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SHEDDING LIGHT ON LIFE: OPTICAL ASSESSMENT OF MITOCHONDRIAL FUNCTION AND TISSUE VITALITY IN BIOLOGY AND MEDICINE

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The involvement of mitochondrial dysfunction in various pathophysiological conditions, developed in experimental and clinical situations, is widely documented. Nevertheless, real time monitoring of mitochondrial function *In-vivo* is very rare. The pressing question is how the mitochondria of intact tissues behave under *In-vivo* conditions as compared to isolated mitochondria that had been described by Chance and Williams over 50 years ago. This subject has been recently discussed in detail (Mayevsky and Rogatsky 2007). We reviewed the subject of evaluating mitochondrial function by monitoring NADH fluorescence together with microcirculatory blood flow, Hemoglobin oxygenation and tissue reflectance. These 4 parameters represent the vitality of the tissue and could be monitored *in vivo*, using optical spectroscopy, in animal models as well as in clinical practice. It is a well known physiological hypothesis that, under emergency conditions, the sympathetic nervous system will give preference to the most vital organs in the body, namely the brain, heart and adrenal glands. The less vital organs, such as the skin, GI-tract, and Urethral wall, will become hypoperfused and their mitochondrial activity will be inhibited. The monitoring of the less vital organs may reveal critical tissue conditions that may manifest an early phase of body deterioration. The aim of the current presentation is to review the experimental and preliminary clinical results accumulated using a new integrated medical device – the "CritiView" which enabled, for the first time, monitoring 4 parameters from the tissue using a single optical probe. The CritiView is a computerized optical device that integrates hardware and software in order to provide real time information on tissue vitality. In preliminary clinical testing, we used a 3-way Foley catheter that includes a bundle of optical fibers enabling the monitoring of the 4 parameters, representing the vitality of the urethral wall (a less vital organ).We found that the exposure of patients to metabolic imbalances in the operation room led to changes in tissue blood flow and inhibition of mitochondrial function in the urethral wall. In conclusion, the new device "CritiView" could provide reliable, real time data on mitochondrial function and tissue vitality in experimental animals as well as in patients.

1. Introduction

Normal physiological function of any organ is dependent on its capacity to produce biological energy from substrates available to it. A main source of energy is glucose which is oxidized through a complex biochemical chain, primarily through a series of

enzymatic reactions known as the Krebs cycle. The end-product of these reactions is the production of ATP by oxidative phosphorylation occurred in the mitochondria. Thus, production of biological energy is dependent upon the functional integrity of the Krebs cycle, the normal mitochondrial function and availability of oxygen. Disruption of oxygen supply may produce disease which is manifested by physiological dysfunction. It is therefore of clinical importance to develop integrative experimental approaches which would make possible elucidation of the interaction between biochemical events, such as mitochondrial function, and their physiological consequences.

In 1914 Barcroft described in details the role of oxygen in the function of cells and tissues and concluded that "there is no instance in which it can be proven that an organ increases its activity under physiological conditions, without also increasing in its call for oxygen, and- "in no organ excited by any form of stimulation can it be shown that positive work is done without the blood supply having to respond to a call for oxygen.[3] Less then ten years earlier, the involvement of adenine nucleotides in yeast fermentation was described.[18] This discovery opened a period of 100 years of intensive research activities on mitochondrial function. Table 1 describes the history of NADH monitoring in studying mitochondrial function. As can be seen in the table, the contribution of Prof. Britton Chance to this important field is obvious. Also, the shift from a single parameter to the multiparametric monitoring approach including NADH redox state is presented.

During the last decades a large number of publications had shown the very wide involvement of mitochondrial dysfunction in many pathological conditions as shown in Fig. 1. It is clear that normal mitochondrial function is a critical factor and need in order to keep the physiological and biochemical activities of all cells, tissues and organs in the body.[36,45]

In this article, the brain will be used as a target organ but the same principles of mitochondrial function could be applied to other organs and systems in the body as well.

The mechanisms behind the development of brain damage during pathological states such as ischemia, hypoglycemia and epilepsia were investigated during the past two decades using various experimental approaches. Also, the mechanisms behind other human brain related diseases such as Alzheimer or alcohol intoxcication and other drug addiction, are poorly understood due to the lack of suitable experimental models and monitoring capabilities.

1.1. *Energy metabolism and brain functions*

Normal brain activity depends upon the continuous supply of oxygen (carried by the blood) due to the high O_2 consumption as well as a very limited reserve of dissolved O_2 in the tissue. The brain consumes approximately 20% of the total O_2 used by the body per unit time. Over 50% of the energy consumed by the brain is utilized for active transport mechanisms (such as Na^+K^+ATPase) responsible for

Table 1. Milestones in Monitoring of Mitochondrial activity using NADH fluorescence and other physiological parameters, from animal studies to clinical use.

Year	Discovery	Author (s)
1906	Involvement of adenine containing nucleotides in yeast fermentation	Harden & Young[20]
1935	Complete structure of "Hydrogen transferring Coenzyme" in erythrocytes	Warburg *et al.*[56]
1936	Definition of the two cofactors DPN and TPN	Warburg[55]
1951	A shift in the absorption spectrum of DPNH with Alcohol dehydrogenase	Theorell & Bonnichsen[53]
1951	Development of a rapid and sensitive Spectrophotometer	Chance & Legallias[10]
1952	Monitoring of pyridine nucleotide enzymes	Chance[5]
1954	Development of the double beam spectrophotometer	Chance[4]
1957	The first detailed study of NADH using Fluorescence spectrophotometry	Duysens & Amesz[15]
1958	Measurement of NADH fluorescence in isolated mitochondria	Chance & Baltscheffsky[6]
1959	Measurement of muscle NADH fluorescence *in vitro*	Chance & Jobsis[9]
1962	*In vivo* monitoring of NADH fluorescence from the brain and kidney	Chance *et al.*[7]
1965	Comparison between NADH fluorescence *in vivo* and enzymatic analysis	Chance *et al.*[8]
1968	Monitoring tissue reflectance in addition to NADH fluorescence	Jöbsis & Stansby[24]
1971	The first attempt to monitor the human brain in neurosurgery	Jöbsis *et al.*[23]
1973	The first fiber optic NADH fluorometer used in the brain of an awake animal	Chance *et al.*[11]; Mayevsky & Chance[37]
1982	Simultaneous monitoring of NADH *in vivo* in four different organs	Mayevsky & Chance[39]
1982	Monitoring of NADH, ECoG, DC potential and extracellular ions *in vivo*	Friedli *et al.*[17]
1985	Monitoring of brain NADH together with 31P NMR Spectroscopy	Mayevsky *et al.*[45]
1991	Monitoring of NADH , CBF, ECoG, and extracellular ions in animals and neurosurgical patients	Mayevsky *et al.*[42]
1996	Detection of cortical spreading depression in a comatose patient	Mayevsky *et al.*[41]
2000	"Tissue Spectroscope" a new device for monitoring of NADH and TBF	Mayevsky *et al.*[44]
2006	Monitoring of tissue vitality (NADH, TBF and HbO_2) by the "CritiView"	Mayevsky *et al.*[35]

the normal distribution of ions around the cell membranes.[15] ATP is the sole form of energy available for cellular functions and is produced by the oxidative phosphorylation process which takes place in the mitochondria. Direct coupling exists between the extracellular levels of various ions (such as K^+, Ca^{2+}, Na^+ or H^+) and the metabolic activity of the mitochondria, reflected in the oxidation reduction state of the respiratory chain enzymes (such as NADH — Nicotine Adenine Dinucleotides).

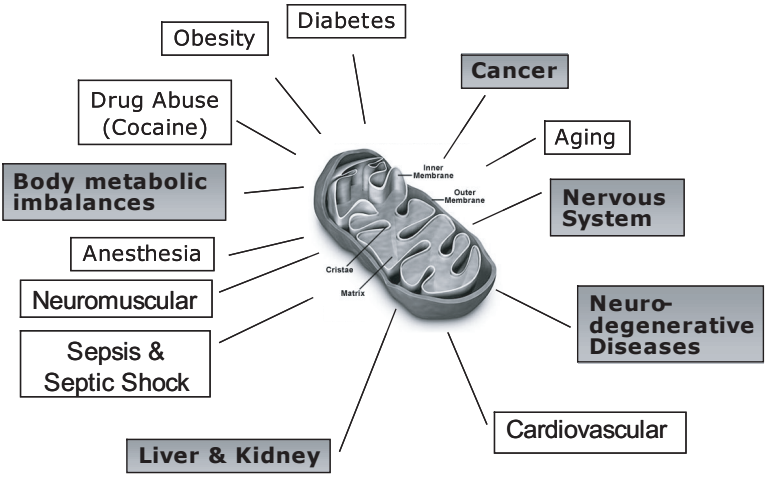

Fig. 1. The involvement of mitochondrial dysfunction in various pathophysiological conditions. Mitochondrial dysfunction was described as a critical step in the development of many states of disease in patients.

A decrease in O_2 supply to the brain will result in a decrease in ATP levels and in the inhibition of active transport mechanisms (such as Na^+ K^+ ATPase). Extracellular K^+ levels will then increase until restoration of normal energy supply. The redox state of the mitochondria is a sensitive indicator of the intracellular metabolic state and can be used for the evaluation of the cellular energy status.[12,30] Any change in the electrical activity of the brain will result in an activation of the ion pumps in an effort to restore normal ion distribution. In the pioneering work described by Chance and Williams,[12] they describe several metabolic states for the isolated mitochondria, which depend upon the availability of O_2, substrate and ADP. The "resting state", state 4, exhibited high O_2 and substrate levels, with the limiting factor being the ADP level. Any increase in the energy demand will result in the acceleration of electron flow through the respiratory chain and the oxidation of the various carriers. The active state, during which O_2 consumption is increased, was defined as state 3. In state 3, cerebral blood flow will increase in order to compensate for the increased O_2 consumption,[47] while in state 4, 99% of the NADH will be in the reduced form. The "resting" brain *in vivo* is probably somewhere between state 4 and state 3.[31,32] In order to evaluate the functional state of the brain *in vivo* it is necessary to monitor numerous parameters relevant to various brain functions. A multiparametric monitoring device was developed for this purpose and applied to an experimental animal model of stroke[33] and to other pathological states.[34]

As shown in Fig. 2, the supply of O_2 to all tissues is based on the same principles namely, that the oxygenated blood flows in the tissue is delivering the O_2 to the cells. Oxygen's demand on the other hand is dependent on the energy needs of every organ. In other words in order to measure O_2 supply to various tissues one can use

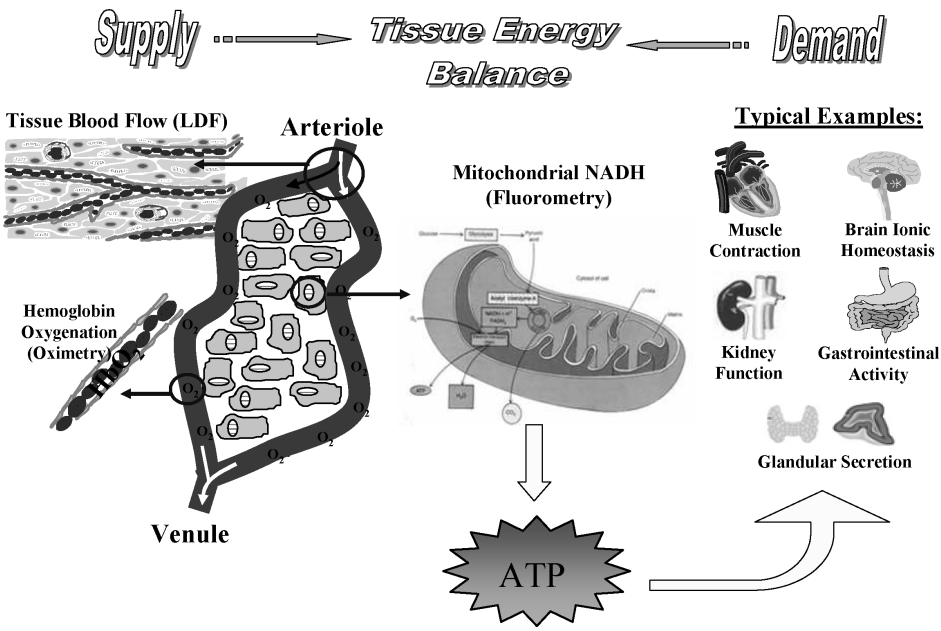

Fig. 2. The relationship between oxygen supply and demand in the various tissues in the body. The redox state of the mitochondria could be used as an indicator to oxygen balance in the tissues.

the same technology while for O_2 demand, different techniques need to be applied to the different organs. In general terms, when the balance between O_2 supply and O_2 demand is negative (oxygen debt is created) the function of the tissue or organ will be affected and a pathological state is developed. Typical clinical examples of conditions characterized by a severe negative O_2 balance are myocardial infarction or stroke where due to occlusion of blood vessel in the heart or the brain the supply of O_2 to a specific region in the vital organ is limited and the function is then inhibited or severely compromised. These two examples represent dramatic and acute pathological events that create very specific manifestations and the diagnosis of such conditions are relatively straight forward.

Conversely, subclinical O_2 delivery insufficiency is a condition that may result from a number of several pathological states in patients with acute critical Illness. The underlying characteristic here can be defined as a circulatory failure and this may result from different conditions associated with Shock such as sepsis, cardiac insufficiency, hemorrhage, neurogenic shock etc.

In order to assess the functioning brain *in vivo*, we have developed a unique approach by which various parameters representing the several functions of the brain are simultaneously monitored from the same area in real-time mode. The key parameter that was developed was the monitoring of the intactness of mitochondrial function. The energy state and metabolism are evaluated by monitoring the cerebral blood flow and volume (Laser Doppler Flowmetry) as well as

intramitochondrial redox state (surface NADH fluorometry reflectometry). The ion homeostasis is determined by measuring the extracellular K^+, Na^+ Ca^{2+} activities (ion selective surface mini-electrodes). The electrical activities are assessed by the spontaneous ECoG activity (bipolar electrodes) as well as by the extracellular direct current steady potential (Ag/AgCl-electrodes).

The various aforementioned transducer probes are located on the surface of the cerebral cortex by using the **Multiprobe Assembly-MPA** which has been adapted for routine usage in experimental animals as well as under operating room conditions.[29,41,45] The MPA is connected to various specific detectors and amplifiers and the data are displayed on a multi-channel computerized recording system. We record up to 16 channels at the same time and only the most advanced computer technology can cope with the need for data acquisition and storage as well as on-line and off-line analyses.

Using the MPA in conjunction with the computerized monitoring and acquisition system will help the neuroscientist and the neurosurgeon to evaluate the status of the brain during various procedures. It is believed that such a system will contribute to the greater success in drug development and usage as well during brain surgery and thus to benefit the patient.

Figure 2 also shows the parameters that can be monitored on-line even in the awake state *in vivo*. Relative CBF is monitored by Laser Doppler Flowmetry.[54] Mitochondrial redox state is measured by monitoring NADH fluorescence.[30] Surface minielectrodes are used for monitoring extracellular levels of K^+, Na^+, Ca^{2+} and H^+. The functional state of the brain is evaluated by monitoring electrical activities (ECoG and DC steady potential).

Our approach is to monitor the brain at the "tissue level" rather than the "cellular level". We believe that the various elements of the brain: neurons, glial cell and capillaries, act as an integrated system. Therefore, we monitor the brain functions using "mini"-probes rather than the "micro"-probes commonly used by other investigators for monitoring ionic homeostasis.[17] The evaluations of other parameters, such as CBF and NADH redox state, were also adapted to the "tissue" level rather than to the "cellular" level. For all these reasons, our strategy was to develop a multiparametric monitoring assembly in which all the probes have the same type of contact with the sampled tissue volume.[13,16,29,40,43] The probes do not penetrate the tissue itself, thus avoiding severe damage to the brain or formation of an artificial environment around the sensor, as is created around a penetrating microelectrode. Also, the surface monitoring approach is more acceptable for clinical applications.

1.2. *Spreading depression and spreading-depression-like depolarization*

Spreading depression (SD), discovered by Leao (1944),[28] is a phenomenon initiated by a rapid depolarization of neuronal tissue in the gray mater of the cerebral cortex

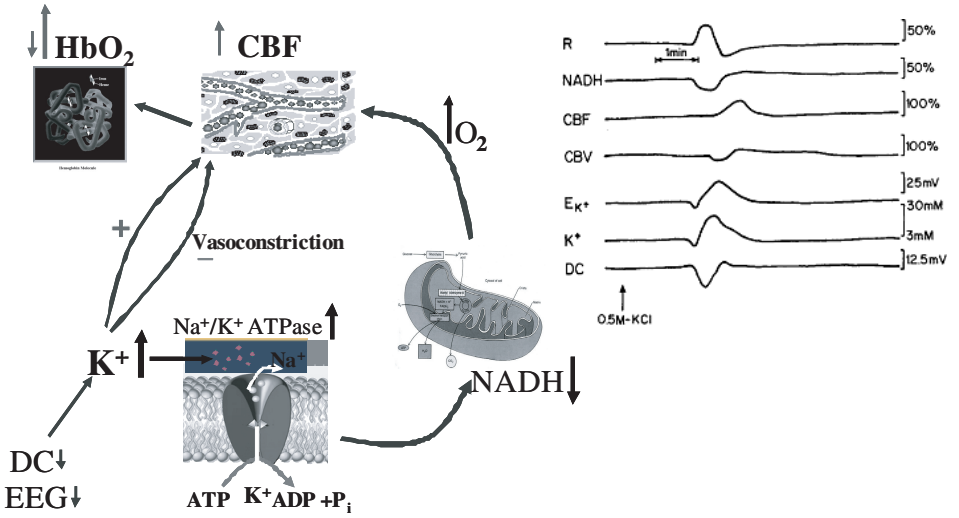

Fig. 3. Hemodynamic, metabolic, ionic and electrical activities of the brain recorded after initiation of a wave of Spreading Depression (right side). A possible mechanism showing the cascade of events is presented in the left side.

(Fig. 3 left side), with a massive redistribution of ions between the intracellular and extracellular spaces, a decrease in the DC steady potential and a depression of the spontaneous electrical activity (EEG or ECoG). No deleterious effects on neuronal tissue function was reported under conditions of increased oxygen supply compensating for the extra oxygen needed by the SD process.[49,51] The SD has a wave (Fig. 3 right side) shape propagating from the point of its initiation to the entire ipsilateral cerebral hemisphere. Under hypoxic or ischemic conditions, depolarization events similar to Spreading Depression may develop, mostly in the ischemic penumbra.[19] These responses are termed, hypoxic or ischemic SD-like depolarization (HSD, ISD) which may start in a small focus or develop simultaneously in few sites depending on the metabolic state of the tissue. The HSD or the ISD may spreads at the same velocity as SD in a normal tissue. It was suggested that ischemic depolarization waves developing after focal cerebral ischemia, cause an expansion of the core-infracted tissue into the penumbra region.[19] Following SD, CBF usually increases above its basal level, while under HSD or ISD, CBF show no hyperemia but rather a clear decrease.[52] Another major difference between SD and HSD or ISD is the state of mitochondrial enzymes which become oxidized under SD, but reduced (increased) after the onset of HSD or ISD.[37,39,48,50] Oxygen is a limiting factor in HSD and the ISD, therefore the time needed for the recovery from depolarization in the ischemic tissue is longer as compared to the normal SD (Fig. 2). It was also hypothesized that normoxic SD may protect the brain from ischemic injury.[23,24] Considering all these aspects, we believe that increasing knowledge in this field, to be accumulated in the proposed project, may contribute to a better

understanding of brain ischemia and/or stroke phenomena. Also, we are proposing to use the SD response as a standard tool for the evaluation of the ischemic damage or the reversibility of the insult which is a new approach.

1.3. *Body homeostatic compensatory mechanisms*

It is well known and documented that the autonomic nervous system (ANS) and mainly its sympathetic branch, including the adrenal gland, play a major role in mounting the compensatory mechanisms of the body to O_2 deficiency. The rapid compensatory reaction to a decrease in blood volume (hypovolemia), for example, includes redistribution of blood flow to various organs and giving preference to the most vital organs in the body, namely to the, brain, heart and adrenal glands.[2] These compensatory mechanisms are overcome if the insult is severe enough or the underlying conditions are not withdrawn within a reasonable time. As a central protection mechanism, blood flow redistribution will occur and the three protected organs (brain, heart and adrenal gland) will receive more blood and O_2, while the peripheral organs or areas (skin and muscles), as well as others non vital visceral organs, will undergo vasoconstriction and subsequent decrease in blood flow and O_2 supply. This mechanism is presented in Fig. 4.

1.4. *Pathophysiology of critically ill patients*

The pattern of the physiopathological cascade of events that may occur in many clinical conditions associated with an imbalance between oxygen delivery and

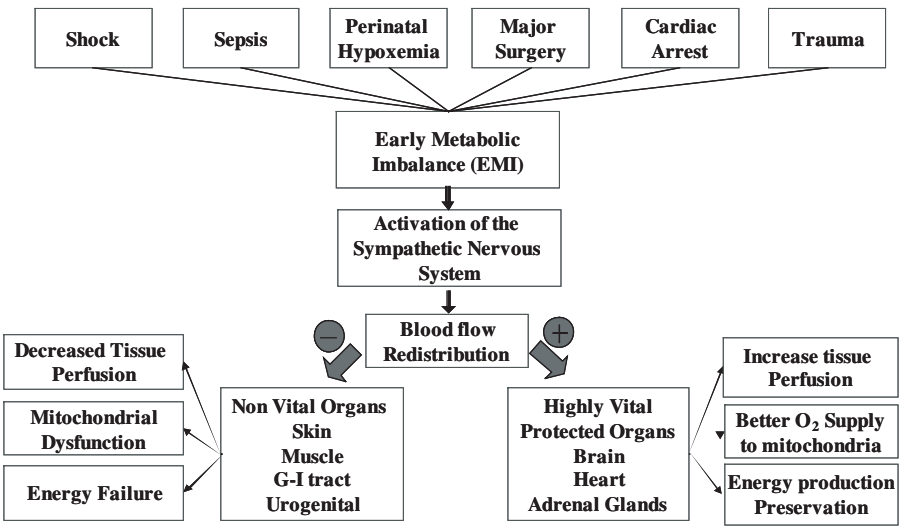

Fig. 4. Schematic presentation of various pathological states developed under various clinical situations, which lead to the development of early emergency imbalance (EMI). As a result, blood flow redistribution will lead to an increase in blood flow to the most vital organs and a decrease in blood flow to the less vital organs.

oxygen consumption is shown in Fig. 2. Tissue hypoxia is the common denominator. Various pathological states as well as major surgery may lead to metabolic disturbances and may end up in cellular energy derangement. Consequently, compromised global O_2 balance can be associated with significantly high morbidity and mortality in large number of patients. The most common example of such a pathological state is sepsis, which is a major cause of death in critically ill patients.[1,20]

Sepsis represents a large group of patient's illnesses in which early identification and accurate quantification of impaired O2 balance is difficult to accomplish and the consequences of the delayed recognition of subclinical shock are associated with increased mortality. Other conditions such as hypotension, systemic hypoxemia, or early acute respiratory distress syndrome (ARDS) may benefit from "precocious" interventions if adequate monitoring techniques capable of identifying reversible critical thresholds of metabolic or energetic failure of the mitochondria could be identified before the onset of irreversible cellular damage has occurred. The same problem of O_2 imbalance may develop in preterm or full term infants hospitalized in the neonatal ICU or even in the newborn during delivery.

The six conditions shown in the upper part of Fig. 4, are some of the most common events associated with impaired O_2 balance in clinical practice. Under all those situations the metabolic state of the body will deteriorate and energy failure will develop if the appropriate corrective measures are not implemented. These clinical conditions could occur in any area of the hospital or in the prehospital field. Current monitoring mechanisms designed to identify manifestations of early metabolic imbalance (EMI) can only be applied in sophisticated ICU environments. Also, patients that undergo major surgery such as cardiac bypass, neurosurgical or organ transplantation as well as newborns during delivery or fragile elderly patients admitted to general internal medicine wards are at an increased risk of EMI.

1.5. *Monitoring of critically ill patients in medical practice*

There is a strong evidence in the literature that the severity of impaired tissue perfusion associated with cardiovascular failure and other states of shock is a major determinant of outcome. The lack of standard endpoints or targets of complete resuscitation after severe hypoperfusion may account for increased incidence of multiple organ dysfunction syndrome (MODS) and poor outcome in critically ill trauma patients. A vast amount of resources in critical care research are being directed to identify and analyze generic markers of incomplete resuscitation such as splanchnic hypoperfusion, tissue acidosis, and impaired systemic oxygen delivery. Early identification of such factors and prompt interventions to correct them may result in reduced incidence of MODS, reduced length of stay and overall improvement of ICU outcomes.

If specific markers of incomplete resuscitation at the cellular level can be identified by means of applying new and less invasive sensors, then perhaps thresholds of irreversible cellular damage can be delineated and interventions be designed to

prevent or ameliorate cellular injury and consequently MODS. The search for the perfect indicator as well as the most representative organ or tissue in the body to be monitored is an ongoing process. Ince and Sinaasappel (1999)[21] concluded that "to evaluate the severity of microcirculatory distress and the effectiveness of resuscitation strategies, new clinical technologies aimed at the microcirculation will need to be developed. It is anticipated that optical spectroscopy will play a major role in the development of such tools". In a recent published paper Kruse summarized the effort done by various investigators regarding the perfect indicator of dysoxia, which could be defined as a state of supply-dependent oxygen consumption.[27]

Figure 5 present's results of a typical experiment showing the responses of a vital organ (brain) and less vital organ (skin of the scalp) to two perturbations. When the 2 common carotid arteries were occluded (left side), blood supply to the 2 organs decreased and therefore TBF decreased to the brain and skin. As a result, the NADH was elevated in the 2 organs indicating a low intramitochondrial oxygen levels. During the reperfusion stage the TBF in the brain showed a typical hyperemic response that was missing in the skin.

When adrenaline was injected IV, a clear differentiation between the responses of the 2 organs was recorded. In the brain a large increase in TBF was followed

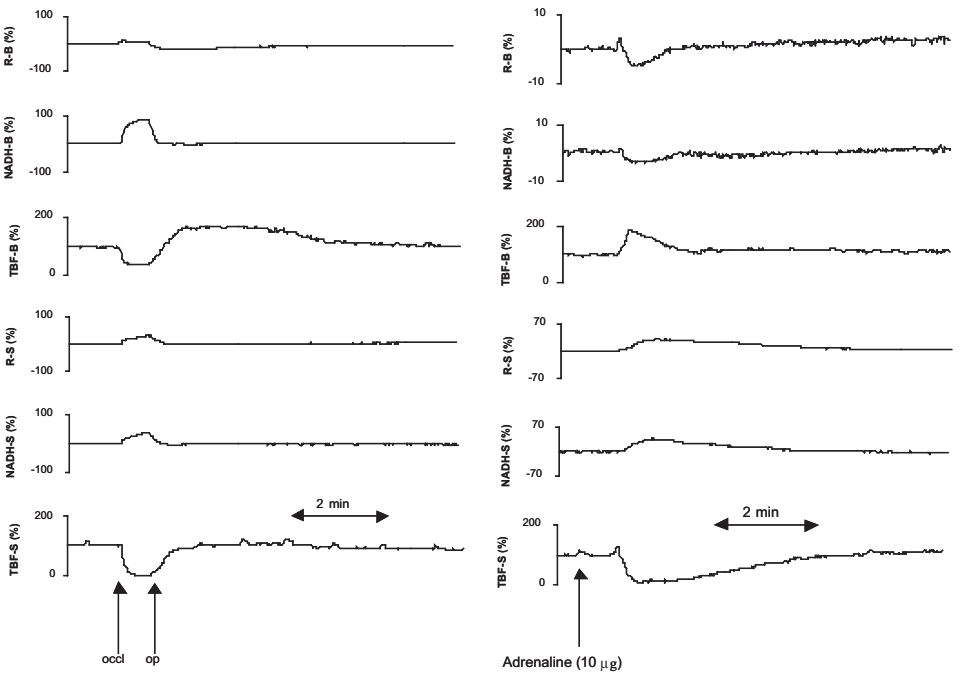

Fig. 5. The responses of the rat brain and scalp skin to ischemia (left) and IV injection of Adrenaline (right). Occl, Op-occlusion and opening of the two common carotid arteries. R-B, NADH-B and TBF-B: reflectance, NADH fluorescence and blood flow in the brain. R-S, NADH-S and TBF-S: reflectance, NADH fluorescence and blood flow in the skin.

by an oxidation of NADH. In the skin ischemic responses were recorded namely, a large decrease in TBF was recorded together with elevation in NADH. Those results were in accordance with the previously published material that show the difference in the responses of vital and less vital organs.[25,26]

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