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Minimally invasive Real Time Monitoring of mitochondrial NADH and tissue blood flow in the urethral wall during hemorrhage and resuscitation

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

Julio A. Clavijo[1ABCDEF], James van Bastelaar[1B], Michael R. Pinsky[2ADEG], Juan Carlos Puyana[1ABCDEFG], Avraham Mayevsky[3ABCDEF]

[1] Department of Critical Care Medicine and Surgery, University of Pittsburgh Medical Center, Presbyterian Hospital, Pittsburgh, PA, U.S.A.
[2] Cardiopulmonary Research Laboratory, University of Pittsburgh Medical Center, Presbyterian Hospital, Pittsburgh, PA, U.S.A.
[3] The Mina & Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Ramat Gan, Israel

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Summary

Background: The ideal endpoint of resuscitation after severe hemorrhage should indicate not only that optimal oxygen delivery has been achieved, but also that oxygen utilization has been restored. A modified Foley catheter for simultaneous assessment of microcirculatory blood flow (TBF) and mitochondrial NADH in the urethral wall was used in the female swine. We hypothesized that changes in mitochondrial NADH and TBF are associated with impaired energy metabolism in the urethra and that these changes correlate with impaired tissue perfusion in the bladder during shock and resuscitation.

Material/Methods: Female swine n=5 underwent laparotomy. TBF was measured by a laser Doppler flowmeter. Mitochondrial function was evaluated by measuring NADH fluorescence *in vivo*. Multiparameter sensors (pH, pCO_2 and pO_2) were placed in the bladder mucosa (BM), and in the skeletal muscle (Sk). Animals underwent hemorrhage and their MAP was maintained at 40 mm Hg by appropriate infusing or withdrawing of blood for 10 min. Animals were resuscitated and observed for 20 min.

Results: Urethral NADH increased during shock and recovered during resuscitation, while TBF showed an opposite effect (r^2=0.74). Skeletal muscle and bladder pO_2 decreased during shock ($p<0.01$) and recovered after resuscitation. NADH increased significantly ($p<0.05$) during shock and decreased after resuscitation.

Conclusions: Changes in TBF and NADH in the urethral mucosa represent novel markers for the energetic state of the tissue. They could be measured *in vivo* by a minimally invasive approach and thus could provide important information on the end-points of resuscitation in hemorrhagic shock.

key words: **microcirculatory blood flow • urethral wall metabolism • mitochondrial NADH • hemorrhagic shock • resuscitation end point**

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Author's address: Prof. Avraham Mayevsky, The Mina & Everard Goodman Faculty of Life Sciences, Bar Ilan University, Ramat Gan 52900, Israel, e-mail: mayevsa@mail.biu.ac.il

BACKGROUND

During hemorrhagic shock, the adequacy of oxygen delivery to the tissues is compromised, resulting in the induction of anaerobic metabolism [1]. Severe shock is characterized by acute general failure of O_2 delivery that manifests itself in acute organ dysfunction and ischemic tissue injury [2]. It has been suggested that this insult, if repeated or not promptly corrected, may subsequently generate organ system dysfunction. Unfortunately, current monitoring techniques for the assessment of O_2 delivery and utilization are invasive, and impractical for implementation in the pre-hospital or trauma care environment. Several monitoring systems have been developed to determine the status of oxygen transport and oxygen utilization, however, they are invasive and only applicable in the intensive care unit. In fact, the only methods clinically used to assess the adequacy of oxygen delivery and metabolism, rely on the indirect assessment of the severity of anaerobic metabolism or acidosis by determining the levels of lactic acid or base deficit [3,4]. Indirect methods, such as gastric tonometry, have been extensively used in the laboratory for the early detection of impaired splanchnic perfusion by determining the intra-mucosal pCO_2. However, this technique is cumbersome and difficult to apply clinically, especially during the acute trauma setting.

Moore et al. demonstrated similar trends using other techniques, as did we in prior studies. He showed that in severe injury victims that are subjected to an established resuscitation protocol aimed at maximizing O_2 delivery, there may be a subgroup of non-responders. These patients were characterized by a failure to achieve the goal of oxygen consumption greater than 150 ml/min.m^2. These non-responding patients had elevated blood lactate levels and proceeded to develop multiple organ failure. The authors suggested that the initial shock insult, if uncorrected, plays a pivotal role in priming the host's subsequent organ failure [5]. Subsequently, Hayes et al., and Gattinoni et al. reported that achieving supranormal DO_2 values for oxygen delivery, did not reduce morbidity or mortality among critically ill surgical patients [6,7]. It was proposed that this flow independent impairment of VO_2 could be caused by either a persistent flow dependency secondary to microcirculatory mal-distribution, or by an inherent impairment in the ability to consume oxygen at the mitochondrial level. Unfortunately, up to date, there are no clinical methods for an early identification or quantification of the deterioration of a patient in critical care medicine.

We have previously demonstrated that hemorrhagic shock induces changes in tissue pH and pCO_2 of the bladder wall mucosa and that these changes are comparable to those measured by direct intestine tonometry [8]. In the search for a more accessible route to determine regional oxygen delivery, to redefine more accurate and physiologically more relevant end points of resuscitation, we propose that a multiparametric, minimally invasive monitoring platform must be designed to place via a urinary catheter into the urethra and the bladder. Such a platform could provide not only surrogate information on the integrity of splanchnic circulation, but also information on regional oxygen utilization. For this purpose, we constructed a device similar to a Foley catheter that includes optical fibers for the direct measurement of mitochondrial NADH and simultaneous assessment of microcirculatory blood flow, previously used to measure oxygen balance in the brain and other organs [9–16].

The use of fluorescent light to interrogate the urethral mucosa, provides a qualitative assessment of tissue NADH. In order to establish whether these changes are related to tissue perfusion, it is necessary to simultaneously use a laser Doppler flowmeter to assess regional blood flow. Cellular substrates donate electrons to the high-energy electron carriers: NADH and $FADH_2$, which interact with the electron transport chain within the mitochondria. As electrons are transferred down the chain and accepted by oxygen at cytochrome a,a_3, protons are extruded across the mitochondrial membrane, forming the proton gradient necessary for the conversion of ADP to ATP (oxidative phosphorylation). This concept was examined in a similar approach using intra-vital microscopy, as described by Puyana et al. [17] in a rat model of shock. In that study, the changes in NADH were correlated to hepatic tissue pO_2 and bile output. The authors reported that hemorrhagic shock was associated with a significant nutritive perfusion failure, which persisted despite resuscitation and accounted for the insufficient energy supply to the cells. In this model, the authors used bile output as a surrogate of hepato-cellular ATP production, insofar as bile excretion in the hepatocytes is ATP dependent [18–20].

The queries of this study were as follows:

1. Does severe hemorrhage induce changes in mitochondrial function and microcirculatory blood flow in one of the less vital organs of the body, namely, the urethral wall?
2. Are these parameters correlated with shock-induced changes in tissue pO_2 and pCO_2 monitored in another less vital organ, the bladder mucosa?

We used the bladder and the muscle as comparable less vital organs while monitoring pO_2 and pCO_2, since few publications appeared on this subject. Also, in order to compare the same parameters in various less vital organs, a more complex monitoring system is required. Such a multisite monitoring system for NADH and tissue blood flow, was not available for the present study.

MATERIAL AND METHODS

All experiments were performed in accordance with the Guidelines of the National Institutes of Health for the Care and Use of Laboratory Animals, and were approved by the Animal Care Committee of Pittsburgh University. Five female domestic pigs (45–50 kg) were used in all the experiments. The protocol of this study is presented schematically in Figure 1.

Anesthesia and animal preparation

The animals were allowed to acclimatize to laboratory conditions for at least 5 days before use, receiving food and water ad libitum throughout the acclimatization period, with day light -night cycles of 12 hours. Anesthesia was induced by ketamine (10 mg/kg) and xylazine (1.5 mg/kg) and was intubated. The animals were maintained on isoflurane (1–4%) to effect. The concentration of isoflurane

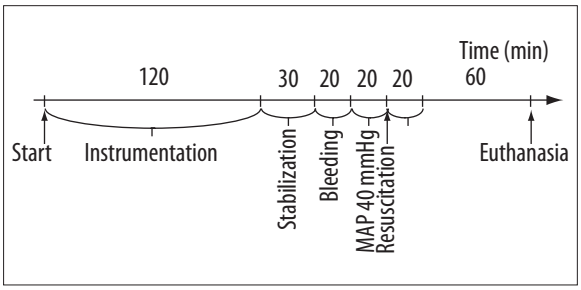

Figure 1. Time scale of the various steps of the animal protocol.

was then decreased to 0–1.5% and animals received alpha chloralose (Sigma-Aldrich Inc, St. Louis, MO) at a dose of 20 mg/kg intravenously every 30 minutes. The respiratory rate and tidal volume were adjusted as necessary to strictly maintain $PaCO_2$ between 40–45 mmHg with 100% FiO_2. A rectal probe was inserted for the measurement of body temperature. Body temperature was maintained between 37.0°C and 38°C during the course of the experiment, using a heated operating table.

The left carotid artery was catheterized and an arterial line was placed for blood gas analysis. The right femoral artery and vein were dissected and cannulated for blood withdrawal and re-infusion. A Millar pressure transducer was placed in the right femoral artery.

The specially designed catheter (similar to the Foley catheter) was placed through the urethra into the bladder space. This probe enabled the monitoring of mitochondrial NADH as well as microcirculatory blood flow, as will be described later on. Laparotomy was performed to confirm the placement of the probe, since the length of the urethra in the female pig is very short. Also, urine was collected directly from the bladder and not via the catheter. After the surgical manipulation, animals were allowed to equilibrate for 30 minutes.

Instrumentation

Monitoring of Tissue Vitality

Urethral Tissue Blood Flow (UTBF)

To measure UTBF in real time, we used the Laser Doppler Flowmetry (LDF) technique [21–23]. In this study, we used PF 2B and PF 3 model manufactured by Perimed Inc, Sweden. The LDF measures relative flow changes, and the readings have been shown to correlate with the relative changes in UTBF measured by the other two quantitative approaches [23].

The LDF monitoring utilizes the Doppler shift, namely, the frequency change undergone by the light reflected from moving red blood cells. A beam of low-power or diode laser light is transmitted by an optical fiber to the tissue. After the multiple light scattering, another optical fiber collects the reflected light that is recorded by a photo-detector. The signal is analyzed by a complex algorithm developed by the manufacturers, and the results are presented as percentage of a full scale (0–100%), thereby providing arbitrary relative flow values. The change in the total back-scattered light is an indirect measure of the blood volume in the sampled tissue.

To quantify and normalize Tissue Blood Flow (TBF) values, we defined the reading value after death as 0 TBF. The 100% value was defined as percent TBF read on the LDF scale during the control period before the treatment.

NADH Redox State Fluorometry

The principle of NADH monitoring from the urethral wall is that excitation light (366 nm from a 70 W metal Halide, double ended lamp, located in the TRI-LIGHT instrument, WPI, Sarasota, USA) is passed from the fluorometer to the tissue via a bundle of optical fibers made of quartz. The emitted light (450 nm), together with the reflected light at the excitation wavelength, is transferred to the fluorometer via another bundle of fibers. The changes in the reflected light (R) are correlated to the changes in tissue blood volume and also serve to correct for hemodynamic artifacts appearing in the NADH measurement. An increase in R is due to a lesser absorption of light by the tissue because of a decrease in blood volume, and vice versa. The changes in fluorescence and reflectance signals are calculated relative to the calibrated signals under normoxic conditions. This type of calibration is not absolute but provides reliable and reproducible results from different animals and for different laboratories. More details of this technique have been published by our group [24–28].

Monitoring of pH, pO_2 and pCO_2

The TrendCare Multiparameter Monitoring System (Diametrics Medical Inc, Roseville, MN) was used in the present study to monitor tissue pH, pCO_2 and pO_2 during hemorrhagic shock and resuscitation [29,30]. Each TrendCare multi-parameter sensor incorporates optical fibers for the measurements of pH, pCO_2, and pO_2, as well as a thermocouple for temperature measurements. The individual sensor elements are located at the tip of the probe (25 mm) and are enclosed within a flexible polyethylene tubing membrane with an average outside diameter of approximately 0.5 mm. The probe is connected via a fiber-optic cable to a TrendCare Monitor, an opto-electronic microprocessor based device incorporating a screen for data display. The sensor elements include different chemical indicators that are sensitive to the concentrations of oxygen, CO_2 or hydrogen ions. The monitor transmits light signals down the optical fibers to the sensor tip, which are modified by the indicator and re-emitted to the monitor. The change in the light signals (either absorbance or fluorescence) is proportional to the concentration of the substance being measured. Upon insertion of the probe, the software-controlled monitor provides a numerical and graphical display of the data measured by all the 4 sensors simultaneously and continuously in real time. Computers were interfaced to the monitors via RS232 serial cables for data collection. Data were collected and stored every 10 seconds using MPC software (MicroSol Corporation, Columbia, MD).

Immediately before use, each sensor was calibrated following manufacturers' guidelines. The sensor response times are typically less than 15 seconds at 37°C. The sensor is capable of measuring the following range values: pO_2 – from

20 to 500 mm Hg, pCO_2 – from 10 to 160 mm Hg, and pH – from 6.80 to 7.80.

A Multi-parameter Fiber-optic Sensor (Paratrend 7, Diametrics Medical, MN) was placed in the bladder by direct cystostomy, as previously described [8]. A second paratrend sensor was placed in the leg skeletal muscle, as previously described [31].

Hemorrhage and resuscitation model

Shock was created by withdrawing blood through the femoral vein line at a maximum rate of 15 ml/min, until reaching a mean arterial pressure of 40 mm Hg within 20 minutes. The animal was maintained in this condition for 20 minutes by withdrawal or re-infusion of blood as needed. Once the target time was reached, the animal was resuscitated with shed blood plus 2 times the shed blood with Lactate Ringers and 20 minutes were allowed for recovery. The animal was monitored for 1 hour after resuscitation. At the completion of the experiment, the animal was euthanized while still under general anesthesia.

Heart rate, mean arterial pressure (MAP), Urethral Tissue Blood Flow (UTBF) and Urethral NADH were recorded at 10 seconds intervals throughout the duration of the experiment (Ponemah version 3.30). Continuous skeletal muscle tissue and bladder tissue monitoring of pH, pCO_2, and pO_2 were recorded at 10 seconds intervals throughout the duration of the experiment (Diametrics Inc. paratrend sensor).

Data analysis

Data are expressed as mean ±SD. Analysis of variance with repeated measures was used to determine the significance of changes in gas tensions at each major time point. A Post-hoc Tukey/Kramer procedure was performed in cases of significance. Paired t-tests were used to compare sensor responses at different sites and at several time points during and after shock and resuscitation. Linear regression analysis was conducted to examine the relationship between UTBF and NADH. Significance was determined at $p<0.05$ (GB-Stat, Dynamic Microsystems Inc, Silver Spring, MD).

RESULTS

Hemodynamic parameters

Animals remained hemodynamically stable throughout the instrumentation phase and before the Base Line (BL) time point. The mean arterial blood pressure decreased sharply from 94±15 mm Hg to 45±7 mm Hg over a 15 minutes hemorrhage period. After resuscitation, the blood pressure recovered to 89±16 mm Hg. Animals became tachycardic during shock (the heart rate rose from 118±45 to 152±54 beats per min, $p<0.01$), but returned to the baseline (122±60) after resuscitation (Figure 2).

Multi-parametric monitoring of the bladder wall

The bladder's pCO_2 and pO_2 remained stable during the instrumentation time (Figure 3A). The pO_2 fell dramatically and was significantly different at the end of the shock

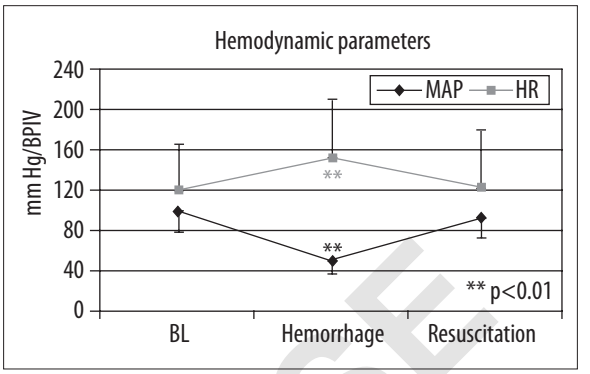

Figure 2. Changes in hemodynamic parameters during shock and resuscitation (MAP – Mean arterial pressure; HR – Heart rate). **p<0.01 for ANOVA (repeated measures) with *post hoc* Tukey's test.

(a decrease from 97±102 to 9±9 mm Hg, $p<0.05$). In most animals, the pO_2 tended towards recovery during resuscitation (Figure 3B).

The monitoring of urethral blood flow and urethral mucosa NADH

Urethral tissue blood flow decreased during hemorrhage and recovered to the BL values following resuscitation (proceeding from 100% at BL to 30±16% at the end of hemorrhage, and to 108±25% during resuscitation, $p<0.01$). During hemorrhage, the urethral NADH values increased significantly as compared to the baseline (from 100% to 424±221%, $p<0.05$), but returned to the baseline with resuscitation (127±9%) (Figure 3C).

Figure 4 exemplifies the continuous data from a single animal. In this chart, we can appreciate the changes described previously in the group analysis (Figure 3C).

When comparing urethral tissue blood flow and NADH through the Pearson correlation, an inverse high correlation is found between the two parameters during hemorrhage and resuscitation (r^2 0.74) (Figure 5). In summary, there is a decrease of the tissue blood flow during shock and an increase in NADH until the beginning of resuscitation.

Bladder and skeletal muscle pCO_2 compared to urethral mucosa NADH

The bladder and skeletal pCO_2 increased during hemorrhage and recovered to the BL values following resuscitation. There was an almost symmetrical response in NADH and pCO_2 in both tissues (skeletal and bladder). Examples of continuous data from single animals are shown in Figure 6AB.

Bladder and skeletal muscle pO_2 compared to urethral mucosa NADH

The bladder and skeletal pO_2 decreased during hemorrhage and recovered to the BL values following resuscitation. Both tissues' pO_2 (skeletal and bladder) showed a mirrored response when compared to urethral NADH. Examples of continuous data from single animals are shown in Figure 7AB.

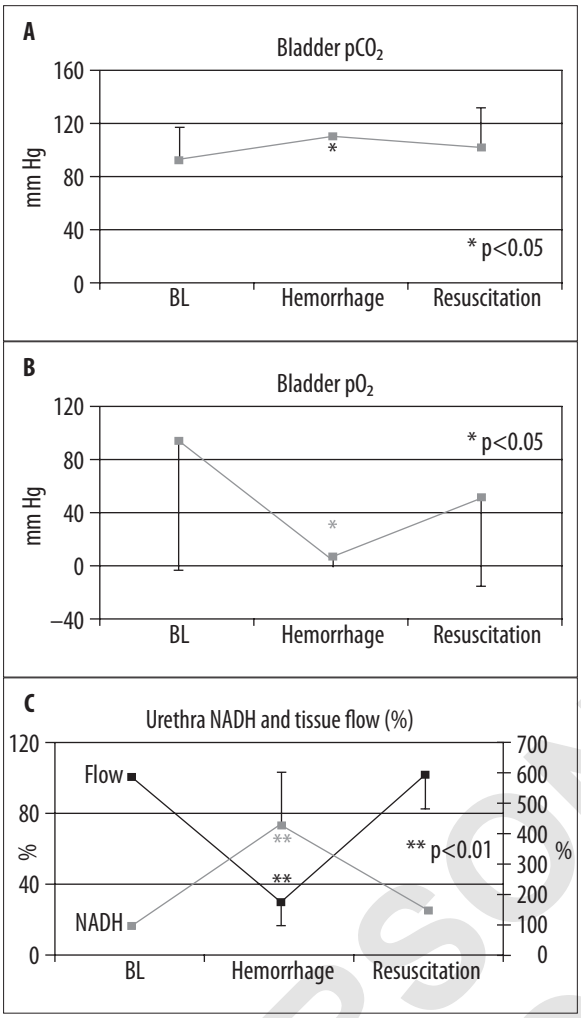

Figure 3. pCO_2 (**A**), and pO_2 (**B**) changes in the bladder compared to Urethral NADH and tissue flow (**C**) during hemorrhagic shock. *p<0.05, **p<0.01 for ANOVA with *post hoc* Tukey's test.

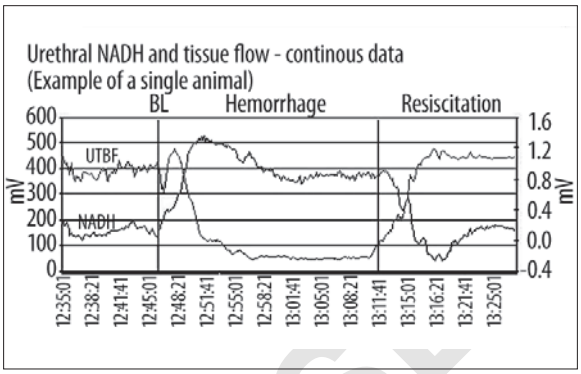

Figure 4. Urethral NADH and UTBF changes in a single animal experiment. There is an almost symmetrical response in NADH accompanied by changes in TBF. NADH data are expressed in millivolts.

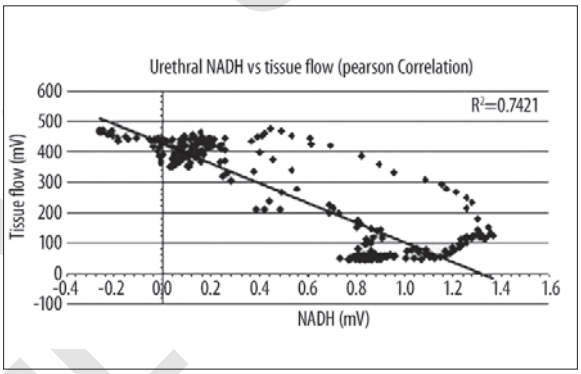

Figure 5. Pearson's correlation between Urethral NADH and UTBF (example of a single animal).

Discussion

Our study demonstrates that the measures of local intracellular energy metabolism and blood flow vary, in a predictable fashion, in response to transient severe hemorrhagic shock. The theoretical basis of the present technique is that, under hemorrhage-induced perfusion impairment, there is an increase in mitochondrial NADH levels due to inadequate O_2 availability. NADH is a naturally occurring fluorophore and is one of the main means of energy transfer from the tricarboxylic acid cycle to the respiratory chain in the mitochondria [25,28,32]. The inhibition of the respiratory chain and the reduction of oxidative phosphorylation due to inadequate oxygen supply, are reflected by increased intracellular NADH levels. NADH, unlike NAD^+, absorbs UV light and fluoresces in the blue range [28,32–34]. NADH fluorescence provides direct information about the activity of mitochondrial respiration and enables the imaging of its spatial distribution on organ surfaces *in vivo*.

In the present study, we constructed a special probe that mimics the clinical Foley catheter. We incorporated the NADH/blood flow monitoring instrument into the catheter-like device and measured the mitochondrial redox state, assessing the adequacy of oxygen balance. Fiber-optic surface fluorometry-reflectometry was used to monitor the NADH redox state of the urethral mucosa. We described the changes in NADH and microcirculatory blood flow, and their relation to impaired tissue perfusion in a standard hemorrhage model of controlled hypotension. In this model, we showed that, in the urethra, the optically measured NADH increased during shock and recovered during resuscitation, while tissue blood flow showed an opposite effect (r^2=0.74). NADH increased significantly ($p<0.05$) during shock and decreased after resuscitation. Our preliminary data suggest that there is a significant correlation between blood flow and mitochondrial NADH in the urethral wall during hemorrhage and resuscitation. These parameters could potentially be measured *in vivo* by a minimally invasive approach. These parameters represent novel markers for the vitality of the tissue and could provide important information for optimizing the end-points of resuscitation in hemorrhagic shock, using a relatively convenient and accessible device.

The unique monitoring methodology

The reported metabolic and flow measurement technique has been previously validated by our group and it represents a promising method for assessing tissue vitality. Its strength

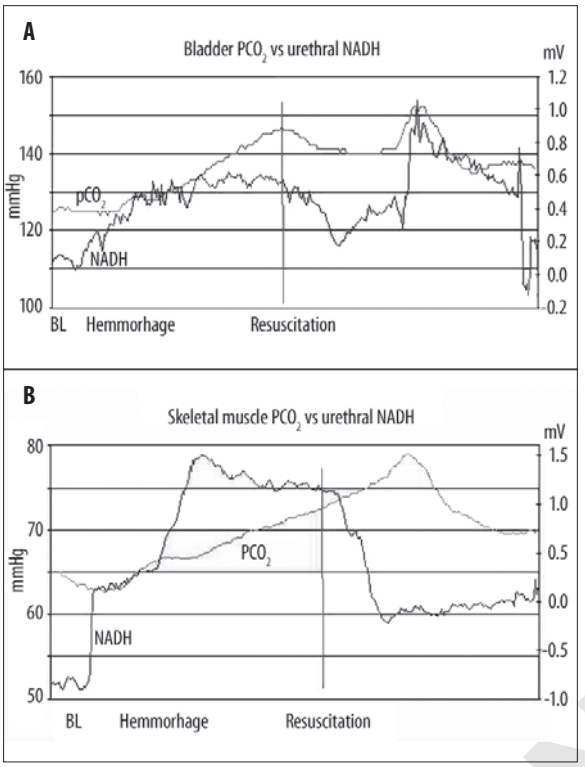

Figure 6. Changes in Urethral NADH and Bladder pCO_2 (**A**), and Skeletal muscle pCO_2 (**B**) in a single animal experiments. There is an almost symmetrical response in NADH and pCO_2 in both tissues (skeletal and bladder). NADH data are expressed in millivolts and pCO_2 in mm Hg.

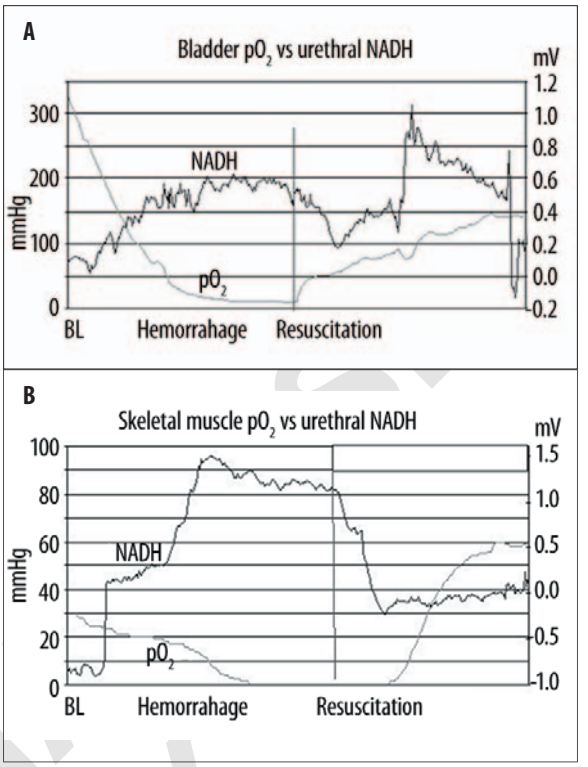

Figure 7. Changes in Urethral NADH and Bladder pO_2 (**A**) and Skeletal muscle pO_2 (**B**) in a single animal experiment. There is an almost mirrored response in NADH and pO_2 in both tissues (skeletal and bladder). NADH data are expressed in millivolts and pO_2 in mm Hg.

lies in the ability to conduct continuous measurements, with a fast reaction time, allowing for real-time trend analysis, in order to define responses to therapy and potentially instruct cardiovascular management.

We chose the urethra mucosal tissue as a sampling site for two reasons: convenience and physiological rationale. This is a commonly instrumented site and it would therefore involve no further invasion for continuous, long-term monitoring. Bladder tissue has a low metabolic rate and does not constitute a privileged site for blood flow, thus its metabolic activity should accurately reflect the final vascular bed flow restoration, without irreversible components, during resuscitation. This contrasts to intestinal mucosa which can easily demonstrate irreversible injury and thus may not be a good marker of global resuscitation effectiveness.

Superior monitoring systems

The concept that a single parameter could provide information on the adequacy of resuscitation, is rapidly loosing validity among clinicians. It is clear that, despite the scores of publications on gastric tonometry, this technique has achieved neither wide acceptance nor clinical applicability. Perhaps even more important is the fact that, presently, there is no state of the art method to assess when an optimal level of cellular homeostasis has been achieved in order to ascertain that there is no further metabolic stress or ongoing energetic failure at the cellular level after a severe shock.

Ideally, a monitoring strategy capable of providing real time information on cell vitality, should fulfill a number of requirements. First, it should be based on highly reliable specific markers. Secondly, the system must allow for an easy and minimally or, preferably, non-invasive placement. It should be of low cost, low risk and should provide fast feedback. Each and every one of these requirements is important. Unfortunately, up to date, such a monitoring strategy is not available. Our group has been exploring the concept that the urinary bladder and the urethral mucosa may be an easily accessible region for tissue perfusion monitoring. We believe that, if the proper sensors are developed for specific markers and incorporated into a "smart" urinary catheter, then real time information can be gained on tissue vitality.

The success in creating such sensors relies on the development of a multi-focal approach that needs to progress simultaneously in three different investigation fields. First, there is a need to investigate the underlying physiological concepts, which, in the present case, should answer such questions as: Is the bladder the appropriate organ to interrogate? If so, is the mitochondrial function the suitable parameter for interrogation? Furthermore, the biomedical engineering research needs to proceed in parallel to these concepts. In this regard, we would like to address the following question: Can NADH and/or blood flow be accurately measured? And finally, and most importantly, we need to develop appropriate animal models and/or clinical scenarios to study whether specific interventions or resuscitation strat-

egies, aimed at modifying or preventing changes in these markers, can actually translate into better outcomes.

We recognize that these are enormous tasks and admit that our current work only begins to addresses some of these issues. Nevertheless, we have adapted techniques that have been used and validated for other clinical applications and have started testing how these new monitoring methods perform in a simple, well-recognized model of pressure-controlled hemorrhagic shock.

NIRS has been used to measure the tissue Hb saturation during resuscitation from traumatic shock [35,36]. This is a non-invasive approach that provides measurements of tissue HbO_2 saturation and it has been developed to report a quantitative clinical variable. McKinley showed that skeletal muscle StO_2 traced systemic O_2 delivery during and after resuscitation in 8 severely traumatized patients. These authors proposed that StO_2 measured by NIR could be a useful parameter to guide resuscitation in the ICU, to monitor resuscitation levels in the operating room and potentially to detect occult shock during the initial assessment of severely traumatized patients in the ER. Our group has subsequently shown that the levels of tissue pCO_2 and pH in the peripheral skeletal muscle are closely correlated to the magnitude of blood volume loss during shock and resuscitation [31]. We have recently measured the same parameters in the subcutaneous tissue and found that they closely correspond to what we previously reported for the muscle [37]. It is important to note that the Hb saturation is a good indicator of O_2 supply, however, the NADH level is an indicator of the O_2 balance in the various compartments of the tissue. Another monitoring approach is the tonometry of the gastric mucosa, but this parameter represents only part of the energy metabolism processes. The NADH represents the events occurring in the most critical point of the energetic activity – the mitochondria.

Study limitations

First of all, the shock injury was transient, even though severe, and may not reflect the events observed in sustained and/or resuscitated states, commonly occurring in the clinic. Secondly, we measured only bladder mucosa and skeletal muscle. Although we would predict that their trends should resemble other non-privileged sites, such information was not collected. We did not examine the impact of vasoactive therapy during shock, which may independently alter non-privileged sites' blood flow. However, if such flow were selectively reduced, then, if anything, our measures would be more sensitive to occult hypoperfusion than measurements from privileged sites, such as the kidney, heart and brain. Finally, although the measurements of intracellular energy reserve tended to correlate with the expected changes in intracellular energy economy, we never measured regional VO_2, lactate production or ATP turnover rates. Thus, we cannot ensure that the measurements of NADH levels by sensitive trend monitors of hypoperfusion, actually reflect a decreased intracellular energy reserve.

We hypothesize that real-time monitoring of urethral mucosal NADH, measured concomitantly with urethral blood flow, may provide a rapid assessment of functional O_2 delivery. If elevated NADH is a sensitive measure of impaired visceral flow and low tissue oxygen consumption, then improvement of these parameters should indicate that other, metabolically more active organs have already replenished their oxygen debt. The same multiparametric monitoring approach was succesfully applied to study simultaneously brain and small intestine exposed to hypoxia and anoxia [38]. We will need to design a different model to test this hypothesis. However, this preliminary work indicates that there are reasonable and easily measurable changes in NADH and blood flow in the targeted tissue, induced by a relatively mild model of hemorrhage. Tissues that are made ischemic by circulatory insufficiency develop an "O_2 debt" wherein they use anaerobic metabolism to sustain energy production. Once the flow of oxygenated blood resumes, the tissues consume more O_2 than is required for their real time energy needs, as they "reimburse" this O_2 debt. With adequate resuscitation, NADH should decrease relatively soon, because: 1) urethral mucosa tissues are not highly metabolically active and thus do not develop significant O_2 debt, and 2) even though the urethra may become well oxygenated early during resuscitation, other tissues, such as the intestine, kidney and contracting muscles, can develop a significant O_2 debt that needs time to compensate. If the patient undergoes a slower or delayed resuscitation, or ongoing bleeding from an uncontrolled or undetected region, such as retroperitoneal hemorrhage or pelvic fracture, then there will be a time delay in urethral NADH recovery that may alert the clinician to an ongoing occult shock.

Conclusions

Further experiments will be needed to identify the thresholds for acute deterioration, as well as early sings of improvement or appropriateness of the response to resuscitation.

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