both on the injured leg and the contralateral leg – at the start of study participation. Further one minute recordings were made if there were missing data errors at the start, or if they developed during the subject's participation. Of the 39 subjects on which 7610 data was collected, 21 had missing data recording issues and 18 did not. Data was obtained from 42 compartments that had recording issues. Five subjects had one problematic compartment, eight had tow, and seven had three. The most frequent compartment having problems was the deep posterior (16), followed by the anterior (14), lateral (11), and superficial posterior (1).

	Compartments Per Injured Extremity with Missing Data – Before Surgery									
	0		1		2		3		4	
	N	%	N	%	N	%	N	%	N	%
Data Missing Due to NIRS Recording issues	57	57.6%	15	15.2%	13	13.1%	13	13.1%	1	1.0%
Data Missing Due to Sensors Not Placed	61	61.6%	25	25.3%	9	9.1%	0	0.0%	4	4.0%
Total Data Missing	33	33.3%	22	22.2%	22	22.2%	12	12.1%	10	10.1%
	Compartments Per Injured Extremity with Missing Data – After Surgery									
Data Missing Due to NIRS Recording issues	29	41.4%	19	27.1%	10	14.3%	12	17.1%	0	0.0%

Table 10: Number of compartments per injured extremity with missing data before and after surgery

Initial analysis of the 7610 data by Nonin engineers confirmed that in many cases there was indeed a reduction in reflected NIR light to an extent that would be outside the 7600 oximeter's lower level of detection. In May 2013, the engineers developed a prototype sensor that had larger photoreceptive diodes that they tested on control muscle compartments and confirmed to function when used with the 7600 and the 7610 oximeters. The purpose of the sensor was primarily to obtain further raw wavelength data using an enhanced photoreceptive diode, but also to see if it could capture sufficient reflected NIR light to allow the 7610 oximeter to generate a reliable oxygenation reading from compartments on which a signal could not previously be captured with the 8003CA sensors connected to the 7600 oximeter.

A batch of 50 enhanced sensors were produced by Nonin and received by the study team at the end of June, 2013. By the close of the study, data from the enhanced sensors had been captured from three subjects, with data recording problems, and a total of eight compartments. The raw wavelength data from these studies is currently being analyzed by Nonin.

Summary of Overall Study Results

Final analysis of almost 30 million data points has been completed. (**Appendix A – Analysis Report**) Despite the immense amount of data, continual monitoring from 12 sensors over 48 hrs in our FDA-IDE trial has proven to be useful and informative for developing final recommendations for the using NIRS monitoring to detect ACS in patients with severe extremity trauma. Critically injured patients with uninjured extremities showed no clinically significant difference between the two extremities (**Figure 3A**). When the uninjured anterior compartment was used as a control for the 4 injured compartments (**Figure 3B**), unilateral injuries showed a hyperemic response on average of

Anterior (A): 4% ($p < 0.001$), Lateral (L): 5% ($p < 0.001$),
Superficial Posterior (SP): 5% ($p < 0.001$), Deep Posterior (DP): 8% ($p < 0.001$).
Alternate Controls: Deltoid (A 3%, L 4%, SP 4%, DP 9%) Volar Forearm (A 2%, L 4%, SP 3%, DP 6%).

Figure 3A (Uninjured)

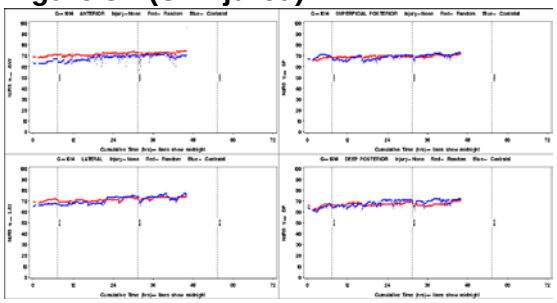
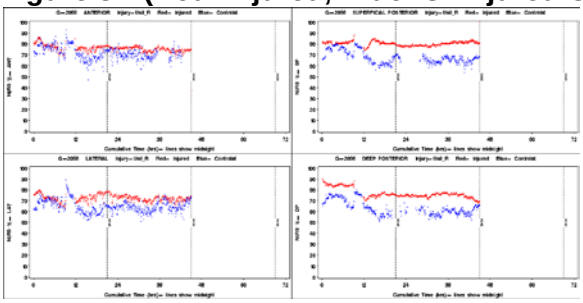


Figure 3B (Red: Injured, Blue: Uninjured Control)



Seven ACS subjects were enrolled. While statistical data was limited due to a small sample size, all subjects demonstrated either crossover from hyperemia to hypo-perfusion (**Figure 4A-B**) or were hypo-perfused at initiation of monitoring (**Figure 4C**). Additionally, hyperemia was seen in post fasciotomy compartments such as in the **Figure 4B** of the three separate ACS patients demonstrated below.

Figure 4A

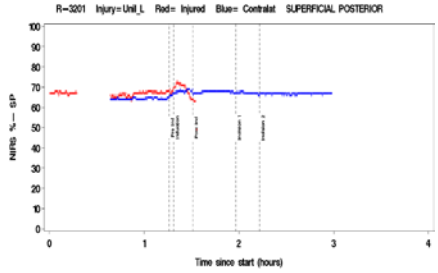


Figure 4B

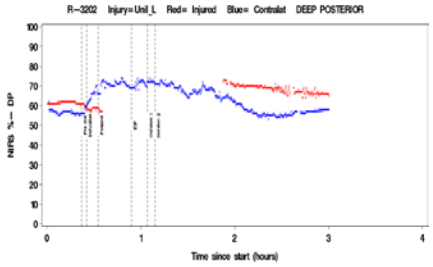
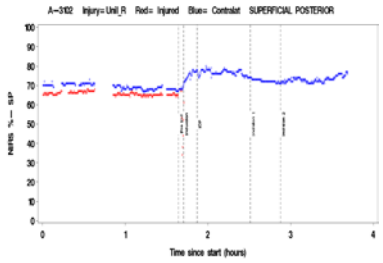


Figure 4C



The analysis demonstrated NIRS continual monitoring is not only possible, but NIRS can also provide continual noninvasive real time perfusion monitoring of extremities. The project identified areas where the technology needs improvement. Missing data was seen in 24% for all causes such as sensor removal, unplugged cord, low light, pad lift off due to sweat, etc. Specifically with significant injury and increased blood flow, low light errors can occur. Low light occurred in an estimated 13% of injured and 27% of ACS subjects. While this number is unacceptable, Nonin is currently working to identify potential solutions including improved light strength, more sensitive light receptors, and potentially, optimized depth penetration. Based on the sum of all data collected and reported on, clinical guidelines are able to be postulated at this time. Injured tissue should show hyperemia when compared to an uninjured control site (uninjured anterior compartment, deltoid, or volar forearm). NIRS as a screening tool can be used to identify well perfused tissue (hyperemia of >= 4%). Close monitoring and possible IMP measurements are indicated in situations concerning for ischemia in the range of hyperperfusion of +3% to hypoperfusion of -2%. Urgent attention, including IMP measurements to confirm ACS, would be indicated when -3% or lower NIRS values are seen when compared to a control site. Additionally, patterns of decreasing values compared to the control of more than 5 percentage points should illicit concerns for increasing risk of ACS. While no technology will replace sound clinical judgment, NIRS has shown the ability monitor injured tissue and identify conditions of muscle ischemia.

Task 2f: Present and Publish Results of Phase 2 Study

Several manuscripts have already been accepted for publication and are included in the published articles. Several more are currently being written based on the data summary above.

Manuscripts:

1. Couch L, Roskosky M, Shuler M, Freedman B. *Correlation between skin pigment and NIRS values: a comparison of three commercially available devices*. **American Journal of Analytical Chemistry.** 2015. 6:911-16.

   The objectives of this study were to 1) collect descriptive data that will allow us to reach a more thorough understanding of factors that contribute to NIRS values and 2) to determine which of three

commercially available NIRS devices are able to accurately provide a NIRS value in the presence of varying skin color (melanin and erythema). The three devices tested were the INVOS by Covidien, the Equanox by Nonin Medical, and the ForeSight by Casmed. Significant correlation of skin pigment and oxygenation was found with the Covidien device, moderate with the Casmed device and no significant correlation detected with the Nonin device. These results indicate that the Nonin device is the best at removing effects of skin pigmentation

2. Johnson A, Roskosky M, Shuler M, Freedman B. *Depth Penetration of Near Infrared Spectroscopy in the Obese*. **Journal of Trauma and Treatment**. (In Press)

   NIRS is able to measure oxygenation to a depth of 2 to 3 cm below the skin, raising concerns over the ability of NIRS to accurately determine oxygenation of leg compartments in the obese. We collected data on 60 healthy participants. Our results indicated that NIRS was able to detect changes in oxygen saturation of muscle with exercise in all 60 participants regardless of BMI. Even in the morbidly obese, depth of subcutaneous fat was less than 2cm in 98% of subjects.

3. Kovalenko B, Roskosky M, Shuler M, Freedman B. *Effects of Ambient Light on Near Infrared Spectroscopy.* **Journal of Trauma and Treatment.** (In Press)

   Through our research we hope to prove, among other points, that NIRS would be a valuable peri-operative tool for patients at risk of developing ACS. Here we investigated the effects of ambient light on the oxygenation readings from the same three commercially available NIRS devices. NIRS readings were recorded for 30 subjects at three different light levels: lights off, lights on, and over-head OR lights on. Significant problems in producing a reading were detected with both covidien and casmed, as they were unable to provide a value in the presence of too much light (OR lights on). It was found that there was no significant difference in oxygenation values displayed between the varying levels of light with the Nonin device. Casmed displayed no significant difference with lights on versus off, although the device was unable to display a reading when OR lights were turned on. Covidien, however, showed a significant difference between values when lights were off vs on (Diff of -0.933; $p=0.0045$) as well as when lights were off vs OR lights on (Diff of -5.0; $p=0.0035$).

4. Reisman W, Shuler M, Roskosky M, Kinsey T, Freedman B. *Use of Near Infrared Spectroscopy to Detect Sustained Hyperaemia Following of Lower Extremity Trauma.* **Military Medicine.** (In Press)

   This study sought to evaluate whether the expected hyperaemic response is present 48 hours post-operatively, using NIRS. Mean rSO_2 values taken 48 hours from surgical stabilization from each compartment of the patients' injured legs were significantly higher than mean values of the contralateral legs (injured = 70, 68, 72, 70; contralateral = 55, 54, 57, 56 for anterior, lateral, deep posterior, and superficial posterior compartments, respectively; $p<0.0001$ for all compartments). These results suggest that the hyperaemic response to injury remains present at 48 hours after surgical stabilization, and that NIRS values in an injured extremity should be expected to remain elevated throughout the window of concern for ACS.

5. Roskosky M, Robinson G, Shuler M, Freedman B. *Subcutaneous Depth in a Traumatized Lower Extremity.* **Journal of Trauma and Acute Care Surgery.** 2014 Sep;77(3 Suppl 2):S190-3. (PMID: 25159354)

   The objective of this study was to ease concerns over the ability of NIRS to accurately determine oxygenation of injured leg compartments in the presence of swelling in the obese. Data was analyzed on 50 patients with severe leg injuries. Distance from skin to fascia in the superficial posterior compartment of both legs was measured on each patient using a portable ultrasound device. No significant correlation was found between the ATT of the injured or uninjured legs and BMI. Mean comparison testing revealed no difference in adipose tissue thickness between the injured and uninjured legs (null hypothesis: equal means; $p>>0.05$). Out of the 50 subjects analyzed, no subject

had a subcutaneous depth of over 2 cm on the injured or uninjured leg.

6. Cole A, Roskosky M, Shuler M, Freedman B. *Near infrared spectroscopy and lower extremity acute compartment syndrome: a review of the literature.* **Journal of Trauma and Treatment.** (In-press) This manuscript is a summary of the literature currently published on NIRS and ACS.

Book Chapter:

1. Shuler MS, Roskosky M, Freedman B. (2015) "Compartment Syndrome." Skeletal Trauma. 5th ed. Ed. Browner B, Jupiter J, Krettek C, Anderson P. Philadelphia: Saunders. 437-463.

Presentations:

1. Shuler M, Roskosky M, Freedman B. Continual Near Infrared Spectroscopy Monitoring in Acute Compartment Syndrome. **Orthopaedic Trauma Association**, Oct 2015.
2. Shuler M, Roskosky M, Freedman B. Continual Near Infrared Spectroscopy Monitoring in Acute Compartment Syndrome. **Military Health Systems Research Symposium**, Aug 2015.
3. Shuler M, Roskosky M, Freedman B. Continual Near Infrared Spectroscopy Monitoring in Acute Compartment Syndrome. **Southern Orthopedic Association**, July 2015, Ashville NC.
4. Reisman W, Cole A, Roskosky M, Shuler M, Andras L, Moore T. Near-Infrared Spectroscopy in the Sub-Acute Setting of Lower Extremity Trauma. **Military Health Systems Research Symposium**, August 2014. (Podium)
5. Roskosky M, Robinson G, Shuler M, Freedman B. Subcutaneous Depth in a Traumatized Lower Extremity. **Society of Military Orthopedic Surgeons 55th Annual Meeting,** December 2013. (Podium)
6. Roskosky M, Robinson G, Shuler M, Freedman B. Subcutaneous Depth in a Traumatized Lower Extremity. **Southern Orthopedic Association 30th Annual Meeting,** July 2013. (Poster)
7. Roskosky M, Robinson G, Shuler M, Freedman B. Subcutaneous Depth in a Traumatized Lower Extremity. **Military Health System Research Symposium**, August 2013. (Podium)
8. Freedman B, Shuler M, Cathcart C, Reynolds L, Budsberg S. NIRS versus direct pressure monitoring of acute compartmental syndrome in a porcine model. **Medical Heath Services Research Symposium**, Fort Lauderdale, FL, Aug 13th-16th, 2012.
9. Jackson K, Cole AL, Potter BK, Kinsey TK, Shuler MS, Smith EK, Freedman BA. Identification of optimal control compartments for near-infrared spectroscopy assessment of lower extremity compartmental perfusion. **Orthopedic Trauma Association 2012 Annual Meeting**, October 2012. (Podium presentation)
10. Cole AL, Herman RA, Heimlich JB, Ahsan S, Freedman BA, Shuler MS. Ability of near infrared spectroscopy to isolate muscle compartments of the upper extremity. **125th Annual Meeting of the American Orthopaedic Association**, Washington D.C., June27-30, 2012. (Poster presentation)
11. Cole AL, Herman RA, Heimlich JB, Ahsan S, Shuler MS, Freedman BA. Ability of near infrared spectroscopy to isolate muscle compartments of the upper extremity. **American Academy of Orthopedic Surgeons Annual Meeting**, San Francisco, CA, February 2012. (Paper presentation)
12. Cathcart C, Shuler M, Freedman B, Reynolds L, Cole A, Budsberg S. Non-invasive NIRS versus invasive direct pressure monitoring of acute compartment syndrome in a porcine model. **Orthopedic Research Society**, San Francisco, CA, February 2012.
13. Herman R, Heimlich B, Cole AL, Shuler MS. Ability of near infrared spectroscopy to isolate muscle compartments of the upper extremity. **Ninth Annual American Medical Association Medical Student Section/Resident & Fellow Section Joint Research Symposium**, New Orleans, LA, November 2011.
14. Shuler MS. Symposium 3: Compartment Syndrome: New Technologies: Non-Invasive Compartment Monitoring. **Orthopaedic Trauma Association**, San Antonio, TX, October 2011. (Forum discussion)
15. Shuler MS, Cole AL, Robinson MA, Freedman BA. Comparison of near infrared spectroscopy values between compartments of the lower extremities. **Advanced Technology Applications for Combat Casualty Care 2011 Conference**, Fort Lauderdale, FL, August 2011. (Poster presentation)
16. Freedman B, Cole A, Shuler M, Jackson K, Owens L, Lackie D. Do skin pigmentation and hair affect near-infrared spectroscopy assessment of leg compartment syndrome. **Advanced Technology**

Applications for Combat Casualty Care 2011 Conference, Fort Lauderdale, FL, August 2011. (Poster presentation)

17. Freedman B, Jackson K, Shuler M, Cole A. Do skin pigmentation and hair affect near-infrared spectroscopy assessment of leg compartment syndrome? **Southern Orthopedic Association 28th Annual Meeting**, Big Island, HI, July 2011. (Poster presentation)

18. MAJ Brett Freedman, MD; CPT Keith Jackson, MD; Michael Shuler, MD; Ashley Cole, MPH Do Skin Pigmentation and Hair Affect Near-Infrared Spectroscopy Assessment of Leg Compartment Syndrome. **American Academy of Orthopaedic Surgeons**, San Diego, CA. Feb 15-19, 2011.

TASK 3: Animal Use Study

Task 3 of this award used porcine models of ACS to further evaluate and validate the clinical utility of using continuous NIRS monitoring to diagnose ACS. The initial experiments using albumin infusion and contusion/albumin infusions models for inducing ACS were successfully completed on target by the end of the second year of this grant. These studies demonstrated that NIRS measurement of hemoglobin oxygen saturation in the tibial compartment provided reliable and sensitive correlation to increases and decreases in intra-compartment pressure and intra-compartment perfusion pressure. In year 3, we built on the success of the animal experiments by employing a second model of tibial compartment syndrome, which uses an inflatable balloon to increase intra-compartment pressure. This model was originally conceived in the US Army Institute of Surgical Research. This demonstrated that NIRS accurately detected a critical hypoperfusion that occurs in the setting of ACS. In the controlled state of an animal model, our series of studies has built on prior knowledge to provide compelling evidence that NIRS can serve as an accurate and reliable noninvasive means for diagnosing ACS. In the final year, we have (under a no-cost extension for the UGA sub-award) designed and will complete animal testing to evaluate the NIRS data obtained when ACS is "missed" or delayed in diagnosis, which is known to be a clinically devastating occurrence. In short, our animal testing and that in existence makes us confident that NIRS can accurately monitor the physiological states associated with severe leg injury and ACS, in the controlled setting of an animal model.

3a: Created UGA IACUC Protocol Application for Animal Studies

The protocol for the missed fasciotomy porcine study was developed during the first quarter of Period 4, in which we evaluated the NIRS response to a "missed" compartment syndrome. This model used NIRS to monitor the changes that take place when a fasciotomy is performed too late and muscle has died. For the final 6 pigs, we induced trauma to recreate a leg injury, then increased intercompartmental pressure up to levels indicative of ACS for 8 hours via an albumin infusion. The 8 hour time period ensured that we would encounter dead muscle at the time of fasciotomy and allowed us to longitudinally monitor the transition from ischemic to dead muscle tissue.

3b: Obtain UGA IACUC and USAMRMC ACURO Approvals for Second Study

The protocol was approved by the UGA IACUC in the first quarter of Period 4, and by ACURO in the second quarter.

3c and d: Initiate and Conduct Animal Studies

In the third quarter of the fourth period, the missed fasciotomy study was initiated and one animal had completed the protocol. The study was completed in the fourth quarter with a sample size of 6 animals.

3e and f: Analyze Data and Prepare for Presentation and Publication

Two manuscripts have already been accepted for publication and are included in the published articles. A third examining NIRS over the entire course of ACS included after 8 hours of ischemia (missed ACS) is currently being written. Copies of manuscripts can be found in Appendix B.

Summary of Animal Study Results

Through our animal studies, with both a balloon and blunt trauma combined with a plasma infusion models, we have shown that using NIRS to measure compartment pressure provides a reliable, sensitive measure of both an increase in tibial intra-compartmental pressure (TICP) and a decrease in tibial intra-compartmental

perfusion pressure (TIPP). Significant negative correlations between muscle degeneration, edema, hemorrhage and NIRS values were found, as well as a rebound in oxygenation following fasciotomy.

Manuscripts:

1. Cathcart C, Shuler M, Freedman B, Reno L, Cole A, Budsberg S. Correlation Of Near Infrared Spectroscopy (NIRS) and Direct Pressure Monitoring In An Acute Porcine Compartmental Syndrome Model. J Ortho Trauma 2014;28(6):365-9. doi: 10.1097/BOT.0b013e3182a75ceb.

2. Budsberg SC, Shuler MS, Hansen M, Uhl E, Freedman BA. Evaluation of NIRS, serum biomarkers and muscle damage in a porcine balloon compression model of acute extremity compartment syndrome. J Ortho Trauma – Submitted Sept 2015.

Presentations:

1. Cathcart C, Shuler M, Freedman B, Reno L, Cole A, Budsberg S. Non-Invasive NIRS Versus Invasive Direct Pressure Monitoring of Acute CompartmentalSyndrome in a Porcine Model. **ORS 2012 Annual Meeting**, San Francisco, California, February, 2012

2. Cathcart C, Shuler M, Freedman B, Reynolds L, Cole A, Budsberg S. Non- invasive NIRS versus invasive direct pressure monitoring of acute compartment syndrome in a porcine model**. American Orthopaedic Association Annual Meeting,** June 2012, Washington, DC.

3. Budsberg S, Shuler M, Freedman B, Hansen M. Evaluation of NIRS, serum biomarkers and muscle damage in a porcine balloon compression model of acute compartment syndrome. **Military Health System Research Symposium (MHSRS)**, August 2013, Fort Lauderdale, Fl

4. Budsberg S, Shuler M, Uhl E, Hansen M, Roskosky M, Freedman B. Evaluation of NIRS, serum biomarkers and muscle damage in a porcine balloon compression model of acute compartment syndrome. **AAOS/OTA/SMOS, ORS Meeting - . Extremity War Injuries Symposium: Reducing disability within the military**. February 2014, Washington DC.

5. Budsberg S, Shuler M, Freedman B, Uhl E, Hansen M. Evaluation of NIRS, serum biomarkers and muscle damage in a porcine balloon compression model of acute compartment syndrome. Abstract # 1398. **Orthopedic Research Society (ORS)** 2014 Annual Meeting, New Orleans LA.

6. Budsberg SC, Shuler MS, Roskosky M, Uhl E, Hansen M, Freedman BA. Correlation of NIRS and histological muscle damage in a prolonged trauma/infusion model of extremity compartment syndrome (ECS) – assessing NIRS ability to detect the clinical consequence of delayed ECS. **AAOS/OTA/SOMOS/ORS Extremity War Injuries X: Return to Health and Function research symposium**. Washington DC, January 2015. (Chosen for additional podium presentation for placing in the top 5 of all posters.)

7. Budsberg SC, Shuler MS, Roskosky M, Uhl E, Hansen M, Freedman BA. Correlation of NIRS and histological muscle damage in a prolonged trauma/infusion model of extremity compartment syndrome (ECS) – assessing NIRS ability to detect the clinical consequence of delayed ECS. Abstract # 1916 **Orthopedic Research Society (ORS)** 2015. Annual Meeting, Las Vegas NV 2015. (Chosen for the Poster Tour at ORS.)

8. Budsberg S, Shuler M, Freedman B, Uhl E, Hansen M. Evaluation of NIRS, serum biomarkers and muscle damage in a porcine balloon compression model of acute compartment syndrome. **Southern Orthopaedic Association Annual Meeting** 2015, Ashville NC.

9. Budsberg SC, Shuler MS, Roskosky M, Uhl E, Hansen M, Freedman BA. Correlation of NIRS and histological muscle damage in a prolonged trauma/infusion model of extremity compartment syndrome

(ECS) – assessing NIRS ability to detect the clinical consequence of delayed ECS. **Southern Orthopaedic Association Annual Meeting 2015**, Ashville NC.

10. Budsberg SC, Shuler MS, Roskosky M, Uhl E, Hansen M, Freedman BA. Correlation of NIRS and histological muscle damage in a prolonged trauma/infusion model of extremity compartment syndrome (ECS) – assessing NIRS ability to detect the clinical consequence of delayed ECS. **Orthopaedic Trauma Association (OTA) Annual meeting. 2015**, San Diego, CA. (The Orthopaedic Trauma Association (OTA) has invited the "best" ORS Trauma poster to be presented at the OTA annual meeting and on behalf of the ORS Program Committee, I am pleased to inform you that your poster has been selected for this honor".)

TASK 4: Reduction to Practice and FDA Approval Process

Task 4a: Finalize Product Development Relationships Between Vendors and J+M Shuler

This task was completed in Year 1. After Somanetics was bought by Covidien, the initial contract was voided by Covidien. Nonin was identified as a not only viable option but in many aspects a more suitable manufacturer. A working relationship has been established and continues to be a strong asset to the developmental process.

Task 4b: Begin Reduction to Practice – This process has been an ongoing task. From initiation of the relationship with Nonin, the research team has been working to improve the existing technology while packaging it in an optimal form for the unique use in ACS. The technology has been expanded to allow for up to 6 sensors at one time to be used allowing for all 4 compartments and at least one control to be monitored. The sensors have been modified to allow more sensitive light reception and improved physical properties. Nonin in conjunction with a new grant application have committed to build a use specific wearable monitor for use in the traumatic and emergency setting for critically injured warriors and civilians.

Task 4c: Produce Final Prototype for Use in Completion of Phase 1, all of Phase 2, and the Investigational Clinical Study to be Supported by a Future Grant

After using the Somanetics INVOS oximeter in the Phase 1 study we determined that it was sufficient to begin investigation of optimization. However, at the end of Year 1 Somanetics was bought out by Covidien Inc who terminated all existing research agreements. During the first quarter of year 2, we attempted to forge a continued relationship with Covidien, but this was unsuccessful. Consequently, for the Phase 2 study we switched vendors to use the Nonin Equanox^{TM} 7600 oximeter which had equivalent functional claims and FDA approval as the Somanetics oximeter. J+M Shuler conducted an IRB-approved study that was the same as Cohort 1 of our Phase 1 study and confirmed that the two devices performed equivalently, and that that data variance was significantly less using the Nonin machine. Further support for using the Nonin device came from its choice as the oximeter to be used in the Major Extremity Trauma Resarch Consortium (METRC) study, which was also funded by USAMRAA.

Consequently, the Nonin Equanox^{TM} 7600 oximeter was used in the Phase 2 study, and the relationship with Nonin has been exceptional. Their contribution to the Phase 2 study, particularly in troubleshooting the issues we had with missed data recording errors, has been significant, and contributed to the success of the Phase 2 study. Additional studies which are now in press have shown Nonin's superiority in areas such as limiting skin pigmentation impact as well as minimizing the effects of ambient light.

Experience with the 7600 oximeter on the Phase 2 study confirms that the oximeter meets our expectations for the ACS diagnosis indication. However, in view of the missing signal errors we have encountered, it is clear that there are some unexpected challenges to using NIRS on traumatized tissue. In some injured muscle compartments there is increased absorption of NIR light such that the amount reflected back to the sensor falls below the sensor's sensitivity. Nonin developed a prototype sensor with larger photoreceptive diodes which

may mitigate this issue. There are also other modifications that could be made to the sensor to improve monitoring of rSO_2 in traumatized tissue, such as increasing NIR light emission. The 7600 oximeter itself fits the intended use, although increasing the number of ports per machine would be make it more functional. However, the current 8003CA sensor is not adequate for monitoring rSO_2 in traumatized tissue.

4d: Respond to provider feedback re: functionality and industrial design – Completed
In our experience with the 7600 oximeter, the only two significant physical improvements needed are:

1. The addition of more ports to a machine, so that a patient can be monitored by a single machine, and
2. "horse-tailing" of leads, such that four (or more) sensors connect via a short cable (one foot) to a common trunk cable that runs to the device. This will cut down on the cable clutter in the current configuration that has led to subjects withdrawing from the study.
3. Improved physical attributes such as a larger photo diode to improve light detection in traumatized tissue.
4. Nonin has initiated and conducted studies at Grady Memorial Hospital to determine if the already approved pediatric sensor would correct the low light situation but still sample the muscle tissue. Based on those studies as well as additional input, Nonin has committed as part of a new grant application to develop a use specific sensor with specific depth design for traumatized tissue.
5. As part of the continued efforts, Nonin will also optimized the build in redundancy using two light sources and two sets of receptors to allow for improved reading acquisition in the traumatized tissue setting in order to maximize readings while maintaining a certain level of quality and accuracy of measurements.

TASK 5: Coordination between study sites

5a: Bi-annual collaborators meeting – Ongoing

5b: Conduct weekly VTC (Telcon) for LRMC/J+M Shuler, and OIF/OEF during Phase 2 – No longer required since Phase 2 study is not being conducted in Georgia and not at LRMC.

5c: Rapid interpretation of weakness in the design and function of sequential NIRS pad prototypes and NIRS monitoring algorithms – Ongoing
The device is in a state where it is and has been fully ready for testing in our studies. As discussed above, the researchers and Nonin are continuing to develop an optimal solution for traumatized tissue that is designed for the optimal depth penetration, with optimal receptors and ideal redundancy within the product. The major improvement will be the design and validation of a diagnostic algorithm based on NIRS values. This process is ongoing and will continue past our grant period. This process will ultimately require validation in a prospective interventional trial. Additional funding has been applied for through the JWMRP funding opportunities.

5d: Coordinate response to FDA requests for information during approval process – Ongoing
LTC David Shoemaker, Marieann Brill and "Decision Gate" are all involved in USAMMDA's sponsorship of this project and the creation/maintenance of an FDA compliant medical monitoring program for the three clinical sites in the Phase 2 study. As a result, this Phase 2 study will be permissible for inclusion in the "burden of proof" submission for our ultimately new FDA 510k approved indication.

5e: Insure mandatory reporting to SAMMC, ISR & USAMRMC is maintained – Ongoing and in good standing.

TASK 6. Future Research Endeavors

The main outcome of this task is to start the next step in the development and validation of NIRS for diagnosis of ACS. Based on results from the animal and clinical studies included under this award, clinical guidelines for

the use of NIRS for the diagnosis and treatment of ACS have been developed and preliminarily validated. The next step is to plan and conduct a prospective, unblinded, clinical trial to calculate the sensitivity and specificity of NIRS to diagnose ACS using a series of comparative benchmarks. Over the current period we have been designing this study. A BAA pre-proposal has been written and submitted to JWMRP for continuation of the final step in the FDA approval process by funding the final unblinded interventional study to test the sensitivity and specificity of the clinical guidelines.

CONCLUSION

The successful completion of this grant represents a resolute determined effort by the researchers to overcome extensive obstacles including the change from in-theater to civilian enrollment of subjects, coordination of three level 1 and 2 trauma centers, the bankruptcy of the initial grant management foundation, and the rescinding of the contract by the original NIRS manufacturer. Despite all the challenges, the grant has been completed and all tasks have been fulfilled. Additionally, the researchers have extensively published and promoted the findings of the studies, which have led to the researchers being invited to author the chapter on ACS in one of the definitive orthopedic textbooks. The concept of using NIRS in the setting of severe leg injury at risk of ACS has been raised from theoretical to realistic, and this promising, novel approach to diagnosing ACS has been well-received from civilian and military trauma surgeons alike. Including the extensive results from this grant and the final work remaining to be covered under a subsequent award, NIRS will become the most validated and proven medical technology for managing ACS to date.

An accurate objective diagnostic tool for evaluating the emergence of ACS in patients with extremity injuries will reduce the number of extremity amputations and deaths that occur in wounded warriors, while also drastically reducing the number of unnecessary fasciotomies and subsequent morbidity associated with this procedure. This reduction in unnecessary fasciotomies will also result in significant reduction in costs not only in unnecessary surgeries (typically at least three surgeries - fasciotomy, repeat washout, and then closure), but also in recovery time as fractures associated with fasciotomies have a significantly increased healing time and nonunion rate. Using NIRS to evaluate muscle oxygenation in addition to standard diagnostic techniques will advance the ability of the treating clinician in diagnosing ACS. Accurate objective monitoring allows for earlier surgical intervention, reduced muscle loss from ischemia, and reduced unnecessary surgeries (fasciotomy and closures). The use of NIRS will improve the patient's subsequent outcomes, period of recovery, and quality of life, while reducing the financial costs to the patient and the treating institution by accurately and appropriately allocating scarce resources in a timely fashion.

Use of NIRS to monitor for ACS development will have the greatest impact in the combat setting, where surgeon inexperience with ACS, austere environments and concerns about evacuation timelines make a difficult situation even more challenging. The JTTS and CCCRP are well aware of this vulnerability and the existing critical unmet need that accurate monitoring and diagnosis of ACS portends. During protracted evacuation through the echelons of care it is difficult to monitor casualties for developing ACS, yet emerging ACS during evacuation can have devastating consequences resulting in increased morbidity and mortality. The end deliverable from our overall development program is a small, self-contained device, that is easy to use and transport, and can be placed near the battlefield by personnel with minimal medical training. Development of the military deployable device was not within the scope of the completed research proposal, but will be supported by the results of the work completed and translation of this work into a military-ready device can be accomplished under a subsequent award.

The JWMRP proposal is a funding opportunity to which this research team has submitted an invited pre-proposal. The pre-proposal covers remaining technological development work and a FDA-IDE clinical study to determine the sensitivity and specificity of using NIRS to monitor and diagnose ACS compared to currently used diagnostic criteria. This new study will build on our currently completed grant and will be the basis for validating the sensitivity and specificity of NIRS oximetry in the setting of ACS to the FDA. The final outcome from our research program will be the translation of an existing technology to monitor oxygenation in cerebral tissue into a new FDA-approved ACS specific decision support tool that will revolutionize military and civilian diagnosis and management of ACS.

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Publications produced under this grant by research team:

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18. Shuler MS, WM Reisman, TE Whitesides, JR, TL Kinsey, EM Hammerberg, MG Davila, TJ Moore. Near Infrared Spectroscopy in Lower Extremity Trauma. **J Bone Joint Surgery Am**. 2009 June; 91(6):1360-68. (PMCID:19487513)

Outside Publications

1. SH Lee, M Padilla, JE Lynch, AR Hargens. Noninvasive Measurements of Pressure for Detecting Compartment Syndromes. *J Orthop Rheumatol*. ; 1(1): 5–20.

2. RM Taylor, MP Sullivan, S Mehta. Acute compartment syndrome: obtaining diagnosis, providing treatment, and minimizing medicolegal risk. Curr Rev Musculoskelet Med(2012) 5:206–213.
3. AG Via, F Oliva, M Spoliti, N Maffulli. Acute compartment syndrome. Muscles, Ligaments and Tendons Journal 18 2015;5 (1):18-22

Book Chapter

1. Shuler MS, Roskosky M, Freedman B. (2015) "Compartment Syndrome." Skeletal Trauma. 5th ed. Ed. Browner B, Jupiter J, Krettek C, Anderson P. Philadelphia: Saunders. 437-463.

Presentations

1. Budsberg SC, Shuler MS, Roskosky M, Uhl E, Hansen M, Freedman BA. Correlation of NIRS and histological muscle damage in a prolonged trauma/infusion model of extremity compartment syndrome (ECS) – assessing NIRS ability to detect the clinical consequence of delayed ECS. **Orthopaedic Trauma Association (OTA) Annual meeting. 2015**, San Diego, CA. (The Orthopaedic Trauma Association (OTA) has invited the "best" ORS Trauma poster to be presented at the OTA annual meeting and on behalf of the ORS Program Committee, I am pleased to inform you that your poster has been selected for this honor".)
2. Budsberg S, Shuler M, Roskosky M, Uhl E, Hansen M, Feedman B. Evaluation Of NIRS, Serum Bipmarker And Muscle Damage In A Porcine Ballroom Compression Model Of ECS. **Orthopaedic Trauma Association**, Oct 2015.
3. Shuler M, Roskosky M, Freedman B. Continual Near Infrared Spectroscopy Monitoring in Acute Compartment Syndrome. **Orthopaedic Trauma Association**, Oct 2015.
4. Shuler M, Roskosky M, Freedman B. Continual Near Infrared Spectroscopy Monitoring in Acute Compartment Syndrome. **Military Health Systems Research Symposium**, Aug 2015.
5. Shuler M, Roskosky M, Freedman B. Continual Near Infrared Spectroscopy Monitoring in Acute Compartment Syndrome. **Southern Orthopedic Association**, July 2015, Ashville NC.
6. Budsberg S, Shuler M, Freedman B, Uhl E, Hansen M. Evaluation of NIRS, serum biomarkers and muscle damage in a porcine balloon compression model of acute compartment syndrome. **Southern Orthopaedic Association,** July 2015, Ashville NC.
7. Budsberg SC, Shuler MS, Roskosky M, Uhl E, Hansen M, Freedman BA. Correlation of NIRS and histological muscle damage in a prolonged trauma/infusion model of extremity compartment syndrome (ECS) – assessing NIRS ability to detect the clinical consequence of delayed ECS. **Southern Orthopaedic Association,** July 2015, Ashville NC.
8. Budsberg S, Shuler M, Feedman B. Assessing NIRS Ability To Detect The Clinical Consequence Of Delayed ECS. **Orthopedic Research Society**, March 2015.
9. Budsberg SC, Shuler MS, Roskosky M, Uhl E, Hansen M, Freedman BA. Correlation of NIRS and histological muscle damage in a prolonged trauma/infusion model of extremity compartment syndrome (ECS) – assessing NIRS ability to detect the clinical consequence of delayed ECS. Abstract # 1916 **Orthopedic Research Society (ORS)** March 2015. Annual Meeting, Las Vegas NV 2015. (Chosen for the Poster Tour at ORS.)
10. Budsberg S, Shuler M, Roskosky M, Uhl E, Hansen M, Feedman B. Evaluation Of NIRS, Serum Bipmarker And Muscle Damage In A Porcine Ballroom Compression Model Of ECS. **Orthopedic Research Society**, March 2015. (**Best Poster Award)**
11. Budsberg SC, Shuler MS, Roskosky M, Uhl E, Hansen M, Freedman BA. Correlation of NIRS and histological muscle damage in a prolonged trauma/infusion model of extremity compartment syndrome (ECS) – assessing NIRS ability to detect the clinical consequence of delayed ECS. **AAOS/OTA/SOMOS/ORS Extremity War Injuries X: Return to Health and Function research symposium**. Washington DC, January 2015. (Chosen for additional podium presentation for placing in the top 5 of all posters.)
12. Reisman W, Cole A, Roskosky M, Shuler M, Andras L, Moore T. Near-Infrared Spectroscopy in the Sub-Acute Setting of Lower Extremity Trauma. **Military Health Systems Research Symposium**, August 2014. (Podium)

13. Budsberg S, Shuler M, Freedman B, Uhl E, Hansen M. Evaluation of NIRS, serum biomarkers and muscle damage in a porcine balloon compression model of acute compartment syndrome. Abstract # 1398. **Orthopedic Research Society (ORS)** 2014 Annual Meeting, New Orleans LA.
14. Budsberg S, Shuler M, Uhl E, Hansen M, Roskosky M, Freedman B. Evaluation of NIRS, serum biomarkers and muscle damage in a porcine balloon compression model of acute compartment syndrome. **AAOS/OTA/SMOS, ORS Meeting - . Extremity War Injuries Symposium: Reducing disability within the military**. February 2014, Washington DC.
15. Roskosky M, Robinson G, Shuler M, Freedman B. Subcutaneous Depth in a Traumatized Lower Extremity. **Society of Military Orthopedic Surgeons 55th Annual Meeting,** December 2013. (Podium)
16. Roskosky M, Robinson G, Shuler M, Freedman B. Subcutaneous Depth in a Traumatized Lower Extremity. **Southern Orthopedic Association 30th Annual Meeting,** July 2013. (Poster)
17. Budsberg S, Shuler M, Freedman B, Hansen M. Evaluation of NIRS, serum biomarkers and muscle damage in a porcine balloon compression model of acute compartment syndrome. **Military Health System Research Symposium (MHSRS)**, August 2013, Fort Lauderdale, Fl
18. Roskosky M, Robinson G, Shuler M, Freedman B. Subcutaneous Depth in a Traumatized Lower Extremity. **Military Health System Research Symposium**, August 2013. (Podium)
19. Freedman B, Shuler M, Cathcart C, Reynolds L, Budsberg S. NIRS versus direct pressure monitoring of acute compartmental syndrome in a porcine model. **Medical Heath Services Research Symposium**, Fort Lauderdale, FL, Aug 13th-16th, 2012.
20. Jackson K, Cole AL, Potter BK, Kinsey TK, Shuler MS, Smith EK, Freedman BA. Identification of optimal control compartments for near-infrared spectroscopy assessment of lower extremity compartmental perfusion. **Orthopedic Trauma Association 2012 Annual Meeting**, October 2012. (Podium presentation)
21. Cathcart C, Shuler M, Freedman B, Reynolds L, Cole A, Whitesides TE, Smith EK, Budsberg S. NIRS vs direct pressure monitoring of acute compartmental syndrome in a porcine model. **Orthopedic Trauma Association 2012 Annual Meeting**, October 2012. (Poster presentation)
22. Cathcart C, Shuler M, Freedman B, Reynolds L, Cole A, Budsberg S, Whitesides TE, Smith EK. Non-invasive NIRS versus invasive direct pressure monitoring of acute compartmental syndrome in a porcine model. **125th Annual Meeting of the American Orthopaedic Association**, Washington D.C., June 27-30, 2012. (Poster presentation)
23. Cole AL, Herman RA, Heimlich JB, Ahsan S, Freedman BA, Shuler MS. Ability of near infrared spectroscopy to isolate muscle compartments of the upper extremity. **125th Annual Meeting of the American Orthopaedic Association**, Washington D.C., June27-30, 2012. (Poster presentation)
24. Cole AL, Herman RA, Heimlich JB, Ahsan S, Shuler MS, Freedman BA. Ability of near infrared spectroscopy to isolate muscle compartments of the upper extremity. **American Academy of Orthopedic Surgeons Annual Meeting**, San Francisco, CA, February 2012. (Paper presentation)
25. Cathcart C, Shuler M, Freedman B, Reynolds L, Cole A, Budsberg S. Non-invasive NIRS versus invasive direct pressure monitoring of acute compartment syndrome in a porcine model. **Orthopedic Research Society**, San Francisco, CA, February 2012.
26. Herman R, Heimlich B, Cole AL, Shuler MS. Ability of near infrared spectroscopy to isolate muscle compartments of the upper extremity. **Ninth Annual American Medical Association Medical Student Section/Resident & Fellow Section Joint Research Symposium**, New Orleans, LA, November 2011.
27. Shuler MS. Symposium 3: Compartment Syndrome: New Technologies: Non-Invasive Compartment Monitoring. **Orthopaedic Trauma Association**, San Antonio, TX, October 2011. (Forum discussion)
28. Shuler MS, Cole AL, Robinson MA, Freedman BA. Comparison of near infrared spectroscopy values between compartments of the lower extremities. **Advanced Technology Applications for Combat Casualty Care 2011 Conference**, Fort Lauderdale, FL, August 2011. (Poster presentation)
29. Freedman B, Cole A, Shuler M, Jackson K, Owens L, Lackie D. Do skin pigmentation and hair affect near-infrared spectroscopy assessment of leg compartment syndrome. **Advanced Technology Applications for Combat Casualty Care 2011 Conference**, Fort Lauderdale, FL, August 2011. (Poster presentation)

30. Freedman B, Jackson K, Shuler M, Cole A. Do skin pigmentation and hair affect near-infrared spectroscopy assessment of leg compartment syndrome? **Southern Orthopedic Association 28th Annual Meeting**, Big Island, HI, July 2011. (Poster presentation)
31. MAJ Brett Freedman, MD; CPT Keith Jackson, MD; Michael Shuler, MD; Ashley Cole, MPH Do Skin Pigmentation and Hair Affect Near-Infrared Spectroscopy Assessment of Leg Compartment Syndrome. **American Academy of Orthopaedic Surgeons**, San Diego, CA. Feb 15-19, 2011.
32. Shuler MS, WM Reisman, TE Whitesides, JR, TL Kinsey, EM Hammerberg, MG Davila, TJ Moore. Correlation between muscle oxygenation and compartment pressure in acute compartment syndrome of the leg: A new screening technique. **American Orthopaedic Association (AOA),** San Diego, CA. June 8-10, 2010.
33. Shuler MS, WM Reisman, TE Whitesides, JR, TL Kinsey, EM Hammerberg, MG Davila, TJ Moore. Correlation between muscle oxygenation and compartment pressure in acute compartment syndrome of the leg: A new screening technique. **Georgia Orthopaedic Society,** Greensboro, GA. Oct 7-10, 2009.
34. Shuler MS, WM Reisman, TE Whitesides, JR, TL Kinsey, EM Hammerberg, MG Davila, TJ Moore. Correlation between muscle oxygenation and compartment pressure in acute compartment syndrome of the leg: A new screening technique. **Southern Orthopaedic Association,** Amelia Island, FL. July 15-18, 2009.
35. Shuler MS, WM Reisman, TE Whitesides, JR, TL Kinsey, EM Hammerberg, MG Davila, TJ Moore. Near Infrared Spectroscopy (NIRS) in Lower Extremity Trauma. **Eastern Orthopaedic Association,** Paradise Island, Bahamas. June 17-20, 2009.
36. Shuler MS, WM Reisman, TE Whitesides, JR, TL Kinsey, EM Hammerberg, MG Davila, TJ Moore. Correlation between muscle oxygenation and compartment pressure in acute compartment syndrome of the leg: A new screening technique. **Orthopaedics Trauma Association OTA** Annual Meeting - 25th Anniversary Meeting, San Diego, CA. Oct 8-10, 2009
37. Shuler M, Reisman W, Whitesides, Jr. T, Hammerberg EM, Andras L, Moore T. Near Infrared Spectroscopy (NIRS) in Lower Extremity Trauma. **Southern Orthopaedic Association,** Hot Springs VA. June 11 - 15, 2008**.**

Patents

ALLOWED PATENTS

- **5,425,643-** Method And System For Monitoring Oxygenation Levels Of A Compartment For Detecting Conditions Of A Compartment Syndrome
- **8,639,309-** Method And System For Monitoring Oxygenation Levels Of Compartments And Tissue
- **8,100,834** - Method And System For Monitoring Oxygenation Levels Of A Compartment For Detecting Conditions Of A Compartment Syndrome
- **12,855,019** – Methods and Dressing System for Promoting Healing of Injured Tissue

PENDING PATENTS

- US 12/855,019- Methods and dressing systems for promoting healing of injured tissue
- US 13/671,861- METHOD AND SYSTEM FOR PROVIDING VERSATILE NIRS SENSORS

APPENDICES

Appendix A: The executive summary of the final analysis for the Phase 2 study.

Appendix B: The collection of full version published and in press manuscripts associated with this grant.

Appendix C: In-Press Articles (*Published articles are available upon request):

- Johnson A, Roskosky M, Shuler M, Freedman B. *Depth Penetration of Near Infrared Spectroscopy in the Obese*. **Journal of Trauma and Treatment**. (In Press)

- Couch L, Roskosky M, Freedman B, Shuler MS, *Effect of Skin Pigmentation on Near Infrared Spectroscopy*, **American Journal of Analytical Chemistry**, (In Press)
- Cole A, Roskosky, M, Freedman B, Shuler MS, *Near infrared spectroscopy and lower extremity acute compartment syndrome: a review of the literature*, (In Press)
- Reisman W, Shuler M, Roskosky M, Kinsey T, Freedman B. *Use of Near Infrared Spectroscopy to Detect Sustained Hyperaemia Following of Lower Extremity Trauma.* **Military Medicine.** (In Press)

"Near-Infrared Spectroscopy to Reduce the Prophylactic Fasciotomies for and Missed Cases of Acute Compartment Syndrome in Soldiers Injured in OEF/OIF":

Summary of Methods and Results for Final Grant Report

Prepared 11/17/2015 from full report (TK)

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Table of Contents

I. Overview and Methods

a. Objectives

The analysis was designed to address the analytic aims that were stated in the protocol:

"For all bivariate comparisons, the average NIRS value over a short period of time (e.g., 30 seconds) will be used. This is meant to minimize random variability (i.e., noise), while preserving true changes that may occur over a longer period of time. Continual NIRS data will be examined and appropriately processed to select an appropriate period of time over which to summarize NIRS data. Due to the observational nature of this preliminary study, no formal hypotheses testing will be done and objectives are largely descriptive and exploratory.

The analysis plan is based on five aims:

1. Describe and contrast values and longitudinal behavior (trends, variability) of NIRS between injured and uninjured extremities, between critically injured patients with and without lower extremity orthopaedic injuries, and between injured subjects and healthy controls (Phase 1 subjects).
    a. NIRS values will be described using summarization (means, variance, percentiles, and percentile ranges) and graphical methods at each of the key clinical time points captured. Values of NIRS will be compared between limbs, compartments, and/or patient groups using parametric or non-parametric (as appropriate for sample size and distributions) tests for differences of central tendency, linear correlation, and/or repeated measures/mixed models for multi-level comparisons and estimation.
    b. Variability of NIRS measurements (i.e., short-term fluctuation) will be characterized graphically and quantitatively. Variability will be compared between patient/injury groups using tests for equality of variance and/or percentiles, as appropriate, to determine whether systematic differences of variability are associated with traumatized vs. non-traumatized physiologic states. Variability of q 4-second NIRS measurements will be evaluated to determine whether the use of optimum smoothing algorithms using an iterative approach is necessary.
    Longitudinal trends of NIRS will be characterized and compared graphically and descriptively, across extremities within patients and across cohorts.
2. Describe the natural course of the clinical process of ACS among patients who develop ACS while under observation with continuous NIRS monitoring.
    a. Longitudinal trends of NIRS and their coincidence with clinical events relevant to the process of ACS will be described narratively and graphically on a case-by-case basis. The relationships between NIRS values and trends and intercompartment pressure measurements will be explored, as well as other quantitative and qualitative clinical correlates of or relevant co-factors to ACS, as available. Using an exploratory approach, clinical process descriptions will be reviewed qualitatively to elucidate characteristics or patterns of potential interest. The purposes are to describe the response of NIRS to the process of clinical ACS as it occurs *in vivo*; to identify factors or characteristics relevant to the use of

NIRS as an aid to the diagnosis of impending ACS; and to identify hypotheses for further study.

3. Characterize diagnostic utility of NIRS for predicting the clinical diagnosis of ACS with fasciotomy among subjects with lower extremity injury (Cohort 2). Identify optimum cut point(s) for diagnostic accuracy with data available, for the purpose of identifying approximate diagnostic criteria that might be appropriate for use in future decision analysis studies or clinical trials.
    a. Differences between patients of Cohort 2 who do and do not receive clinical diagnoses of lower extremity ACS with fasciotomy will be described in terms of NIRS values relative to internal control values (contralateral lower extremity and/or baseline values), and changes of same over time. NIRS values at various time points will be compared, including prior to and following fasciotomy, among subjects who receive a fasciotomy, and the minimum value among patients who do not receive a fasciotomy.
    b. Possible effect of skin pigmentation (colorimeter measures, skin type scale, and/or race), fat thickness, time to injury, smoking history, or physiologic variation such as vasopressor use) may be explored and described, as available sample sizes warrant.
    c. Sensitivity, specificity, and receiver operating curve characteristics will be calculated for and compared across a range of potential diagnostic criteria determined from the observed data.

4. Evaluate utility of uninjured contralateral limb and alternative sites (ipsilateral and contralateral foot, upper extremity) as internal control sites among various patient types.
    a. Characterize the relationships between NIRS values of muscle compartments of injured (or selected) leg and various potential control sites using correlation plots, Pearson or Spearman correlation coefficient, and comparison of delta values between injured and control sites. Describe and compare these relationships among and between uninjured (Phase 1) and injured subjects with (Phase2 cohort 2) and without (Phase 2 cohort 1) lower extremity trauma.
    b. Among Cohort 2a patients (those with unilateral LE trauma) we will recalculate and compare diagnostic accuracy statistics described in objective 3 between methods using the corresponding compartment of contralateral leg as an internal control, and methods using alternative control compartments. Agreement statistics (Kappa) will be calculated for agreement of ACS diagnosis between methods that utilize different control compartments.

5. Elucidate and describe findings of interest regarding situation-specific behavior of NIRS as observed opportunistically among these critically injured patients (e.g., hypotension/hypovolemia/shock states; respiratory disequilibrium; induction of anesthesia)
    a. Descriptive analyses as indicated. “

b. Data sources

Prepared datasets were received from the Project Manager in the form of eighteen (18) .sas7bdat files representing the processed data from patient case report forms ('CRF datafiles'). 122 individual patients were represented in the CRF datafiles. 121 raw datasets in .csv format were also received from the Project Manager, each representing the sequential recordings output from the Nonin NIRS monitoring device of a unique patient ('NIRS datafiles'). Three (3) of the provided NIRS data files (labeled 2002, 2003, and 2004) had no corresponding record in the CRF dataset.

In consultation with the project manager, 114 of the 122 patients contained in the CRF dataset were included in the analyses after the following exclusions:

Study ID	NIRS dataset provided?	Exclusion reason
1022	No	Did not complete minimum monitoring for inclusion
1024	No	Did not complete minimum monitoring for inclusion
2017	Yes	Had been initially enrolled but found afterward to be ineligible
2019	Yes	Did not complete minimum monitoring for inclusion
2063	Yes	Did not complete minimum monitoring for inclusion
2070	No	Withdrawn by PI – no monitoring obtained after 12 hours
2108	Yes	Corrupt NIRS data file (irreconcilable error in the recording of time)
2109	No	Did not complete minimum monitoring for inclusion

The 114 included patients comprised the following cohorts according to the study design outlined in the protocol:

Cohort:	Group description	Number patients
Cohort 1	Critical controls ('Uninjured group')*	23
Cohort 2	Lower extremity (LE) injury without compartment syndrome	84
	Unilateral LE injury = 79	
	Bilateral LE injury = 5	
Cohort 3	LE injury with compartment syndrome	7
	Unilateral LE injury = 6	
	Bilateral LE injury = 1 (ACS in one leg)	

* Use of term 'uninjured' group in this report refers to lack of major injury to lower extremities

c. Data cleaning and preparation

CRF datasets provided to the data analysts had been prepared by a contract research organization (CRO) according to terms of agreement between the investigator and the CRO, which included procedures for data verification and quality control. Data dictionaries and formats were included in typical formats for use by the analysts.

NIRS data files represented raw, direct output from the monitoring device generated in longitudinal ('long file') electronic form via custom software modifications to the device with no secondary process for systematic verification or cleaning. NIRS data consisted of one row of measurements for all 12 measured compartments every 4 seconds for the duration of monitoring with exception of 2 patients having one row every 16 seconds (ID# 2201, 2202) and 1 patient having one row every 8 seconds (ID# 3003).

For the purposes of all analyses and descriptive statistics, the alternate legs of each individual patient in the study were classified one as the 'test' leg (primary limb of interest for the individual) and the 'contralateral' leg (served as an internal control value for interpretation of values of the test leg). Test and contralateral status of the legs were typically indicated in variable names as T_ and C_ or similar notation, respectively. Classification was determined as follows, thus creating these discrete patient groups:

-Patients with unilateral injuries:	Test = injured side;	Contralateral = uninjured side
-Patients without LE injury:	Test = randomly selected;	Contralateral = opposite side
-Patients with bilateral injuries:	Test = randomly selected;	Contralateral = opposite side
-Patients with ACS:	Test = ACS side;	Contralateral = opposite side

Thus for the patients with bilateral injuries, including the one with unilateral ACS, the contralateral side was an injured leg without ACS; For the unilateral injured group (Cohort 2) and uninjured group (Cohort 1), the contralateral limbs were uninjured. For the uninjured group (Cohort 1), the test leg was also uninjured, randomly selected. From this data structure, legs with injury (e.g., Cohort 2) can be compared to legs without injury (Cohort 1) using a 2-sample design with internal control values available from the corresponding compartments of the opposite leg for all patients.

Random selections of limbs were computer generated in SAS software using random sort method. A uniformly distributed random number between 0 and 1 was generated for each patient using a pre-specified seed after data were reproducibly sorted. The resulting distribution was halved (< 0.5 and => 0.5) and test and contralateral side designations, respectively, were arbitrarily assigned.

Extensive initial qualitative and graphical inspections were performed to evaluate data capture and other qualitative features. Examples of 72-hour tracings from 10 randomly selected patients from the uninjured, unilateral injured, and bilateral injured group *after correction for verified lead switches (described subsequently)* are shown in **Figures 1a-j.** Graphical data from the 7 patients with ACS are shown in their entirety in Volume 2, Section II of this report. The 72-hour plots were reduced by a ratio of 60:1 data points (one reading every 4 minutes instead of every 4

seconds) by systematic selection of every n^{th} row to improve readability of the condensed plot. In addition, plots were generated to a scale of 20:1 data point reduction (every 80 seconds) over sequential 24 hour periods, and at 8:1 reduction (every 30 seconds) over sequential 8-hour periods for the ability to examine any particular section more closely. The complete battery of all longitudinal graphics for all patient-compartments are provided in the Electronic Appendix, delivered to the Project Manager on electronic disk media.

From initial qualitative inspection, several issues were noted and discussed with the investigative team:

1. Loss of data capture was frequently observed. Qualitatively, data capture was most consistent among the critical control (uninjured legs) patients and least consistent among injured leg compartments. Compartment syndrome patients (Cohort 3) typically demonstrated very short overall times on monitor with frequent non-capture.
2. Some periods of data loss appeared to constitute complete disconnection; these could usually be correlated to a disconnection event noted in the Field Data Sheets. In addition, there were frequent instances of data loss in individual compartments for varying lengths of time. It was noted that longer periods of random disconnection were seen during night hours, commonly followed by restoration of tracings around ~8:00am. These observations are consistent with the surveillance schedule for checking of leads by the research team (q ~2 hour rounds were made during daytime hours).
3. There were frequent instances of ceiling level values (~100%) which were sometimes (but not always) associated with hard escalation and de-escalation patterns. This was a known issue to the investigative team. These patterns were seen most commonly in the leg compartments of patients with LE injury, more commonly in the injured than uninjured leg. Graphics for representative sample of 12 compartments are shown to illustrate the variety of patterns associated with these readings (**Figures 2a-l**)
4. Patterns suggestive of possible reversal of one or more leads (resulting in mislabeling) could be seen for at least10 patients. These cases were investigated by the Project Manager and hardcode data changes were made for cases for which lead switches could be confirmed against Field Data Sheets.

Considerable effort was given to investigation of the issue of validity of readings in the high range. A battery of graphics of patient tracings of NIRS data with sensor alert and signal quality errors plotted individually as well as overlaid over patient NIRS data were produced for qualitative inspection. These graphics revealed no clear patterns. Sensor alert and signal quality errors appeared to be uniformly distributed across the entire range of NIRS values, not concentrated at high or low values. These plots were delivered to the Project Manager for consultation with the engineering team. Patient graphics including Figures 2a-l were submitted to the manufacturer for consultation with the engineering team. A plan of action was regarding the handling of high (>~85-90%) and low range values in consultation with all members of the investigative team after extensive graphical analysis and further discussion with the device manufacturer. The consensus of the investigative team was that instrumentation error is prevalent in at least some significant proportion of readings over ~85%, and highly likely in all readings above ~95%. In the data analysis stage of this study, there is no clear way to diagnose the validity of any given measurement. No objective and systematic method could be determined to

distinguish valid from invalid measurements that might be coexistent within some range of values (e.g., between 85% and 95%) based solely on observation of the recorded values themselves. The decision was made in consultation with the investigators to omit or 'trim' values (i.e., impute to missing) above 85% from the analyses as being of questionable biologic plausibility, therefore having significant likelihood of being erroneous. As well, values lower than 30 were omitted (imputed to missing) as biologically implausible, based on observations of this group in previous porcine studies (Steven Budsberg and Michael Shuler, personal communication).

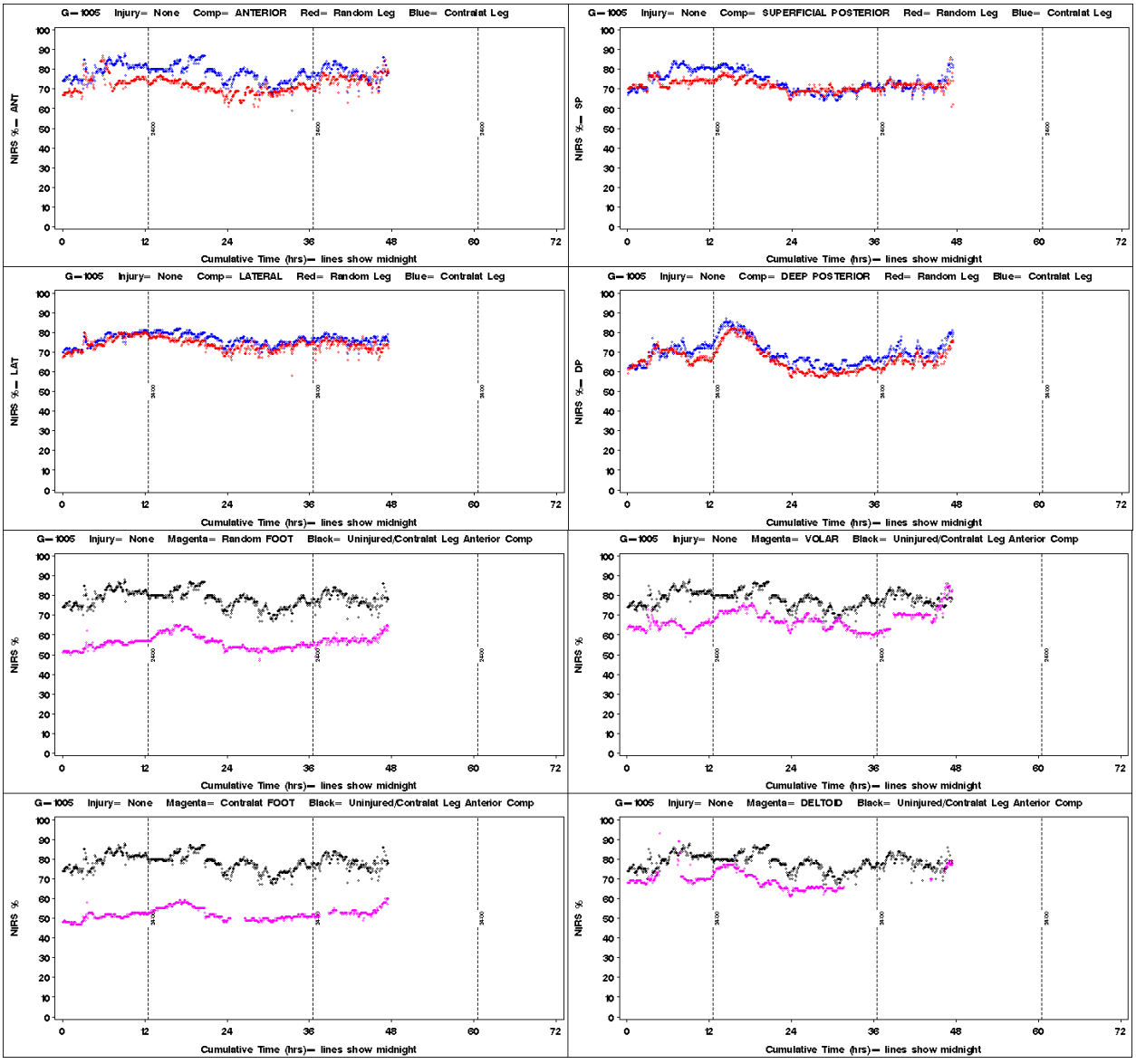
Finally, plots of all compartments of all patients were review again on a case-by-case basis to identify artifact from loose leads, which generated a very characteristic pattern of wide dispersal of values during a period otherwise evidencing disconnection. Sections of values exhibiting these qualitative patterns with a range of at least 50 percentage points fluctuation of NIRS values were imputed to missing by hardcode programming after determination of start and stop times from graphics and/or inspection of raw data files. An example of this type of characteristic artifact can be seen in Figures 1c-d below at approximately hours 18 through 22.

SAS version 9.3 software (SAS Institute, Cary, NC) was used for all datastep procedures described in sections 1b-d and section II of this report. All SAS programs for all datastep procedures including all hardcode modifications described in this section are submitted to the Project Manager in an Electronic Appendix for complete transparency. The SAS programs are the final authoritative source for all programmatic and hardcode changes of values of raw data. All changes or imputations to values of data were decided and implemented in close consultation with the Project Manager and/or Investigators.

Table 1 provides a quantitative summary of missing vs. non-missing data in the raw NIRS datafiles, before the data cleaning-related modifications or imputations described above.

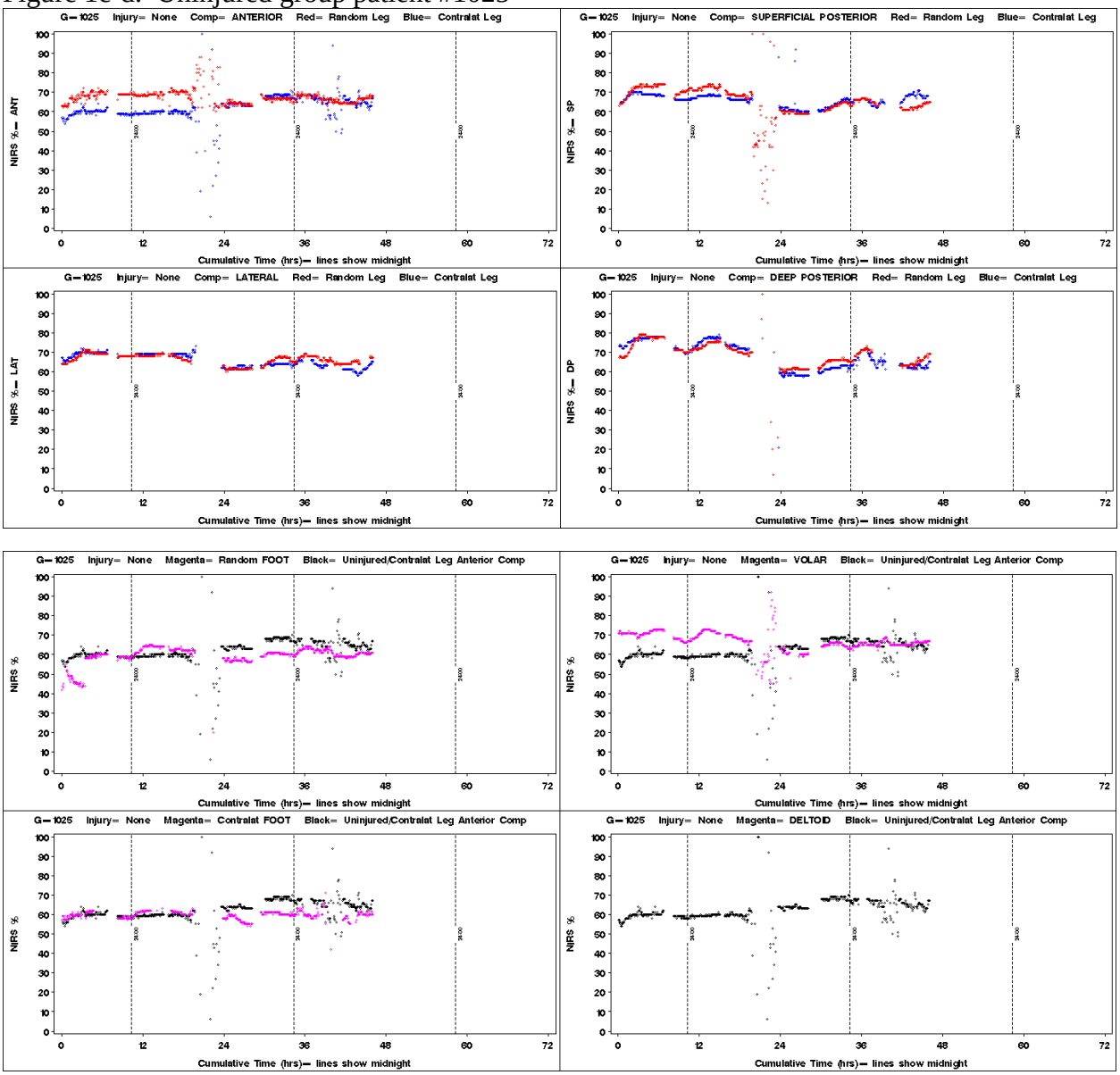
Figures 1a-t. 72-hour NIRS tracings for all compartments of 10 randomly selected patients

Figures 1a-b. Uninjured group patient #1005

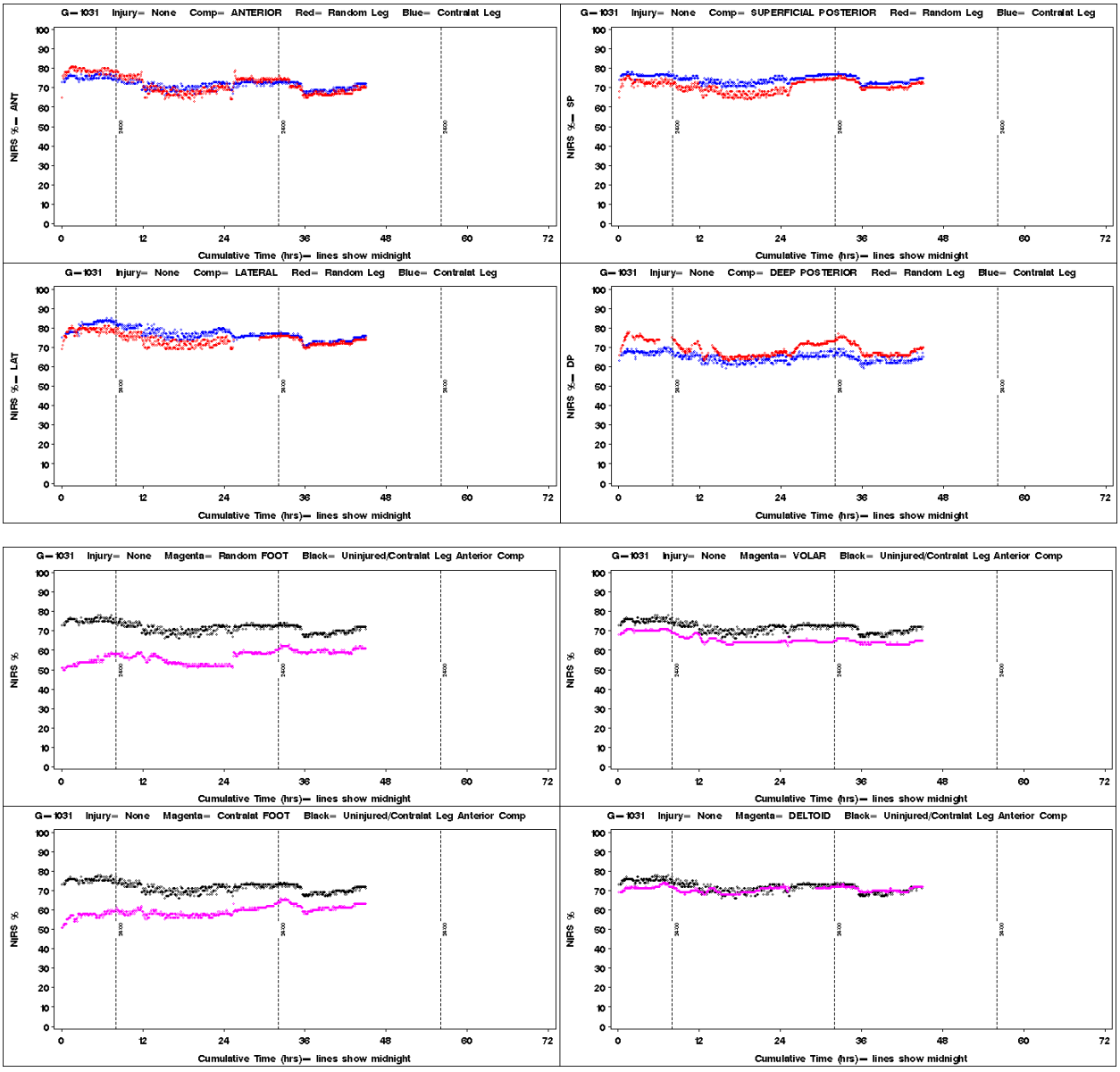


*Note: The second composite graphic for all figures in this section utilizes the same 'control' compartment for tracings of foot and arm compartments (Contralateral leg anterior compartment). The first composite graphics plot each compartment of the test leg (red) against the corresponding compartment of the contralateral let (black).

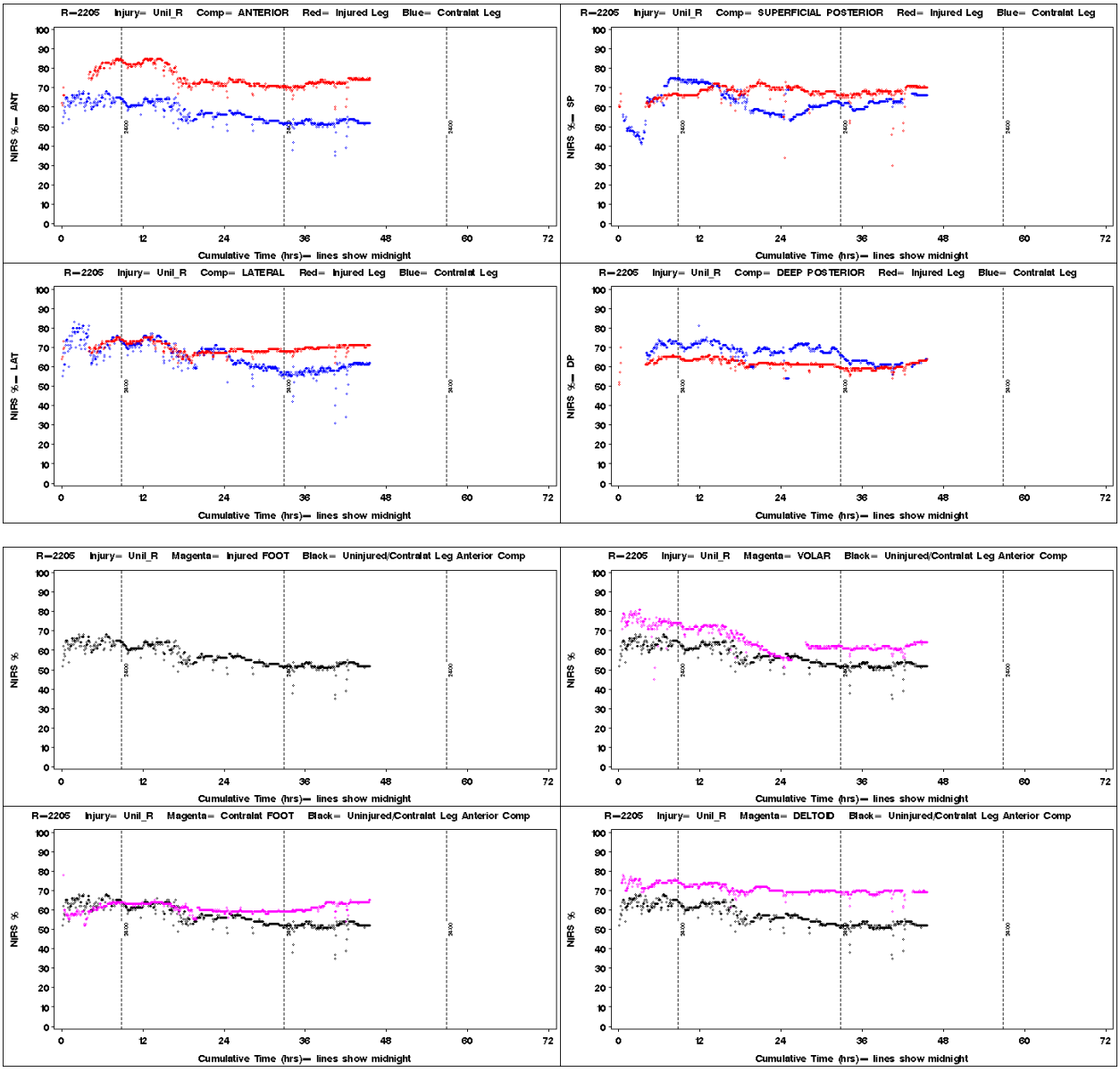
Figure 1c-d. Uninjured group patient #1025



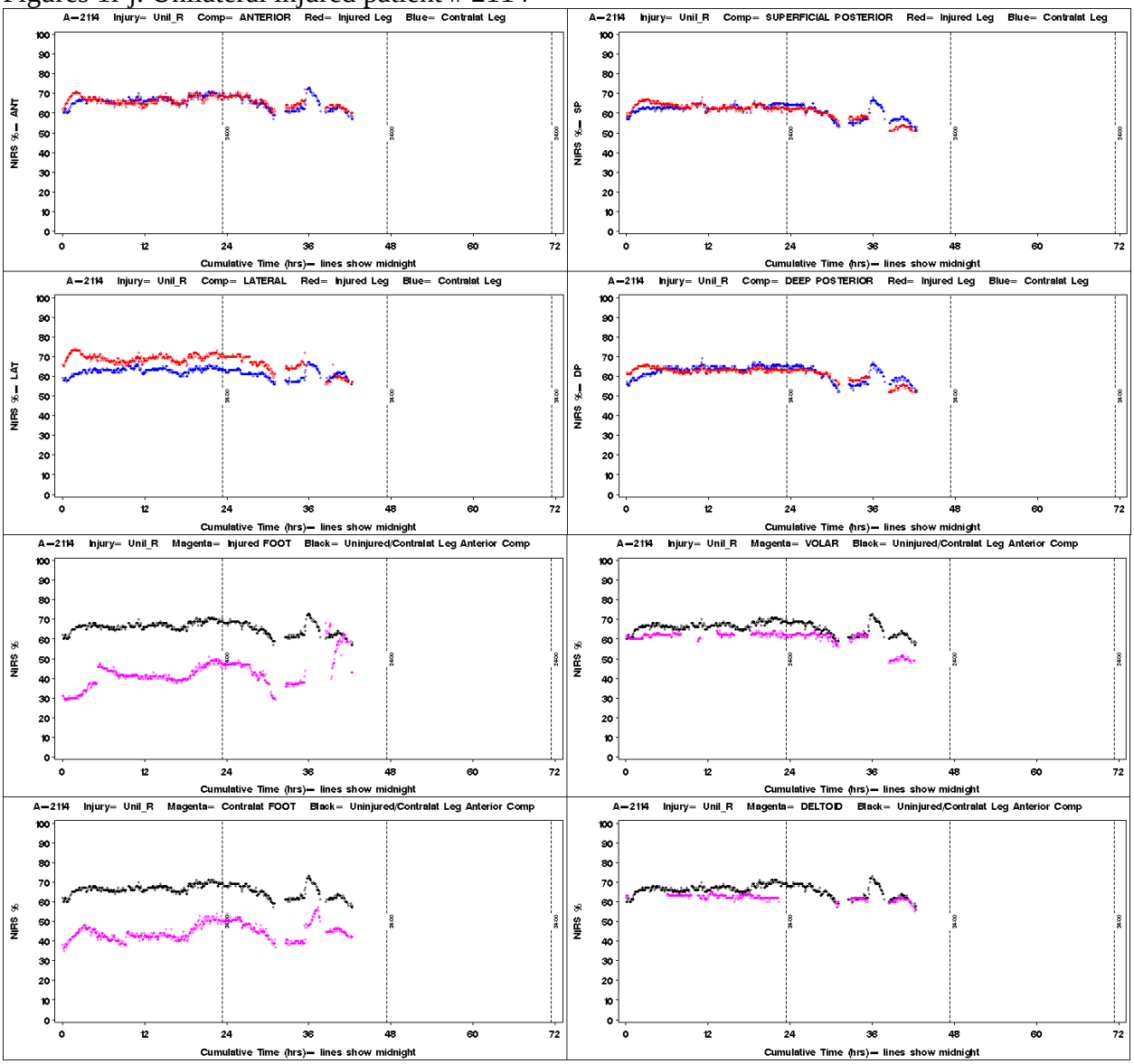
Figures 1e-f. Uninjured group patient #1031



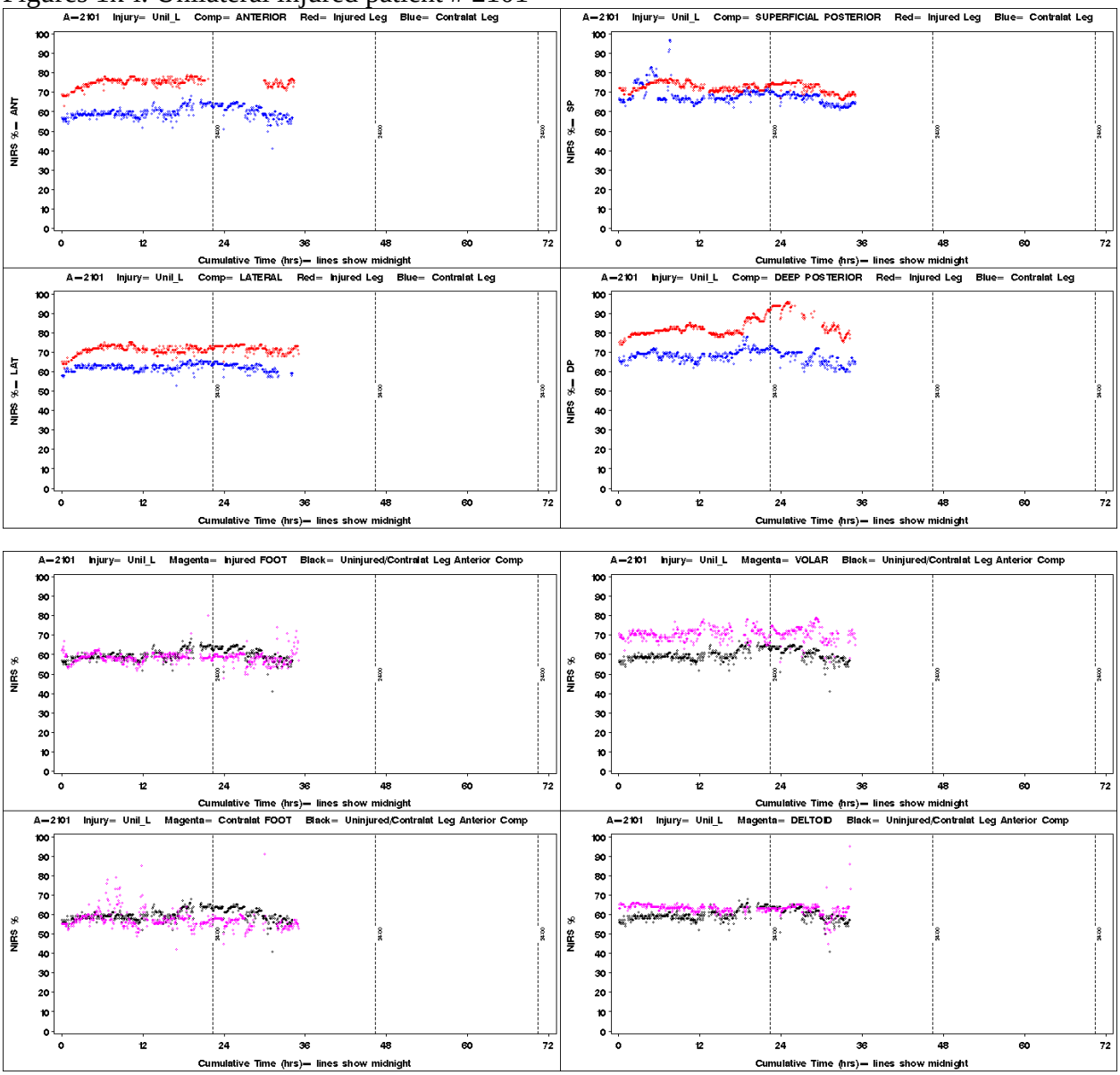
Figures 1g-h. Unilateral injured patient # 2205



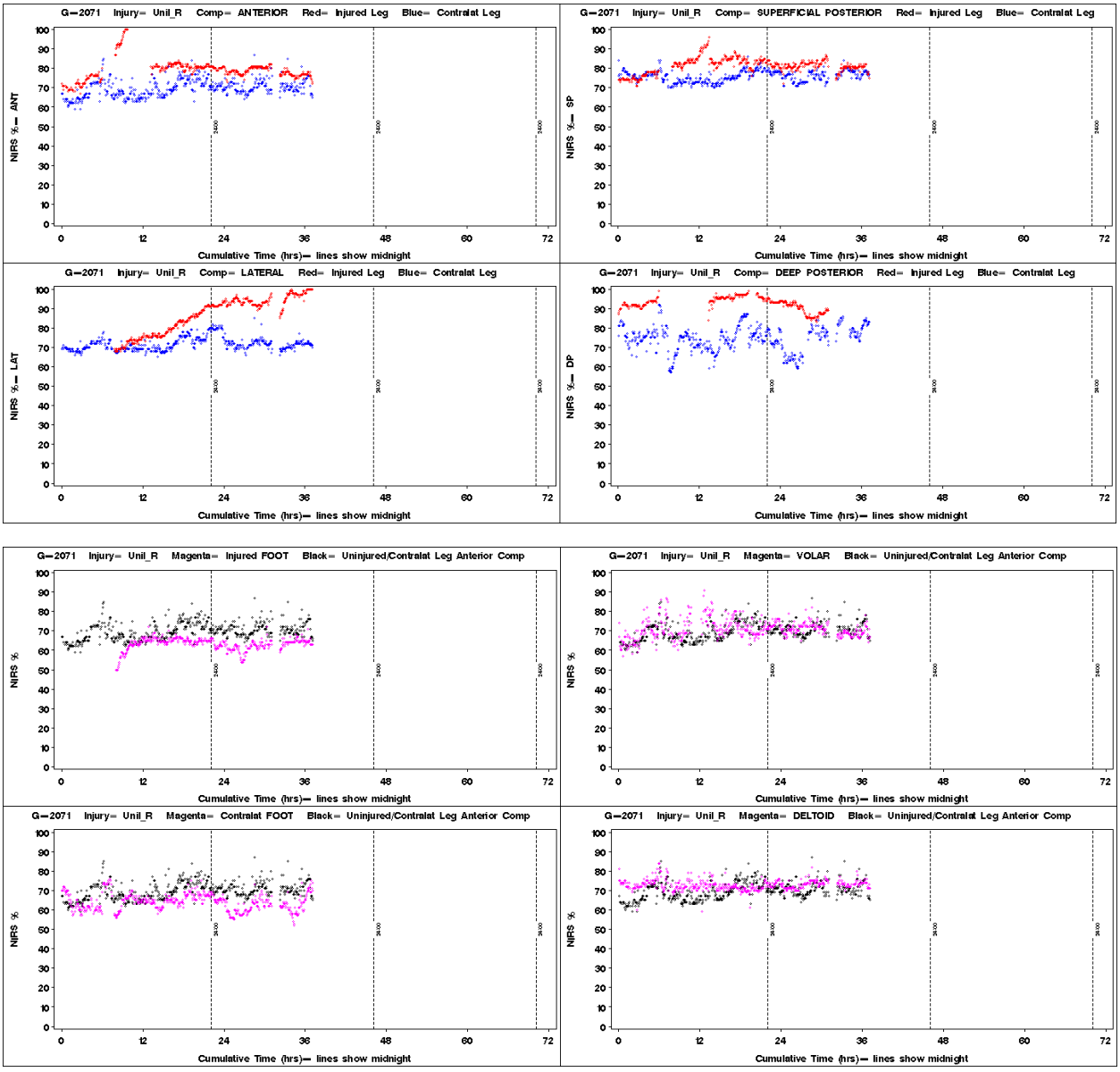
Figures 1i-j. Unilateral injured patient # 2114



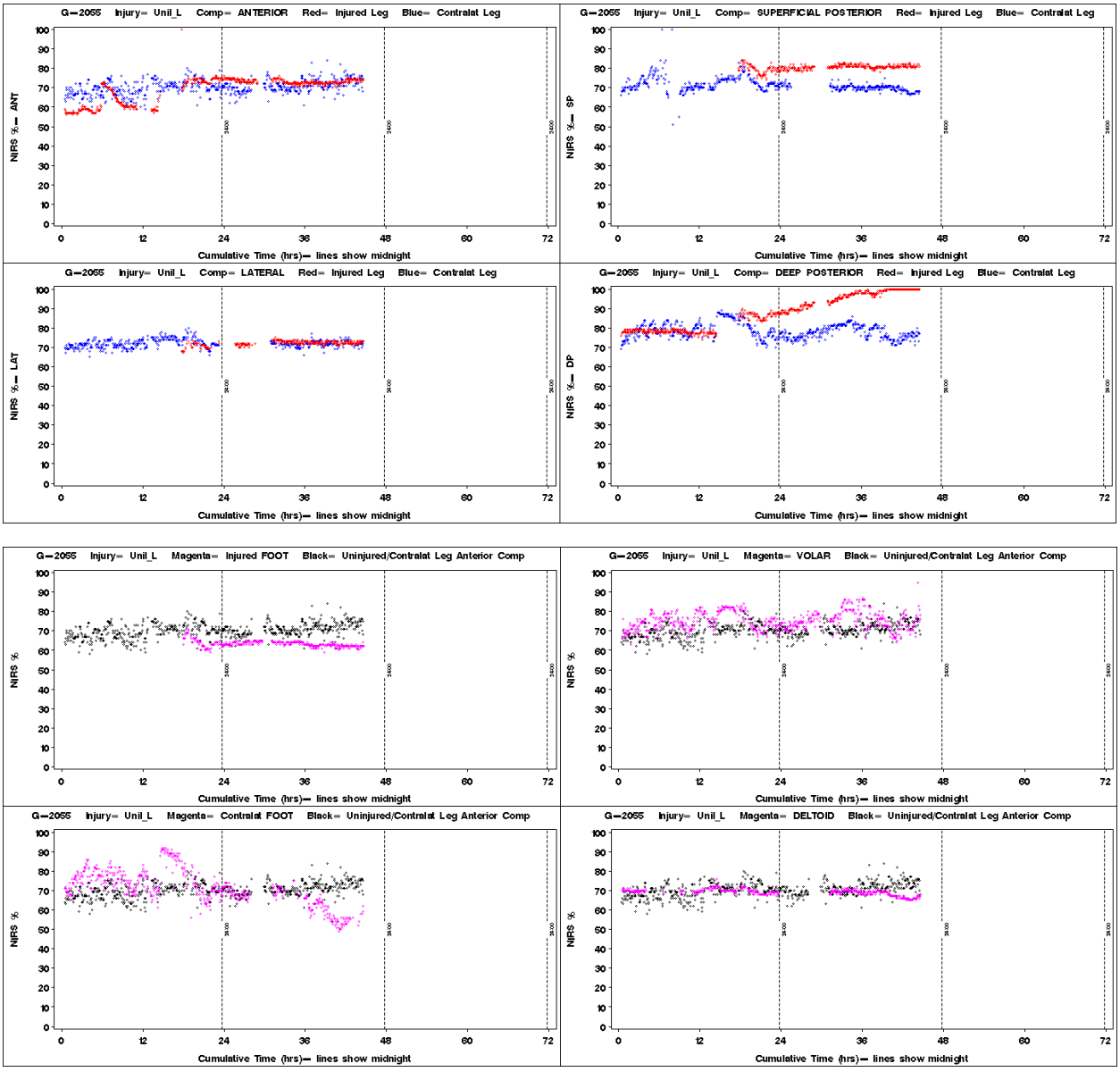
Figures 1k-l. Unilateral injured patient # 2101



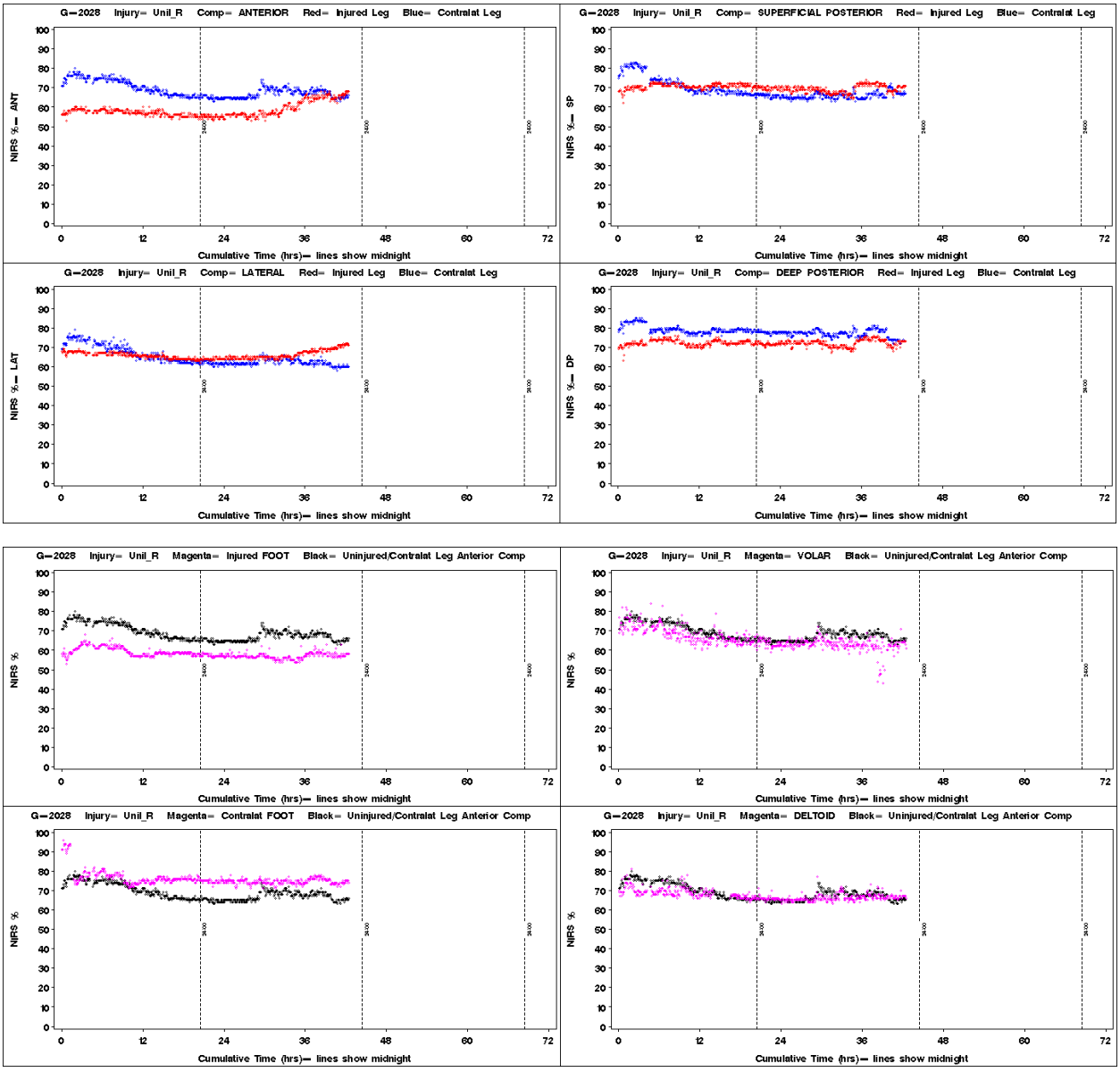
Figures 1m-n Unilateral injured patient # 2071



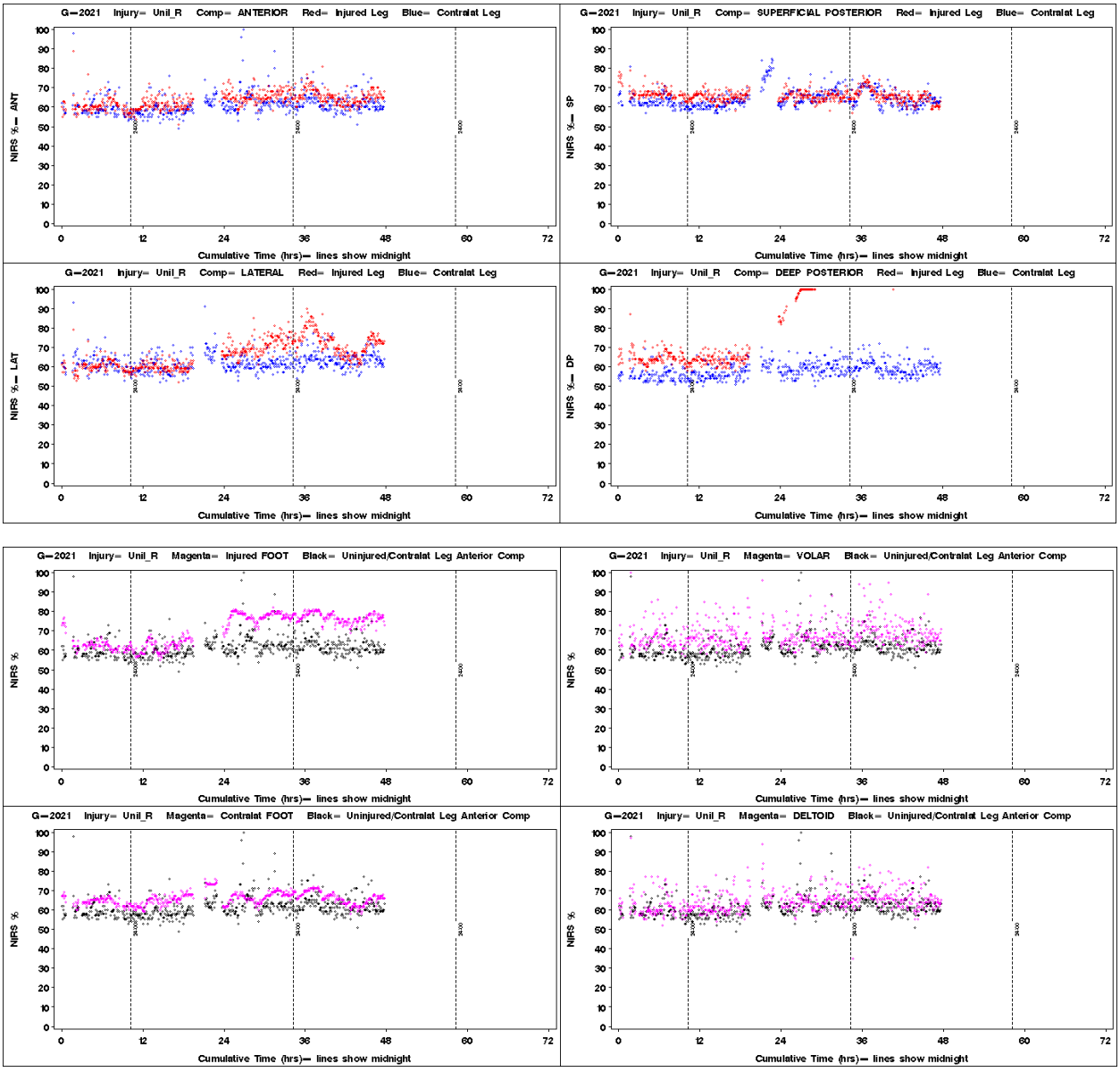
Figures 1o-p Unilateral injured patient # 2055



Figures 1q-r. Unilateral injured patient # 2028

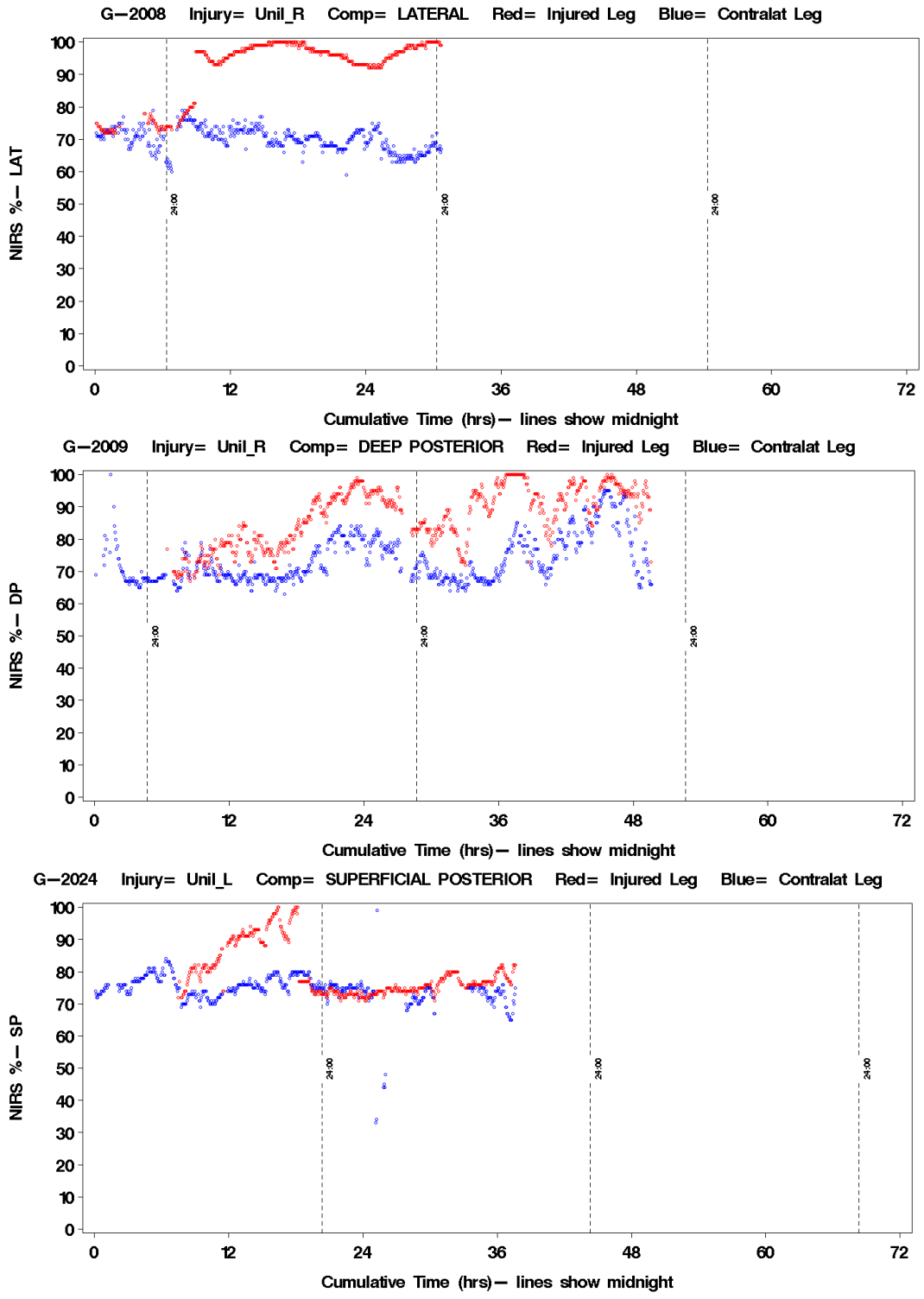


Figures 1s-t. Unilateral injured patient # 2021

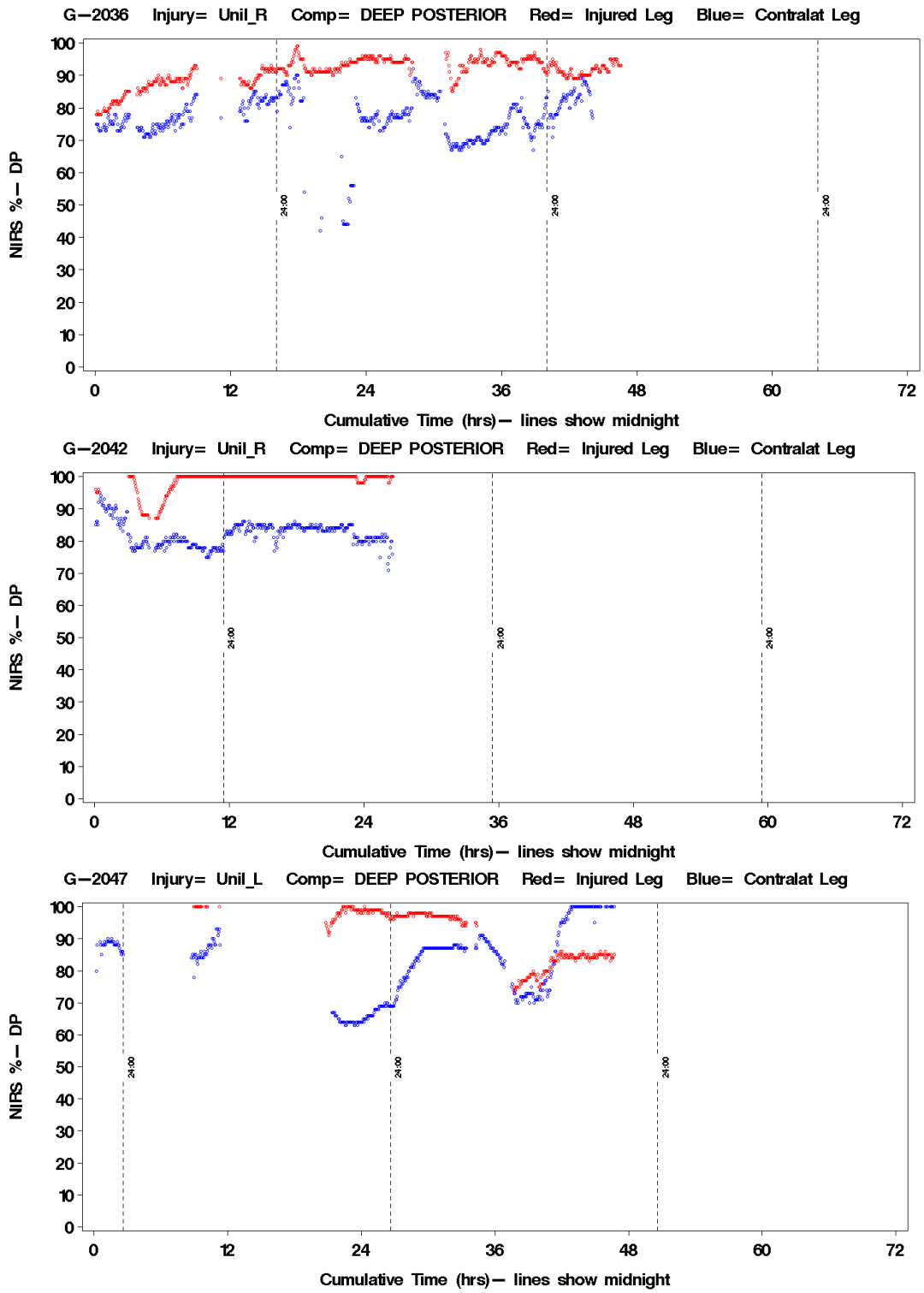


Figures 2a-l. Selected examples of selected compartments demonstrating 'ceiling' NIRS values

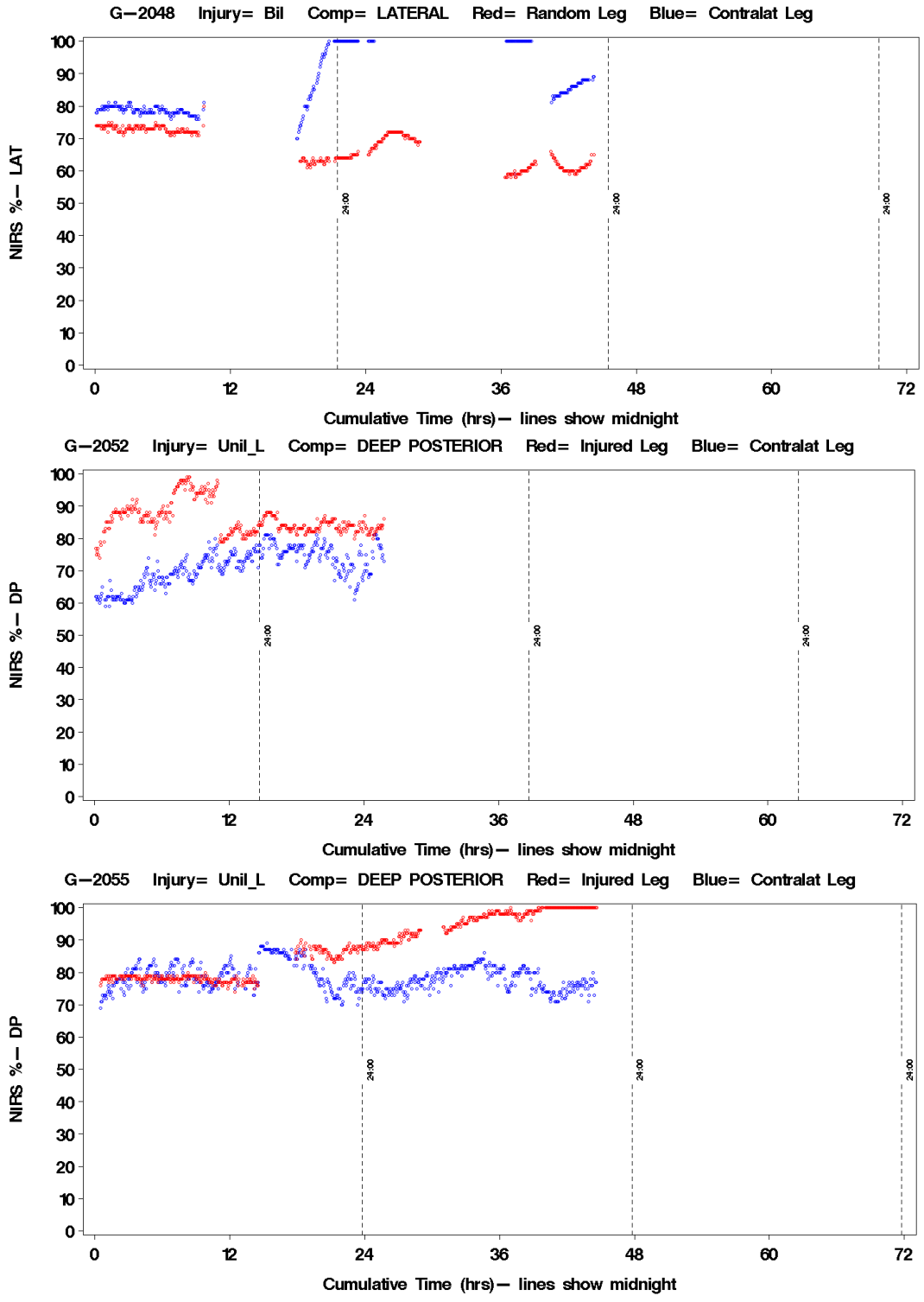
Figures 2a-c.



Figures 2d-f.


G—2036 Injury= Unil_R Comp= DEEP POSTERIOR Red= Injured Leg Blue= Contralat Leg
NIRS %— DP
Cumulative Time (hrs)— lines show midnight
G—2042 Injury= Unil_R Comp= DEEP POSTERIOR Red= Injured Leg Blue= Contralat Leg
NIRS %— DP
Cumulative Time (hrs)— lines show midnight
G—2047 Injury= Unil_L Comp= DEEP POSTERIOR Red= Injured Leg Blue= Contralat Leg
NIRS %— DP
Cumulative Time (hrs)— lines show midnight

Figures 2g-i.


G—2048 Injury= Bil Comp= LATERAL Red= Random Leg Blue= Contralat Leg
NIRS %— LAT
Cumulative Time (hrs)— lines show midnight
G—2052 Injury= Unil_L Comp= DEEP POSTERIOR Red= Injured Leg Blue= Contralat Leg
NIRS %— DP
Cumulative Time (hrs)— lines show midnight
G—2055 Injury= Unil_L Comp= DEEP POSTERIOR Red= Injured Leg Blue= Contralat Leg
NIRS %— DP
Cumulative Time (hrs)— lines show midnight

Figures 2j-l.

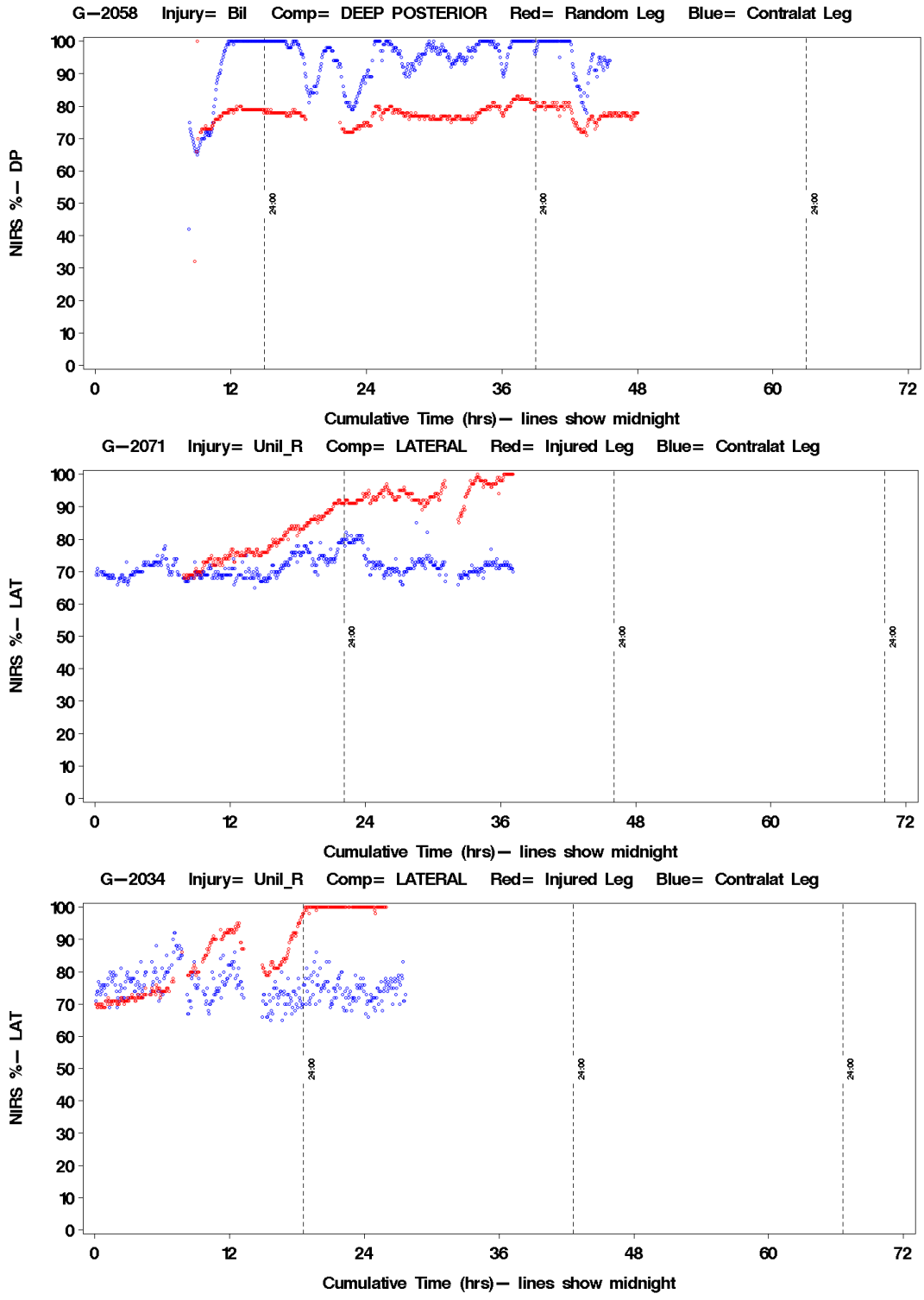

G—2058 Injury= Bil Comp= DEEP POSTERIOR Red= Random Leg Blue= Contralat Leg
NIRS %— DP
Cumulative Time (hrs)— lines show midnight
G—2071 Injury= Unil_R Comp= LATERAL Red= Injured Leg Blue= Contralat Leg
NIRS %— LAT
Cumulative Time (hrs)— lines show midnight
G—2034 Injury= Unil_R Comp= LATERAL Red= Injured Leg Blue= Contralat Leg
NIRS %— LAT
Cumulative Time (hrs)— lines show midnight

Table 1. Quantity of NIRS data points recorded in raw files with percent of missing values by injury type.

Table 1. Quantity of NIRS data points recorded in raw files with percent of missing values by injury type															
	All patients combined (n=114 patients)			Uninjured group (n=23 patients)			Unilateral injury group (n=79 patients)			Bilateral injured group (n=5 patients)			ACS group (n=7 patients)		
Compartment:	Total # data cells	Total # missing values	% cells w/missing values	Total # data cells	Total # missing values	% cells w/missing values	Total # data cells	Total # missing values	% cells w/missing values	Total # data cells	Total # missing values	% cells w/missing values	Total # data cells	Total # missing values	% cells w/missing values
T_Ant	3,210,771	1,134,405	35%	706,313	63,258	9%	2,314,422	997,147	43%	159,827	46,799	29%	30,209	27,201	90%
T_Lat	3,210,771	1,019,927	32%	706,313	70,936	10%	2,314,422	891,140	39%	159,827	32,211	20%	30,209	25,640	85%
T_DP	3,210,771	1,252,615	39%	706,313	106,609	15%	2,314,422	1,089,014	47%	159,827	35,807	22%	30,209	21,185	70%
T_DP	3,210,771	932,462	29%	706,313	77,791	11%	2,314,422	810,192	35%	159,827	28,728	18%	30,209	15,751	52%
C_Ant	3,210,771	431,312	13%	706,313	59,058	8%	2,314,422	328,272	14%	159,827	39,454	25%	30,209	4,528	15%
C_Lat	3,210,771	399,114	12%	706,313	80,919	11%	2,314,422	266,736	12%	159,827	41,511	26%	30,209	9,948	33%
C_DP	3,210,771	507,500	16%	706,313	76,526	11%	2,314,422	380,488	16%	159,827	41,754	26%	30,209	8,732	29%
C_SP	3,210,771	376,891	12%	706,313	63,012	9%	2,314,422	283,302	12%	159,827	26,475	17%	30,209	4,102	14%
T_F	3,210,771	1,188,212	37%	706,313	146,233	21%	2,314,422	986,306	43%	159,827	40,476	25%	30,209	15,197	50%
C_F	3,210,771	650,748	20%	706,313	197,920	28%	2,314,422	415,313	18%	159,827	33,660	21%	30,209	3,855	13%
Vol	3,210,771	595,060	19%	706,313	84,726	12%	2,314,422	456,772	20%	159,827	47,784	30%	30,209	5,778	19%
Del	3,210,771	666,977	21%	706,313	147,958	21%	2,314,422	491,679	21%	159,827	22,750	14%	30,209	4,590	15%
Combined	38,529,252	9,155,223	24%	8,475,756	1,174,946	14%	27,773,064	7,396,361	27%	1,917,924	437,409	23%	362,508	146,507	40%
Total rows of data recorded*			3,210,771			706,313			2,314,422			159,827			30,209
Total non-missing NIRS values recorded*			29,374,029			7,300,810			20,376,703			1,480,515			216,001

*Data were captured from Nonin devices with data of all 12 compartments on each row every 4.5 seconds (exception: 33 patients were recorded q8 or q16 seconds)

*Missing data includes all cells recorded by the devices as empty cells, which includes periods of intentional and unintentional disconnection. This represents the status of the raw data as originally recorded, and does not reflect values later imputed to missing for analytic purposes (e.g., setting implausible values (>85 or <20) to missing, removal of clear loose lead artifact, etc)

d. Construction of the analytic dataset

After modifications to the data as previously described and contained in the associated datastep programs submitted with the Electronic Appendix, an aggregate dataset was constructed which was designed to provide effective reduction of the longitudinal NIRS data to a set of sequential summary values for each patient which could be used for the production of informative summary statistics and growth modeling analyses. The SAS program(s) producing these manipulations is also submitted with the Electronic Appendix.

2-minute samples of NIRS readings for all compartments were taken every 15 minutes for each patient's duration of monitoring beginning with minute 1 of monitoring. The initial (first) reading for each patient was collected from minutes 4-6 after connection, and subsequent samples were obtained every 15 minutes starting minutes 14-16. For patients with values recorded every 4 minutes (all but 3 patients) with no missing data, the 2-minute sample would provide 29 or 30 values. The seven cases with ACS were evaluated on a case-by-case basis for selection of appropriate 2-minute samples to ensure that reasonable samples were obtained since those patients were characterized by very short periods of monitoring of the injured limbs for some patients (less than 5 minutes total for some) and frequent loose lead artifact.

The median value of each 2-minute sample was used as the summary value to represent NIRS value for that segment of time in further analysis. Summary statistics for the distribution underlying each median value were generated and included on the analytic dataset. In other words, for each 2-minute sample of each compartment the n, mean, median, standard deviation, minimum, maximum, and interquartile range (IQR) were computed. For 2-minute samples with less than 10 non-missing values all summary statistics were set to zero (including the median value). Therefore, at least 10 values within the 2-minute period were required for inclusion of data from that period. For patients with less frequent than q 4-second recordings, the minimum requirement was relaxed to a minimum of 5 for # 3003 (q 8 second recordings) and min of 4 for # 2201 and 2202 (q 16 second recordings). These three patients were furthermore omitted from all subsequent analyses of sample distributions for the purpose of examining short-term variability, therefore all summary statistics for the distributions were set to missing for these patients except for the median if the minimum n was met.

The reduced analytic dataset contained a total of 6,557 rows of data each representing data for all compartments of a distinct patient (total of 114 distinct patients) at a distinct time, after omission of rows containing no summary data for any compartment. This analytic dataset was used for the primary analysis that is reported in detail in sections III – XVII of this report; this analytic dataset was created from raw NIRS data that were trimmed of all data with values ≥ 85% as described previously. Analytic datasets were also constructed using trim cutoffs of ≥ 90% and ≥ 95% in order to repeat the key aspects of the analysis using alternative cutoffs.

e. Statistical methods

With respect to the structure of this report, before capturing and analyzing intra- and inter-individual variability, descriptives (with graphics) and correlational statistics will be reported for the aggregated data (i.e., all data points for each patient aggregated into a single median value) and disaggregated data (i.e., all observations computed in the statistical reporting) so as to detail the distributional properties of the variables and familiarize the audience with the primary outcomes. Moreover, between group comparisons (i.e., ACS cases, injured unilateral, uninjured, etc.) on each of the compartments (i.e., anterior, lateral, etc.) will be conducted, for the test and contralateral leg.

The 12 compartments are as follows:

T_Ant_MED_Agg NIRS: Anterior (test leg) agg median
C_Ant_MED_Agg NIRS: Anterior (contralateral leg) agg median
T_Lat_MED_Agg NIRS: Lateral (test leg) agg median
C_Lat_MED_Agg NIRS: Lateral (contralateral leg) agg median
T_SP_MED_Agg NIRS: Superficial Posterior (test leg) agg median
C_SP_MED_Agg NIRS: Superficial Posterior (contralateral leg) agg median
T_DP_MED_Agg NIRS: Deep Posterior (test leg) agg median
C_DP_MED_Agg NIRS: Deep Posterior (contralateral leg) agg median
T_F_MED_Agg NIRS: Foot (test leg) agg median
C_F_MED_Agg NIRS: Foot (contralateral leg) agg median
Vol_MED_Agg NIRS: Volar agg median
Del_MED_Agg NIRS: Deltoid agg median

Thus, one-way ANOVA will be conducted for these set of analyses with level of significance set at $\alpha = .05$. Given the small sample sizes for some of the groups (e.g., ACS) even if significance for the omnibus *F* test is obtained, post hoc test such as the Tukey HSD (or the Games-Howell if homogeneity of variance was not met) will be deferred (though if requested, this can be performed subsequent to report review). The eta-squared (η^2) variance explained effect size will be reported, and though what constitutes small/medium/large is highly context dependent (Plonsky & Oswald, 2014), for this study 1%, 5.9%, and 13.8% will be judged as small/medium/large. Much of this analysis will address **Aim 4.**

A mixed linear modeling approach (often referred to by the umbrella term, multilevel modeling-MLM) will be performed for this project (Singer, & Willet, 2003; Raudenbush & Bryk, 2002) given the repeated measures/multiple waves of data collection for this design (**Aims 1 and 2)**. As well, this technique is appropriate for nested structures as per the design for the proposed study, where occasion (Level 1 = micro level) is nested within the individual (level 2 = macro level). The flexibility of a MLM approach is in evidence insofar there can be varying patterns of missing data as well as varying timing of the measures (which can be coded and incorporated as a time-varying parameter). And there is, indeed, much variation between patients with respect to the number of

NIRS measures, with the number of measures over time ranging from $t = 1$ to 73 (t = time).

Moreover, for MLM both time-varying (eq. 1 with one time varying covariate, that being time (a_{ti}) in this example)) and time-invariant covariates (eq. 2 for the random intercept (u_{0i}) and one time-invariant predictor (Z_i)) can be included.

$$y_{ti} = \pi_{0i} + \pi_{1i} a_{ti} + e_{ti} \quad \text{(eq 1).}$$

$$\pi_{0i} = \beta_{00} + \beta_{01} Z_i + u_{0i} \quad \text{(eq. 2)}$$

Additionally, if theoretically/clinically of interest, cross-level interactions (time x individual, i.e., level 1 x level 2) can be tested. As well, examining the random (stochastic) parameters (level one residual: e_{ti} and level 2 variance components for the intercept and/or slope (u_{0i} and/or u_{1i}) can help identify interindividual differences in intraindividual change (Nesselroade, 1991; Rovine & Lo, 2012). For normally distributed, quantitative outcomes the restricted maximum likelihood (REML) estimator will be used in this study.

A sequence of model testing will be conducted with the unconditional intraclass correlation coefficient (ICC) computed for the null (i.e., no predictor) model which will capture inter-individual variability, i.e., variance explained between the individual intercepts (eq. 3).

$$u_{oj}/(u_{oj} + r_{ij}) \quad \text{(eq. 3)}$$

where u_{oj} is the variance component for the intercepts, and r_{ij} is the level one residual. The null model will be labeled as M1 with two, increasingly more complex models to be tested, as follows:

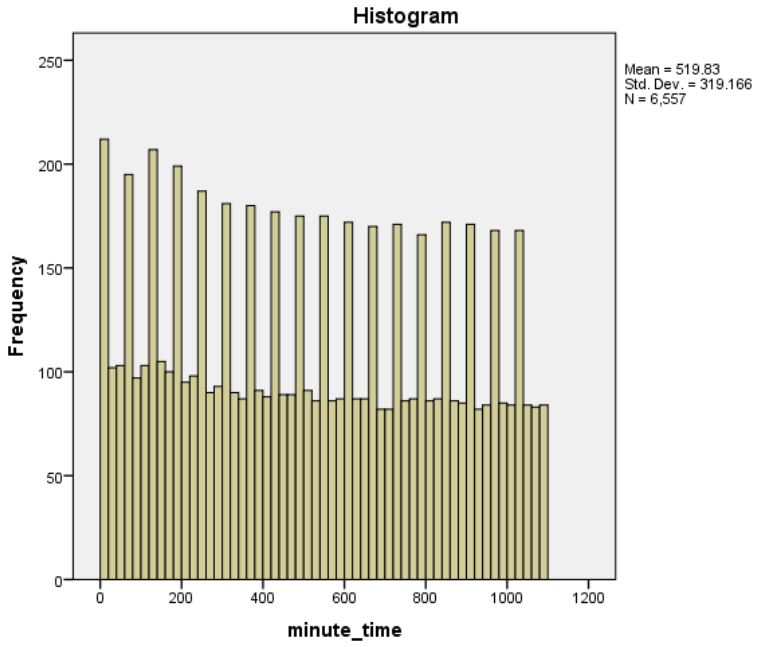
M1: null model
M2: growth model adding time as a time-varying predictor
M3: adding time varying (contralateral measure) and time-invariant (injury type group, age, race, and sex) variables (time still included as time-varying covariate).

Time is captured in minutes ranging from 1 to 1080.

Statistics

minute_time

N	Valid	6557
	Missing	0
Mean		519.83
Std. Error of Mean		3.942
Median		510.00
Mode		5
Std. Deviation		319.166
Variance		101866.809
Skewness		.076
Std. Error of Skewness		.030
Kurtosis		-1.221
Std. Error of Kurtosis		.060
Range		1079
Minimum		1
Maximum		1080
Sum		3408526
Percentiles	25	240.00
	50	510.00
	75	795.00



Note that for the 4-category grouping variable, the injured unilateral group (n = 79) will serve as the reference group when testing the model in HLM. Thus, three dummy coded vectors will be created to capture between-group variability (Cohen, Cohen, West, & Aiken, 2003). Hence, the partial regression coefficients for the dummy coded vectors will capture the mean difference between the coded group (e.g., ACS) and the reference group (uninjured unilateral). In SPSS, when testing the same model pairwise

comparisons between the groups using the Sidak Multiple comparison procedure (Toothaker, 1991) will also be obtained and reported in the narrative.

Injtype_4cat

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	1.00 ACS Bilat/Unilat	7	6.1	6.1	6.1
	2.00 Injured Bilateral	5	4.4	4.4	10.5
	3.00 Injured Unilateral	79	69.3	69.3	79.8
	4.00 Uninjured	23	20.2	20.2	100.0
	Total	114	100.0	100.0	

Also, for the level 2 explanatory variables, race was dichotomized as follows (54.4%, n = 62 are Black).

RACE Race

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	AMER/ALAS	1	.9	.9	.9
	ASIAN	1	.9	.9	1.8
	BLACK	62	54.4	54.4	56.1
	NATI/PACI	1	.9	.9	57.0
	WHITE	49	43.0	43.0	100.0
	Total	114	100.0	100.0	

RaceDich

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	.00 Not Black	52	45.6	45.6	45.6
	1.00 Black	62	54.4	54.4	100.0
	Total	114	100.0	100.0	

For sex, 77.2% (n = 88) are males.

sex_rec

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	.00 F	26	22.8	22.8	22.8
	1.00 M	88	77.2	77.2	100.0
	Total	114	100.0	100.0	

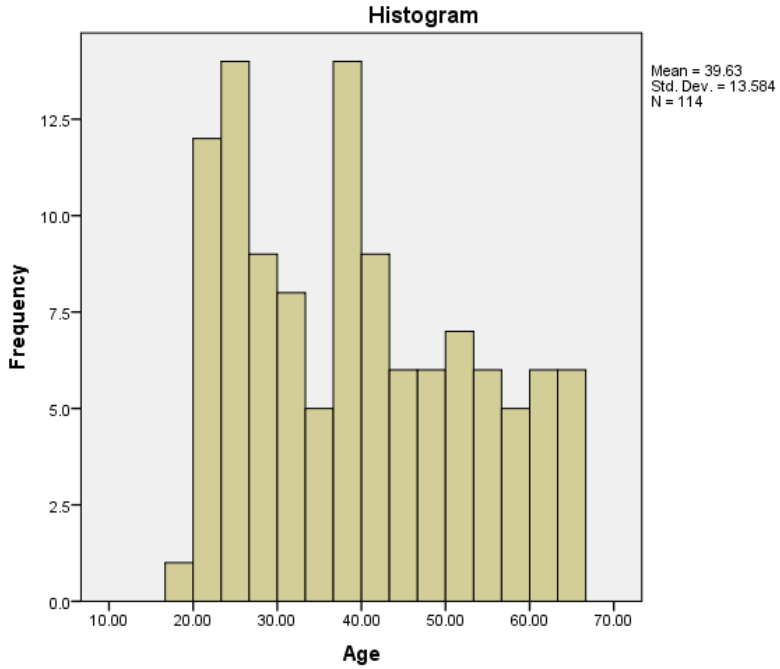
And for age the mean = 39.63 (*SD* = 13.58)

Statistics

AGE Age

N	Valid	114
	Missing	0
Mean		39.6275
Std. Error of Mean		1.27229
Median		38.6950
Mode		39.87[a]
Std. Deviation		13.58431
Variance		184.533
Skewness		.318
Std. Error of Skewness		.226
Kurtosis		-1.000
Std. Error of Kurtosis		.449
Range		47.54
Minimum		18.40
Maximum		65.94
Sum		4517.54
Percentiles	25	26.9725
	50	38.6950
	75	50.5050

a. Multiple modes exist. The smallest value is shown



For each successive model (M1 to M3), model selection will be demonstrated via change in the variance components, significance of micro- and/or macro-level explanatory variables, and information –theoretic indices (e.g., AIC or BIC, as well as the deviance

statistic: -2LL) of which the lower value (between competing models) is indicative of a better-fitting model (Burnham & Anderson, 1998). For all data analysis, data cleaning will be conducted including examination of outliers, non-normality, and homoscedasticity/heterogeneous variances, and missing data (Enders; Graham, 2009). All analysis will be conducted using SPSS 22.0, HLM 7.01, and/or Mplus 7.31.

f. References

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II. Qualitative Analysis of Compartment Syndrome Cases (Cohort 3)

Seven (7) patients in the study had outcomes of compartment syndrome evidenced by decision for fasciotomy. This resultant sample size was short of the intended sample size for cohort 3 (n=25). Unfortunately, this precluded the quantitative objectives related to detection of ACS (Aim 3 of the analytic objectives). 'Case-series' style qualitative analyses are provided in this section, which comprises the extent of informative analyses that were possible of those patients. Analyses presented in this section directly address Aims 2 and 5 of the analytic objectives. Cases with ACS were included in all longitudinal analyses and multilevel models for completeness, though estimates for those groups were unstable with wide confidence intervals due to the inadequate sample size.

Data collected around the time of fasciotomy from the 7 patients with ACS are shown on Tables 1-5 in this section as recorded on the case report forms. Data collection was incomplete for many patients. Intracompartment pressures (ICP) were obtained for 6 of the 7 Cohort 3 patients. Five of those had ICP recorded for all 4 leg compartments and 1 had values for 2 compartments. One patient with fasciotomy (#3003) had no ICP measurement (this was confirmed by the Project Manager).

Qualitative analyses are provided in the form of graphical representation on a case-by-case basis. All recorded data are presented in this qualitative analysis, including all NIRS values recorded between 0 and 100% inclusively ('trimming' of values above x% cutoff was not applied to this section of the report).

a. Summary of Field Data

DATA FROM CRFs / FDS

*Note: These were the data exactly as recorded on the CRF forms. NIRS values were not imputed with any data from the monitor.

1. Anesthetic Induction data (F1, F2, F3)

	Pre-induction				Induction				Post-Induction			
Subject	t1	Syst	Diast	MAP*	t2	Syst	Diast	MAP	t3	Syst	Diast	MAP
3002	11:19 AM	160	84	109	11:21 AM	146	84	109	11:43 AM	123	77	105
3003	12:51 AM	148	95	113	12:52 AM	108	80	103	12:59 AM	147	104	119
3101	6:22 AM	151	65	94	6:27 AM	128	62	92	6:37 AM	152	70	97
3102	5:55 PM	132	82	99	5:59 PM	132	82	99		132	82	99
3201	8:37 PM	205	97	133	8:40 PM	188	93	130	8:52 PM	119	67	113
3202	7:41 PM	153	77	102	7:45 PM	82	45	81	7:52 PM	135	71	98
3203	9:40 AM	128	89	102	9:45 AM	81	58	81	9:51 AM	100	60	83

* Mean arterial pressures (MAP) were calculated because it often wasn't recorded

2. Pre-release ICP measurement (F5)

	Time	BP			ICP				Perfusion Pressure, calculated (DBP - ICP)				NIRS			
Subject	t5	Syst	Diast	MAP	ICP Ant	ICP Lat	ICP DP	ICP SP	PP Ant	PP Lat	PP DP	PP SP	NIRS Ant	NIRS Lat	NIRS DP	NIRS SP
3002	12:05 PM	91	45	60	62	74	65	63	-17	-29	-20	-18	54	50	50	0
3003*																
3101**	7:14 AM	113	48	70	44		23		4		25		69	80	83	69
3102	6:09 PM	132	82	99	38	40	52	43	44	42	30	39				
3201***		162	82	109	46	90	90	70	36	-8	-8	12			66	66
3202	8:14 PM	104	78	87	101	73	68	75	-23	5	10	3	100	84	57	
3203	10:09 AM	104	63	77	27	47	45	44	36	16	18	19				34

*No ICP measurements were obtained for this patient

**ICP for this patient were measured 5 hours before release

***Time of measurement unclear

3. Incisions (F7)

	Incision 1						Incision 2					
Subject	Inc1 Time	Inc1 Side	NIRS Ant	NIRS Lat	NIRS DP	NIRS SP	Inc2 Time	Inc2 Side	NIRS Ant	NIRS Lat	NIRS DP	NIRS SP
3002		LAT						MED				
3003												
3101	7:47 AM	LAT	98	61	59	32	7:47 AM	MED	98	61	59	32
3102	6:47 PM	LAT	57	58			7:09 PM	MED			0	0
3201	9:20 PM	LAT		71			9:35 PM	MED			60	67
3202	8:24 PM	MED			69	62	8:28 PM	LAT	63	59		
3203	10:19 AM	LAT				73	(*single incision procedure)					

*No data recorded

*No data were collected for this patient

4. Stabilization (F8)

	Operative Leg						Contralateral Leg							
Subject	Time	Sensors Attached?	NIRS Ant	NIRS Lat	NIRS DP	NIRS SP	NIRS Ant	NIRS Lat	NIRS DP	NIRS SP	NIRS LF	NIRS RF	NIRS Vol	NIRS Del
3002	12:41 PM	N	0	85	0	0								
3003		Y	0	0	0	47								
3101		Y												
3102		Y												
3201		Y												
3202		Y												
3203		Y												

*This is what was recorded on the forms. (Unclear why zeros)

5. Stryker Measurements Logged (recorded either on the Fasciotomy details form, or on the Stryker log*)

					BP				ICP				Perfusion Pressure, calculated (DPB - ICP)				NIRS (spot check recorded)			
Form	Subject	date	time	Sequence	Syst	Diast	MAP	MAP (calc)	ICP Ant	ICP Lat	ICP DP	ICP SP	PP Ant	PP Lat	PP DP	PP SP	NIRS Ant	NIRS Lat	NIRS DP	NIRS SP
FA	3002	01/23/13	12:05:00 PM	1	91	45		60	62	74	65	63	-17	-29	-20	-18	54	50	50	0
FA	3003			0																
SM	3101**	02/10/13	2:32:00 AM	1					44		23									
FA	3101	02/10/13	7:14:20 AM	-	113	48		70									69	80	83	69
FA	3102	06/29/13	6:09:04 PM	1	132	82		99	38	40	52	43	44	42	30	39				
FA	3201***	**	**	(?)	162	82		109	46	90	90	70	36	-8	-8	12			66	66
SM	3201	11/13/12	7:45:00 PM	(?)	162	82		109	46	90	90	70	36	-8	-8	12				
FA	3202	04/16/13	8:14:00 PM	1	104	78		87	101	73	68	75	-23	5	10	3	100	84	57	
SM	3203****	08/01/13	9:36:00 AM***	1	109	76		87	43	32	41	35	33	44	35	41				
FA	3203	08/02/13	10:09:00 AM	2	104	63		77	27	47	45	44	36	16	18	19				34

*They were often (but not always) recorded in both places. Clear duplicates were omitted.

**This patient apparently had 2 compartments measured about 5 hours before surgery

*** There is some unresolved confusion about times for this pt as these seem to be duplicated entries, and the time listed was about an hour before surgery. The missing times were on the fasciotomy form. There was not an electronic event mark.

****This patient was on monitor for about 13 hours and had an ICP measurement done on the previous date, but this time was before the time of admission. So, unclear, but could have been 9:30pm which was about the time enrolled.

b. Qualitative analyses

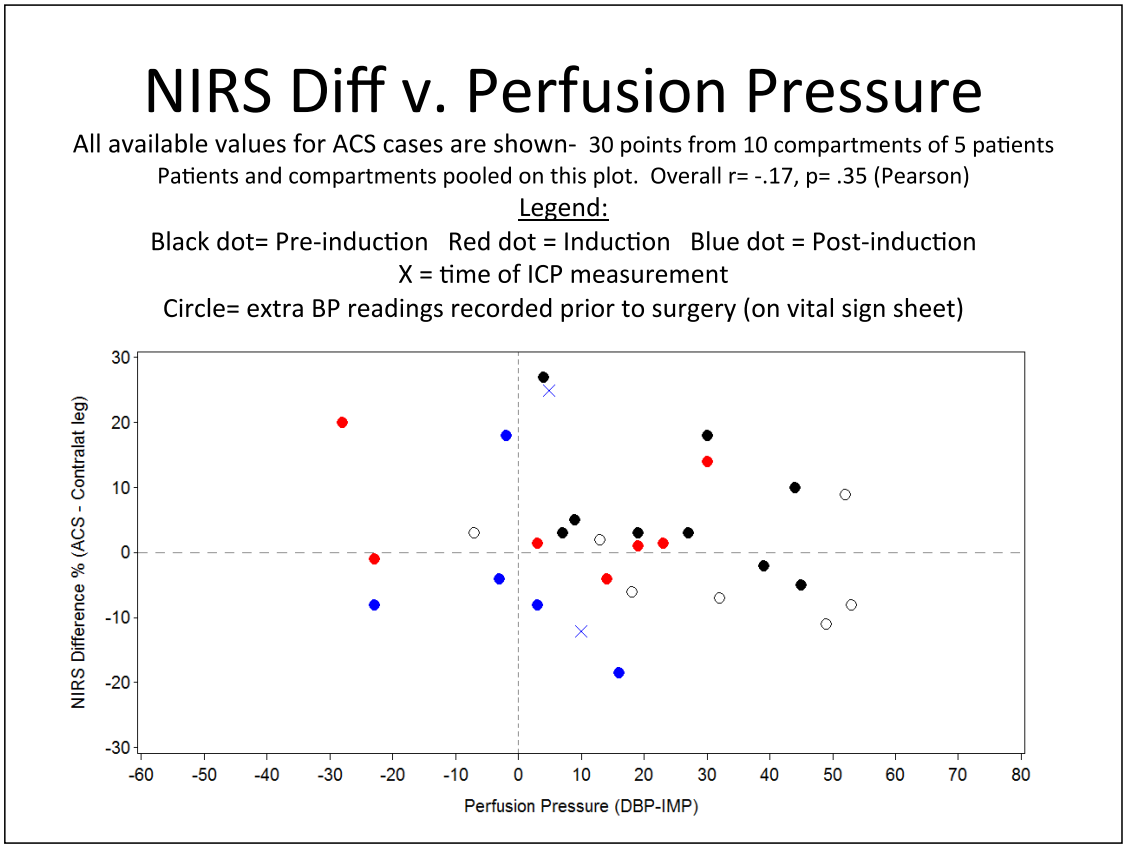
NIRS vs. PP in ACS limbs

- These slides show all available data points where NIRS of both legs, BP, and an ICP measurement reasonably close in time could be related to get a value for perfusion pressure and NIRS difference.
- Methods:
  1. All recorded BP measurements were obtained.
  2. Monitor data were sampled at each BP measurement for all compartments of both legs. Median value over the 1 minute just prior to the BP time was used (sample starts at time – 1 minute). All samples were reviewed by one by one against graphics to exclude or correct bad samples (artifact, etc.)
  3. All recorded spot check data were obtained. Values of 0%, >95%, and one value of 34% were excluded as implausible.
  4. NIRS difference was calculated for all times for which there were valid non-missing data for both legs, using monitor data first. Spot check values for the ACS leg, if available, were substituted for missing monitor data (this was done for 2 observations).

NIRS vs. PP in ACS limbs

- Methods (continued):
  5. IMP data were obtained and assigned to each observation. IMP was usually measured once, and the value was used for all times. There were two exceptions decided:
     1. 3101- These IMP data were originally thought missing, but I found values obtained for two compartments at time of enrollment after which the pt. was observed for ~ 5 hours before releasing. Measurements were not done at the time of surgery. I did not carry these values forward to normalize the surgery BPs due to the amount of time elapsed and the assumption that the pt's condition must have changed to inspire a surgery decision 5 hours later.
     2. 3203- This pt had IMP measurements at enrollment time, then was observed about 12 hours before having a release. IMP were repeated in surgery. I normalized data earlier than 3 hours pre-release to the first IMPs, and data from within 3 hours pre-release and forward to the pre-release values (this is shown on the graphic).
  6. Perfusion pressure was calculated for times that had values for both BP and ICP. (Diastolic BP – IMP)
  7. 30 viable values were found from 10 compartments of 5 patients
  8. Scatterplot was created. Pearson correlation was not significant overall. r= -.17, p=.37. If you want them subsetted differently let me know.
  9. All compartments with any viable data are shown graphically with complete annotations. This is an exhaustive qualitative analysis.

Figure Iia. Correlation of NIRS difference between leg compartments and perfusion pressure

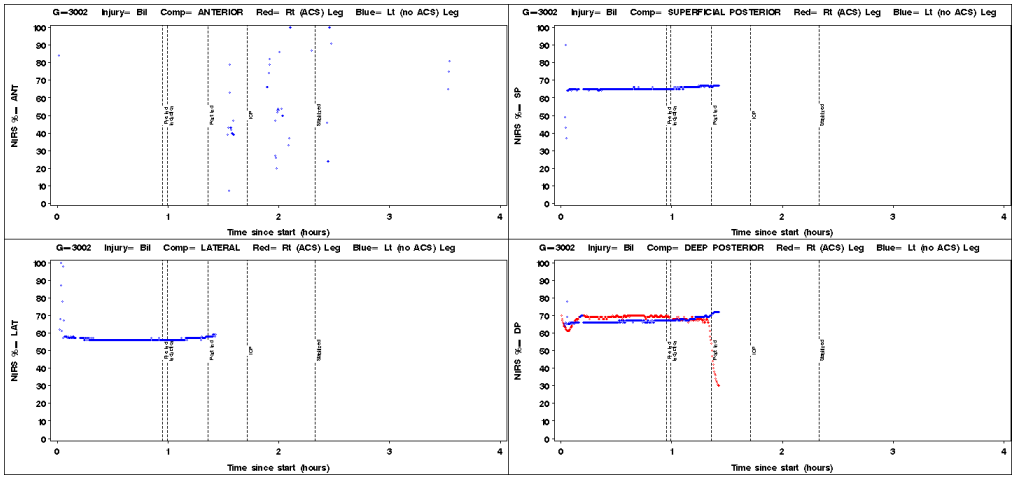


Pearson correlation coefficient for this scatterplot (all data combined) was r= -.17, p=.37.

Figures IIb-x Case-by-case graphical representations of clinical events surrounding fasciotomy

SUBJECT # 3002

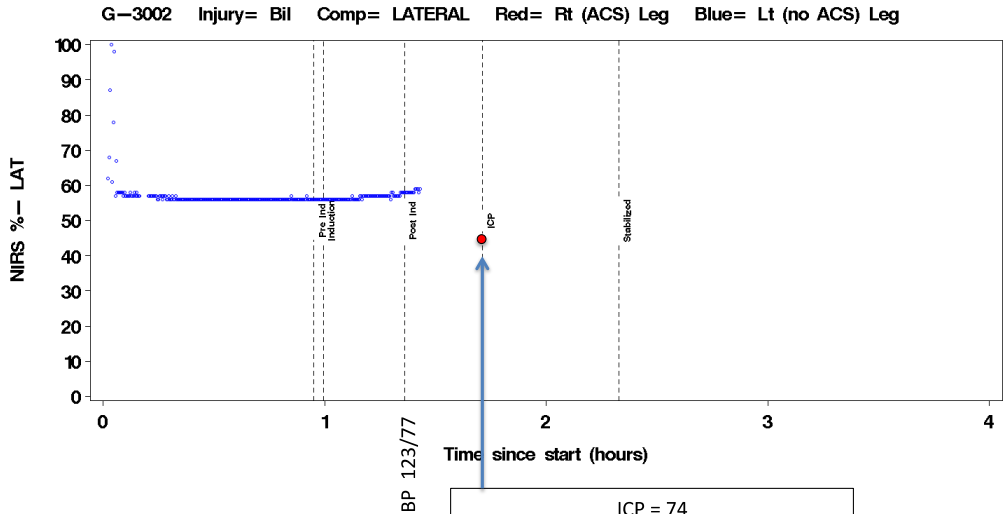
3002%



Note:%This%aBent%ad%ilateral%ractures%with%ACS%n%ne%ide.%%
There%s%o%iable%ontralateral%eg%data%n%he%anterior%ompartment%
Had%NIRS%pot%heck%data%t%he%Bme%f%CP%or%A,%,%and%DP%nly%(“0”%ero%was%ecorded%or%he%SP)%ut%ad%o%ontrol%mb%alues%t%hat%Bme.%%
%

3002 Lateral

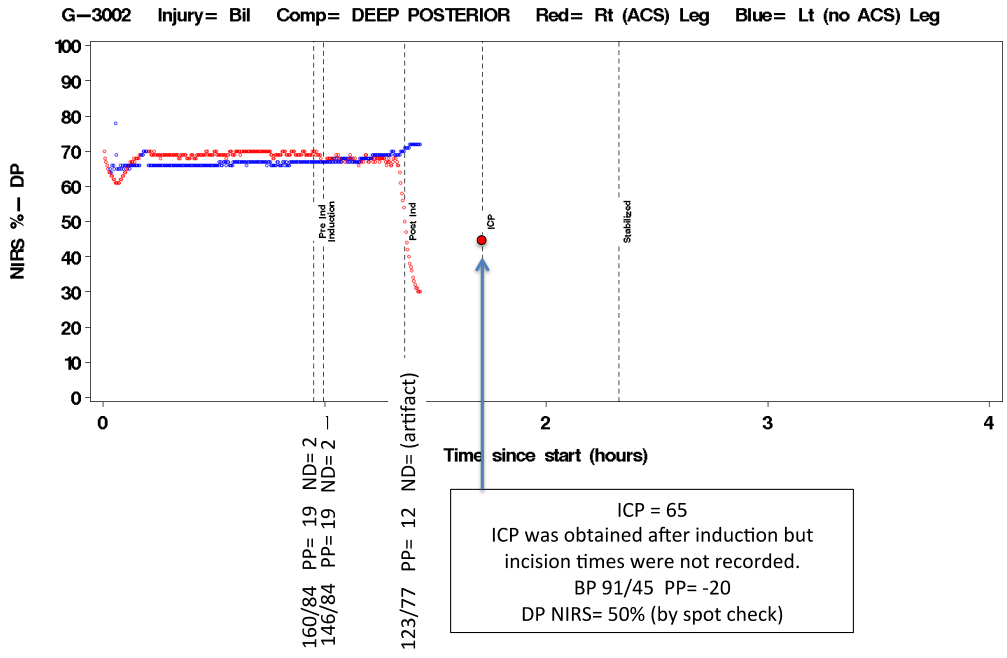
(Note: This patient had bilateral fractures)



ICP = 74
Incision times were not recorded.
BP 91/45 PP -29
Lat NIRS= 50% (by spot check)

3002 Deep Post

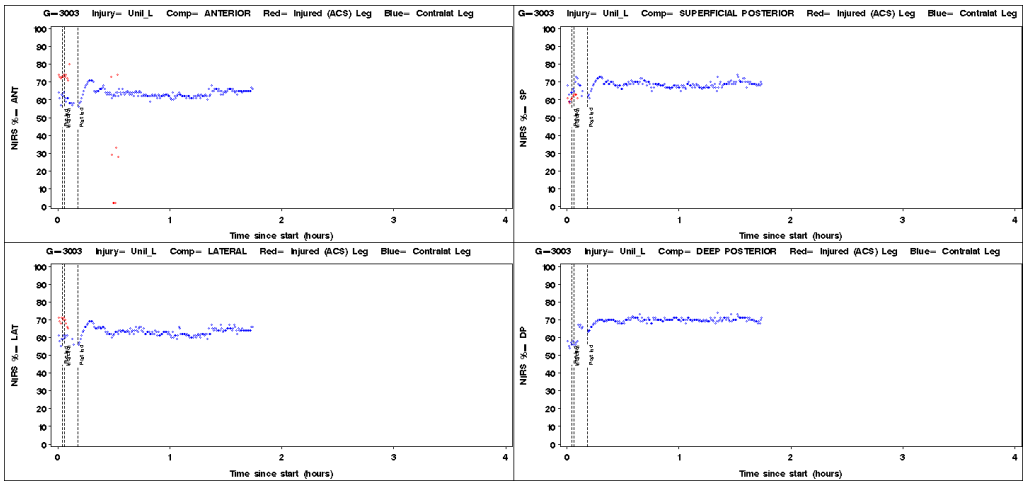
(Note: This patient had bilateral fractures)



ICP = 65
ICP was obtained after induction but incision times were not recorded.
BP 91/45 PP= -20
DP NIRS= 50% (by spot check)

SUBJECT # 3003

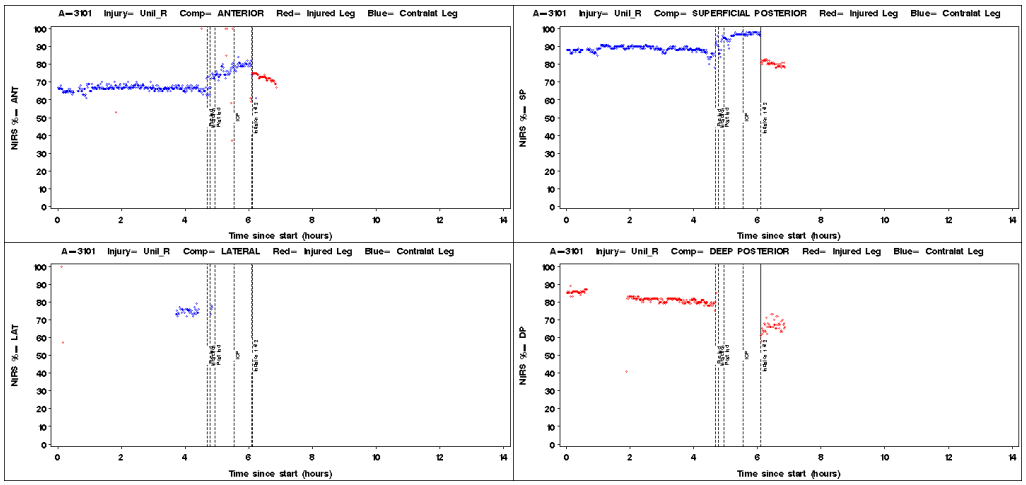
3003%



This%aBent%as%o%CP%measurements%ecorded%nd%most%data%ollecBon%uring%urgery%were%omi` ed.%There%was%about%%minutes%NIRS%data%apture%f%he%ACS%mb.%

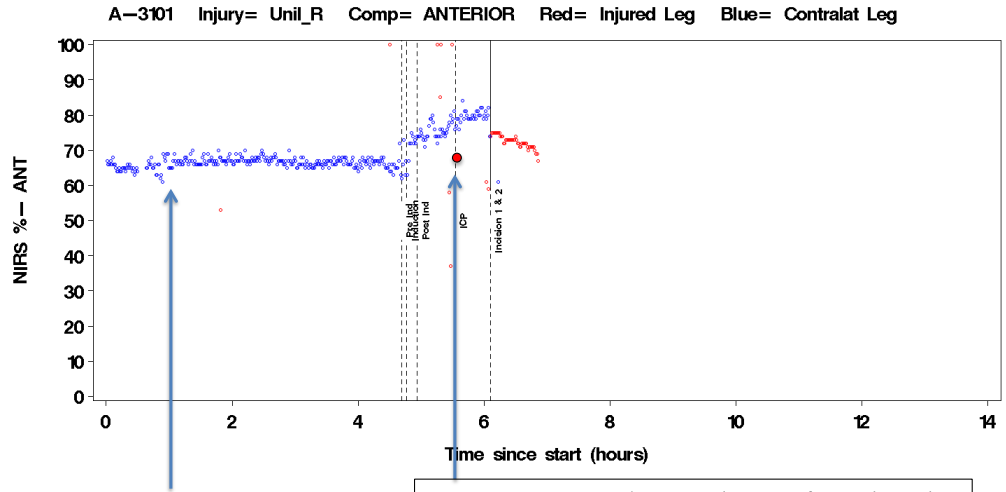
SUBJECT # 3101

3101



This patient has little viable data. ICP measurements were done about 5 hours pre-release in A and DP compartments only. IMPs were not done at time of release according to the records. There are no tracings of the ACS limb during induction and no simultaneous tracings of both limbs anywhere. There was one spot check with a BP in the Anterior comp which could be correlated to its control limb (next slide).

#3101 Anterior

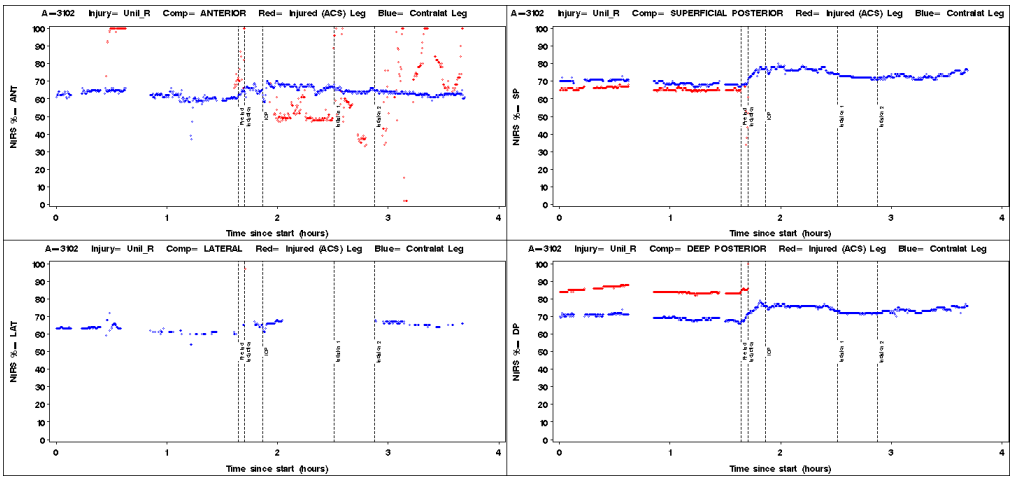


ICP = 44
ICP obtained ~ 5 hours prior to release in Anterior and DP compartments only

ICP was NOT measured again at the time of pre-release, but a NIRS spot check and BP were recorded.
BP 113/48 NIRS 69 NIRS diff = -8
PP= 4 calculated using earlier ICP, but it seems likely ICP could be higher by this time. Therefore I did not include this one as a viable PP.

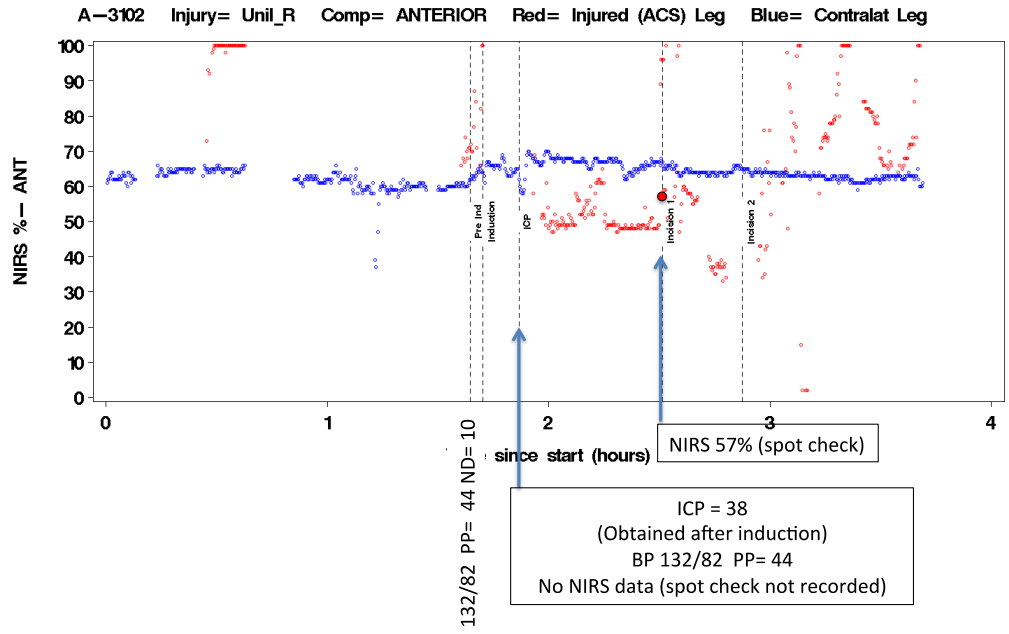
SUBJECT # 3102

3102

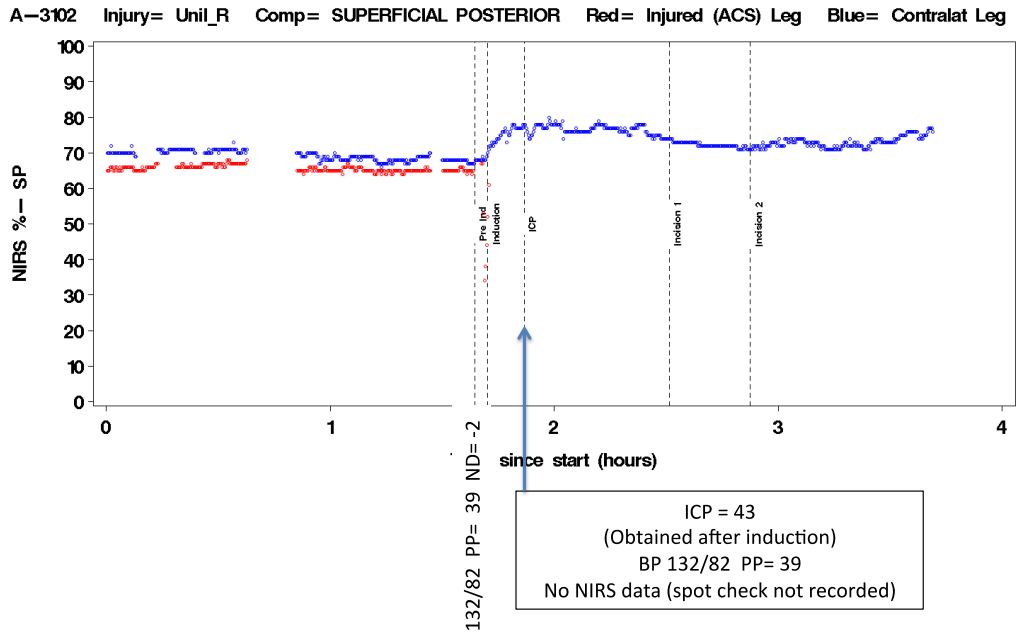


This patient had no post-induction time recorded (either on the form or by event mark) and had no spot check data at time of ICP. Had spot check of A & L comp only at incision 1 time. It is also noted the exact same BP (132/82) was recorded for every surgery time point: pre-induction, induction, post-induction, and pre-release ICP (?)

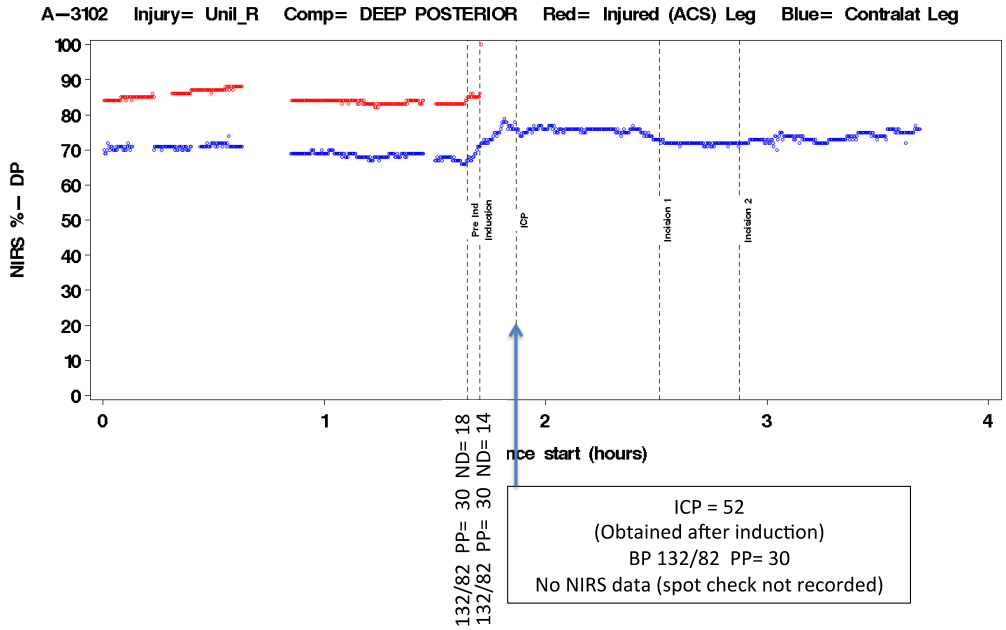
#3102 Anterior



#3102 Superficial Post

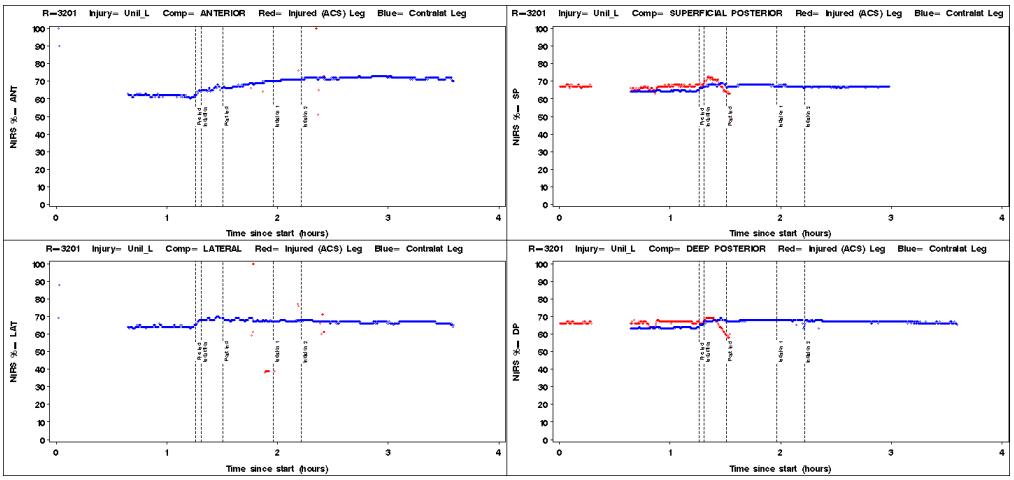


#3102 Deep Post



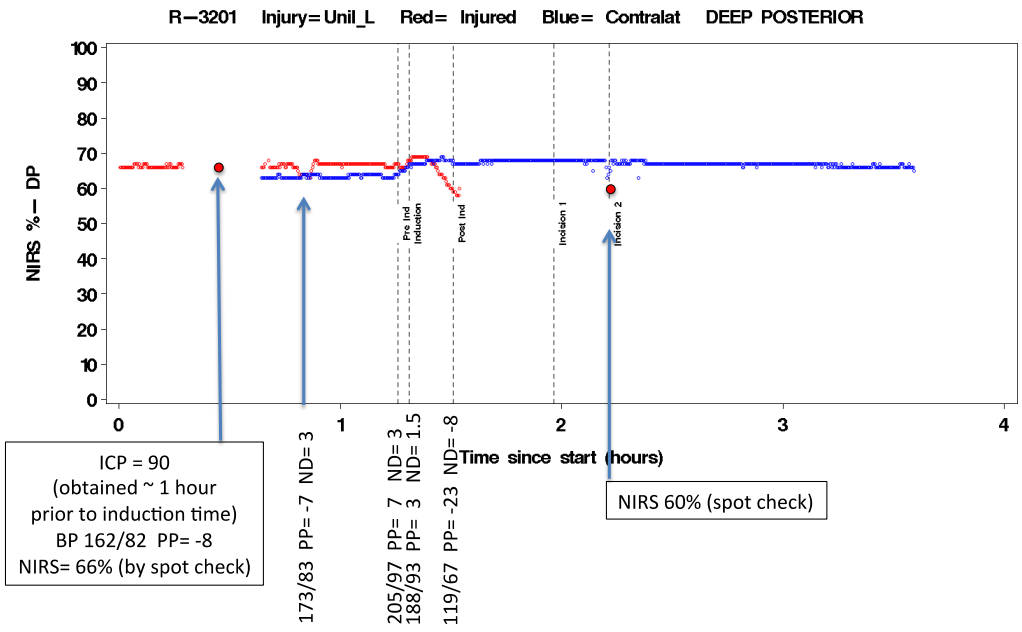
SUBJECT # 3201

3201

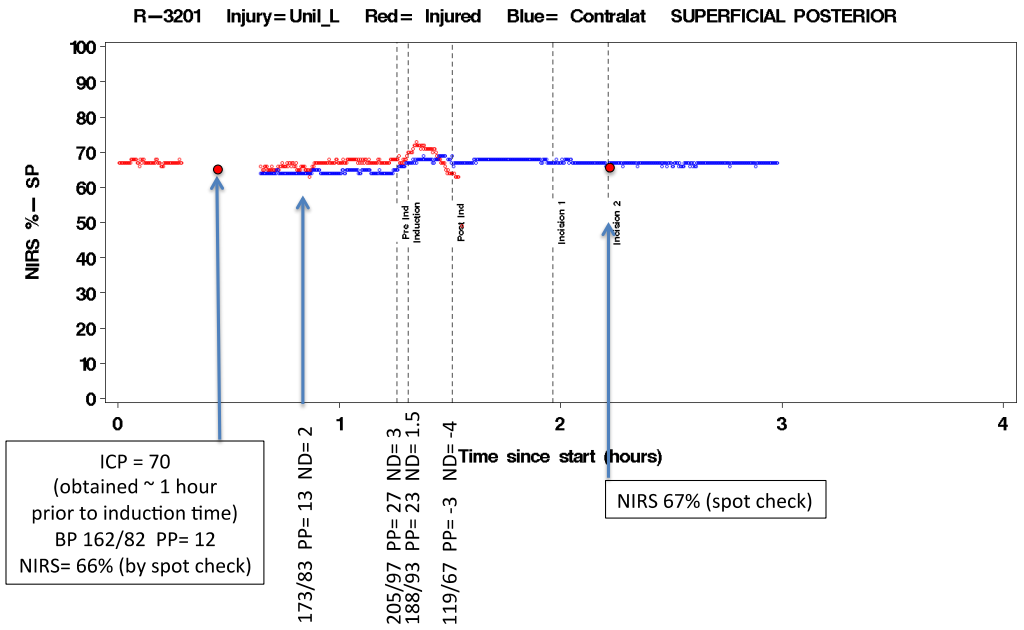


This patient had great views of the SP and DP compartments with good recording of corroborating data (BPs and spot checks). ICP measurement was obtained about an hour before surgery according to the record.

#3201 Deep Posterior

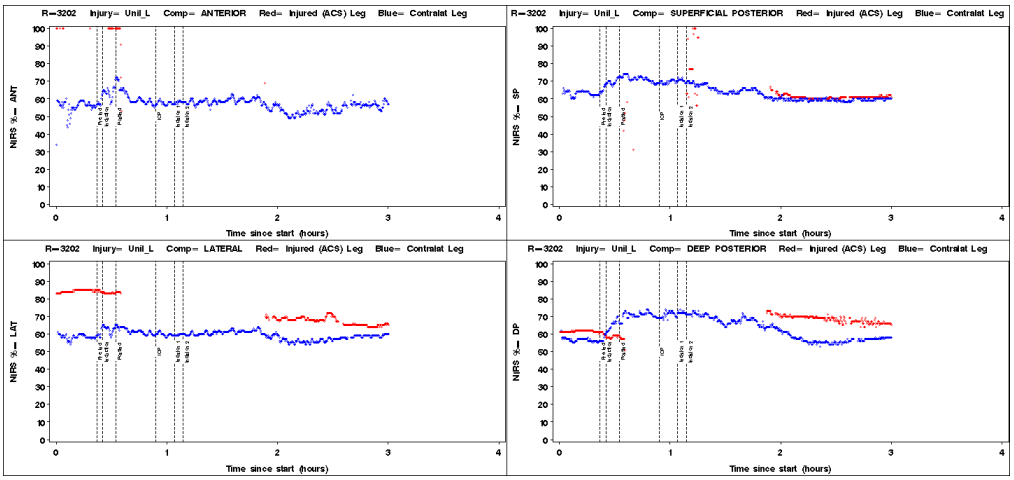


#3201 Superficial Post



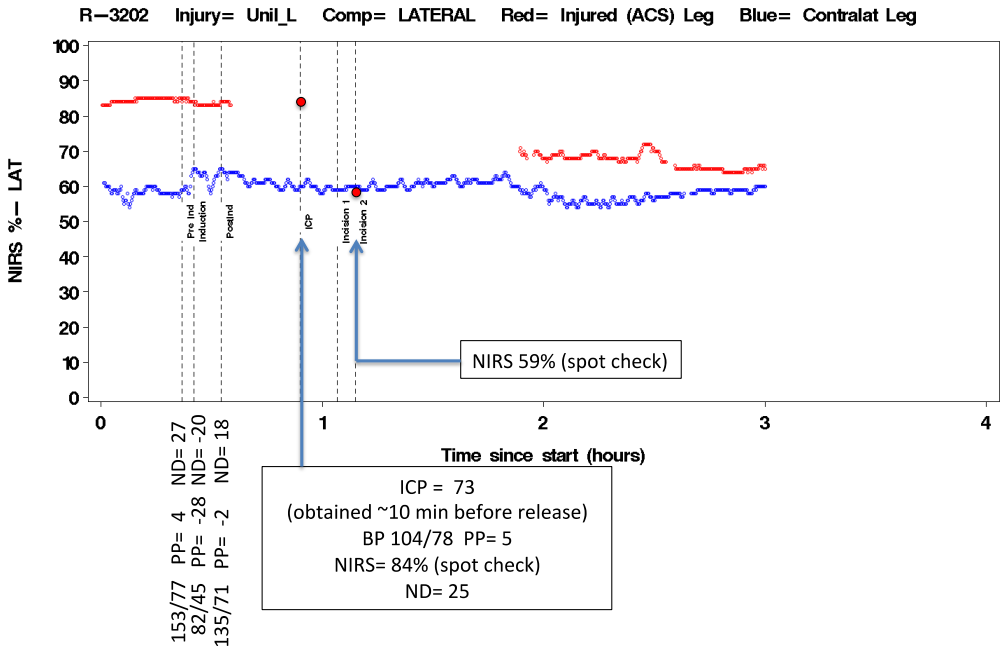
SUBJECT # 3202

3202%



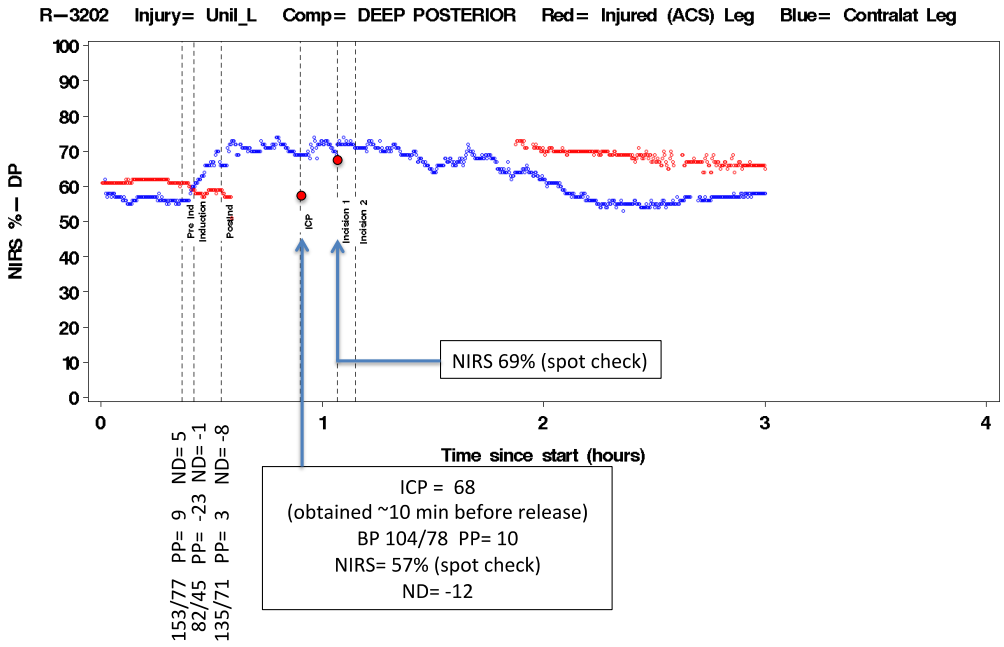
Good%racings%were%obtained%or%his%aBent%in%%nd%DP%compartments%with%ery%omplete%recording%f%orroboraBng%data.%%

#3202 Lateral



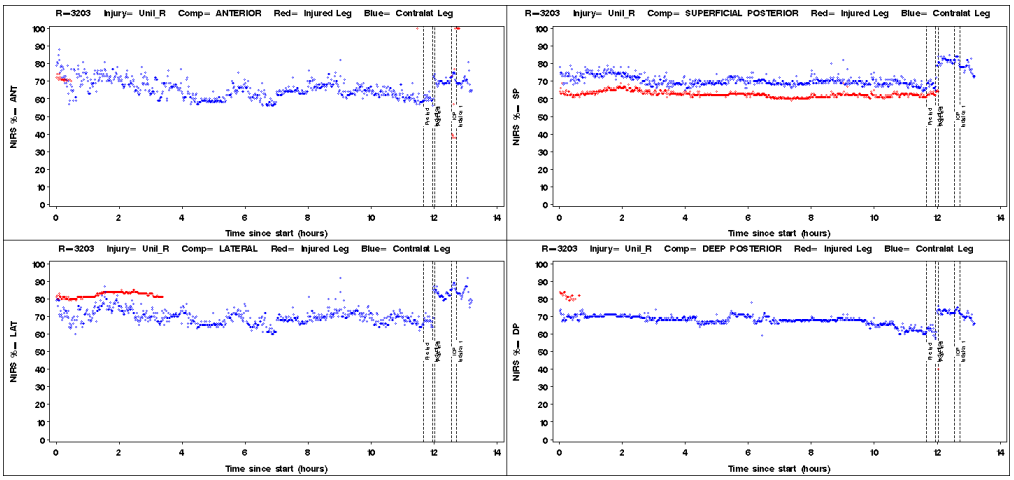
#3202 Deep Posterior

ICP= 68



SUBJECT #3203

3203

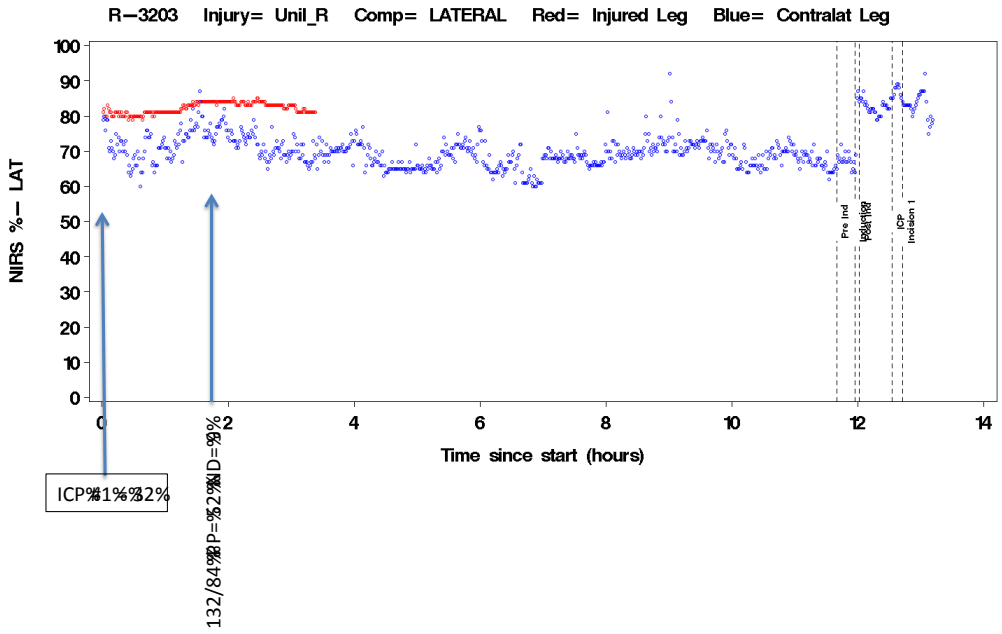


This patient was on monitor for about 13 hours. IMP was done around time of enrollment and again pre-release during surgery. A number of extra BPs were recorded.

*Note: The first IMP values were used to normalize data for the first ~8 hours, and the pre-release IMP values were used to normalize data from ~ 3 hours before surgery and forward

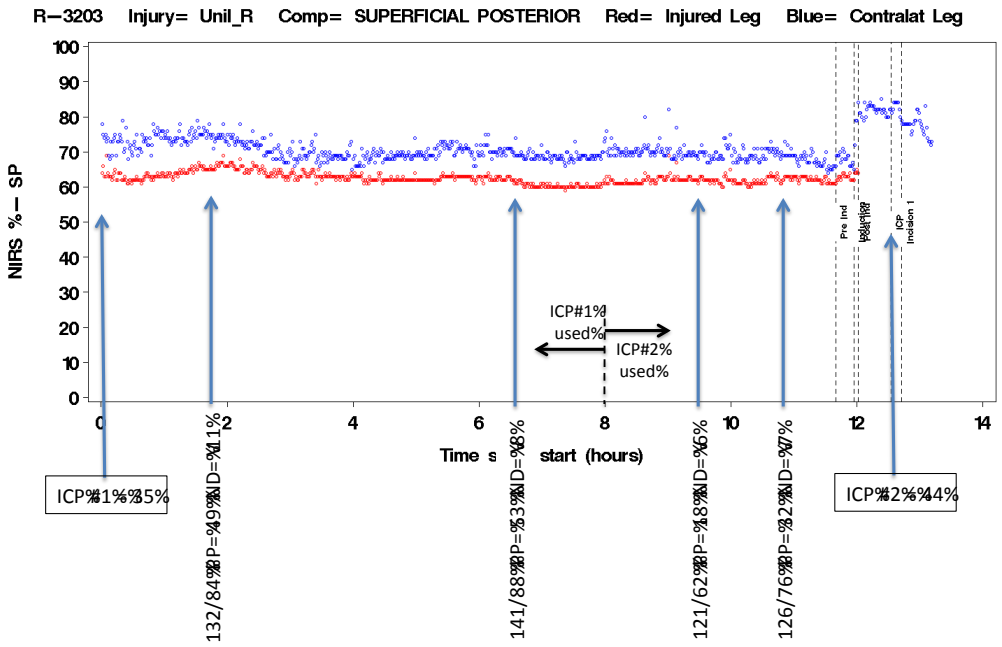
No viable spot check data were obtained. One value was recorded of 34% for the SP, which was excluded as erroneous (value seems implausible and data entry sheet was messy)

#3203%ateral%



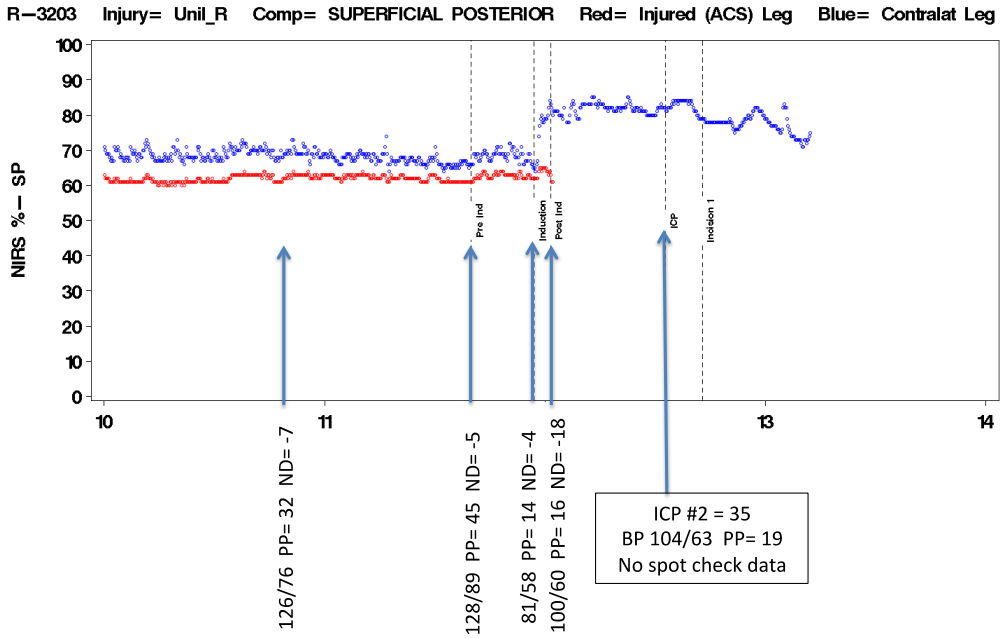
#3203 Superficial Post

EnBre monitor me, with prev op data



#3203 Superficial Post

Surgery events (This is a closer view of the later portion)



III. Key Results of Quantitative Analyses Using 3 Trimming Thresholds

a. Methodology

This section presents the key results of the quantitative analysis in an 'Executive Summary' format. In the spirit of sensitivity analysis, and in an effort to locate an optimal (upper value) NIRS trim point, the analysis was repeated using: (1) 85%, (2) 90%, and (3) 95% NIRS cutoffs (See Section I, Methods). 'NIRS cutoff' herein refers to defining a value threshold above which all values were set to missing (aka, 'trimming'). This approach was recommended by the investigators due to the belief that many if not most NIRS values that were recorded in the highest ranges (above ~90%) were biologically unlikely and much more likely to represent limitations of measurement.

In consultation with the Investigators, it was determined that the key clinically relevant aspects of the analysis include compartment correlations, comparison of adjusted group means (unilateral injured vs. uninjured) from the multilevel modeling (MLM) analyses, and the parameter estimates for the full (and final) model. The methodology and analytical (statistical) strategy is described above in Section 1 (Methods) of this report, and the implementation of the analysis is described in Sections IV-XIX of the full two-part analysis report titled "*Near-Infrared Spectroscopy to Reduce the Prophylactic Fasciotomies for and Missed Cases of Acute Compartment Syndrome in Soldiers Injured in OEF/OIF": Statistical and Summary Report, Volume 2 of 2*".

b. Executive Summary of Results (85% NIRS cutoff)

1) Correlations for each of the compartments (anterior, lateral, etc.) were computed with the uninjured/contralateral leg anterior, lateral, superficial posterior (SP), volar, and deltoid (in part to discern potential control compartments). Results of correlations computed separately by injury type are shown. Given small sample sizes for ACS and Bilateral injured, the following summary is for only the unilateral injured and injured group. Moreover, analysis was done for **aggregated data** (computing correlations based on composite of all median times for each case; in other words, each patient receives one single summary value for each compartment, which is the mean of all median values collected) and **disaggregated data** (computing correlations using all median values of all patients, hence ignoring non-independence (e.g., repeated measurements of the same patient), though the nonindependence is explicitly modeled in the multilevel modeling). It is important to understand that aggregated data assigns a single summary value to the patient for each compartment which represents the 'average' experience of all captured data from the patient for that compartment; the derivation of the aggregate values for two separate compartments being correlated may include values for each that were not measured at the same time in the other. Disaggregated data includes all median values at all times collected, therefore correlations using disaggregated data are restricted only to observations where both compartments were measured at the same time.

Table 1. NIRS Compartment Correlations for Unilateral Injury Group (aggregated and disaggregated)- 85% cutoff.

Unilateral Injury Group--85% cutoff

Aggregated (one summary value per patient*)

			Injured Leg				Uninjured Contralateral Leg				Feet		Arm	
			Anterior	Lateral	SP	DP	Anterior	Lateral	SP	DP	Injured Leg	Contral Leg	Volar	Deltoid
			T_Ant_MED_Agg	T_Lat_MED_Agg	T_SP_MED_Agg	T_DP_MED_Agg	C_Ant_MED_Agg	C_Lat_MED_Agg	C_SP_MED_Agg	C_DP_MED_Agg	T_F_MED_Agg	C_F_MED_Agg	Vol_MED_Agg	Del_MED_Agg
Uninjured Leg Anterior	C_Ant_MED_Agg	r	0.447	0.454	0.348	0.258	1.000	0.781	0.456	0.476	0.264	0.390	0.479	0.552
Uninjured Leg Lateral	C_Lat_MEDIAN	r	0.529	0.454	0.525	0.224	0.781	1.000	0.592	0.639	0.367	0.474	0.579	0.612
Uninjured Leg SP	C_SP_MEDIAN	r	0.214	0.362	0.657	0.669	0.456	0.592	1.000	0.748	0.479	0.542	0.458	0.546
Volar	Vol_MED_Agg	r	0.193	0.390	0.294	0.177	0.479	0.579	0.458	0.496	0.426	0.348	1.000	0.548
Deltoid	Del_MED_Agg	r	0.493	0.529	0.516	0.266	0.552	0.612	0.546	0.634	0.399	0.486	0.548	1.000

* Aggregated value for each patient is the mean of all available 2-minute median values collected every 15 minutes

Disaggregated (multiple values (q 15 min) included for each patient**)

			Injured Leg				Uninjured Contralateral Leg				Feet		Arm	
			Anterior	Lateral	SP	DP	Anterior	Lateral	SP	DP	Injured Leg	Contral Leg	Volar	Deltoid
			T_Ant_MED_DisAg	_Lat_MED_DisAg	T_SP_MED_DisAgg	T_DP_MED_DisAgg	C_Ant_MED_DisAg	C_Lat_MED_DisAgg	C_SP_MED_DisAgg	_DP_MED_DisAgg	T_F_MED_DisAgg	F_MED_DisAgg	Vol_MED_DisAgg	Del_MED_DisAgg
Uninjured Leg Anterior	C_Ant_MED_Agg	r	0.482	0.445	0.328	0.204	1.000	0.726	0.456	0.405	0.271	0.358	0.375	0.474
Uninjured Leg Lateral	C_Lat_MEDIAN	r	0.490	0.514	0.426	0.218	0.726	1.000	0.527	0.534	0.319	0.402	0.489	0.515
Uninjured Leg SP	C_SP_MEDIAN	r	0.259	0.418	0.509	0.465	0.456	0.527	1.000	0.655	0.419	0.449	0.371	0.449
Volar	Vol_MED_Agg	r	0.210	0.301	0.231	0.185	0.375	0.489	0.371	0.404	0.366	0.309	1.000	0.457
Deltoid	Del_MED_Agg	r	0.502	0.508	0.484	0.210	0.474	0.515	0.449	0.523	0.306	0.373	0.457	1.000

For the unilateral uninjured group, correlation between injured and contralateral leg for the Anterior, Lateral, and Superficial Posterior compartments were for the aggregate data, respectively, .447, .454, and .657 and for the disaggregated data: .482, .514, and .509.

Table 2. NIRS Compartment Correlations for Uninjured Group (aggregated and disaggregated)- 85% cutoff.

Uninjured Group--85% cutoff

Aggregated (one summary value per patient*)

			Test' Leg (randomly selected)				Contralateral Leg				Feet		Arm	
			Anterior	Lateral	SP	DP	Anterior	Lateral	SP	DP	Injured Leg	Contral Leg	Volar	Deltoid
			T_Ant_MED_Agg	T_Lat_MED_Agg	T_SP_MED_Agg	T_DP_MED_Agg	C_Ant_MED_Agg	C_Lat_MED_Agg	C_SP_MED_Agg	C_DP_MED_Agg	T_F_MED_Agg	C_F_MED_Agg	Vol_MED_Agg	Del_MED_Agg
Contral Leg Anterior	C_Ant_MED_Agg	r	0.834	0.752	0.567	0.462	1.000	0.757	0.608	0.378	0.503	0.354	0.137	0.665
Contral Leg Lateral	C_Lat_MEDIAN	r	0.772	0.815	0.787	0.624	0.757	1.000	0.756	0.529	0.293	0.385	0.045	0.607
Contral Leg SP	C_SP_MEDIAN	r	0.579	0.746	0.853	0.751	0.608	0.756	1.000	0.754	0.387	0.530	0.303	0.237
Volar	Vol_MED_Agg	r	0.304	0.317	0.289	0.210	0.137	0.045	0.303	0.256	0.644	0.643	1.000	0.206
Deltoid	Del_MED_Agg	r	0.590	0.458	0.266	0.194	0.665	0.607	0.237	0.327	0.442	0.460	0.206	1.000

* Aggregated value for each patient is the mean of all available 2-minute median values collected every 15 minutes

Disaggregated (multiple values (q 15 min) included for each patient**)

			Test' Leg (randomly selected)				Contralateral Leg				Feet		Arm	
			Anterior	Lateral	SP	DP	Anterior	Lateral	SP	DP	Injured Leg	Contral Leg	Volar	Deltoid
			T_Ant_MED_DisAg	_Lat_MED_DisAg	T_SP_MED_DisAgg	T_DP_MED_DisAgg	C_Ant_MED_DisAg	C_Lat_MED_DisAgg	C_SP_MED_DisAgg	_DP_MED_DisAgg	T_F_MED_DisAg	F_MED_DisAgg	Vol_MED_DisAgg	Del_MED_DisAgg
Contral Leg Anterior	C_Ant_MED_Agg	r	0.718	0.650	0.497	0.327	1.000	0.635	0.502	0.335	0.349	0.243	0.077	0.549
Contral Leg Lateral	C_Lat_MEDIAN	r	0.683	0.744	0.690	0.525	0.635	1.000	0.683	0.460	0.203	0.253	0.059	0.498
Contral Leg SP	C_SP_MEDIAN	r	0.489	0.662	0.834	0.623	0.502	0.683	1.000	0.649	0.360	0.414	0.248	0.216
Volar	Vol_MED_Agg	r	0.249	0.212	0.281	0.232	0.077	0.059	0.248	0.278	0.555	0.613	1.000	0.199
Deltoid	Del_MED_Agg	r	0.468	0.374	0.268	0.201	0.549	0.498	0.216	0.279	0.365	0.300	0.199	1.000

For the uninjured group, correlation between 'test' (randomly selected) and contralateral leg for the Anterior, Lateral, and Superficial Posterior compartments were, for the aggregated data, respectively: .834, .815, and .853, and for the disaggregated data: .718, .744, and .834.

2) From the multilevel modeling analysis (MLM) which examined both intra- and inter-individual variability, the pattern of estimated means in Table 3 (based on the full model with all covariates/predictors) is presented below for the designated test compartment (note: given small sample sizes for ACS and injured bilateral group, those two groups were omitted from analysis.). The time- varying covariates included the contralateral compartment as well as time (measured in minutes) and the time-invariant covariates included the injury-type grouping variable (injured unilateral vs. uninjured.), race (dichotomized as black vs. not black), sex, and age (continuous level).

The values on Table 3 represent the estimated values with 95% confidence intervals for NIRS of the 'test' limb for the specified compartment among patients in the specified injury group, controlling for age, sex, race, and the contralateral compartment (except no contralateral compartment for Volar and Deltoid). These are, loosely speaking, the overall 'average' values given all the data of each patient and the covariates mentioned.

The overall pattern of findings are as follows:

- For anterior, lateral, superficial posterior, deep posterior, and foot a higher NIRS value was obtained for the injured unilateral group.
- For volar and deltoid, a higher NIRS value was obtained for the uninjured group.

Table 3. Adjusted Group Means from Multilevel Modeling (MLM)- 85% cutoff.

	Anterior	95% CI (around mean)			Lateral	95% CI (around mean)			Superficial Posterior	95% CI (around mean)	
	Mean	LB	UB		Mean	LB	UB		Mean	LB	UB
Uninjured	69.31	66.76	71.87		70.70	68.49	72.91		71.53	69.51	73.56
Injured Unilateral	71.70	70.15	73.24		73.61	72.35	74.87		72.84	71.64	74.03
CI around Difference	2.38	-0.64	5.41		2.91	0.32	5.49		1.30	-1.07	3.68
	Deep Posterior	95% CI (around mean)			Foot	95% CI (around mean)			Volar	95% CI (around mean)	
	Mean	LB	UB		Mean	LB	UB		Mean	LB	UB
Uninjured	71.04	68.90	73.19		59.72	57.04	62.40		70.68	68.47	72.89
Injured Unilateral	76.01	74.53	77.49		60.94	59.31	62.57		69.65	68.50	70.80
CI around Difference	4.97	2.27	7.66		1.22	-1.97	4.42		-1.03	-3.55	1.50
	Deltoid	95% CI (around mean)									
	Mean	LB	UB								
Uninjured	69.91	67.59	72.23								
Injured Unilateral	68.69	67.48	69.90								
CI around Difference	-1.22	-3.86	1.423								
Note: CI = confidence interval; LB = lower bound; UB = upper bound											

3) Even though a sequence of models were tested for the multilevel modeling (MLM) from the null (no predictors, of which the ICC > .592 for all outcomes, indicative of substantive between individual variability on the intercepts) model to the growth model (including time) and then the full model which includes both time-varying [such as the contralateral compartment] and time-invariant [such as injury-type group, sex, race, and age] predictors, for brevity sake only the full model will be summarized (see Table 4):

- For all outcomes (e.g., test leg for anterior, lateral, etc.) time was significant except for Anterior (p = .253), though coefficients should be interpreted with consideration of clinical significance (for example, the estimated coefficient for time in the lateral compartment 0.000384 indicates the model predicts 0.000384 of a percentage point increase of NIRS value of the test leg for each progressive minute of time, controlling for injury type, age, race, and sex. This corresponds to 0.02304 percentage points increase for each hour, and .553 percentage points increase for each 24 hour day).
- The contralateral compartment was significant for all outcomes, though there was no such variable used in the model for Volar and Deltoid.
- The overall effect for the injury-type grouping variable was significant for Lateral and Deep Posterior.
- Age was significant for Anterior, Superficial Posterior, Deep Posterior and Deltoid. The overall pattern was a small (~0.10 percentage points) decrease of mean NIRS value of the 'test' leg for each year increase of age which corresponds roughly to ~1 percentage point decrease for each 10 years increase of age.
- Race was not significant for any of the outcomes
- Sex was significant for Volar with females obtaining a 3.18% higher NIRS than males.

Table 4. Parameter Estimates from Full Model for Multilevel Modeling (MLM)- 85% cutoff.

	DV = Anterior (Test leg)			**DV = Lateral (Test leg)**		**DV = Superficial Posterior (Test leg)**	
		M3: Time Varying/Invariant Predictors		**M3: Time Varying/Invariant Predictors**		**M3: Time Varying/Invariant Predictors**	
		b/SE	p-value	*b/SE*	p-value	*b/SE*	p-value
	Fixed effects						
Level 1							
	intercept	70.77/1.61	<0.001	70.89/1.38	<0.001	71.27/1.27	<0.001
	time	-.0002/.00016	0.253	0.000384/.00014	0.007	0.00093/.00016	<0.001
	Contralateral limb	0.318/.014	<0.001	0.396/.014	<0.001	0.264/.0156	<0.001
Level 2							
	Inj_grp[1]	2.38/1.52	0.121	2.91/1.3	0.028	1.30/1.19	0.278
	Age	-.103/.047	0.031	-.034/.04	0.402	-.137/.038	<0.001
	Race	-1.36/1.35	0.316	-1.32/1.13	0.243	.302/1.04	0.773
	Sex	-.458/1.52	0.764	.54/1.28	0.676	-.0008/1.18	0.999
	Random effects						
	intercept (u0)	34.50	< .001	25.08	< .001	21.18	< .001
	residual (rij)	8.27	< .001	6.84	< .001	8.27	< .001
	Unconditional ICC						
	-2LL	19031.31		19592.80		18986.44	
	AIC	19035.31		19596.80		18990.44	
	BIC	19047.77		19609.40		19002.90	
	[1]Dummy coded vector:						
	0 = uninjured; 1 = injured unilateral						

	DV = Deep Posterior (Test leg)			DV = Foot(Test leg)		DV = Volar	
		M3: Time Varying/Invariant Predictors		M3: Time Varying/Invariant Predictors		M3: Time Varying/Invariant Predictors	
		b/SE	p-value	*b/SE*	p-value	*b/SE*	p-value
	Fixed effects						
Level 1							
	intercept	71.70/1.33	<0.001	60.26/1.69	<0.001	74.50/1.36	<0.001
	time	0.00051/.00016	<0.001	0.00119/.00023	<0.001	-.001/.00019	<0.001
	Contralateral Limb	0.426/.014	<0.001	0.532.014	<0.001		
Level 2							
	Inj_grp[1]	4.96/1.35	<0.001	1.22/1.60	0.447	-1.03/1.27	0.421
	Age	-.101/.044	0.023	-.042/.05	0.405	-.031/.037	0.406
	Race	-1.07/1.29	0.41	-.23/1.42	0.871	-1.30/1.04	0.217
	Sex	.21/1.32	0.877	-.70/1.64	0.671	-3.18/1.22	0.01
	Random effects						
	intercept (u0)	21.62	< .001	35.61	< .001	25.02	< .001
	residual (rij)	5.92	< .001	14.06	< .001	18.44	< .001
	Unconditional ICC						
	-2LL	13445.86		18498.03		30346.53	
	AIC	13449.86		18502.03		30350.53	
	BIC	13461.76		18514.23		30363.64	
	[1]Dummy coded vector:						
	0 = uninjured; 1 = injured unilateral						

	DV = Deltoid		
		M3: Time Varying/Invariant Predictors	
		b/SE	p-value
	Fixed effects		
Level 1			
	intercept	72.59/1.47	<0.001
	time	-.0017/.00013	<0.001
	Contralateral Limb		
Level 2			
	Inj_grp[1]	-1.22/1.33	0.362
	Age	-.08/.039	0.039
	Race	-1.56/1.10	0.159
	Sex	-1.05/1.28	0.414
	Random effects		
	intercept (u0)	28.08	< .001
	residual (rij)	8.44	< .001
	Unconditional ICC		
	-2LL	27013.19	
	AIC	27017.19	
	BIC	27030.36	
	[1]Dummy coded vector:		
	0 = uninjured; 1 = injured unilateral		

c. Executive Summary of Results (90% NIRS cutoff)

1) Correlations for each of the compartments (anterior, lateral, etc.) were computed with the uninjured/contralateral leg anterior, lateral, superficial posterior (SP), volar, and deltoid (in part to discern potential control compartments). Results of correlations computed separately by injury type are shown. Given small sample sizes for ACS and Bilateral injured, the following summary is for only the unilateral injured and injured group. Moreover, analysis was done for **aggregated data** (computing correlations based on composite of all median times for each case; in other words, each patient receives one single summary value for each compartment, which is the mean of all median values collected) and **disaggregated data** (computing correlations using all median values of all patients, hence ignoring non-independence (e.g., repeated measurements of the same patient), though the nonindependence is explicitly modeled in the multilevel modeling). It is important to understand that aggregated data assigns a single summary value to the patient for each compartment which represents the 'average' experience of all captured data from the patient for that compartment; the derivation of the aggregate values for two separate compartments being correlated may include values for each that were not measured at the same time in the other. Disaggregated data includes all median values at all times collected, therefore correlations using disaggregated data are restricted only to observations where both compartments were measured at the same time.

Table 1. NIRS Compartment Correlations for Unilateral Injury Group (aggregated and disaggregated)- 90% cutoff.

Unilateral Injury Group--90% cutoff

Aggregated (one summary value per patient*)

			Injured Leg				Uninjured Contralateral Leg				Feet		Arm	
			Anterior	Lateral	SP	DP	Anterior	Lateral	SP	DP	Injured Leg	Contral Leg	Volar	Deltoid
			T_Ant_MED_Agg	T_Lat_MED_Agg	T_SP_MED_Agg	T_DP_MED_Agg	C_Ant_MED_Agg	C_Lat_MED_Agg	C_SP_MED_Agg	C_DP_MED_Agg	T_F_MED_Agg	C_F_MED_Agg	Vol_MED_Agg	Del_MED_Agg
Uninjured Leg Anterior	C_Ant_MED_Agg	r	0.415	0.440	0.350	0.263	1.000	0.786	0.434	0.468	0.249	0.369	0.479	0.548
Uninjured Leg Lateral	C_Lat_MEDIAN	r	0.448	0.459	0.528	0.191	0.786	1.000	0.578	0.571	0.357	0.474	0.581	0.611
Uninjured Leg SP	C_SP_MEDIAN	r	0.230	0.411	0.627	0.649	0.434	0.578	1.000	0.677	0.467	0.548	0.437	0.533
Volar	Vol_MED_Agg	r	0.140	0.407	0.279	0.245	0.479	0.581	0.437	0.500	0.412	0.347	1.000	0.550
Deltoid	Del_MED_Agg	r	0.468	0.530	0.489	0.329	0.548	0.611	0.533	0.565	0.399	0.484	0.550	1.000

* Aggregated value for each patient is the mean of all available 2-minute median values collected every 15 minutes

Disaggregated (multiple values (q 15 min) included for each patient**)

			Injured Leg				Uninjured Contralateral Leg				Feet		Arm	
			Anterior	Lateral	SP	DP	Anterior	Lateral	SP	DP	Injured Leg	Contral Leg	Volar	Deltoid
			T_Ant_MED_DisAg	_Lat_MED_DisAg	T_SP_MED_DisAgg	T_DP_MED_DisAgg	C_Ant_MED_DisAg	C_Lat_MED_DisAgg	C_SP_MED_DisAgg	_DP_MED_DisAgg	T_F_MED_DisAgg	_F_MED_DisAgg	Vol_MED_DisAgg	Del_MED_DisAgg
Uninjured Leg Anterior	C_Ant_MED_Agg	r	0.467	0.438	0.344	0.258	1.000	0.750	0.454	0.423	0.255	0.336	0.406	0.471
Uninjured Leg Lateral	C_Lat_MEDIAN	r	0.469	0.478	0.432	0.209	0.750	1.000	0.540	0.548	0.325	0.409	0.500	0.526
Uninjured Leg SP	C_SP_MEDIAN	r	0.257	0.386	0.507	0.488	0.454	0.540	1.000	0.665	0.430	0.466	0.364	0.443
Volar	Vol_MED_Agg	r	0.219	0.274	0.206	0.127	0.406	0.500	0.364	0.429	0.372	0.320	1.000	0.478
Deltoid	Del_MED_Agg	r	0.506	0.470	0.464	0.273	0.471	0.526	0.443	0.528	0.301	0.369	0.478	1.000

** Includes all available summary values for each patient collected q 15 minutes as the median value over a 2-minute period. Note: these results to not account for non-independence of repeated observations of the same patient

For the unilateral uninjured group, correlation between injured and contralateral leg for the Anterior, Lateral, and Superficial Posterior compartments were for the aggregate data, respectively, .415, .459, and .627, and for the disaggregated data: .467, .478, and .507.

Table 2. NIRS Compartment Correlations for Uninjured Group (aggregated and disaggregated)- 90% cutoff.

Uninjured Group--90% cutoff

Aggregated (one summary value per patient*)

			Test' Leg (randomly selected)				Contralateral Leg				Feet		Arm	
			Anterior	Lateral	SP	DP	Anterior	Lateral	SP	DP	Injured Leg	Contral Leg	Volar	Deltoid
			T_Ant_MED_Agg	T_Lat_MED_Agg	T_SP_MED_Agg	T_DP_MED_Agg	C_Ant_MED_Agg	C_Lat_MED_Agg	C_SP_MED_Agg	C_DP_MED_Agg	T_F_MED_Agg	C_F_MED_Agg	Vol_MED_Agg	Del_MED_Agg
Contral Leg Anterior	C_Ant_MED_Agg	r	0.839	0.780	0.593	0.588	1.000	0.773	0.693	0.536	0.519	0.385	0.368	0.667
Contral Leg Lateral	C_Lat_MEDIAN	r	0.782	0.813	0.785	0.668	0.773	1.000	0.778	0.626	0.284	0.390	0.264	0.602
Contral Leg SP	C_SP_MEDIAN	r	0.643	0.809	0.876	0.830	0.693	0.778	1.000	0.819	0.526	0.637	0.480	0.347
Volar	Vol_MED_Agg	r	0.459	0.513	0.468	0.496	0.368	0.264	0.480	0.475	0.729	0.716	1.000	0.373
Deltoid	Del_MED_Agg	r	0.581	0.456	0.256	0.311	0.667	0.602	0.347	0.454	0.409	0.442	0.373	1.000

* Aggregated value for each patient is the mean of all available 2-minute median values collected every 15 minutes

Disaggregated (multiple values (q 15 min) included for each patient**)

			Test' Leg (randomly selected)				Contralateral Leg				Feet		Arm	
			Anterior	Lateral	SP	DP	Anterior	Lateral	SP	DP	Injured Leg	Contral Leg	Volar	Deltoid
			T_Ant_MED_DisAgg	T_Lat_MED_DisAgg	T_SP_MED_DisAgg	T_DP_MED_DisAgg	C_Ant_MED_DisAgg	C_Lat_MED_DisAgg	C_SP_MED_DisAgg	C_DP_MED_DisAgg	T_F_MED_DisAgg	C_F_MED_DisAgg	Vol_MED_DisAgg	Del_MED_DisAgg
Contral Leg Anterior	C_Ant_MED_Agg	r	0.721	0.701	0.553	0.399	1.000	0.675	0.546	0.410	0.394	0.300	0.151	0.573
Contral Leg Lateral	C_Lat_MEDIAN	r	0.674	0.761	0.701	0.530	0.675	1.000	0.681	0.497	0.216	0.266	0.112	0.500
Contral Leg SP	C_SP_MEDIAN	r	0.504	0.697	0.852	0.664	0.546	0.681	1.000	0.672	0.436	0.486	0.256	0.262
Volar	Vol_MED_Agg	r	0.291	0.267	0.361	0.290	0.151	0.112	0.256	0.302	0.590	0.611	1.000	0.216
Deltoid	Del_MED_Agg	r	0.469	0.428	0.269	0.247	0.573	0.500	0.262	0.364	0.329	0.279	0.216	1.000

** Includes all available summary values for each patient collected q 15 minutes as the median value over a 2-minute period. Note: these results to not account for non-independence of repeated observations of the same patient

For the uninjured group, correlation between 'test' (randomly selected) and contralateral leg for the Anterior, Lateral, and Superficial Posterior compartments were, for the aggregated data, respectively: .839, .813, and .876, and for the disaggregated data: .721, .761, and .852.

2) From the multilevel modeling analysis (MLM) which examined both intra- and inter-individual variability, the pattern of estimated means in Table 3 (based on the full model with all covariates/predictors) is presented below for the designated test compartment (note: given small sample sizes for ACS and injured bilateral group, those two groups were omitted from analysis.). The time- varying covariates included the contralateral compartment as well as time (measured in minutes) and the time-invariant covariates included the injury-type grouping variable (injured unilateral vs. uninjured.), race (dichotomized as black vs. not black), sex, and age (continuous level).

The values on Table 3 represent the estimated values with 95% confidence intervals for NIRS of the 'test' limb for the specified compartment among patients in the specified injury group, controlling for age, sex, race, and the contralateral compartment (except no contralateral compartment for Volar and Deltoid). These are, loosely speaking, the overall 'average' values given all the data of each patient and the covariates mentioned.

The overall pattern of findings are as follows:

- For anterior, lateral, superficial posterior, deep posterior, and foot a higher NIRS value was obtained for the injured unilateral group.
- For volar and deltoid, a higher NIRS value was obtained for the uninjured group.

Table 3. Adjusted Group Means from Multilevel Modeling (MLM)- 90% cutoff.

	Anterior	95% CI (around mean)			Lateral	95% CI (around mean)			Superficial Posterior	95% CI (around mean)	
	Mean	LB	UB		Mean	LB	UB		Mean	LB	UB
Uninjured	69.79	66.99	72.58		70.90	68.39	73.42		71.99	69.90	74.09
Injured Unilateral	72.82	71.15	74.49		74.67	73.25	76.08		73.26	72.01	74.51
CI around Difference	3.04	-0.26	6.34		3.77	0.84	6.69		1.27	-1.19	3.73
	Deep Posterior	95% CI (around mean)			Foot	95% CI (around mean)			Volar	95% CI (around mean)	
	Mean	LB	UB		Mean	LB	UB		Mean	LB	UB
Uninjured	71.78	69.16	74.40		59.90	57.15	62.65		71.49	69.14	73.83
Injured Unilateral	77.91	76.26	79.57		61.10	59.43	62.78		70.07	68.83	71.30
CI around Difference	6.13	2.96	9.31		1.21	-2.07	4.48		-1.42	-4.10	1.27
	Deltoid	95% CI (around mean)									
	Mean	LB	UB								
Uninjured	69.96	67.63	72.28								
Injured Unilateral	68.73	67.52	69.94								
CI around Difference	-1.23	-3.872	1.42								
Note: CI = confidence interval; LB = lower bound; UB = upper bound. Difference: injured unilateral - uninjured.											

3) Even though a sequence of models were tested for the multilevel modeling (MLM) from the null (no predictors, of which the ICC > .575 for all outcomes, indicative of substantive between individual variability on the intercepts) model to the growth model (including time) and then the full model which includes both time-varying [such as the contralateral compartment] and time-invariant [such as injury-type group, sex, race, and age] predictors, for brevity sake only the full model will be summarized (see Table 4):

- For all outcomes (e.g., test leg for anterior, lateral, etc.) time was significant except for Anterior (p = .253), though coefficients should be interpreted with consideration of clinical significance (for example, the estimated coefficient for time in the lateral compartment 0.001 indicates the model predicts 0.001 of a percentage point increase of NIRS value of the test leg for each progressive minute of time, controlling for injury type, age, race, and sex. This corresponds to 0.06 percentage points increase for each hour, and 1.44 percentage points increase for each 24 hour day).
- The contralateral compartment was significant for all outcomes, though there was no such variable used in the model for Volar and Deltoid.
- The overall effect for the injury-type grouping variable was significant for Lateral and Deep Posterior.
- Age was significant for Superficial Posterior, Deep Posterior and Deltoid. The overall pattern was a small (~0.10 percentage points) decrease of mean NIRS value of the 'test' leg for each year increase of age which corresponds roughly to ~1 percentage point decrease for each 10 years increase of age.
- Race was not significant for any of the outcomes
- Sex was significant for Volar with females obtaining a 3.22% higher NIRS than males.

Table 4. Parameter Estimates from Full Model for Multilevel Modeling (MLM)-90% cutoff.

	DV = Anterior (Test leg)			**DV = Lateral (Test leg)**		**DV = Superficial Posterior (Test leg)**	
		M3: Time Varying/Invariant Predictors		**M3: Time Varying/Invariant Predictors**		**M3: Time Varying/Invariant Predictors**	
		b/SE	p-value	*b/SE*	p-value	*b/SE*	p-value
	Fixed effects						
Level 1							
	intercept	70.84/1.76	<0.001	71.24/1.56	<0.001	71.45/1.32	<0.001
	time	-.00018/.00017	0.274	0.001043/.000164	<0.001	0.00107/.00016	<0.001
	Contralateral Limb	0.283/.014	<0.001	0.363/.015	<0.001	0.278/.0153	<0.001
Level 2							
	Inj_grp[1]	3.04/1.66	0.071	3.77/1.47	0.012	1.27/1.24	0.308
	Age	-.085/.0514	0.102	-.057/.045	0.209	-.148/.04	<0.001
	Race	-1.79/1.46	0.225	-1.48/1.27	0.248	.507/1.09	0.643
	Sex	.375/1.66	0.822	-.086/1.43	0.952	.0686/1.24	0.956
	Random effects						
	intercept (u0)	41.53	< .001	32.79	< .001	23.52	< .001
	residual (rij)	9.64	< .001	9.45	< .001	8.81	< .001
	Unconditional ICC						
	-2LL	20717.27		21837.63		20144.99	
	AIC	20721.27		21841.63		20148.99	
	BIC	20733.84		21854.31		20161.54	
	[1]Dummy coded vector:						
	0 = uninjured; 1 = injured unilateral						

	DV = Deep Posterior (Test leg)			DV = Foot(Test leg)		DV = Volar	
		M3: Time Varying/Invariant Predictors		M3: Time Varying/Invariant Predictors		M3: Time Varying/Invariant Predictors	
		b/SE	p-value	*b/SE*	p-value	*b/SE*	p-value
	Fixed effects						
Level 1							
	intercept	72.31/1.63	<0.001	60.42/1.73	<0.001	75.78/1.45	<0.001
	time	0.00062/.00017	<0.001	0.00127/.00024	<0.001	-.0018/.000219	<0.001
	Contralateral Limb	0.35/.014	<0.001	0.514/014	<0.001		
Level 2							
	Inj_grp[1]	6.13/1.59	<0.001	1.21/1.64	0.466	-1.42/1.35	0.297
	Age	-.14/.05	0.007	-.044/.052	0.4	-.049/.04	0.224
	Race	-.838/1.47	0.57	-.17/1.45	0.906	-1.51/1.12	0.18
	Sex	.15/1.54	0.924	-.86/1.68	0.61	-3.22/1.30	0.015
	Random effects						
	intercept (u0)	34.24	< .001	37.53	< .001	28.85	< .001
	residual (rij)	7.57	< .001	15.02	< .001	22.79	< .001
	Unconditional ICC						
	-2LL	16156.87		18942.16		32483.49	
	AIC	16160.87		18946.16		32487.49	
	BIC	16173.04		18958.38		32500.66	
	[1]Dummy coded vector:						
	0 = uninjured; 1 = injured unilateral						

	DV = Deltoid		
		M3: Time Varying/Invariant Predictors	
		b/SE	p-value
	Fixed effects		
Level 1			
	intercept	72.60/1.47	<0.001
	time	-.0018/.00014	<0.001
	Contralateral Limb		
Level 2			
	Inj_grp[1]	-1.23/1.33	0.36
	Age	-.084/.039	0.036
	Race	-1.00/1.29	0.162
	Sex	-1.05/1.28	0.438
	Random effects		
	intercept (u0)	28.17	< .001
	residual (rij)	9.05	< .001
	Unconditional ICC		
	-2LL	27451.70	
	AIC	27455.70	
	BIC	27468.87	
	[1]Dummy coded vector:		
	0 = uninjured; 1 = injured unilateral		

d. Executive Summary of Results (95% NIRS cutoff)

1) Correlations for each of the compartments (anterior, lateral, etc.) were computed with the uninjured/contralateral leg anterior, lateral, superficial posterior (SP), volar, and deltoid (in part to discern potential control compartments). Results of correlations computed separately by injury type are shown. Given small sample sizes for ACS and Bilateral injured, the following summary is for only the unilateral injured and injured group. Moreover, analysis was done for **aggregated data** (computing correlations based on composite of all median times for each case; in other words, each patient receives one single summary value for each compartment, which is the mean of all median values collected) and **disaggregated data** (computing correlations using all median values of all patients, hence ignoring non-independence (e.g., repeated measurements of the same patient), though the nonindependence is explicitly modeled in the multilevel modeling). It is important to understand that aggregated data assigns a single summary value to the patient for each compartment which represents the 'average' experience of all captured data from the patient for that compartment; the derivation of the aggregate values for two separate compartments being correlated may include values for each that were not measured at the same time in the other. Disaggregated data includes all median values at all times collected, therefore correlations using disaggregated data are restricted only to observations where both compartments were measured at the same time.

Table 1. NIRS Compartment Correlations for Unilateral Injury Group (aggregated and disaggregated)- 95% cutoff.

Unilateral Injury Group--95% cutoff

Aggregated (one summary value per patient*)

			Injured Leg				Uninjured Contralateral Leg				Feet		Arm	
			Anterior	Lateral	SP	DP	Anterior	Lateral	SP	DP	Injured Leg	Contral Leg	Volar	Deltoid
			T_Ant_MED_Agg	T_Lat_MED_Agg	T_SP_MED_Agg	T_DP_MED_Agg	C_Ant_MED_Agg	C_Lat_MED_Agg	C_SP_MED_Agg	C_DP_MED_Agg	T_F_MED_Agg	C_F_MED_Agg	Vol_MED_Agg	Del_MED_Agg
Uninjured Leg Anterior	C_Ant_MED_Agg	r	0.402	0.447	0.354	0.254	1.000	0.779	0.407	0.459	0.249	0.362	0.472	0.547
Uninjured Leg Lateral	C_Lat_MEDIAN	r	0.421	0.441	0.521	0.160	0.779	1.000	0.556	0.554	0.351	0.469	0.589	0.613
Uninjured Leg SP	C_SP_MEDIAN	r	0.222	0.441	0.659	0.625	0.407	0.556	1.000	0.660	0.478	0.531	0.433	0.536
Volar	Vol_MED_Agg	r	0.133	0.428	0.289	0.235	0.472	0.589	0.433	0.504	0.395	0.343	1.000	0.554
Deltoid	Del_MED_Agg	r	0.438	0.534	0.503	0.340	0.547	0.613	0.536	0.551	0.406	0.478	0.554	1.000

* Aggregated value for each patient is the mean of all available 2-minute median values collected every 15 minutes

Disaggregated (multiple values (q 15 min) included for each patient**)

			Injured Leg				Uninjured Contralateral Leg				Feet		Arm	
			Anterior	Lateral	SP	DP	Anterior	Lateral	SP	DP	Injured Leg	Contral Leg	Volar	Deltoid
			T_Ant_MED_DisAg	T_Lat_MED_DisAg	T_SP_MED_DisAgg	T_DP_MED_DisAgg	C_Ant_MED_DisAg	C_Lat_MED_DisAgg	C_SP_MED_DisAgg	C_DP_MED_DisAgg	T_F_MED_DisAgg	F_MED_DisAgg	Vol_MED_DisAgg	Del_MED_DisAgg
Uninjured Leg Anterior	C_Ant_MED_Agg	r	0.448	0.398	0.355	0.282	1.000	0.755	0.427	0.438	0.245	0.331	0.426	0.479
Uninjured Leg Lateral	C_Lat_MEDIAN	r	0.444	0.443	0.445	0.241	0.755	1.000	0.528	0.554	0.320	0.409	0.515	0.531
Uninjured Leg SP	C_SP_MEDIAN	r	0.252	0.333	0.503	0.540	0.427	0.528	1.000	0.663	0.425	0.459	0.379	0.452
Volar	Vol_MED_Agg	r	0.210	0.298	0.214	0.140	0.426	0.515	0.379	0.452	0.369	0.325	1.000	0.492
Deltoid	Del_MED_Agg	r	0.467	0.449	0.457	0.311	0.479	0.531	0.452	0.531	0.298	0.373	0.492	1.000

** Includes all available summary values for each patient collected q 15 minutes as the median value over a 2-minute period. Note: these results to not account for non-independence of repeated observations of the same patient

For the unilateral uninjured group, correlation between injured and contralateral leg for the Anterior, Lateral, and Superficial Posterior compartments were for the aggregate data, respectively, .402, .441, and .659, and for the disaggregated data: .448, .443, and .503.

For the uninjured group results are as follows (see Table 2):

Table 2. NIRS Compartment Correlations for Uninjured Group (aggregated and disaggregated)- 95% cutoff.

Uninjured Group--95% cutoff

Aggregated (one summary value per patient*)

			Test' Leg (randomly selected)				Contralateral Leg				Feet		Arm	
			Anterior	Lateral	SP	DP	Anterior	Lateral	SP	DP	Injured Leg	Contral Leg	Volar	Deltoid
			T_Ant_MED_Agg	T_Lat_MED_Agg	T_SP_MED_Agg	T_DP_MED_Agg	C_Ant_MED_Agg	C_Lat_MED_Agg	C_SP_MED_Agg	C_DP_MED_Agg	T_F_MED_Agg	C_F_MED_Agg	Vol_MED_Agg	Del_MED_Agg
Contral Leg Anterior	C_Ant_MED_Agg	r	0.857	0.788	0.593	0.598	1.000	0.773	0.703	0.554	0.520	0.381	0.421	0.667
Contral Leg Lateral	C_Lat_MEDIAN	r	0.789	0.815	0.786	0.632	0.773	1.000	0.747	0.626	0.283	0.387	0.307	0.598
Contral Leg SP	C_SP_MEDIAN	r	0.706	0.816	0.870	0.858	0.703	0.747	1.000	0.825	0.562	0.646	0.564	0.343
Volar	Vol_MED_Agg	r	0.583	0.571	0.481	0.570	0.421	0.307	0.564	0.556	0.719	0.709	1.000	0.398
Deltoid	Del_MED_Agg	r	0.599	0.459	0.251	0.297	0.667	0.598	0.343	0.459	0.407	0.435	0.398	1.000

* Aggregated value for each patient is the mean of all available 2-minute median values collected every 15 minutes

Disaggregated (multiple values (q 15 min) included for each patient**)

			Test' Leg (randomly selected)				Contralateral Leg				Feet		Arm	
			Anterior	Lateral	SP	DP	Anterior	Lateral	SP	DP	Injured Leg	Contral Leg	Volar	Deltoid
			T_Ant_MED_DisAg	T_Lat_MED_DisAg	T_SP_MED_DisAgg	T_DP_MED_DisAgg	C_Ant_MED_DisAg	C_Lat_MED_DisAgg	C_SP_MED_DisAgg	C_DP_MED_DisAgg	T_F_MED_DisAgg	F_MED_DisAgg	Vol_MED_DisAgg	Del_MED_DisAgg
Contral Leg Anterior	C_Ant_MED_Agg	r	0.746	0.720	0.565	0.506	1.000	0.687	0.616	0.485	0.405	0.307	0.174	0.577
Contral Leg Lateral	C_Lat_MEDIAN	r	0.693	0.775	0.714	0.546	0.687	1.000	0.688	0.543	0.221	0.263	0.131	0.497
Contral Leg SP	C_SP_MEDIAN	r	0.590	0.757	0.870	0.783	0.616	0.688	1.000	0.737	0.558	0.574	0.359	0.340
Volar	Vol_MED_Agg	r	0.298	0.293	0.374	0.408	0.174	0.131	0.359	0.331	0.597	0.597	1.000	0.233
Deltoid	Del_MED_Agg	r	0.517	0.441	0.268	0.315	0.577	0.497	0.340	0.414	0.323	0.264	0.233	1.000

** Includes all available summary values for each patient collected q 15 minutes as the median value over a 2-minute period. Note: these results to not account for non-independence of repeated observations of the same patient

For the uninjured group, correlation between 'test' (randomly selected) and contralateral leg for the Anterior, Lateral, and Superficial Posterior compartments were, for the aggregated data, respectively: .857, .815, and .87, and for the disaggregated data: .746, .775, and .87.

2) From the multilevel modeling analysis (MLM) which examined both intra- and inter-individual variability, the pattern of estimated means in Table 3 (based on the full model with all covariates/predictors) is presented below for the designated test compartment (note: given small sample sizes for ACS and injured bilateral group, those two groups were omitted from analysis.). The time- varying covariates included the contralateral compartment as well as time (measured in minutes) and the time-invariant covariates included the injury-type grouping variable (injured unilateral vs. uninjured.), race (dichotomized as black vs. not black), sex, and age (continuous level).

The values on Table 3 represent the estimated values with 95% confidence intervals for NIRS of the 'test' limb for the specified compartment among patients in the specified injury group, controlling for age, sex, race, and the contralateral compartment (except no contralateral compartment for Volar and Deltoid). These are, loosely speaking, the overall 'average' values given all the data of each patient and the covariates mentioned.

The overall pattern of findings are as follows:

- For anterior, lateral, superficial posterior, deep posterior, and foot a higher NIRS value was obtained for the injured unilateral group.
- For volar and deltoid, a higher NIRS value was obtained for the uninjured group.

Table 3. Adjusted Group Means from Multilevel Modeling (MLM)- 95% cutoff.

	Anterior	95% CI (around mean)			Lateral	95% CI (around mean)			Superficial Posterior	95% CI (around mean)	
	Mean	LB	UB		Mean	LB	UB		Mean	LB	UB
Uninjured	70.20	67.22	73.18		70.95	68.12	73.78		72.26	70.13	74.38
Injured Unilateral	73.18	71.40	74.95		75.44	73.86	77.03		73.69	72.42	74.96
CI around Difference	2.98	-0.54	6.50		4.49	1.21	7.78		1.43	-1.08	3.95
	Deep Posterior	95% CI (around mean)			Foot	95% CI (around mean)			Volar	95% CI (around mean)	
	Mean	LB	UB		Mean	LB	UB		Mean	LB	UB
Uninjured	72.09	69.22	74.95		59.97	57.16	62.77		72.73	70.25	75.21
Injured Unilateral	79.16	77.37	80.94		61.16	59.45	62.87		70.98	69.41	72.54
CI around Difference	7.07	3.60	10.54		1.19	-2.15	4.54		-1.76	-4.61	1.10
	Deltoid	95% CI (around mean)									
	Mean	LB	UB								
Uninjured	69.97	67.63	72.30								
Injured Unilateral	68.80	67.58	70.01								
CI around Difference	-1.17	-3.831	1.488								
Note: CI = confidence interval; LB = lower bound; UB = upper bound. Difference: injured unilateral - uninjured.											

3) Even though a sequence of models were tested for the multilevel modeling (MLM) from the null (no predictors, of which the ICC > .59 for all outcomes, indicative of substantive between individual variability on the intercepts) model to the growth model (including time) and then the full model which includes both time-varying [such as the contralateral compartment] and time-invariant [such as injury-type group, sex, race, and age] predictors, for brevity sake only the full model will be summarized (see Table 4):

- For all outcomes (e.g., test leg for anterior, lateral, etc.) time was significant except for Anterior (p = .668), though coefficients should be interpreted with consideration of clinical significance (for example, the estimated coefficient for time in the lateral compartment 0.0015 indicates the model predicts 0.0015 of a percentage point increase of NIRS value of the test leg for each progressive minute of time, controlling for injury type, age, race, and sex. This corresponds to 0.09 percentage points increase for each hour, and 2.22 percentage points increase for each 24 hour day).
- The contralateral compartment was significant for all outcomes, though there was no such variable used in the model for Volar and Deltoid.
- The overall effect for the injury-type grouping variable was significant for Lateral and Deep Posterior.
- Age was significant for Superficial Posterior, Deep Posterior and Deltoid. The overall pattern was a small (~0.10 percentage points) decrease of mean NIRS value of the 'test' leg for each year increase of age which corresponds roughly to ~1 percentage point decrease for each 10 years increase of age.
- Race was not significant for any of the outcomes
- Sex was significant for Volar with females obtaining a 2.99% higher NIRS than males.

Table 4. Parameter Estimates from Full Model for Multilevel Modeling (MLM)-95% cutoff.

	DV = Anterior (Test leg)			**DV = Lateral (Test leg)**		**DV = Superficial Posterior (Test leg)**	
		M3: Time Varying/Invariant Predictors		**M3: Time Varying/Invariant Predictors**		**M3: Time Varying/Invariant Predictors**	
		b/SE	p-value	*b/SE*	p-value	*b/SE*	p-value
	Fixed effects						
Level 1							
	intercept	70.95/1.88	<0.001	71.25/1.76	<0.001	71.45/1.32	<0.001
	time	-.000077/.00018	0.668	0.00154/.000176	<0.001	0.001/.00017	<0.001
	Contralateral Limb	0.284/.015	<0.001	0.354/.016	<0.001	0.308/.0147	<0.001
Level 2							
	Inj_grp[1]	2.98/1.77	0.096	4.49/1.65	0.008	1.43/1.26	0.259
	Age	-.090/.0547	0.104	-.066/.051	0.197	-.141/.04	<0.001
	Race	-1.92/1.555	0.22	-2.10/1.41	0.141	.93/1.11	0.404
	Sex	.798/1.77	0.654	.027/1.60	0.987	.238/1.26	0.851
	Random effects						
	intercept (u0)	47.10	< .001	41.70	< .001	24.49	< .001
	residual (rij)	11.21	< .001	11.25	< .001	9.33	< .001
	Unconditional ICC						
	-2LL	21816.26		23320.59		20926.49	
	AIC	21820.26		23324.59		20930.49	
	BIC	21832.88		23337.34		20943.10	
	[1]Dummy coded vector:						
	0 = uninjured; 1 = injured unilateral						

	DV = Deep Posterior (Test leg)			DV = Foot(Test leg)		DV = Volar	
		M3: Time Varying/Invariant Predictors		M3: Time Varying/Invariant Predictors		M3: Time Varying/Invariant Predictors	
		b/SE	p-value	*b/SE*	p-value	*b/SE*	p-value
	Fixed effects						
Level 1							
	intercept	72.93/1.79	<0.001	60.34/1.77	<0.001	76.28/1.55	<0.001
	time	0.00046/.00018	0.009	0.0014/.00025	<0.001	-.002/.000219	<0.001
	Contralateral Limb	0.33/.014	<0.001	0.479/014	<0.001		
Level 2							
	Inj_grp[1]	7.07/1.74	<0.001	1.19/1.68	0.479	-1.75/1.44	0.226
	Age	-.14/.054	0.014	-.047/.053	0.378	-.055/.043	0.203
	Race	-2.12/1.58	0.184	-.14/1.49	0.923	-1.72/1.2	0.154
	Sex	.65/1.68	0.699	-.82/1.71	0.636	-2.99/1.39	0.034
	Random effects						
	intercept (u0)	41.29	< .001	39.12	< .001	33.07	< .001
	residual (rij)	9.29	< .001	16.02	< .001	24.21	< .001
	Unconditional ICC						
	-2LL	18170.65		19240.28		33027.74	
	AIC	18174.65		19244.28		33031.74	
	BIC	18186.97		19256.52		33044.93	
	[1]Dummy coded vector:						
	0 = uninjured; 1 = injured unilateral						

	DV = Deltoid		
		M3: Time Varying/Invariant Predictors	
		b/SE	p-value
	Fixed effects		
Level 1			
	intercept	72.57/1.48	<0.001
	time	-.0017/.00014	<0.001
	Contralateral Limb		
Level 2			
	Inj_grp[1]	-1.17/1.34	0.384
	Age	-.084/.04	0.036
	Race	-1.59/1.11	0.153
	Sex	-.94/1.29	0.467
	Random effects		
	intercept (u0)	28.43	< .001
	residual (rij)	9.96	< .001
	Unconditional ICC		
	-2LL	28018.53	
	AIC	28022.53	
	BIC	28035.70	
	[1]Dummy coded vector:		
	0 = uninjured; 1 = injured unilateral		

VI. NIRS Short-term Variability

Variability of NIRS values for the 2-minute periods of sampled data was summarized in terms of standard deviation, and interquartile range (IQR) for all samples of the 12 monitored compartments by patient type. The dependent variables for these analyses are the calculated standard deviation and IQR of each 2-minute sample of each compartment of each patient that was included in the longitudinal analyses (See Section 1 Methods). NIRS data were captured electronically for each compartment every 4 seconds, of which a 2-minute sample was obtained every 15 minutes for the duration the patient was on the monitor. NIRS values ≥ 85% were omitted (set to missing) for these analyses (See Methods). The sample standard deviation and IQR were calculated for all samples with 10 or more non-missing values during the 2 minutes.

*Key to variable names on output: example "T_Ant_STD"

T_ or C_ prefix indicates 'test' leg or contralateral leg
Ant, Lat, SP, DP, Vol, Del, Foot indicate compartment
_STD or _IQR indicates the dependent variable - SD or IQR of the 2-minute sample

Summary of Standard Deviations By Group:

For the **ACS** group, the highest mean NIRS Standard Deviation was for Anterior-Contralateral (*M* = 1.39, *SD* = 1.25) and the lowest was for Lateral-Test (*M* = .27, *SD* = .287).

For the **Injured Bilateral** group, the highest mean NIRS Standard Deviation was for Volar (*M* = 1.48, *SD* = 1.62) and the lowest was for Superficial Posterior-Contralateral (*M* = .31, *SD* = .273).

For the **Injured Unilateral** group, the highest mean NIRS Standard Deviation was for Volar (*M* = 1.49, *SD* = 1.43) and the lowest was for Deep Posterior-Test (*M* = .47, *SD* = .554).

For the **Uninjured** group, the highest mean NIRS Standard Deviation was for Anterior-Test (*M* = .91, *SD* = .941) and the lowest was for Deltoid (*M* = .47, *SD* = .800).

Summary of IQR By Group:

For the **ACS** group, the highest mean NIRS IQR was for Volar (*M* = 2.01, *SD* = 1.73) and the lowest was for Lateral-Test (*M* = .27, *SD* = .458.

For the **Injured Bilateral** group, the highest mean NIRS IQR was for Volar (*M* = 1.48, *SD* = 1.62) and the lowest was for Superficial Posterior-Contralateral (*M* = .31, *SD* = .273).

For the **Injured Unilateral** group, the highest mean NIRS IQR was for Volar (*M* = 2.02, *SD* = 2.36) and the lowest was for Deep Posterior-Test (*M* = .58, *SD* = .976).

For the **Uninjured** group, the highest mean NIRS IQR was for Anterior-Test (*M* = 1.15, *SD* = 1.43) and the lowest was for Deltoid (*M* = .58, *SD* = 1.20).

a. Standard Deviation

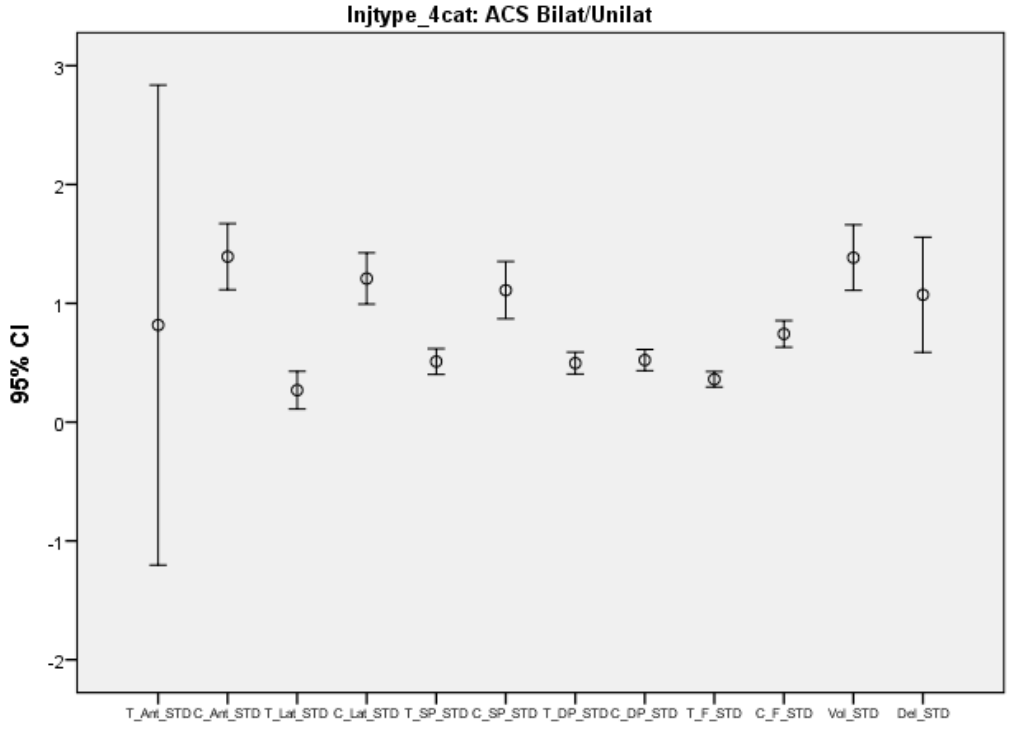
Following are the descriptive statistics for the standard deviations of the 2-minute sample data by group for the disaggregated (time-based) data.

Report

Injtype_4cat		T_DP_STD	C_DP_STD	T_F_STD	C_F_STD	Vol_STD	Del_STD
1.00 ACS Bilat/Unilat	Mean	.4964	.5223	.3611	.7426	1.3845	1.0720
	Median	.5299	.4960	.4302	.6778	1.1657	.6394
	Std. Deviation	.24177	.35699	.25260	.50642	1.00933	2.16351
	Minimum	.00	.00	.00	.00	.00	.00
	Maximum	1.12	1.70	1.06	2.74	5.17	19.07
	Range	1.12	1.70	1.06	2.74	5.17	19.07
	N	29	64	61	82	54	79
2.00 Injured Bilateral	Mean	.5605	.5489	.5956	.5222	1.4777	.5587
	Median	.4576	.3844	.4837	.4611	.9130	.4600
	Std. Deviation	.85600	1.62874	.61401	.67229	1.61698	.60615
	Minimum	.00	.00	.00	.00	.00	.00
	Maximum	7.63	18.45	4.54	7.92	9.95	3.71
	Range	7.63	18.45	4.54	7.92	9.95	3.71
	N	165	141	173	185	184	199
3.00 Injured Unilateral	Mean	.4726	.7224	.5481	.7190	1.4866	.8782
	Median	.4355	.5092	.4708	.5061	1.0171	.5092
	Std. Deviation	.55399	.71021	.57478	.75874	1.43001	1.06481
	Minimum	.00	.00	.00	.00	.00	.00
	Maximum	11.30	7.43	6.53	13.47	18.43	12.65
	Range	11.30	7.43	6.53	13.47	18.43	12.65
	N	1824	3989	2492	4108	3857	4015
4.00 Uninjured	Mean	.5691	.7638	.5562	.5506	.7221	.4671
	Median	.4901	.6336	.4708	.4837	.4549	.3509
	Std. Deviation	.54602	.65488	.64536	.54969	1.04261	.80099
	Minimum	.00	.00	.00	.00	.00	.00
	Maximum	9.10	6.94	7.83	6.93	11.82	12.83
	Range	9.10	6.94	7.83	6.93	11.82	12.83
	N	1220	1311	1228	1117	1276	1247
Total	Mean	.5136	.7255	.5498	.6785	1.3037	.7770
	Median	.4661	.5222	.4708	.5012	.8193	.5012
	Std. Deviation	.57006	.73357	.59623	.71858	1.38966	1.03780
	Minimum	.00	.00	.00	.00	.00	.00
	Maximum	11.30	18.45	7.83	13.47	18.43	19.07
	Range	11.30	18.45	7.83	13.47	18.43	19.07
	N	3238	5505	3954	5492	5371	5540

Report

Injtype_4cat		T_DP_STD	C_DP_STD	T_F_STD	C_F_STD	Vol_STD	Del_STD
1.00 ACS Bilat/Unilat	Mean	.4964	.5223	.3611	.7426	1.3845	1.0720
	Median	.5299	.4960	.4302	.6778	1.1657	.6394
	Std. Deviation	.24177	.35699	.25260	.50642	1.00933	2.16351
	Minimum	.00	.00	.00	.00	.00	.00
	Maximum	1.12	1.70	1.06	2.74	5.17	19.07
	Range	1.12	1.70	1.06	2.74	5.17	19.07
	N	29	64	61	82	54	79
2.00 Injured Bilateral	Mean	.5605	.5489	.5956	.5222	1.4777	.5587
	Median	.4576	.3844	.4837	.4611	.9130	.4600
	Std. Deviation	.85600	1.62874	.61401	.67229	1.61698	.60615
	Minimum	.00	.00	.00	.00	.00	.00
	Maximum	7.63	18.45	4.54	7.92	9.95	3.71
	Range	7.63	18.45	4.54	7.92	9.95	3.71
	N	165	141	173	185	184	199
3.00 Injured Unilateral	Mean	.4726	.7224	.5481	.7190	1.4866	.8782
	Median	.4355	.5092	.4708	.5061	1.0171	.5092
	Std. Deviation	.55399	.71021	.57478	.75874	1.43001	1.06481
	Minimum	.00	.00	.00	.00	.00	.00
	Maximum	11.30	7.43	6.53	13.47	18.43	12.65
	Range	11.30	7.43	6.53	13.47	18.43	12.65
	N	1824	3989	2492	4108	3857	4015
4.00 Uninjured	Mean	.5691	.7638	.5562	.5506	.7221	.4671
	Median	.4901	.6336	.4708	.4837	.4549	.3509
	Std. Deviation	.54602	.65488	.64536	.54969	1.04261	.80099
	Minimum	.00	.00	.00	.00	.00	.00
	Maximum	9.10	6.94	7.83	6.93	11.82	12.83
	Range	9.10	6.94	7.83	6.93	11.82	12.83
	N	1220	1311	1228	1117	1276	1247
Total	Mean	.5136	.7255	.5498	.6785	1.3037	.7770
	Median	.4661	.5222	.4708	.5012	.8193	.5012
	Std. Deviation	.57006	.73357	.59623	.71858	1.38966	1.03780
	Minimum	.00	.00	.00	.00	.00	.00
	Maximum	11.30	18.45	7.83	13.47	18.43	19.07
	Range	11.30	18.45	7.83	13.47	18.43	19.07
	N	3238	5505	3954	5492	5371	5540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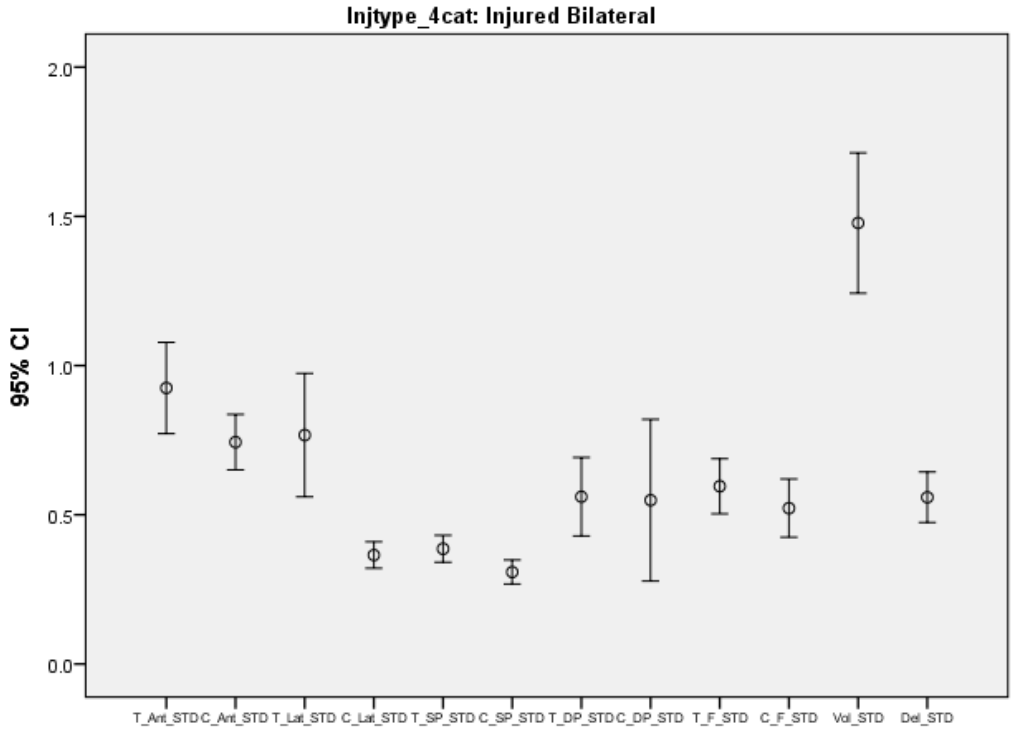
C_SP_STD
T_DP_STD
C_DP_STD
T_F_STD
C_F_STD
Vol_STD
Del_STD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T_F_STD
C_F_STD
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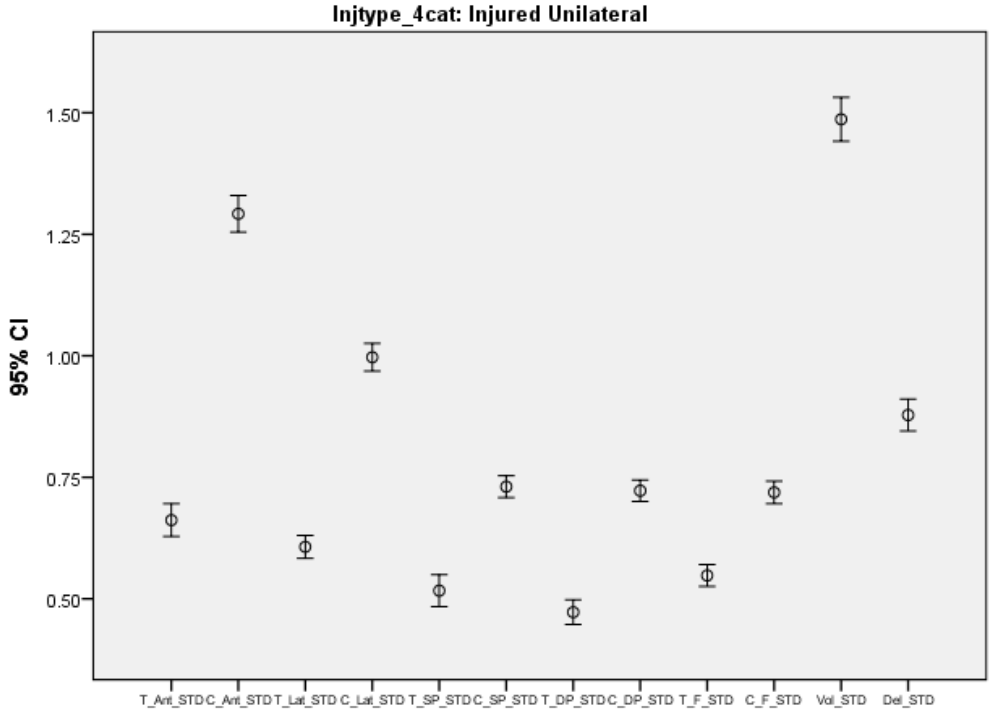
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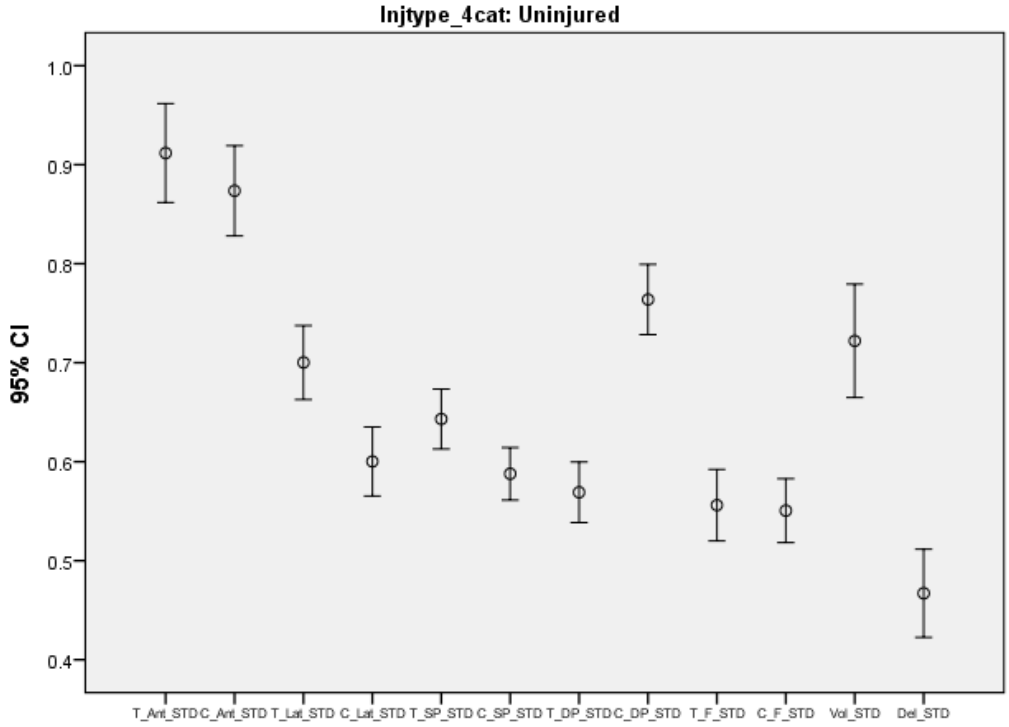
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C_Lat_STD
T_SP_STD
C_SP_STD
T_DP_STD
C_DP_STD
T_F_STD
C_F_STD
Vol_STD
Del_STD

b. IQR

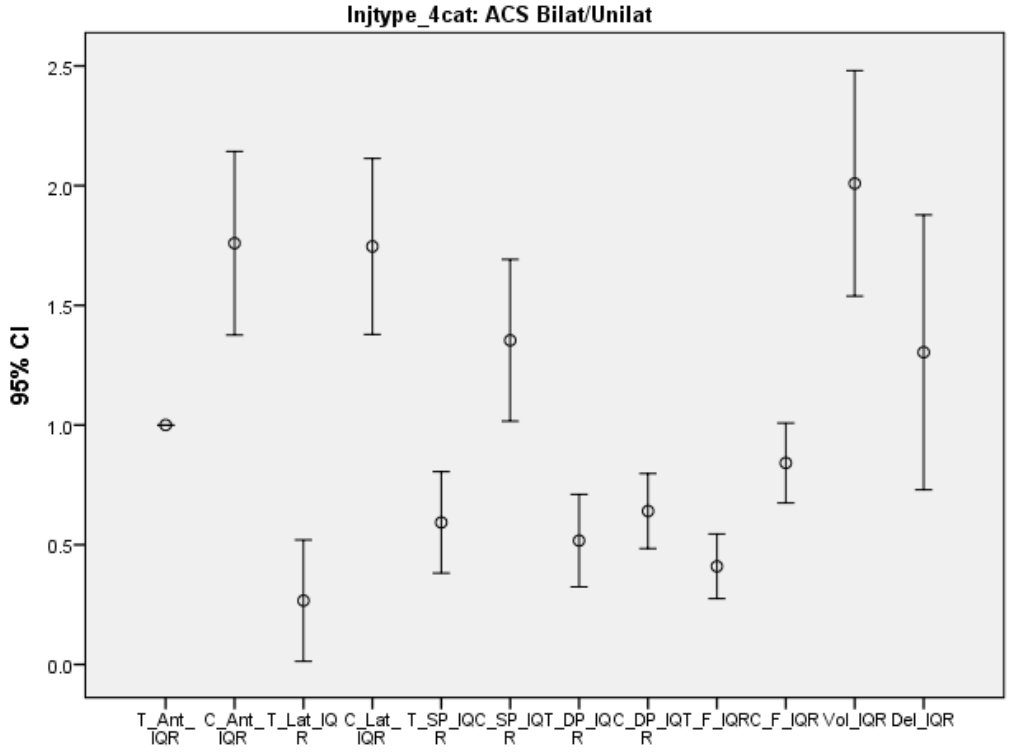
Following are the descriptive statistics for the IQR of the 2-minute sample data by group for the disaggregated (time-based) data.

Report

Injtype_4cat		T_Ant_IQR	C_Ant_IQR	T_Lat_IQR	C_Lat_IQR	T_SP_IQR	C_SP_IQR
1.00 ACS Bilat/Unilat	Mean	1.0000	1.7595	.2667	1.7460	.5932	1.3538
	Median	1.0000	1.0000	.0000	2.0000	.0000	1.0000
	Std. Deviation	.00000	1.71131	.45774	1.45877	.81195	1.36280
	Minimum	1.00	.00	.00	.00	.00	.00
	Maximum	1.00	10.00	1.00	7.00	5.00	5.00
	Range	.00	10.00	1.00	7.00	5.00	5.00
	N	2	79	15	63	59	65
2.00 Injured Bilateral	Mean	1.0854	.9676	.9892	.4695	.3912	.3389
	Median	1.0000	1.0000	1.0000	.0000	.0000	.0000
	Std. Deviation	1.37793	1.05211	1.88479	.58427	.60685	.53025
	Minimum	.00	.00	.00	.00	.00	.00
	Maximum	7.00	8.00	15.00	2.00	4.00	2.00
	Range	7.00	8.00	15.00	2.00	4.00	2.00
	N	158	170	139	131	170	180
3.00 Injured Unilateral	Mean	.8642	1.6509	.7690	1.3104	.6401	.9198
	Median	1.0000	1.0000	1.0000	1.0000	.0000	1.0000
	Std. Deviation	1.59940	1.84079	.98079	1.53084	1.56384	1.31347
	Minimum	.00	.00	.00	.00	.00	.00
	Maximum	40.00	19.00	11.00	16.00	50.00	34.00
	Range	40.00	19.00	11.00	16.00	50.00	34.00
	N	2559	4289	2775	4451	2633	4275
4.00 Uninjured	Mean	1.1520	1.1210	.9011	.7221	.8109	.7044
	Median	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
	Std. Deviation	1.42809	1.23118	1.19059	.95668	1.01249	.84269
	Minimum	.00	.00	.00	.00	.00	.00
	Maximum	13.00	11.00	10.00	10.00	10.00	6.00
	Range	13.00	11.00	10.00	10.00	10.00	6.00
	N	1372	1347	1380	1342	1351	1304
Total	Mean	.9693	1.5113	.8167	1.1647	.6842	.8584
	Median	1.0000	1.0000	1.0000	1.0000	.0000	1.0000
	Std. Deviation	1.54112	1.71616	1.09243	1.43195	1.37498	1.21383
	Minimum	.00	.00	.00	.00	.00	.00
	Maximum	40.00	19.00	15.00	16.00	50.00	34.00
	Range	40.00	19.00	15.00	16.00	50.00	34.00
	N	4091	5885	4309	5987	4213	5824

Report

Injtype_4cat		T_DP_IQR	C_DP_IQR	T_F_IQR	C_F_IQR	Vol_IQR	Del_IQR
1.00 ACS Bilat/Unilat	Mean	.5172	.6406	.4098	.8415	2.0093	1.3038
	Median	1.0000	1.0000	.0000	1.0000	1.0000	1.0000
	Std. Deviation	.50855	.62659	.52843	.76125	1.72520	2.56382
	Minimum	.00	.00	.00	.00	.00	.00
	Maximum	1.00	3.00	2.00	3.00	7.00	22.00
	Range	1.00	3.00	2.00	3.00	7.00	22.00
	N	29	64	61	82	54	79
2.00 Injured Bilateral	Mean	.6939	.7305	.8353	.6486	2.0326	.7362
	Median	.0000	.0000	1.0000	.0000	1.0000	.0000
	Std. Deviation	1.29444	3.27737	1.14515	1.11721	2.58918	.98831
	Minimum	.00	.00	.00	.00	.00	.00
	Maximum	11.00	38.00	8.00	11.50	15.00	6.00
	Range	11.00	38.00	8.00	11.50	15.00	6.00
	N	165	141	173	185	184	199
3.00 Injured Unilateral	Mean	.5757	.8869	.6894	.9489	2.0231	1.1303
	Median	.0000	1.0000	.0000	1.0000	1.0000	1.0000
	Std. Deviation	.97558	1.11770	1.02979	1.34772	2.36072	1.71051
	Minimum	.00	.00	.00	.00	.00	.00
	Maximum	17.00	11.00	14.00	28.00	41.00	26.00
	Range	17.00	11.00	14.00	28.00	41.00	26.00
	N	1824	3989	2492	4108	3857	4015
4.00 Uninjured	Mean	.6734	.9043	.6600	.6804	.9557	.5778
	Median	1.0000	1.0000	.0000	1.0000	.0000	.0000
	Std. Deviation	.86358	.97965	1.06982	.88668	1.73029	1.20158
	Minimum	.00	.00	.00	.00	.00	.00
	Maximum	11.50	11.00	15.00	7.00	25.00	24.00
	Range	11.50	11.00	15.00	7.00	25.00	24.00
	N	1220	1311	1228	1117	1276	1247
Total	Mean	.6180	.8842	.6823	.8826	1.7697	.9942
	Median	.0000	1.0000	.0000	1.0000	1.0000	1.0000
	Std. Deviation	.95212	1.18856	1.04267	1.25785	2.27529	1.62135
	Minimum	.00	.00	.00	.00	.00	.00
	Maximum	17.00	38.00	15.00	28.00	41.00	26.00
	Range	17.00	38.00	15.00	28.00	41.00	26.00
	N	3238	5505	3954	5492	5371	5540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C_Ant_IQR
T_Lat_IQR
C_Lat_IQR
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T_DP_IQR
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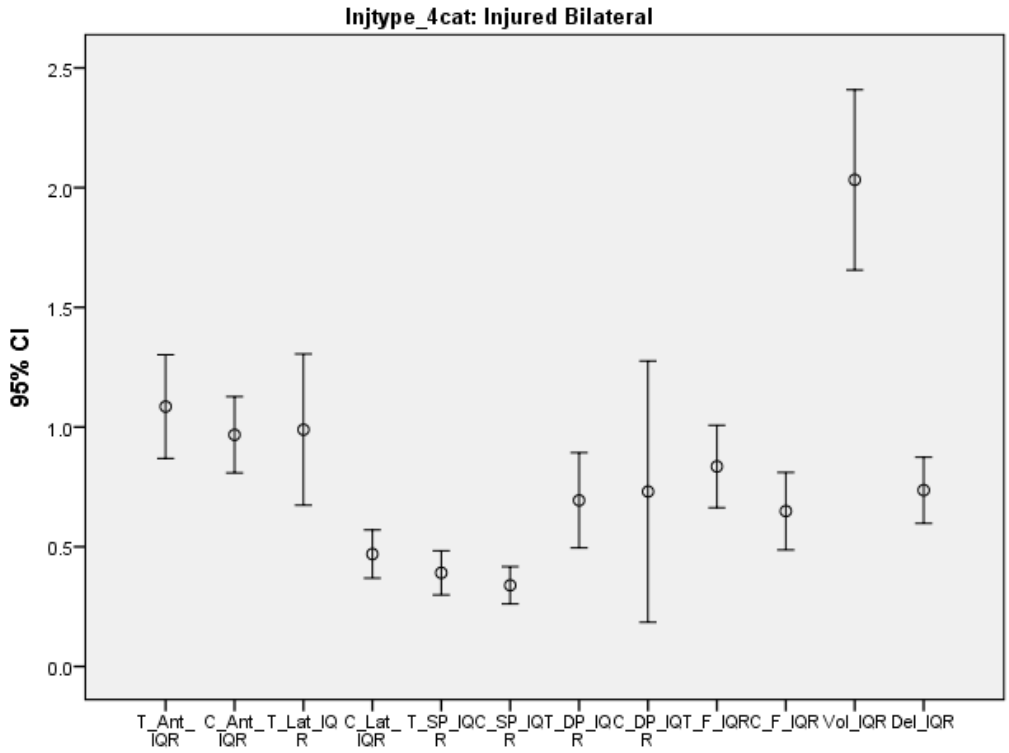
Vol_IQR
Del_IQR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T_Lat_IQR
C_Lat_IQR
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T_F_IQR
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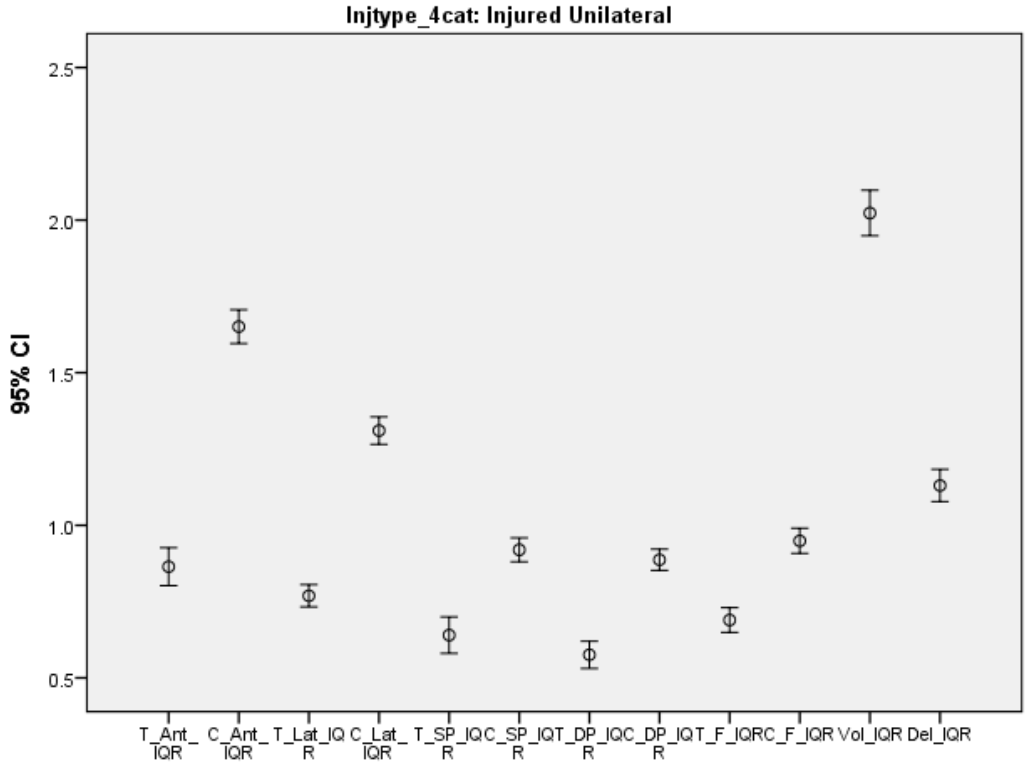
Vol_IQR
Del_IQR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C_Ant_IQR
T_Lat_IQR
C_Lat_IQR
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C_SP_IQR
T_DP_IQR
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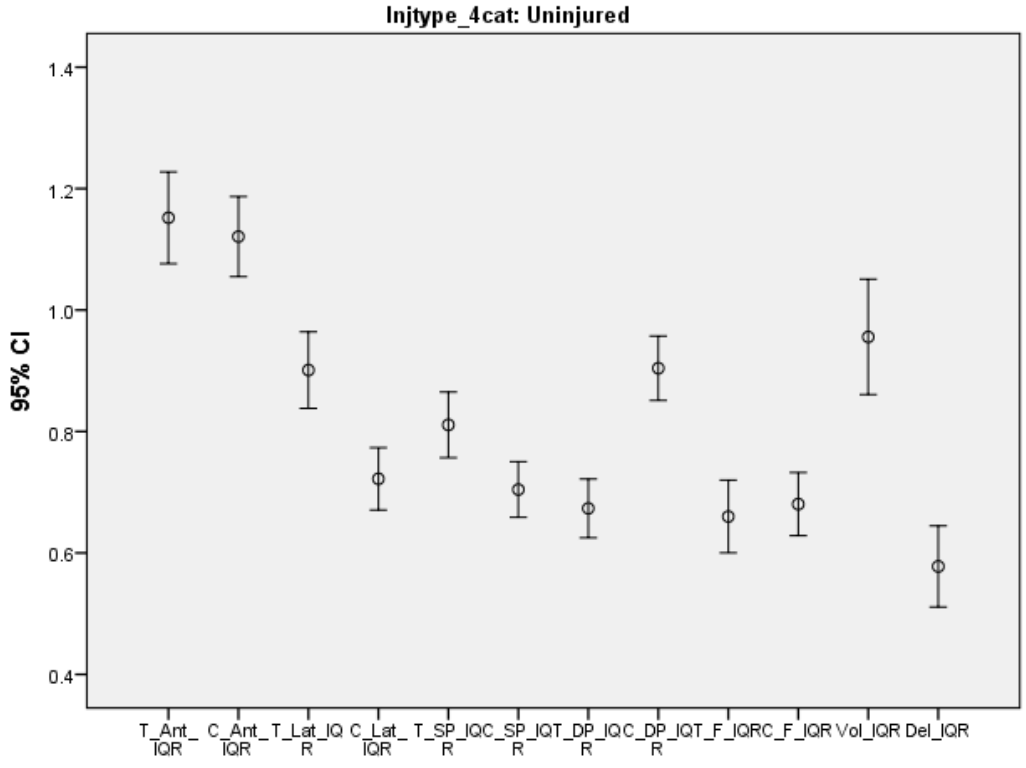
T_F_IQR
C_F_IQR
Vol_IQR
Del_IQR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1.2
1.0
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0.6
0.4
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T_Lat_IQR
C_Lat_IQR
T_SP_IQR
C_SP_IQR
T_DP_IQR
C_DP_IQR
T_F_IQR
C_F_IQR
Vol_IQR
Del_IQR

Appendix B:

Publications produced under this grant by research team:

1. Budsberg SC, Shuler MS, Hansen M, Uhl E, Freedman BA. Evaluation of NIRS, serum biomarkers and muscle damage in a porcine balloon compression model of acute extremity compartment syndrome. J Ortho Trauma – Submitted Sept 2015.

2. Couch L, Roskosky M, Shuler M, Freedman B. Correlation between skin pigment and NIRS values: a comparison of three commercially available devices. **American Journal of Analytical Chemistry.** 2015. 6:911-16.

3. Johnson A, Roskosky M, Shuler M, Freedman B. Depth Penetration of Near Infrared Spectroscopy in the Obese. **Journal of Trauma and Treatment**. (In Press)

4. Kovalenko B, Roskosky M, Shuler M, Freedman B. Effects of Ambient Light on Near Infrared Spectroscopy. **Journal of Trauma and Treatment.** (In Press)

5. Reisman W, Shuler M, Roskosky M, Kinsey T, Freedman B. Use of Near Infrared Spectroscopy to Detect Sustained Hyperaemia Following of Lower Extremity Trauma. **Military Medicine.** (In Press)

6. Roskosky M, Robinson G, Shuler M, Freedman B. Subcutaneous Depth in a Traumatized Lower Extremity. **Journal of Trauma and Acute Care Surgery.** 2014 Sep;77(3 Suppl 2):S190-3. (PMID: 25159354)

7. Cole A, Roskosky M, Shuler M, Freedman B. Near infrared spectroscopy and lower extremity acute compartment syndrome: a review of the literature. **Journal of Trauma and Treatment.** (In-press)

8. Cathcart, CC, MS Shuler, BA Freedman, LR Reno, SC Budsberg. Correlation of near infrared spectroscopy (NIRS) and direct pressure monitoring in an acute porcine compartmental syndrome model. **J Ortho Trauma**. 2014; June 28(6):365-9. (PMID:24857905)

9. Jackson K 2nd, Cole A, Potter BK, Shuler M, Kinsey T, Freedman B. Identification of optimal control compartments for serial near-infrared spectroscopy assessment of lower extremity compartmental perfusion. **J Surg Orthop Adv**. 2013; Spring;22(1):2-9.

10. Reisman WM, Shuler MS, Kinsey TK, Cole AL, Whitesides TE, Davila MG, Smith EK, Moore TJ. Relationship between near infrared spectroscopy and intracompartmental pressures. **J Emerg Med**. 2013; 44(2):292-298. (PMID: 22921857)

11. Harvey EJ, Sanders DW, Shuler MS, Lawendy A, Cole AL, Al Qahtani SM, Schmidt AH. What's new in acute compartment syndrome? **J Orthop Trauma**. Dec, 2012. 26(12):699-702. (PMID: 22913965)

12. Cole AL, Herman RA, Heimlich JB, Ahsan S, Freedman BA, Shuler MS. Ability of near infrared spectroscopy to measure oxygenation in isolated upper extremity compartments. **J Hand Surg**. 2012; 37(2): 297-302. (PMID: 22189186)

13. Cole AL, Smith EK, Austin AV, Freedman BA, Shuler MS. Near Infrared Spectroscopy Monitoring for Compartment Syndrome. **Techniques in Orthopaedics.** 2012; 27(1):15-21.

14. Potter BK, Freedman BA, Shuler MS. Fasciotomy Wound Management and Closure. **Techniques in Orthopaedics.** 2012; 27(1): 62-6.

15. Desai MJ, Shuler MS, Seiler JG. Compartment Syndrome of the Forearm. **Techniques in Orthopaedics.** 2012; 27(1):30-7.

16. Shuler MS, WM Reisman, AL Cole, TE Whitesides, JR, TJ Moore. Near-infrared spectroscopy in acute compartment syndrome: Case report. **Injury**. 2011;42:1506-8. (PMCID:21489528)

17. Shuler MS, WM Reisman, TL Kinsey, TE Whitesides, JR, EM Hammerberg, MG Davila, TJ Moore. Correlation between Muscle Oxygenation and Compartment Pressures in Acute Compartment Syndrome of the Leg. **J Bone Joint Surgery Am**. 2010;92:863-870.

18. Shuler MS, WM Reisman, TE Whitesides, JR, TL Kinsey, EM Hammerberg, MG Davila, TJ Moore. Near Infrared Spectroscopy in Lower Extremity Trauma. **J Bone Joint Surgery Am**. 2009 June; 91(6):1360-68. (PMCID:19487513)

Outside Publications

1. SH Lee, M Padilla, JE Lynch, AR Hargens. Noninvasive Measurements of Pressure for Detecting Compartment Syndromes. *J Orthop Rheumatol*. ; 1(1): 5–20.
2. RM Taylor, MP Sullivan, S Mehta. Acute compartment syndrome: obtaining diagnosis, providing treatment, and minimizing medicolegal risk. Curr Rev Musculoskelet Med(2012) 5:206–213.
3. AG Via, F Oliva, M Spoliti, N Maffulli. Acute compartment syndrome. Muscles, Ligaments and Tendons Journal 18 2015;5 (1):18-22

Book Chapter

1. Shuler MS, Roskosky M, Freedman B. (2015) "Compartment Syndrome." Skeletal Trauma. 5th ed. Ed. Browner B, Jupiter J, Krettek C, Anderson P. Philadelphia: Saunders. 437-463.

Presentations

1. Budsberg SC, Shuler MS, Roskosky M, Uhl E, Hansen M, Freedman BA. Correlation of NIRS and histological muscle damage in a prolonged trauma/infusion model of extremity compartment syndrome (ECS) – assessing NIRS ability to detect the clinical consequence of delayed ECS. **Orthopaedic Trauma Association (OTA) Annual meeting. 2015**, San Diego, CA. (The Orthopaedic Trauma Association (OTA) has invited the "best" ORS Trauma poster to be presented at the OTA annual meeting and on behalf of the ORS Program Committee, I am pleased to inform you that your poster has been selected for this honor".)

2. Budsberg S, Shuler M, Roskosky M, Uhl E, Hansen M, Feedman B. Evaluation Of NIRS, Serum Bipmarker And Muscle Damage In A Porcine Ballroom Compression Model Of ECS. **Orthopaedic Trauma Association**, Oct 2015.
3. Shuler M, Roskosky M, Freedman B. Continual Near Infrared Spectroscopy Monitoring in Acute Compartment Syndrome. **Orthopaedic Trauma Association**, Oct 2015.
4. Shuler M, Roskosky M, Freedman B. Continual Near Infrared Spectroscopy Monitoring in Acute Compartment Syndrome. **Military Health Systems Research Symposium**, Aug 2015.
5. Shuler M, Roskosky M, Freedman B. Continual Near Infrared Spectroscopy Monitoring in Acute Compartment Syndrome. **Southern Orthopedic Association**, July 2015, Ashville NC.
6. Budsberg S, Shuler M, Freedman B, Uhl E, Hansen M. Evaluation of NIRS, serum biomarkers and muscle damage in a porcine balloon compression model of acute compartment syndrome. **Southern Orthopaedic Association,** July 2015, Ashville NC.
7. Budsberg SC, Shuler MS, Roskosky M, Uhl E, Hansen M, Freedman BA. Correlation of NIRS and histological muscle damage in a prolonged trauma/infusion model of extremity compartment syndrome (ECS) – assessing NIRS ability to detect the clinical consequence of delayed ECS. **Southern Orthopaedic Association,** July 2015, Ashville NC.
8. Budsberg S, Shuler M, Feedman B. Assessing NIRS Ability To Detect The Clinical Consequence Of Delayed ECS. **Orthopedic Research Society**, March 2015.
9. Budsberg SC, Shuler MS, Roskosky M, Uhl E, Hansen M, Freedman BA. Correlation of NIRS and histological muscle damage in a prolonged trauma/infusion model of extremity compartment syndrome (ECS) – assessing NIRS ability to detect the clinical consequence of delayed ECS. Abstract # 1916 **Orthopedic Research Society (ORS)** March 2015. Annual Meeting, Las Vegas NV 2015. (Chosen for the Poster Tour at ORS.)
10. Budsberg S, Shuler M, Roskosky M, Uhl E, Hansen M, Feedman B. Evaluation Of NIRS, Serum Bipmarker And Muscle Damage In A Porcine Ballroom Compression Model Of ECS. **Orthopedic Research Society**, March 2015. (**Best Poster Award)**
11. Budsberg SC, Shuler MS, Roskosky M, Uhl E, Hansen M, Freedman BA. Correlation of NIRS and histological muscle damage in a prolonged trauma/infusion model of extremity compartment syndrome (ECS) – assessing NIRS ability to detect the clinical consequence of delayed ECS. **AAOS/OTA/SOMOS/ORS Extremity War Injuries X: Return to Health and Function research symposium**. Washington DC, January 2015. (Chosen for additional podium presentation for placing in the top 5 of all posters.)
12. Reisman W, Cole A, Roskosky M, Shuler M, Andras L, Moore T. Near-Infrared Spectroscopy in the Sub-Acute Setting of Lower Extremity Trauma. **Military Health Systems Research Symposium**, August 2014. (Podium)
13. Budsberg S, Shuler M, Freedman B, Uhl E, Hansen M. Evaluation of NIRS, serum biomarkers and muscle damage in a porcine balloon compression model of acute compartment syndrome. Abstract # 1398. **Orthopedic Research Society (ORS)** 2014 Annual Meeting, New Orleans LA.
14. Budsberg S, Shuler M, Uhl E, Hansen M, Roskosky M, Freedman B. Evaluation of NIRS, serum biomarkers and muscle damage in a porcine balloon compression model of acute compartment syndrome. **AAOS/OTA/SMOS, ORS Meeting - . Extremity War Injuries Symposium: Reducing disability within the military**. February 2014, Washington DC.
15. Roskosky M, Robinson G, Shuler M, Freedman B. Subcutaneous Depth in a Traumatized Lower Extremity. **Society of Military Orthopedic Surgeons 55th Annual Meeting,** December 2013. (Podium)
16. Roskosky M, Robinson G, Shuler M, Freedman B. Subcutaneous Depth in a Traumatized Lower Extremity. **Southern Orthopedic Association 30th Annual Meeting,** July 2013. (Poster)

17. Budsberg S, Shuler M, Freedman B, Hansen M. Evaluation of NIRS, serum biomarkers and muscle damage in a porcine balloon compression model of acute compartment syndrome. **Military Health System Research Symposium (MHSRS)**, August 2013, Fort Lauderdale, Fl
18. Roskosky M, Robinson G, Shuler M, Freedman B. Subcutaneous Depth in a Traumatized Lower Extremity. **Military Health System Research Symposium**, August 2013. (Podium)
19. Freedman B, Shuler M, Cathcart C, Reynolds L, Budsberg S. NIRS versus direct pressure monitoring of acute compartmental syndrome in a porcine model. **Medical Heath Services Research Symposium**, Fort Lauderdale, FL, Aug 13th-16th, 2012.
20. Jackson K, Cole AL, Potter BK, Kinsey TK, Shuler MS, Smith EK, Freedman BA. Identification of optimal control compartments for near-infrared spectroscopy assessment of lower extremity compartmental perfusion. **Orthopedic Trauma Association 2012 Annual Meeting**, October 2012. (Podium presentation)
21. Cathcart C, Shuler M, Freedman B, Reynolds L, Cole A, Whitesides TE, Smith EK, Budsberg S. NIRS vs direct pressure monitoring of acute compartmental syndrome in a porcine model. **Orthopedic Trauma Association 2012 Annual Meeting**, October 2012. (Poster presentation)
22. Cathcart C, Shuler M, Freedman B, Reynolds L, Cole A, Budsberg S, Whitesides TE, Smith EK. Non-invasive NIRS versus invasive direct pressure monitoring of acute compartmental syndrome in a porcine model. **125th Annual Meeting of the American Orthopaedic Association**, Washington D.C., June 27-30, 2012. (Poster presentation)
23. Cole AL, Herman RA, Heimlich JB, Ahsan S, Freedman BA, Shuler MS. Ability of near infrared spectroscopy to isolate muscle compartments of the upper extremity. **125th Annual Meeting of the American Orthopaedic Association**, Washington D.C., June27-30, 2012. (Poster presentation)
24. Cole AL, Herman RA, Heimlich JB, Ahsan S, Shuler MS, Freedman BA. Ability of near infrared spectroscopy to isolate muscle compartments of the upper extremity. **American Academy of Orthopedic Surgeons Annual Meeting**, San Francisco, CA, February 2012. (Paper presentation)
25. Cathcart C, Shuler M, Freedman B, Reynolds L, Cole A, Budsberg S. Non-invasive NIRS versus invasive direct pressure monitoring of acute compartment syndrome in a porcine model. **Orthopedic Research Society**, San Francisco, CA, February 2012.
26. Herman R, Heimlich B, Cole AL, Shuler MS. Ability of near infrared spectroscopy to isolate muscle compartments of the upper extremity. **Ninth Annual American Medical Association Medical Student Section/Resident & Fellow Section Joint Research Symposium**, New Orleans, LA, November 2011.
27. Shuler MS. Symposium 3: Compartment Syndrome: New Technologies: Non-Invasive Compartment Monitoring. **Orthopaedic Trauma Association**, San Antonio, TX, October 2011. (Forum discussion)
28. Shuler MS, Cole AL, Robinson MA, Freedman BA. Comparison of near infrared spectroscopy values between compartments of the lower extremities. **Advanced Technology Applications for Combat Casualty Care 2011 Conference**, Fort Lauderdale, FL, August 2011. (Poster presentation)
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Patents

ALLOWED PATENTS

- **5,425,643-** Method And System For Monitoring Oxygenation Levels Of A Compartment For Detecting Conditions Of A Compartment Syndrome
- **8,639,309-** Method And System For Monitoring Oxygenation Levels Of Compartments And Tissue
- **8,100,834** - Method And System For Monitoring Oxygenation Levels Of A Compartment For Detecting Conditions Of A Compartment Syndrome
- **12,855,019 –** Methods and Dressing System for Promoting Healing of Injured Tissue

PENDING PATENTS

- US 12/855,019- Methods and dressing systems for promoting healing of injured tissue
- US 13/671,861- METHOD AND SYSTEM FOR PROVIDING VERSATILE NIRS SENSORS

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Depth Penetration of Near Infrared Spectroscopy in the Obese

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Abstract

Near-Infrared Spectroscopy (NIRS) measures to a depth of 2 to 3 cm below the skin, raising concern over the utility of NIRS in the obese patient. The purpose of this prospective study is to investigate the effect of overlying adipose tissue thickness (ATT) on NIRS oxygenation measurements of skeletal muscle. ATT was measured by ultrasound. NIRS sensors were placed over the anterior and superficial posterior compartments of one leg during exercise and the change in regional oxygen saturation was calculated for each compartment. There was a decreasing trend in change of rSO_2 from baseline with increasing ATT. Extremely obese patients (BMI >40) had significantly smaller changes in rSO_2 from baseline as compared to otherwise similar patients in both the compartments ($p<0.01$). As ATT increased, the change of the NIRS values from baseline decreased. There was not a specific BMI or ATT determined to be incapable of being monitored.

Keywords: Near infrared spectroscopy; Adipose tissue thickness; Compartment syndrome

Introduction

Clinical diagnosis of Acute Compartment Syndrome (ACS) is customarily made based on clinical symptoms and occurs when increased pressure within a muscle compartment causes muscle ischemia and ultimately death if left untreated [1,2]. If the diagnosis of ACS is uncertain after clinical evaluation, the Intramuscular Pressure (IMP) within the compartment can be measured to identify the area of high pressure [2]. However, the procedure is invasive and can lead to inaccurate values if not performed correctly [3]. Near-infrared spectroscopy (NIRS) allows for continuous, non-invasive measurement of tissue oxygen saturation [3,4]. NIRS uses light transmission and absorption to measure the percentage of hemoglobin saturated with oxygen in the tissue roughly 2 to 3 cm below the skin [3,5]. This technology has the capacity to provide data on oxygen perfusion in an affected compartment. Skeletal muscles also deoxygenate during exercise and NIRS can be used to monitor these metabolic changes as well [6]. The ability of NIRS to measure only to a depth of 2 to 3 cm below the skin has raised concern over its utility in the obese patient [5,7]. Adipose tissue metabolism is lower than muscle metabolism, leading to an inaccurate estimation of muscle oxygen consumption. The subcutaneous adipose tissue layer can fluctuate among individuals and may confound NIRS measurements made in muscles underlying the adipose layer [7]. Prior research has made mention that ATT could impact NIRS values [8,9]. A study was designed to investigate how varying depths of overlying adipose tissue affect the ability of NIRS to measure muscle oxygenation. By measuring the adipose depth in both the anterior and superficial posterior compartments of the leg and then measuring the decrease in tissue oxygenation caused by muscle contraction during exercise in each compartment, the ability of NIRS to measure muscle oxygenation and not adipose tissue was determined. The hypothesis that NIRS values of the activated compartment would decrease significantly from baseline if the adipose depth is less than 2 cm was examined.

Materials and Methods

The study population consisted of 120 uninjured volunteers between 18 and 60 years of age, who provided written informed consent in accordance with institutional review board approval. Exclusion criteria included subjects with a diagnosis of peripheral vascular disease or pulmonary disease, subjects with type I or type II diabetes mellitus, tattoos over the area the NIRS sensors placement, or a prior diagnosis of compartment syndrome. Subjects were categorized according to the National Institutes of Health Classification of Overweight and Obesity by body mass index (BMI). There were 24 subjects in each of the five classifications (<25, 25-29.99, 30-34.99, 35-39.99 and greater than 40 kg/m^2). Potential subjects were randomly screened for eligibility based on their age, height, and weight to ensure equal numbers of subjects within each BMI classification, and gender to ensure equal numbers of males and females [10-12].

Subjects were screened for eligibility based on age, height, and weight. Once enrolled, gender, race, and BMI were recorded. An ultrasound was conducted using a BodyMetrix Professional – BX2000 (IntelaMetrix, Livermore, CA) ultrasound device on the anterior and superficial posterior compartments to measure the adipose tissue depth overlying the muscle groups [5,13-17]. NIRS values were obtained using an Equinox 7600 Oximeter (Nonin Medical, Inc, Plymouth, MN). The sensor used in this study has two sensor depths, which by design allow the superficial depth to be subtracted from the deeper values in order to isolate oxygenation values in the deeper tissue. Values are displayed as the percentage of hemoglobin saturated with oxygen (rSO_2). Consequently, a higher reading indicates a higher tissue oxygenation level. The device was calibrated during manufacturing and did not require recalibration before each use. The NIRS sensors were placed over the middle one-third of the tibia for the anterior and superficial posterior compartments of the leg (directly posterior). The anterior compartment was located by palpating the anterior tibial ridge and placing the sensor lateral approximately 2 cm [5,13-17]. The superficial posterior compartment measurement was located by placing

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the sensor directly posterior. Based on adipose tissue distribution, the largest deposit of subcutaneous adipose tissue is located over the superficial posterior compartment. The least amount of subcutaneous tissue is located over the anterior compartment. Therefore, the anterior compartment would be used as an internal control for the posterior compartment. Additionally, each of these compartments is easily isolated through specific and simple exercises. The deep posterior and lateral compartments were not monitored in this study.

The sensors were applied to the leg and rSO_2 was monitored for approximately thirty to sixty seconds to obtain a stable reading to serve as a baseline measurement for each compartment. In order to isolate the compartments, subjects were asked to perform specific exercises intended to activate only the desired muscle group. The exercises were performed with the subject sitting with the legs extended on an exam table. A 6-foot length special heavy resistance exercise band (Thera-Band, Akron, OH) was folded once and used to provide resistance to enhance muscle activation. The participant performed each exercise for 30 to 60 seconds followed by a period of about 60 seconds of rest to allow the rSO_2 values to return to baseline. To activate the anterior compartment, the exercise band was placed around the dorsal aspect of the toes and pulled about two feet away from the feet of the subject. Subjects were instructed to quickly pump the foot in a dorsiflexed position (pull their toes towards their nose) to isolate the anterior compartment. The exercise was repeated until the subject fatigued or the time of exercising reached one minute. The superficial posterior compartment was activated by placing the exercise band around the plantar aspect of the toes and then pulling the exercise band to the knee of the patient. Subjects were instructed to quickly pump the foot in a plantar flexed motion (similar to a calf raise against resistance) against the resistance to isolate the superficial posterior compartment muscle group. The exercise was again repeated until the subject fatigued or the time of exercising reached one minute. For each exercise the lowest NIRS measurement reached during activation and the duration of exercise were recorded.

Statistical analysis

The change in rSO_2 was calculated separately for each compartment based on the difference between NIRS values pre and post exercise. Significance of this pre- and post-test difference in rSO_2 was tested using ANOVA. BMI was calculated using the following equation (weight (lbs.) / 2.20462262) / (Height (in) / 39.3700787)^2. The change in rSO_2 was plotted across increasing BMI and fat-depth. Subjects were divided into BMI groups of <25, 25-30, 30-35, 35-40 and >40. Fat-depth categories were <5 mm, 5-10 mm, 10-15 mm and >15 mm. ANOVA testing with Bonferroni correction for multiple comparisons was used to test whether the mean change in rSO_2 differed significantly by BMI group or fat-depth category. Pearson correlation was calculated for comparing rSO_2 and ATT. All statistical calculations were performed using STATA statistical software version 12.

Results

One hundred twenty adult volunteers were recruited to participate with ages ranging from 18 to 60 (mean: 39.4) years (Table 1). There were 60 male and 60 female patients. The majority of patients were Caucasian (approximately 86%). No significant trends were found based on age or race. Males were found to show a greater change in rSO_2 after exercise for both the anterior and poster compartments (p=0.000). Subjects were asked to perform the exercises until fatigue. On average patients completed 49.4 (range: 14-60) seconds of exercise for superficial posterior activation and 36.3 (range: 13-67) seconds for anterior activation.

Characteristics	Mean (SD)	
Age	39.38 (12.99)	
Gender	Male (N=60)	Female (N=60)
BMI	32.81 (6.92)	32.38 (8.94)
Anterior Fat Depth	4.12 (1.54)	7.05 (3.03)
Posterior Fat Depth	5.65 (2.63)	10.00 (5

Table 1: Subject characteristics.

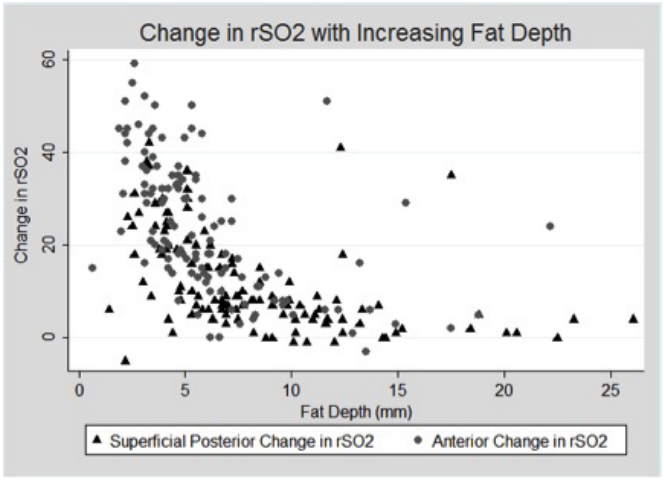

Figure 1: Change in rSO_2 with increasing adipose tissue thickness. As the depth of fat increases the average change in muscle oxygenation after exercise decreases (r=0.5105; p=0.000). Outliers are generally males, with BMI greater than 34 kg/m^2.

Anterior Compartment rSO_2			
F-Stat			0.000
FD Group	**Comparison FD Group**	**Mean difference in change in** rSO_2	**P-value**
<5 mm	5-10 mm	-13.885*	0.000
	>10 mm	-20.477*	0.000
5-10 mm	>10 mm	-6.592	0.252
* The mean difference is significant at the 0.05 Level			

Table 2: Fat-depth category rSO_2 comparison – anterior compartment.

Superficial Posterior Compartment rSO_2			
F-Stat			0.000
FD Group	**Comparison FD Group**	**Mean difference in change in** rSO_2	**P-value**
<5 mm	5-10 mm	-9.480*	0.000
	>10 mm	-14.819*	0.000
5-10 mm	>10 mm	-5.339*	0.026
* The mean difference is significant at the 0.05 Level			

Table 3: Fat-depth Category rSO_2 Comparison – Superficial Posterior Compartment.

The change in rSO_2 as fat-depth increases can be seen in Figure 1. Both anterior (r=-0.5312) and posterior (r=-0.5105) changes in NIRS values after exercise show a negative correlation with moderate magnitude. Tables 1-4 show the results of the ANOVA analysis. No statistically significant difference was found between fat-depth and NIRS values after 1 cm of subcutaneous fat. However, a statistically significant drop in the average change in rSO_2 was seen when the subject's subcutaneous-depth was greater than 5 mm in the anterior compartment (Table 2). In the superficial compartment, a significant drop in NIRS values was seen when fat-depth increased from <5 mm to

5-10 mm (-9.480; p=0.000) and again when greater than 1 cm (-5.339; p<0.05) as seen in Table 3. Change in NIRS values after exercise in subjects with BMI greater than 40 kg/m^2 differed significantly than those with BMI less than 25 kg/m^2 in the superficial posterior compartment (-9.25, p<0.05; Table 4) and was also significantly less in those less than 30 kg/m^2 in the anterior compartment (<25: -15.583, p<0.01; 25-30: -13.083, p<0.05; Table 5).

Discussion

Near infrared spectroscopy has previously been validated to measure tissue oxygenation in humans [10,11] and further described by several studies as a method of correlating muscle oxygenation to compartment pressures in acute compartment syndrome of the leg [5,12-16]. It has also been shown that NIRS has the ability to isolate among specific muscle groups with proper sensor placement over individual compartments [17]. The displayed NIRS values are unique for each compartment; contracted compartments have decreasing NIRS values while compartments at rest show little or no change [17]. However, previous studies do not address the concern about the efficacy of the device's penetration depth. The ability of NIRS to be used in obese patients is still unknown [9].

It was expected that the change in rSO_2 for the obese groups would be smaller than the changes in the normal and overweight groups based upon physiologic differences. More lean muscle enables better stimulation of the muscle compartment and further lowers rSO_2 from baseline. The ability to elicit a stronger contraction increases energy costs and lowers oxygen saturation values [18]. Also, intense exercise training increases mitochondrial gene expression [19], leading to a metabolic advantage in leaner and more trained individuals which would be expected to be found in lower BMI subjects. This finding possibly influences the data to show lower rSO_2 levels in the subjects with less adipose tissue or lower BMI. Additionally, subjects in the Class II and Class III obesity groups likely do not have the effects of increased mitochondrial expression attributed to training, contributing to the expected decrease in observed change of NIRS values from baseline in these groups. Therefore, obese subject would not be expected to have as great of a drop in NIRS values due to their inability to perform exercises efficiently nor extract the supplied oxygen effectively. This trend was observed in this study. The more obese the subjects, the less the drop in NIRS values was observed. The reduced effect of exercise in the obese could be explained in part by the inability of NIRS to measure changes in muscle in the obese. While this factor may play a partial role in the observed results, a complete failure of NIRS to monitor muscle oxygenation would result in no change in rSO_2 values. If measurements came from solely subcutaneous fat, not change (decrease in rSO_2) would be seen in these subjects as subcutaneous fat does not play a role in exercise. The fact that in all subjects, a decrease in NIRS values was recorded with exercise indicates the NIRS device was monitoring muscle below the subcutaneous fat at least in part.

Anterior Compartment rSO_2			
F-Stat			0.0007
BMI Group	**Comparison BMI Group**	**Mean difference in change in rSO_2**	**P-value**
<25 kg/m^2	25-30 kg/m^2	2.500	1.000
	30-35 kg/m^2	-5.667	1.000
	35-40 kg/m^2	-10.667	0.072
	>40 kg/m^2	-15.583*	0.001
25-30 kg/m^2	30-35 kg/m^2	-3.167	1.000
	35-40 kg/m^2	-8.167	0.384
	>40 kg/m^2	-13.083*	0.011
30-35 kg/m^2	35-40 kg/m^2	-5.000	1.000
	>40 kg/m^2	-9.917	0.123
35-40 kg/m^2	>40 kg/m^2	-4.917	1.000
* The mean difference is significant at the 0.05 Level			

Table 4: BMI category rSO_2 comparison – anterior compartment.

Superficial Posterior Compartment rSO_2			
F-Stat			0.0096
BMI Group	**Comparison BMI Group**	**Mean difference in change in rSO_2**	**P-value**
<25 kg/m^2	25-30 kg/m^2	2.125	1.000
	30-35 kg/m^2	-3.917	1.000
	35-40 kg/m^2	-7.958	0.078
	>40 kg/m^2	-9.25*	0.021
25-30 kg/m^2	30-35 kg/m^2	-1.792	1.000
	35-40 kg/m^2	-5.833	0.494
	>40 kg/m^2	-7.125	0.168
30-35 kg/m^2	35-40 kg/m^2	-4.042	1.000
	>40 kg/m^2	-5.333	0.720
35-40 kg/m^2	>40 kg/m^2	-1.292	1.000
* The mean difference is significant at the 0.05 level			

Table 5: BMI category rSO_2 comparison– superficial posterior compartment.

The group with the lowest BMI has distinct physiologic characteristics hindering their use as the reference standard in the study. Tanner et al. found that leaner muscle has more type I (slow-twitch) fibers and tends to be oxidative and vascularized [20] while Gavin et al. found that obese muscle has a lower capillary density than lean skeletal muscle [21]. Capillary density and muscle fiber recruitment can contribute to deoxygenation levels and affect NIRS readings [18,22]. Subjects with a BMI of 25-30 have less lean muscle (along with fewer of the corresponding physiological changes) and therefore have less of a drastic impact on the NIRS readings, enabling their use as a reference standard to best represent the general population. Despite an attempt to recruit extremely obese subjects, 95% of the study population had a fat depth of less than 2 cm in either compartment. This finding suggests that having a depth of adipose tissue greater than 2 cm is very rare. The deposition of fat in the lower leg region is quite low in humans; therefore, demonstrating the lower leg is an ideal location to monitor muscle perfusion. The NIRS device displayed rSO_2 changes at increasing fat depths beyond 2 cm indicating the ability of NIRS to measure muscle rSO_2 changes despite the extreme amounts of subcutaneous fat that was specifically sought and selected for in this study. This type of subject is not typically found in the general population.

Figure 1 shows a moderate correlation between ATT and the change in rSO_2 from baseline following exercise (r= -0.5105). As expected, there was a trend showing that as ATT increased, the change in rSO_2 from baseline decreased. Increasing ATT was expected to correlate with less muscle training. These results are statistically significant and can be seen in Tables 1 and 2. A mean decrease in change of approximately 9.5 percentage points in rSO_2 can be seen when subjects have <5 mm of adipose tissue versus 5-10 mm, and decreases an average 5.3 points above 10 mm in the superficial posterior compartment. There was also a decrease in change of rSO_2 from baseline with increasing BMI. This difference became statistically significant between the overweight group (BMI between 25 and 30) and the extremely obese group (BMI >40) in the anterior compartment (p<0.05), as well as between the normal BMI and extremely obese groups in both compartments (p<0.05). The study also found that the baseline NIRS reading is not a predictor

of ATT and cannot be used to assess the utility of NIRS in a given patient. Baseline values varied across all thicknesses of adipose tissue in both compartments studied. There are some limitations to this study. While the study did stratify age and gender, there still could be innate physiological differences in the BMI groups that contributed to the data that were not accounted for, such as varying amounts of myoglobin and hemoglobin in skeletal muscle [19,22] or differences in vascular supply [23]. Individuals may have inherent differences in muscle fiber types, such as in aging [24], and humans have a vast blend of muscle fiber types within a given muscle group [20]. However, the study recruited a similar age distribution and mean age for each BMI category. The group factors of gender and race/ethnicity were not tested for differences and would be of interest in the future.

The inherent error of the BodyMetrix BX-2000 (IntelaMetrix Inc., Livermore, CA) used to measure the ATT is ± 3.5%. Although this is an acceptable range of error, this could influence the range of the data and cause the measured adipose thickness to be less than the actual value. There are several different NIRS devices and while this specific device (Nonin Medical, Inc) detected changes up to 2 cm and beyond, this finding may not be able to be extrapolated to other manufactures based on specific sensor configurations and settings. This study was performed in uninjured subjects which do not correlate to the acute injury setting. However, in the acute injury setting, subcutaneous fat depths have been shown to be reduced, not increased, and the swelling occurs within the compartment itself and not in the subcutaneous tissue [25]. Further research is needed to examine muscle perfusion and NIRS values in the traumatized population in a longitudinal fashion. Additionally, guidelines need to be established for normal and abnormal perfusion in the injured extremity on a continual basis in order to use NIRS as a diagnostic tool for ACS. In summary, the purpose of this study was to determine if NIRS was capable of monitoring rSO_2, muscle oxygenation, in the general population as well as in obese subjects. First, this study found few people with an ATT of over 2 cm in either the anterior or superficial compartment, even among the Class II and Class III obesity groups indication subcutaneous fat deposits in this region of the body remain quite shallow despite extremely obese subjects being selected in this study. Second, despite specifically selecting an unnatural population of extremely obese subjects, NIRS still recorded decreased rSO_2 values with exercise indicating the ability of this specific NIRS device to monitor muscle perfusion in the most extreme patient population. These findings indicate that NIRS is capable of monitoring muscle perfusion in not only the general population, but also in the extremely obese subjects that occur quite rarely in the general population. Although the change in NIRS readings was significantly smaller in patients with >40 kg/m^2 BMI compared to other groups, even extremely obese subjects registered substantial changes in NIRS values during exercise. These two results indicate NIRS is not only useful in the general population where obesity is distributed in a more standard distribution, but it also recorded changes in a purposefully manipulated subset of the extremely obese population. Additionally, specifically the lower leg does not have significant depositions of subcutaneous fat depositions indicating the lower leg is an ideal location for monitoring muscle perfusion.

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Effect of Skin Pigmentation on Near Infrared Spectroscopy

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Abstract

The purpose of this study was to determine the effects of skin pigmentation regarding Near Infrared Spectroscopy (NIRS) tissue oxygen saturation values (StO_2). The study examined NIRS values in individuals with varying skin pigmentation on the anterior compartment of the lower leg and volar forearm to determine if correlation exists among three NIRS devices, the EQUANOX, Casmed, and INVOS. Skin pigmentation was measured on the anterior lower leg (AL) and volar forearm (VF) of participants using a noninvasive colorimeter that employed reflective spectroscopy to produce a quantitative value for erythema (skin "redness") and melanin (skin pigment). Muscle oxygenation was measured using three oximetry devices with sensors placed in the same areas. The EQUANOX device showed no significant correlation with skin pigmentation, while the Casmed and INVOS devices showed moderate and significant correlation with skin pigmentation, respectively. Different devices have different abilities to remove confounding variables, such as skin pigmentation and erythema, which may affect clinical decision-making, and affect the use of NIRS technology.

Keywords

Near Infrared Spectroscopy, Skin Chromophores, Confounding Factors, Variability between Manufacturers

1. Introduction

Near-Infrared Spectroscopy (NIRS) is a useful technology that allows for noninvasive measurement of percentage of oxygenated hemoglobin as well as local blood flow and oxygen consumption [1] [2]. NIRS devices have

three primary components: 1) A light source; 2) An optode to detect unabsorbed light; 3) A computer to perform calculations related to absorption based on diffusion theory (Beer-Lambert Law). This theory correlates absorbance to the extinction coefficient, path length, and concentration of light absorbers in the sample. Devices used in medicine typically consist of a sensor pad, containing both the light source and optode, connected by wire to a nearby computer. The sensor pad is typically coated with an adhesive and may be placed superficially on the skin. Devices utilize two or more wavelengths of light with sensors in the pad to detect the absorption of each. The depth of penetration of the light is roughly half of the distance between the light source and the sensor. Melanin has also been shown to impair light penetration and reported rSO_2 values [3]-[5].

The values reported by NIRS indicate the relative oxygenation of hemoglobin under the sensor and is mostly confined to the microcirculation as larger vessels absorb the light completely. The microcirculation consists of arterioles, capillaries, and venules. Because roughly sixty percent of blood volume is found in the venous system, values tend to be lower and reflect hemoglobin that has off-loaded it's oxygen to tissues in contrast to pulse-ox monitoring which reflects the saturation of arterial blood.

NIRS has found application in medicine and several NIRS devices are currently FDA approved for monitoring cerebral perfusion. Typical clinical settings where this could prove useful include those undergoing cardiac surgery, or who are at risk of systemic shock and resulting cerebral hypoperfusion. An additional utilization of the technology is its application in the detection and diagnosis of acute compartment syndrome.

Gianotti *et al*. found that NIRS StO_2 values were significantly lower than control values in limb compartments of trauma patients with compartment syndrome [6]. Shuler *et al*. have demonstrated that NIRS values decrease significantly with decreasing lower limb perfusion pressures in patients with lower limb trauma [7]. Studies have also shown differences in NIRS values between injured vs. non-injured limbs. A non-injured contralateral limb may serve as a control in evaluating and detecting a possible compartment syndrome [3]. Because skin pigmentation is a factor known to confound NIRS values, it is vital to understand its effects on device measurements. Wassenaar & Van den Brand demonstrated a relationship between dark skin color and loss of signal in NIRS devices in 2005 [5].

This study seeks to determine skin pigmentation's effects on NIRS readings. NIRS has the potential to provide non-invasive, real time data to the clinician to aid them in the diagnosis and subsequent treatment of compartment syndrome. Because skin pigmentation varies so widely among individuals, it is vital to understand and to account for these differences as well as determine differences between technologies before the data can be integrated into the decision making process. The hypothesis is that there is there are no differences between technologies for skin pigment.

2. Materials and Methods

2.1. Study Participants

Approval for the study was received from the local Institutional Review Board. Enrollment came from a clinical patient population between the dates of May 20, 2013 and May 28, 2014. Participants were otherwise healthy and excluded if they were under the age of 18, over 65, or pregnant.

Participants were patients, screened and recruited during regular clinical visits. Eligible subjects were males and non-pregnant females between the ages of 18 and 65 who were able and willing to participate. Data on age, race, body mass index (BMI), and gender were collected.

2.2. Measuring Skin Color

Once patients were deemed eligible and went through the informed consent process, their skin pigmentation was measured on the anterior compartment of the lower leg (AL) and volar forearm (VF) using the Cortex Industries DSM-II (Cortex Technology ApS, Denmark). The DSM_II is a noninvasive colorimeter that employs reflective spectroscopy to produce a quantitative value for erythema (skin redness) and melanin (skin pigment). Participants were in the seated position as measurements were recorded. Three measurements were obtained over each compartment and averaged for each subject.

2.3. Measuring Muscle Oxygenation

Following determination of pigmentation, muscle oxygenation was measured using three oximetry devices with

sensors placed in the same position. This study employed the INVOS5100C (Somanetics, Troy, MI), EQUANOXTM Model 7600 (Nonin Medical Inc., Plymouth, MN), and the CASMED MC-2030C (CASMED, Branford, CT). Sensors were placed over the same area where pigmentation was measured. Data was recorded following 4 cycles of a stable value. The INVOS cycles every 6 seconds, versus every 1.5 seconds for the EQUANOXTM and CASMED machines. Oxygenation values were obtained after approximately 60 seconds with each machine. Again, subjects were in the seated position.

2.4. Data Analysis

Final skin color measures for each patient were the average of the three recorded measurements for each variable (melanin, erythema, red, green, and blue). Correlations were calculated using Pearson's correlation coefficients and associated p-values. Means testing was conducted using ANOVA. All calculations were performed using STATA statistical software.

3. Results

Over a two-month period, 196 subjects agreed to participate in the study. The characteristics of the study population can be seen in **Table 1**.

The colorimeter was able to detect differences in mean skin color measures (mealanin, erythema, red, blue and green colors) by race for both the anterior leg and volar forearm compartments as seen in **Table 2**. The correlations between mean skin pigmentation measures (melanin and erythema) were analyzed by ethnicity and anatomical location in **Table 3**. In both the anterior leg and volar forearm compartments there was a clear trend

Table 1. Subject demographic characteristics.

Characteristic	N = 196	
	Mean (SD)	Range
Age (years)	45.70 (14.10)	18 - 65
BMI (kg/m^2)	30.14 (6.94)	17.85 - 50.21
Gender	Males (%)	Females (%)
	94 (47.96)	102 (52.04)
Race/Ethnicity	Frequency	Percent
Caucasian	139	70.92
African-American	42	21.43
Hispanic	12	6.12
Asian	2	1.02
Native American	1	0.51

Table 2. Differences in skin color between races.

	Hispanic (12)	Caucasian	African-American	Asian
Caucasian (140)	a, b, c, d, e, f, g, h, i, j			
African-American (41)	a, b, e, f, h, j	a, b, c, d, e, f, g, h, i, j		
Asian (2)	-	-	*b, f, h	
Native American (1)	*c, e, g, h, i, j	-	*a, b, c, d, e, f, g, h, i, j	-

Significant differences are indicated by letters in each cell. Each letter corresponds to a different measure as follows: [a]Melanin (Anterior), [b]Melanin (Volar), [c]Erythema (Anterior), [d]Erythema (Volar), [e]Red (Anterior), [f]Red (Volar), [g]Green (Anterior), [h]Green (Volar), [i]Blue (Anterior), [j]Blue (Volar). A dash line indicates that no significant differences were found. Numbers in parentheses represent sample size for each race. Sample sizes for Asian and Native American participants are too small for definitive conclusions (*).

Table 3. (a) Summary statistics of skin color by race (anterior leg compartment); (b) Summary statistics of skin color by race (volar forearm compartment).

(a)

Race/Ethnicity [N]	Melanin	Erythema	Red	Green	Blue
Caucasian [139]					
Mean (SD)	41.87 (7.05)	12.78 (3.09)	98.49 (15.49)	74.18 (15.88)	70.83 (17.83)
Range	29.31 - 61.02	7.51 - 19.85	62.67 - 130.00	40.33 - 108.33	36.67 - 111.00
African-American [42]					
Mean (sd)	68.31 (13.38)	17.35 (1.44)	55.00 (15.78)	37.36 (11.40)	34.31 (15.26)
Range	47.36 - 95.53	14.72 - 20.14	28.33 - 85.67	19.33 - 61.33	18.00 - 106.33
Hispanic [12]					
Mean (sd)	56.33 (7.28)	15.94 (2.20)	71.97 (12.21)	50.17 (10.72)	44.58 (12.15)
Range	42.00 - 67.60	11.37 - 18.44	54.00 - 97.00	35.33 - 74.67	30.67 - 72.00

(b)

Race/Ethnicity [N]	Melanin	Erythema	Red	Green	Blue
Caucasian [139]					
Mean (SD)	42.15 (5.38)	14.73 (3.03)	97.78 (10.98)	70.35 (12.12)	65.51 (13.36)
Range	30.07 - 59.77	6.88 - 22.28	65.67 - 127.67	39.67 - 109.00	35.00 - 108.00
African-American [42]					
Mean (sd)	67.72 (10.44)	19.28 (1.92)	55.18 (13.17)	35.69 (9.99)	29.47 (8.67)
Range	48.59 - 90.21	14.34 - 22.55	30.67 - 83.67	19.33 - 60.00	16.67 - 50.33
Hispanic [12]					
Mean (sd)	52.10 (8.57)	17.35 (2.86)	78.14 (14.30)	52.94 (12.40)	46.06 (12.02)
Range	41.61 - 69.88	13.49 - 22.55	50.67 - 97.66	32.00 - 71.67	26.00 - 65.00

in both melanin and erythema with changes in skin color. Caucasians had the lowest values followed by Hispanic and African American participants.

Oximetry values (rSO_2) were recorded for each device and correlated against the five measures of skin color (**Table 4(a)**, **Table 4(b)**). The EQUANOXTM device showed no significant correlation with skin pigmentation, while the Casmed and INVOS devices showed moderate and significant correlation with skin pigmentation, respectively. **Table 5** shows correlation of rSO_2 between devices by location. There was a moderate degree of correlation seen, with the highest being between the INVOS and Casmed readings over the anterior leg compartment ($r = 0.6948$).

4. Discussion

NIRS has potential application in a variety of clinical settings. Broadly, oximetry monitoring could be utilized to detect patients who are deteriorating rapidly. Such states could include systemic shock, neurologic problems, or the development of a compartment syndrome.

In each of these examples, NIRS provides the clinician a means to monitor the patient in a continuous, non-invasive manner that also has the value of being in real time. However, there are several variables that have potential to affect the accuracy of reported values. Trauma, subcutaneous adipose tissue, and skin pigmentation are three factors that vary among patients [3] [8] [9]. In addition, manufacturers of NIRS devices use proprietary algorithms and different wavelengths of light in determining stO_2 values. This study sought to determine if skin

Table 4. (a) Correlation of rSO_2 values and skin color by device (anterior leg compartment); (b) Correlation of rSO_2 values and skin color by device (volar forearm compartment).

(a)

Device	Melanin	Erythema	Red	Green	Blue
INVOS	−0.4281**	−0.3647**	0.4262**	0.4230**	0.3970**
EQUANOX	−0.0574	0.0092	0.0259	0.0114	−0.0011
CASMED	−0.3514**	−0.2010**	0.3075**	0.2866**	0.2458**

(b)

Device	Melanin	Erythema	Red	Green	Blue
INVOS	−0.4900**	−0.4446**	0.5044**	0.5079**	0.4971**
EQUANOX	−0.1342	−0.0645	0.1576	0.1406	0.1348
CASMED	−0.1376	−0.1456*	0.1329	0.1489*	0.1323

*significant at $\alpha = 0.05$; **significant at $\alpha = 0.01$.

Table 5. (a) Correlation of rSO_2 values between devices (anterior leg compartment); (b) Correlation of rSO_2 values between devices (volar forearm compartment).

(a)

	INVOS	EQUANOX
EQUANOX	0.6727	
CASMED	0.6948	0.5210

(b)

	INVOS	EQUANOX
EQUANOX	0.5865	
CASMED	0.6107	0.4280

pigmentation correlated with reported values between devices made by three different manufacturers. This study sought to answer this question by quantitatively measuring skin pigmentation and erythema prior to measuring stO_2 values with the three devices.

The findings in this study demonstrate that all technologies are not created equally. Different technologies have different algorithms which have different capabilities to remove pigment and erythema from a reading. In this study, the Equinox device was least affected by variations in pigment or erythema. Of note, all devices were moderately well correlated and indicated all were reading a similar variable (tissue oxygenation).

There were several limitations in this study. A broad spectrum of ethnicities was sought to reflect differences in skin pigmentation. In actuality however, there was an under representation of intermediate skin tones. Roughly 25% of our population sample was non-Caucasian. Future studies could include more participants with intermediate skin tones. Additionally, volunteers were not traumatized and these findings may not completely translate to a traumatized setting. Erythema especially may or may not affect values of NIRS in a traumatized setting.

In summary, this study shows that NIRS manufacturers all show reasonable correlation to tissue perfusion; however, some devices are more capable of removing confounding variables such as skin pigmentation. If controls on the same subject are used, this variability should cancel each other out assuming a similar pigmentation profile at the two sites. In cases where small variations or no control is use, these differences may have significant effects on clinical decision-making.

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Near infrared spectroscopy and lower extremity acute compartment syndrome: a review of the literature

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INTRODUCTION

Similar to pulse-oximetry, near infrared spectroscopy (NIRS) uses the proportionate differential in reflection and absorption of different wavelengths of light (Beer-Lambert Law) to estimate the proportion of hemoglobin saturated with oxygen approximately 2-3 cm below the sensor[1-4]. The depth of readings is based on the separation between the light source(s) and the receptor(s); the farther the receptor and source are separated, the deeper the arc of penetration. With NIRS, light travels in a banana shaped pathway coming out on the same side of the tissue in order to sample deep tissue without the need of traveling straight through an extremity. This effect allows NIRS to be used on a broader array of tissues other than just fingers, toes and ear lobes as in the case of pulse-oximetry. This technology is currently FDA approved for the non-invasive, continuous monitoring of cerebral and somatic tissue regional perfusion. This makes NIRS an ideal technology to consider when seeking a best means for diagnosing acute compartment syndrome (ACS), which is a condition that is physiologically defined as a regional hypoperfused/ischemic state due to excessive pressure within a muscle compartment.

Recent advancements in devices using NIRS technology have made them better suited for use in patients at risk for ACS. Newer devices utilize standardized calibration settings, so timely calibration prior to each use is unnecessary. Smaller, flatter sensors provide easier application and are more maneuverable around dressings and splints. Deeper penetration of light (2-3 cm) permits measurement of oxygen saturation in anatomic locations which were previously much more difficult to isolate. Additionally, by utilizing multiple wavelengths, some NIRS devices have been able to minimize the effects of skin pigment on NIRS values.

Over the past decade, studies using NIRS have consistently supported its use as a diagnostic tool for ACS. While certain clinical factors still require further research, these studies, combined with the recent advancements in NIRS-based monitoring, make NIRS a promising candidate for the next generation of diagnostic tools for ACS.

DISCUSSION

Over the last 20 years ongoing research in both animal models and human participants has reinforced the usefulness of this technology in the trauma setting. The series of support begins with validation of its ability to identify changes in perfusion pressure in

the leg[5] and then, more specifically, ischemic state in a porcine model[6]. This laid the groundwork for validation in humans[7], superiority over perfusion pressure[8] and establishing normal NIRS values[9]. We have now entered a stage of identifying potential sources of error in the technique and responding with relevant research[10,11].

In 1999, Garr et al induced compartment syndrome in 9 anesthetized landrace swine to test the hypothesis that NIRS was a superior predictor of neuromuscular dysfunction than intra-compartment pressure[5]. Both hind legs of each pig were monitored using pressure transducers to record intra-compartment pressures and a Hutchinson NIRS probe (Hutchinson, Minnesota) to measure regional oxyhemoglobin saturation (rSO_2). Compartment syndrome was induced via albumin infusion. A nerve stimulator attached to the peroneal nerve caused dorsiflexion of the lower limb, and compartment syndrome was defined as complete loss of twitch. Perfusion pressure (PP) was calculated as mean arterial pressure (MAP) minus intra-compartmental pressure (ICP). Loss of dorsiflexion was observed at mean compartment pressures of 43.1 mmHg, mean PP of 13.6mmHg, and mean rSO_2 of 19.8%. Within 10 minutes of fasciotomy, pressures and rSO_2 values returned to 75% of baseline values. The authors found that rSO_2 values were significantly correlated with both ICP and PP, suggesting that NIRS can correctly identify changes in perfusion in a leg with compartment syndrome. Incidentally, this study also demonstrates that NIRS may be able to detect the appropriateness of a fasciotomy, as there was a significant increase in rSO2 following successful fasciotomy in the state of critical hypoperfusion with resultant neuromuscular dysfunction (i.e. ACS).

Arbabi and colleagues tested the ability of NIRS to differentiate between hypotension / hypoxia and ACS in a follow-up study, using the same compartment syndrome model and NIRS device described previously[6]. Hypotension (MAP at 60% of baseline) was induced in 9 landrace swine for 30 minutes, followed by the addition of hypoxia for 30 minutes, after which compartment syndrome was added by incrementally increasing compartment pressures with albumin infusion until a compartment syndrome ensued (loss of dorsiflexion twitch). Fasciotomies were performed and measurements were repeated.

All conditions (hypotension, hypotension + hypoxemia, hypotension + hypoxemia + compartment syndrome) produced significant decreases in rSO_2 values from baseline; however, mean rSO_2 values during compartment syndrome in hypotensive, hypoxemic animals were significantly lower, compared to values observed during hypotension + hypoxemia without ACS($p<0.0001$), as well as hypotension alone ($p<0.0001$). The authors found that hypotension and hypoxia did result in small decreases in NIRS values; however, ACS was easily differentiated and had a significantly more profound effect on NIRS values. Compared to mean rSO_2 values of the control leg, values of the test leg only differed significantly once compartment syndrome was induced ($p=0.0002$). This study suggests that NIRS is capable of discriminating between regional ischemic changes caused by compartment syndrome and global changes observed in a severe shock state. Additionally, this study shows the potential value of a control site, which ideally is the contra-lateral like compartment, to differentiate the two conditions in an injured subject. Control leg values when compared in both uninjured and injured subjects have shown

high correlation.[9,12,13] Injured extremities typically demonstrate elevated values compared to uninjured contralateral extremities. With the development of ACS, the increase seen in injured extremities is diminished or falls below the control indicating impaired perfusion.[9,13]

In a 2000 study, Giannotti et al. used NIRS to monitor 9 trauma patients with a clinical diagnosis of lower extremity ACS, as well as 33 randomly selected trauma patients without evidence of ACS[7]. From the 33 control patients, 9 patients were matched based on injury to the patients with ACS. Among compartment syndrome patients, rSO_2 was measured using a NIRS device (Hutchinson Technology, Hutchinson, MN) pre and post fasciotomy in the affected lower extremity and compared to control readings taken from the deltoid, the contralateral leg, and matched controls. Mean rSO_2 values of affected compartments were significantly lower when compared to both the uninjured contralateral leg ($p=0.015$) and the matched extremity of control patients ($p=0.002$). In addition, mean rSO_2 values of the affected compartments before fasciotomy were significantly lower when compared to the same compartment following fasciotomy ($p=0.017$). This clinical study redemonstrated the previous results, that a predictable increase in oxygenation occurs after fasciotomy is performed in patients with ACS.

Although the sample size was small, this study was the first to demonstrate successful use of NIRS in a clinical trauma setting, and it corroborated results observed in previous animal studies. Mean rSO_2 values accurately reflected ischemic changes in ACS patients compared to controls, as well as reperfusion following fasciotomy. However, as the authors note, the study population consisted wholly of patients with definitive ACS, so further study is needed to investigate the ability of NIRS to detect the early evolution of ACS. Additionally, the study was inconclusive regarding ideal control sites, as values observed in the deltoid and contralateral lower extremity were observed to be variable and were not consistent across all subjects, relative to the limb with ACS.

In 2001, Gentilello and colleagues used a cuff compression model to investigate the relationship between NIRS, ICP, and perfusion pressures (PP)[8].Using a 21-cm wide blood pressure cuff placed around the leg, a compartment syndrome was simulated in the lower extremity of 15 volunteers. A NIRS sensor (Hutchinson Technology, Hutchinson, MN) was placed over the anterior compartment and cuff pressures were increased until rSO_2 decreased to 60%, at which point rSO_2 was further decreased at 30 minute intervals until reaching 40%, 20% and <10%. rSO_2 was maintained at <10% for 30 minutes before the cuff was released and measures were repeated after a recovery period. ICP was calculated from cuff pressure using a validated formula and PP was calculated as MAP minus ICP. Ischemia was defined using changes in neuromuscular function measured by deep peroneal nerve conduction studies, monofilament sensitivity, and visual analog pain scale.

In regression modeling, the authors observed a significant association between rSO_2 values and PP for all ischemia measures. Moreover, when plotting various cutoff values of rSO_2 and PP using receiver operating characteristic (ROC) curves, rSO_2 demonstrated

higher sensitivity for detecting ischemia measures at all cutoff values. These results suggest that NIRS is not only a useful tool for detecting ischemia, but that it may be superior to PP, which provides only an indirect measure of ischemia, and in common use PP is obtained only as a painful spot check, making it difficult and/or painful to evaluate the evolution of this process over time.

In order to interpret data in the setting of ACS, a complete understanding of normal NIRS values in both uninjured and injured patients without ACS is required. Shuler et al published a study in 2009, which evaluated 26 patients with unilateral tibial fractures that did not progress to ACS and 25 healthy volunteers[9]. rSO_2 values were collected using an INVOS cerebral oximeter (Somanetics, Troy, MI) from the anterior, lateral, deep and superficial posterior compartments of each lower extremity. rSO_2 values obtained from like compartments of alternate legs among uninjured subjects were found to be extremely well correlated. In linear regression modeling, fracture status combined with rSO_2 values from the contralateral limb explained a large proportion of the variance observed among the test limbs (R^2 = 0.74 to 0.80), suggesting that the contralateral uninjured lower extremity provides a useful internal control against which rSO_2 values of injured extremities can be compared.

Furthermore, a repeated measures model used to evaluate the effect of trauma on NIRS values revealed that rSO_2 values of injured extremities were an estimated 15.4 percentage points higher compared to those of uninjured extremities, supporting the hypothesis of a hyperemic response to injury. The model controlled for rSO_2 of the contralateral limb as well as race, which was found to be a significant covariate with this particular device.

This study provides important insight into NIRS, the pathophysiology of ACS, and the hyperemic response to trauma, which the authors suggest may play a protective role against the development of ACS. Giannotti et al observed that, among some ACS patients, rSO_2 values of the affected leg were nearly equal to those of the uninjured contralateral leg[7]. This study suggests that this lack of a hyperemic response may be indicative of an impending compartment syndrome, not a shortcoming of the NIRS device. Shuler et al also observed significant differences between mean rSO_2 values obtained from black compared to white subjects. The authors surmise that differences in melanin concentration, a chromophore, can explain the lower values seen in more pigmented individuals. However, when using a control within the same subject, such as a contralateral leg, the affects of pigment are removed as pigment levels are similar throughout these sites.

In 2010, a subsequent study was published by Shuler and colleagues, using the same NIRS device as previously mentioned and consisting of 14 patients with clinically diagnosed ACS[13]. rSO_2 and ICP values were collected from each subject. PP was calculated as diastolic blood pressure minus ICP. All rSO_2 values were normalized based on values from the contralateral uninjured side (rSO_2 of the injured leg minus rSO_2 of the contralateral leg).

Compared to the previous study, in which rSO_2 values of injured legs were approximately 14 to 17 percentage points higher, on average, than the contralateral uninjured leg[9], rSO_2 values of legs with ACS were an average of 9 to 16 percentage points lower than the contralateral uninjured leg, indicating a perfusion deficit. The authors also observed significant positive correlations between rSO_2 and PP values.

In 2011, Bariteau et al reported a study in which 7 patients with clinically diagnosed ACS had rSO_2 (using the InSpectra Tissue Spectrometer [Hutchinson Technology, Hutchinson, MN]) and ICP values collected from each compartment of the affected lower extremity prior to fasciotomy[14]. Mixed linear growth modeling was used to test for associations between rSO_2 and ICP and rSO_2 and PP in the affected lower extremity. In both models, no statistically significant association was observed between rSO_2 and each explanatory variable. A simple linear regression model was also constructed to calculate R^2 values for these associations. The model, which contained all observations from all compartments, yielded an R^2 =0.2452 for the model of rSO_2=ICP and R^2=0.0233 for the model of rSO_2=PP.

This study shows that the use of NIRS in ACS is not straight-forward. The negative results from this study may stem from differences between commercially available NIRS devices. The device used in this study is limited in its depth and is commercially available to monitor superficial muscles such as the thenar eminence. Additionally, several design flaws can explain the negative findings. Raw NIRS values were correlated with ICP and PP. Based on the hyperemia associated with trauma, elevated NIRS values would be expected up until approximately 10mmHg of perfusion pressure. A linear correlation between absolute ICP and NIRS would not be expected. Additionally, no control data was obtained to interpret the raw NIRS values obtained in this study.

A case report published in 2011 outlined the longitudinal use of NIRS in 3 cases of lower extremity ACS[15]. Although NIRS monitoring over time requires study in larger samples prior to being generalized to clinical use, the report provided important insight into NIRS' ability as a monitor for ACS in various situations. First, NIRS successfully differentiated between adequately perfused lateral compartment and poorly perfused deep posterior compartments of an individual with ACS. rSO_2 values reflected hyperperfusion followed by gradual decreases in perfusion pressure over time within the affected compartment. Furthermore, NIRS was able to detect perfusion deficits in an unresponsive, hypotensive patient, who was unable to provide clinical feedback. In an additional patient, NIRS' response to changes in perfusion due to induction was seen within seconds. In all three patients, NIRS changes were observed well before permanent muscle damage or necrosis occurred. This study demonstrated the potential power of NIRS as a continual, noninvasive monitoring device that detects perfusion changes in real time.

These positive results were also seen in a 2013 porcine-model study correlating NIRS and tibial intra-compartmental perfusion pressure (TIPP) in two groups of landrace swine with induced ACS[16]. Tibial intra-compartmental pressure (TICP) was increased via albumin infusion in both groups, which was recorded alongside blood pressures and

percent oxygenation in each leg. One group also received blunt trauma to the monitored site. NIRS was able to accurately reflect decreases in TIPP over time (decreased oxygenation) and increases in TIPP after fasciotomy (rebound in oxygenation) in both groups. These results provide another illustration of the responsiveness of NIRS to changes in perfusion. Additionally, this is the first ACS study to attempt to reproduce the actual trauma setting by traumatizing the porcine leg prior to infusion. NIRS was able to record oxygenation without difficulty in the setting of trauma.

Some criticism has been raised regarding anatomic sensor placement of NIRS sensors, particularly in the deep posterior compartment. In order to provide a useful solution for ACS monitoring, anatomic pad placement must be validated to ensure that rSO2 values are specific to the intended compartment. This was done for the upper extremity in a 2012 study published in the Journal of Hand Surgery[10]. 63 volunteers were asked to perform exercises to sequentially isolate muscle groups of each compartment of the forearm. Significant decreases in rSO_2 in compartments being activated by exercise were observed, while neighboring compartments showed no clinically relevant changes. These findings suggest that NIRS can provide a sensitive and specific measure of tissue oxygenation for the upper extremity. Similar studies are planned to validate anatomic placement of the lower extremity; however, the ability to accurately differentiate between the smaller structures and spaces in the forearm, bodes well for the capabilities of this technology in the larger compartments of the leg and this has borne out in clinical experience with NIRS in evaluating ACS in legs.

Concerns of have been raised about possible limitations inherent to NIRS. Those limitations include the effects of skin pigmentation, subcutaneous fat and hematomas. Skin pigmentation does affect raw values in some devices, while others can overcome this confounding variable. However, by using an uninjured control site such as the contralateral leg or forearm, which has shown high correlation as well, any pigment affects can be removed. A control of some sort, will likely be needed in all cases to differentiate perfusion changes based on systemic factors (hypotension / hypoxia) and local changes such as ACS.

Subcutaneous fat was thought to be a limiting factor in the severely obese; however, a recent study of 50 patients with traumatic leg injuries found that symptoms commonly associated with these injuries do not affect the mean subcutaneous adipose tissue thickness (ATT)[11]. The distance from skin to fascia was never more than 2.5 cm, regardless of the presence of swelling, edema, or high body mass index. This study showed that while leg circumference increases in the traumatize leg, this occurs within the compartment and not superficial too it, which is intuitive, since it is the swelling that produces ACS.

Lastly, hematomas can in some instances block the signal of NIRS. Hematomas are so concentrated with hemoglobin, that they act as light sinks, absorbing the emitted light; thereby, reducing the amount of light that is reflected back to the sensor to thresholds that are below the specified parameters for the device. While this fact seems to be a limiting factor, it may be a strength. If a signal is lost due to an expanding hematoma, this loss of

signal can be a warning for the clinician of an expanding hematoma. Additionally, the inability to read through a hematoma, prevents NIRS from inadvertently monitoring a hematoma instead of the desired muscle and masking a potential developing ACS. There has been some evidence to also suggest in some settings increase perfusion or blood flow due to trauma can potentially absorb enough light to prevent readings. These challenges in traumatized tissue are currently being examined.

Evidence in the literature up to this point (summarized in Table 1) supports the consideration of NIRS for the diagnosis of ACS in the trauma setting; however, several factors necessitate further study and as a result this technology is not currently ready for wide-spread use. Current understanding indicates that the lack of hyperemia when comparing an injured extremity to an uninjured extremity may indicate a compartment syndrome[15]. Additionally, a change in values over time of roughly 10 percentage points indicates a significant change in perfusion. Still, this change needs to be examined in conjunction with a control sensor to determine if this change is a systemic versus local change in perfusion.

Large-scale longitudinal observational studies are currently underway to better delineate clinical guidelines for the use of NIRS in the setting of ACS. A fully validated diagnostic device that could accurately detect ACS could lead to a dramatic reduction in the number of unneeded/prophylactic fasciotomies, in addition to reducing morbidity due to missed or delayed diagnosis. Additionally, this device could reduce the burden on medical staff who are monitoring trauma patients for a condition that could develop over a course of hours or even days. NIRS has the potential to offer a continual, noninvasive monitoring system in real time that more accurately estimates tissue perfusion than intracompartmental pressures which do not account for other factors such as hemoglobin concentration, cardiac output, vasospasm and cellular metabolism which can all play a part in tissue perfusion and ischemia.

CONCLUSIONS

The evidence to date shows that the physiological principles behind NIRS are sound and can be feasibly applied, but on-going work is needed to validate a specific technology for this task. Additionally, the parameters with which to interpret the data need to be validated. The tremendous potential NIRS portends as a non-painful, non-invasive continuous measure of the parameter that matters most in ACS (perfusion), means that we must devote the further research needed to vet and translate this technology into common use.

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Table 1. Summary of current literature on near infrared spectroscopy and lower extremity acute compartment syndrome

First Author	Year	Aim/hypothesis	Setting	Study Population	Device Manufacturer	Findings
Garr[5]	1999	NIRS can detect changes in venous oxyhemoglobin levels attributable to compartment syndrome.	Infusion model for ACS	9 landrace swine	Hutchinson Technology, Hutchinson, MN	rSO_2 was significantly inversely correlated with ICP and significantly positively correlated with PP; no significant changes in rSO_2 or ICP observed in control legs
Arbabi[6]	1999	NIRS can differentiate between ischemia due to severe shock and ischemia due to compartment syndrome.	Infusion model for ACS	8 landrace swine	Hutchinson Technology, Hutchinson, MN	Mean rSO_2 during compartment syndrome + shock was significantly lower compared to both hypotension and hypotension + hypoxia, suggesting that NIRS is capable of differentiating between ischemia due to ACS and severe shock.
Giannotti[7]	2000	Describe characteristics NIRS findings in patients with compartment syndrome to better understand the usefulness of NIRS	Trauma center	9 ACS patients + 33 trauma patients without ACS	Hutchinson Technology, Hutchinson, MN	Mean pre-fasciotomy rSO_2 of legs with ACS were significantly lower than both mean rSO_2 in limbs of matched controls and post-fasciotomy measurements
Gentilello[8]	2001	NIRS is more accurate than perfusion pressure at detecting ischemia.	Cuff ischemia model	15 uninjured volunteers	Hutchinson Technology, Hutchinson, MN	rSO_2 and PP were significantly correlated with all ischemia measures; in ROC curves, rSO_2 was a more sensitive measure of ischemia than PP when specificity was the same.
Shuler[9]	2009	Describe the expected alteration of normal tissue oxygenation in the lower leg in the setting of ACS without compartment syndrome and examine the utility of the contralateral leg as a control.	Trauma center	25 tibia fractures without ACS + 26 uninjured volunteers	Somanetics, Troy, MI	rSO_2 values of injured legs were an estimate 15.4 percentage points higher than uninjured legs, indicating a hyperemic response to injury; Together, controlling for fracture status and the contralateral uninjured leg explained an extremely high proportion of variation in rSO_2.
Shuler[13]	2010	Decreased in muscle oxygenation measured with NIRS will be correlated with PP among patients with ACS.	Trauma center	14 ACS patients	Somanetics, Troy, MI	rSO_2 and PP were significantly correlated; mean rSO_2 in legs with ACS were 9 to 16 percentage points lower than the contralateral uninjured leg.
Bariteau[14]	2011	Assess the association between NIRS and compartment pressure in patients with lower extremity compartment syndrome.	Trauma center	7 ACS subjects	Hutchinson Technology, Hutchinson, MN	No significant associations observed between rSO_2 and ICP or rSO_2 and PP. Limited depth of measurement, no control used for NIRS. Hyperemia not accounted for in analysis
Shuler[15]	2011	Describe the longitudinal use of NIRS for monitoring 3 cases of ACS.	Case report	3 ACS subjects	Somanetics, Troy, MI	NIRS differentiated between adequately perfused and poorly perfused compartments within the same leg; NIRS demonstrated real-time changes in perfusion; NIRS detected perfusion deficits in unresponsive, intubated patient.

Jackson[12]	2013	Describe correlation in rSO_2 between varying extremity control sites in lower extremity trauma	Trauma Center	44 uninjured volunteers	Somanetics, Troy, MI	Correlations in rSO_2 suggest corresponding compartments of the contralateral leg serve as the best control sites for one another; As an upper extremity control, the volar forearm displayed the highest correlation
Cathcart[16]	2013	Correlate NIRS and TIPP in ACS	Infusion model for ACS	31 Landrace swine	Nonin, Plymouth, MN	NIRS accurately detected increases in TICP and decreases in TIPP in both the blunt trauma+infusion and infusion only models

Abbreviations: NIRS, near infrared spectroscopy; ACS, acute compartment syndrome; ICP, intracompartmental pressure; PP, perfusion pressure; ROC, receiver operating characteristics; TIPP, tibial intra-compartmental perfusion pressure; TICP, tibial intracompartmental pressure

NUMBER 1 OF 1

AUTHOR QUERIES

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AU1: Please confirm whether "Hyperaemia" should be changed to "Hyperemia" here and throughout the article.

AU2: Please confirm whether edit made to the sentence "NRIS . . . internal control." provides intended meaning.

AU3: Please confirm whether edit made to the sentence "In a subset . . . the hospital." provides intended meaning.

AU4: Please confirm whether the manufacturer added for "SAS version 9.2" is ok or else please provide the correct one.

AU5: Description has been deleted in Figures 1 and 2. Please confirm.

Use of Near-Infrared Spectroscopy to Detect Sustained Hyperaemia Following Lower Extremity Trauma

William M. Reisman, MD; Michael S. Shuler, MD†; Mellisa Roskosky, MSPH†; Tracy L. Kinsey, MSPH†; LTC Brett A. Freedman, MC USA‡*

ABSTRACT Introduction: Patients who sustain lower extremity trauma are at highest risk for acute compartment syndrome (ACS) during the first 48 hours after surgical stabilization. Near-infrared spectroscopy (NIRS) may be a useful monitoring tool for ACS during this period; however, expected normal values have yet to be established. This study sought to evaluate whether the expected hyperaemic response is present 48 hours postoperatively, using NIRS. Materials and Methods: Participants consisted of 25 cases with acute unilateral lower extremity fractures. NIRS measurements for hemoglobin saturated with oxygen (rSO_2) were taken approximately 48 hours after surgical stabilization for each compartment bilaterally, using the contralateral (uninjured) leg as an internal control. Results: Mean rSO_2 values taken 48 hours from surgical stabilization from each compartment of the patients' injured legs were significantly higher than the mean values of the contralateral legs (injured = 70, 68, 72, 70; contralateral = 55, 54, 57, 56 for anterior, lateral, deep posterior, and superficial posterior compartments, respectively; $p < 0.0001$ for all compartments). Conclusions: These results suggest that the hyperaemic response to injury remains present at 48 hours after surgical stabilization, and that NIRS values in an injured extremity should be expected to remain elevated throughout the window of concern for ACS. NIRS may be a valuable tool in monitoring leg injuries during this critical time period.

INTRODUCTION

Extremity trauma remains a major concern in the management of the wounded warrior. Traumatized tissue results in a predictable hyperaemic effect in the injured extremity.[1–4] This response is critical in understanding the development of acute compartment syndrome (ACS), which is most commonly associated with lower extremity trauma. The physiologic changes of trauma-induced hyperaemia have been under-appreciated in the understanding of ACS.

Near-infrared spectroscopy (NIRS), a noninvasive method of quantifying tissue oxygenation and perfusion, has been examined as a monitoring tool for ACS. It has been used in studies of muscle perfusion, exertional compartment syndrome, and ACS[4–12] and has shown promising results. A previous study has demonstrated the ability of NIRS to detect acute increases in perfusion after lower extremity trauma, however the duration of the hyperaemia is not known.[4]

The development of ACS typically occurs within the first 3 days after injury and after initial fracture stabilization.[13,14] This time frame coincides with initial evacuation from the battle field to an in theatre facility to a regional medical facility depending on the severity of the injury. Understanding the expected NIRS response to injury in the absence of a compartment syndrome must be understood before NIRS can be applied in the setting of potential ACS. Therefore, determining the duration of this hyperaemic response to trauma is vital for interpreting the utility of NIRS in the setting of ACS. The purpose of this study was to evaluate patients with lower extremity trauma without compartment syndrome in a subacute setting to determine whether hyperaemia persists approximately 48 hours after surgical stabilization. We hypothesize that NIRS will demonstrate sustained hyperaemic effects in the subacute period for tibia fractures that do not develop ACS.

MATERIALS AND METHODS

Participants were recruited from a level I trauma center. All participants provided written informed consent for the study after approval from the institutional review board at the medical facility.

The study sample consisted of 25 cases, ages 18 to 64, with acute unilateral, open or closed, lower extremity fractures requiring surgical stabilization, including proximal intra-articular (plateau), tibial shaft, as well as distal intra-articular (pilon) or fibula fractures. Patients were excluded if they presented with bilateral lower extremity injuries, a previous diagnosis of pulmonary or vascular disease, or a suspected or confirmed compartment syndrome at initial evaluation or at any point during the study. Nonoperative fractures were excluded secondary to the fact that they were typically discharged before 48 hours after injury.

For each study participant, demographic, clinical, and injury characteristics were obtained. At the time of NIRS measurements, the number of hours elapsed from injury and from completion of surgery was recorded. The surgical record and surgical end time was used to determine the time of completion of surgical intervention.

NIRS measurements were obtained using an INVOS cerebral oximeter (model 41000; Somanetics, Troy, MI), a device

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currently used to monitor cerebral oxygenation of patients under anesthesia.[15,16] This device measures the proportion of hemoglobin saturated with oxygen (rSO_2) 2 to 3 cm below the skin.[15,17,18]

NIRS measurements were taken at the mid-diaphyseal region for each of the four muscle compartments from both the injured and contralateral legs. Previous studies have established the contralateral leg as a valid internal control.[4] The sensor was applied to the leg for 30 to 60 seconds, to allow the device to generate a stable reading for at least 24 seconds or four cycles. The protocol for obtaining NIRS readings has been previously described and this technique was followed in this study.[4]

In all study participants, measurements were obtained approximately 2 days after surgical stabilization for a unilateral fracture, generally within 3 days following admission to the trauma center. Surgical stabilization included intramedullary nailing, external fixation, or plate fixation. AU3 In a subset of participants ($n = 11$), it was possible to obtain readings at initial presentation to the hospital.

Paired two-sample t tests were used to detect significant differences in mean rSO_2 between compartments of the injured and contralateral (uninjured) legs of study subjects, to test the hypothesis of a hyperaemic effect present at 48 hours postsurgical stabilization. Among patients for whom presurgical NIRS values were available, paired two-sample t tests were used to test whether statistically significant differences existed between pre- and postsurgery NIRS gradients (rSO_2 in the injured leg minus rSO_2 in the contralateral leg) in each muscle compartment. All statistical tests were two-sided using $p \leq 0.05$ as the criterion for AU4 significance, and performed using SAS version 9.2 (SAS Institute, Cary, NC).

RESULTS

Baseline characteristics of the study population are shown T1 in Table I. The most common mechanism of injury was a motor vehicle crash ($n = 11$), with the remaining injuries resulting from a fall from height ($n = 6$), pedestrian versus auto ($n = 5$), gunshot wound ($n = 2$), and assault ($n = 1$). The most common type of fracture was tibial shaft ($n = 17$), followed by pilon ($n = 4$), plateau ($n = 3$), and fibular ($n = 1$). Of the 12 subjects who presented with open fractures, 1 was classified as Gustillo grade I, 8 were grade II, and 3 were grade IIIA. Postoperative rSO_2 measurements were taken at an average of 70.5 (standard deviation [SD] = 24.0) hours after injury and 44.6 (SD = 17.3) hours after surgery. The most common type of surgery was intramedullary nailing ($n = 15$), followed by external fixation ($n = 7$), and open reduction internal fixation ($n = 3$).

TABLE I. Selected Demographic and Clinical Characteristics of 25 Subjects With Lower Extremity Trauma

Characteristic	*N*	%
Gender		
Male	19	76.0
Female	6	24.0
Race		
Black	17	68.0
White	7	28.0
Hispanic	1	4.0
	Mean	SD
Age	35.8	13.4
BMI	25.2	2.9
SBP	128.0	17.6
DBP	73.5	12.3

BMI, body mass index (kg/m^2); SBP, systolic blood pressure (mmHg); DBP, diastolic blood pressure (mmHg); SD, standard deviation.

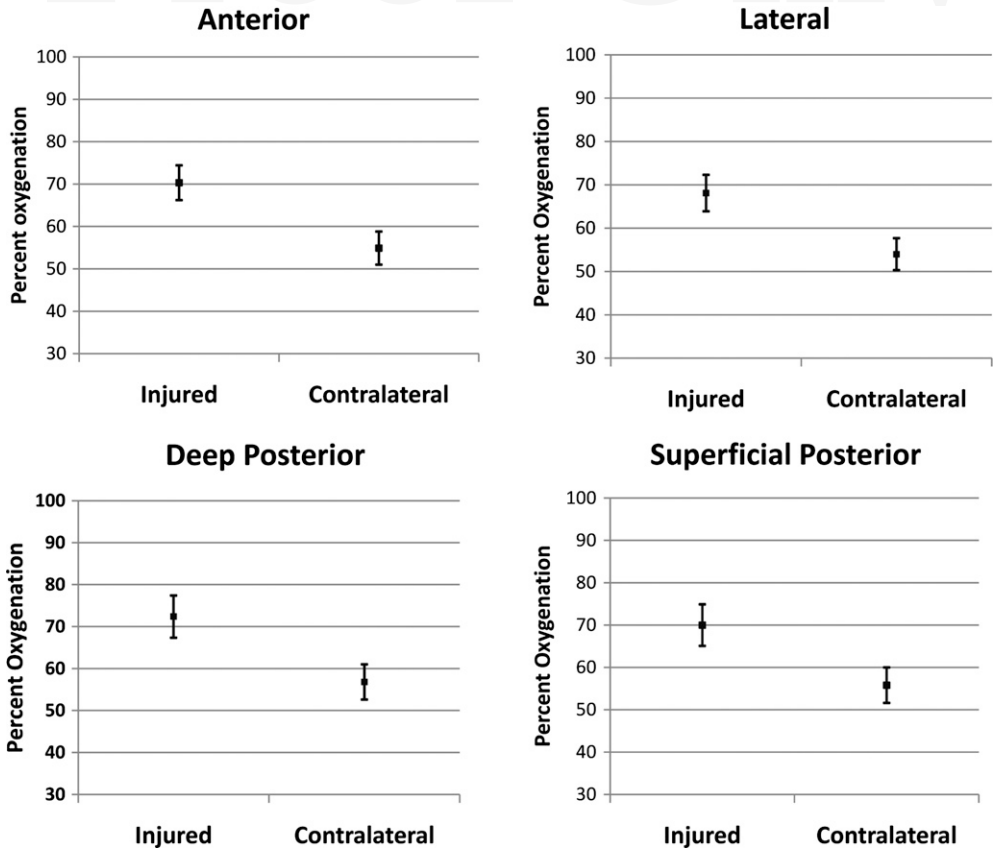
TABLE II. Near-Infrared Spectroscopy (rSO_2) Values Taken Approximately 48 Hours After Surgery From Injured and Contralateral Legs of 25 Subjects With Lower Extremity Trauma

	Anterior	Lateral	Deep Posterior	Superficial Posterior
Injured Leg				
Mean	70	68	72	70
Median	71	68	71	67
SD	9.9	10.1	12.1	11.9
Range	45–85	44–90	46–95	50–93
Contralateral Leg				
Mean	55	54	57	56
Median	51	54	58	54
SD	9.5	9.0	10.2	10.2
Range	42–74	30–69	33–75	41–80
Difference Between Injured and Contralateral Leg				
Mean	15	14	16	14
SD	9.1	8.8	8.5	9.1
Range	2–30	−11 to 31	4–37	3–38
p value†	<0.0001	<0.0001	<0.0001	<0.0001

SD, standard deviation. All data are presented as percent oxygenation (rSO_2). †p value for paired t test.

Mean percent tissue oxygenation (rSO_2) in each compartment approximately 48 hours after surgery is displayed in Table II. Mean rSO_2 values in the anterior (A), T2 lateral (L), superficial (SP), and deep posterior (DP) compartments of the injured leg were 15, 14, 16, and 14 percentage points higher, respectively, compared to the contralateral leg ($p < 0.0001$ for all compartments; Table II). Figure 1 shows the mean and 95% confidence F1 interval (CI) for rSO_2 values in the injured leg compared to the contralateral uninjured leg.

For 11 study subjects, tissue oxygenation values were available before and after fracture stabilization, allowing for comparison of initial and postoperative perfusion. Among these patients, the mean time between injury and preoperative measurements was 14.9 (range = 2–32) hours and the average time between pre- and postsurgical measurements was 54.0 (range = 24–117) hours. A comparison of rSO_2 values, measured before and approximately 48 hours after surgery, is presented in Table III. Over- T3 all, no statistically significant differences were observed



AU5 **FIGURE 1.** Mean and 95% CI for rSO_2 values (percent oxygenation) of injured and contralateral legs of 25 subjects with unilateral lower extremity trauma, taken approximately 48 hours postoperatively. rSO_2 values were measured approximately 48 hours postoperatively. The square represents the mean rSO_2 value, whereas the bars above and below show the upper and lower confidence limits.

TABLE III. Comparison of Pre- and Postsurgical Near-Infrared Spectroscopy Difference

	Anterior		Lateral		Deep Posterior		Superficial Posterior	
	Pre	Post	Pre	Post	pre	post	Pre	Post
Injured leg								
Mean	71	71	71	71	74	75	70	74
Median	70	71	68	66	76	73	69	74
SD	8.7	9.0	9.4	10.0	10.3	9.5	10.4	10.6
Range	54–82	60–84	60–90	61–90	54–87	64–88	57–87	62–88
Contralateral (Uninjured) Leg								
Mean	55	56	56	56	57	60	56	57
Median	53	55	55	55	54	60	55	57
SD	9.7	7.8	12.0	5.7	8.4	5.4	8.6	7.6
Range	44–73	47–70	43–81	47–67	45–72	51–71	44–71	43–72
Difference Between Injured and Contralateral Legs								
Mean	15.8	15.6	15.1	15.5	16.5	14.8	14.2	17.6
SD	10.2	8.5	7.7	8.5	7.2	6.7	6.0	11.3
Range	5–34	8–30	7–33	4–31	2–28	5–25	5–23	3–38
p value†	0.92		0.92		0.61		0.25	

SD, standard deviation. Between Injured and Contralateral Legs for 11 Subjects With Lower Extremity Trauma. All data are presented as percent oxygenation (rSO_2). †*p* value for paired *t* test.

between NIRS gradients (rSO_2 in the injured leg minus rSO_2 in the contralateral leg) after surgery, compared to measurements taken before surgery ($p > 0.05$ in all compartments; Table III).

Figure 2 gives a visual representation of the means and F2 95% CI for NIRS values taken before and after surgical stabilization among 11 study subjects. Dotted lines connect the pre- and postoperative measurements of each leg, representing

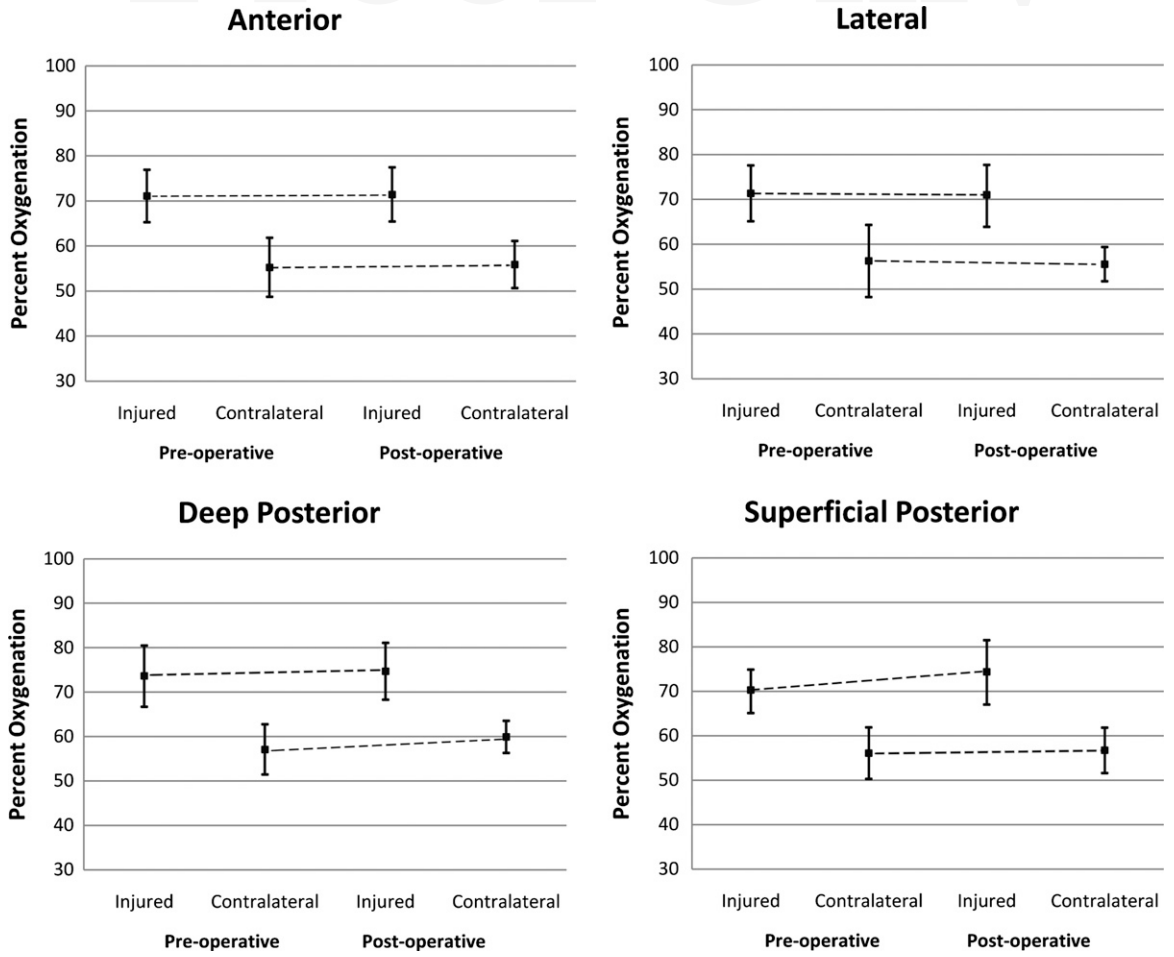


FIGURE 2. Mean and 95% CI for rSO_2 values (percent oxygenation) of injured and contralateral legs of 11 subjects with unilateral lower extremity trauma, taken at hospital admission (preoperatively) and approximately 3 days later (postoperatively). The square represents the mean rSO_2 value, whereas the bars above and below show the upper and lower confidence limits. Dotted lines show the difference in mean rSO_2 values pre- and postoperatively.

observed differences in NIRS values over an average period of 54.0 hours.

DISCUSSION

The implications of this study are two2-fold. First, the increased perfusion in the injured leg, compared to the contralateral leg, demonstrates a hyperaemic effect present at approximately 48 hours after surgical stabilization and 3 days after injury. Second, although the sample size was limited, the similar observed responses between pre- and postoperative NIRS values suggests that this hyperaemic effect remains consistently elevated over the initial 72 hours from injury, and perhaps longer. These two findings are vital for NIRS to be used as a monitoring tool in the development of ACS as well as in the evacuation of wounded warriors.

Prior studies have utilized NIRS technology to demonstrate hyperaemia up to approximately 24 hours from injury.[3,4] In combination with these previous findings, the results of this study suggest that this hyperaemic response is present from the time of injury to at least 3 days after injury, coinciding with the typical window of development of ACS. This period is also the typical period for wounded warrior evacuation. Results from the subset of patients with readings pre- and postoperatively corroborate this observation. This data lead to the utility of NIRS in the evacuation of wounded warriors as a monitoring system for ACS. Of note, typical warrior evacuation includes air transport in partial pressurized cabins, which may also lead to ACS as pressure plays a vital role in the development of ACS.[19,20]

This study was not without limitations. The sample size was limited and preoperative NIRS measurements were unavailable for a proportion of the study sample; consequently, statistical tests of this subsample may have been underpowered. Although measurements spanned a significant time frame after injury and can allow for some speculation, continual values were not obtained in this study. Continual monitoring will help to elucidate the natural history of lower extremity injuries that do and do not develop into ACS. A single NIRS monitor was used in this study. Different NIRS technologies may demonstrate

different characteristics with regard to variables such as ambient light shielding, skin pigment, and signal stability.

Future studies will examine NIRS values over a continual period of time. NIRS monitoring of extremity injuries, especially in obtunded patients, could potentially allow early detection of ACS by recording the absence of hyperaemia in the post-traumatic period from initial injury on the battle field to air evacuation to definitive treatment and transport to regional medical facilities. In addition, NIRS may offer a vital service in monitoring injured extremities in a vulnerable time during air evacuation, which has been shown to have a higher risk of development secondary to altitude increases and shifts in pressure.[19,20]

CONCLUSIONS

This study, and the evidence of prior studies, suggests that, in the absence of ACS, NIRS values of the injured extremity should be elevated when compared to the contralateral uninjured extremity. These elevated values are consistent in the setting of stabilization procedures. Within the first 3 days, the time of measurement from injury or stabilization did not affect the presence of hyperaemia, and the presence of hyperaemia was consistent throughout a wide range of time ranges. Understanding expected norms allows for interpretation of data and the definition of abnormal findings. If NIRS values are not elevated during the first 3 days after injury, a heightened suspicion for poor perfusion should be present.

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