Monitoring mitochondrial PO_2: the next step

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Purpose of review
To fully exploit the concept of hemodynamic coherence in resuscitating critically ill one should preferably take into account information about the state of parenchymal cells. Monitoring of mitochondrial oxygen tension (mitoPO_2) has emerged as a clinical means to assess information of oxygen delivery and oxygen utilization at the mitochondrial level. This review will outline the basics of the technique, summarize its development and describe the rationale of measuring oxygen at the mitochondrial level.

Recent findings
Mitochondrial oxygen tension can be measured by means of the protoporphyrin IX-Triplet State Lifetime Technique (PpIX-TSLT). After validation and use in preclinical animal models, the technique has recently become commercially available in the form of a clinical measuring system. This system has now been used in a number of healthy volunteer studies and is currently being evaluated in studies in perioperative and intensive care patients in several European university hospitals.

Summary
PpIX-TSLT is a noninvasive and well tolerated method to assess aspects of mitochondrial function at the bedside. It allows doctors to look beyond the macrocirculation and microcirculation and to take the oxygen balance at the cellular level into account in treatment strategies.

Keywords
hemodynamic coherence, mitochondrial oxygen tension, mitochondrial respiration, tissue oxygenation

INTRODUCTION

Resuscitating critically ill patients from different states of shock is a key strategy in critical care but remains a challenge. Targeting the normalization of systemic hemodynamic parameters does not lead to improved outcomes [1–5]. Over the last two decades, considerable attention has been given to the role of microcirculatory dysfunction as substrate for such failure, leading to the concept of 'hemodynamic coherence' [6,7].

Hemodynamic coherence is the coherence between the macrocirculation, microcirculation and ultimately the parenchymal cells, leading to an optimal balance of supply and demand of oxygen and nutrients to the tissues. Loss of hemodynamic coherence is associated with increased morbidity and mortality [8–10], as recently confirmed again in cardiogenic shock patients [11▪]. The treatment strategy can have an effect on the occurrence of loss of hemodynamic coherence [12▪].

As the ultimate goal of optimizing macrocirculatory and microcirculatory hemodynamics is providing parenchymal cells with an optimal milieu intérieur, a missing piece of the puzzle remains information from the tissue cells. Especially information from the mitochondria, a key cell organelle and ultimate destination of oxygen could be very helpful. Using an optical technique, it is now possible to get quantitative information about the oxygen tension in mitochondria and their oxygen utilization.

This review will describe the rationale of taking into account mitochondrial measurements in perioperative and intensive care medicine and summarize the development of a clinically applicable technique for assessing mitochondrial oxygen tension and respiration.

MITOCHONDRIAL FUNCTION

Mitochondria are double-membrane organelles that play pivotal roles in cellular physiology. Our

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KEY POINTS

- Mitochondria are important energy-producing organelles at risk in perioperative and intensive care medicine.
- Mitochondrial oxygen tension can be noninvasively and safely measured using the optical properties of protoporphyrin IX.
- Mitochondrial oxygen monitoring is feasible at the bedside and provides unique parameters and information.
- Mitochondrial oxygen monitoring provides a new tool for research in resuscitation, transfusion, and pathophysiology.

understanding of their functions and complex interplay with their surrounding has been boosted in the last two decades and is still growing [13]. Mitochondria are well known as the powerhouses of the cells but they take part in other important cellular processes as well. For example, mitochondria are involved in programmed cell death via opening of the permeability transition pore and cytochrome c release [14,15]. Also, mitochondria might play a role in intracellular calcium homeostasis [16] as they possess calcium uniporters [17,18] and mitochondrially generated reactive oxygen species (ROS) act as cell-signaling molecules involved in metabolic adaptation [19], apoptosis [20] and autophagy [21].

Notwithstanding all other important functions, it is the ATP production by oxidative phosphorylation that is clinically in the foreground. Mitochondria are the primary consumers of oxygen and are responsible for approximately 98% of total body oxygen consumption. Oxygen is ultimately used at complex IV of the electron transport chain in the inner mitochondrial membrane. Reduced nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FADH2), generated in the Krebs cycle, are transferred from carrier molecules to the electron transport chain on complex I and II, respectively. The resulting electron transport through the chain causes protons to be pumped to the intermembrane space. This proton pumping causes an electrochemical potential over the inner membrane that is used to convert ADP to ATP by ATP synthase. ATP is the energy currency of the cells and used for driving cellular processes like maintaining membrane potentials, protein synthesis and replication.

THREATS TO MITOCHONDRIAL FUNCTION

In the perioperative and intensive care setting, many factors pose a threat to mitochondrial integrity and function, as set out in a recent review [22]. Both internal and external threats can be identified (Fig. 1). Such altered mitochondrial function, for example, diminished respiration and ATP-production, does not necessarily mean dysfunction because of damage. It can be an adaptive response to threats, for example, prolonged hypoxia because of oxygen-conformance or metabolic reprogramming [23,24], which extends seamless to a dysfunctional state and responds to resuscitation [25]. The functional consequences of such oxygen-dependent adaptation for cell and organ functions remain largely unknown, as well as its effects on microvascular perfusion. Thus, it remains unclear whether microvascular perfusion disturbances in critical illness are caused by dysfunction and should be a target of treatment, or merely are an epiphenomenon caused by altered cellular metabolism and diminished oxygen demand. Direct measurement of aspects of mitochondrial function could, therefore, be helpful and mitochondrial oxygen is a parameter of great interest in this respect.

MEASURING MITOPO$_2$

The measurement of mitoPO$_2$ has been made possible by the introduction of an optical technique, called the Protoporphyrin IX – Triplet State Lifetime Technique (PpIX-TSLT). Protoporphyrin IX is the final precursor in the heme biosynthetic pathway and is synthesized in the mitochondria [26] and shows a bright red prompt fluorescence when illuminated with blue or green light. This fluorescence is, for example, used in photodynamic diagnosis to visualize tumor during surgical resection [27]. Key in the development of PpIX-TSLT was the discovery of the existence of a more long lived red emission from protoporphyrin IX, called delayed fluorescence [28]. Although prompt fluorescence intensity decays with a nanosecond lifetime, delayed fluorescence lasts microseconds to milliseconds.

OXYGEN-DEPENDENT DELAYED FLUORESCENCE

The delayed fluorescence lifetime is dependent on the oxygen concentration. Higher oxygen concentrations result in a shorter lifetimes, whereas low oxygen concentrations leads to long lifetimes. The molecular mechanisms involved in this oxygen-dependent quenching of delayed fluorescence have been described elsewhere [29]. In short, photoexcitation of PpIX leads to population of an excited triplet state. Relaxation to the ground state can be spontaneous and result in the emission of a photon (delayed fluorescence). Alternatively, the energy can

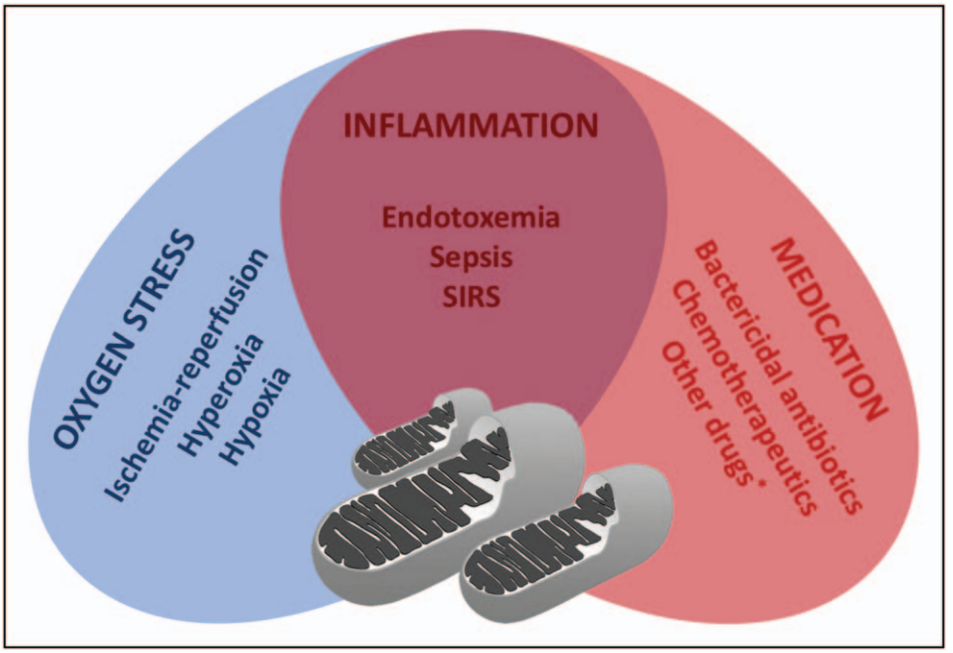

FIGURE 1. Threats to mitochondria in perioperative and intensive care medicine. *Drugs like statins, metformin, propofol, amiodarone and many others.

be transferred to an oxygen molecule upon collision and relaxation occurs without emission of a photon. More oxygen leads to more collisions and a higher collision rate, and therefore, results in a faster decaying delayed fluorescence signal (quenching). The delayed fluorescence lifetime can be converted to partial pressure of oxygen by the Stern–Volmer equation [30].

FROM CULTURED CELLS TO IN VIVO

In 2006, the technique for measuring mitochondrial PO_2 by delayed fluorescence of protoporphyrin IX was first described [28]. In this pivotal study, 5-aminolevulinc acid (ALA) was administered to several types of cell cultures and the mitochondrial localization of ALA-induced PpIX was demonstrated, together with the presence of oxygen-dependent delayed fluorescence from cell suspensions. Also, direct simultaneous measurement of mitoPO_2 and extracellular PO_2 showed that only shallow oxygen gradients exist over the cell membrane. Some years later, it was demonstrated that the technique could be extended to in-vivo use [31]. Intravenous administration of ALA led to detectable oxygen-dependent delayed fluorescence in rat liver [31] and heart [32]. The technique has been used in several preclinical pathophysiological studies [23,33–35].

As the technique was feasible in humans, but systemic administration of ALA was considered an obstacle, topical administration of ALA was tested for mitoPO_2 measurements (Fig. 2). For practical and clinical reasons, the skin was considered an ideal target organ for such measurements. Indeed, topical application of ALA to skin induced sufficient oxygen-dependent delayed fluorescence [36] and allowed local mitoPO_2 measurements [37] in rats. In a pig model, we demonstrated that, unlike tissue oxygenation measured with near-infrared spectroscopy, cutaneous mitoPO_2 is a sensitive parameter for detecting the physiologic limit of hemodilution on an individual level [34]. The skin is especially interesting since, like the gastrointestinal tract [38], it can be regarded as the canary of the body.

HUMAN USE (CELLULAR OXYGEN METABOLISM)

A clinical prototype of PpIX-TSLT was successfully tested in a healthy volunteer study [39] and triggered the development of the COMET system. COMET is an acronym of Cellular Oxygen METabolism and is a monitoring system developed by Photonics Healthcare in Utrecht, The Netherlands. The system is CE-marked and allows, in combination with its SkinSensor, repetitive noninvasive measurements of mitoPO_2 in human skin [40]. To prime the skin for delayed fluorescence measurements, a ALA-containing plaster is applied to the skin (Alacare, photonamic & Co. KG, Pinneberg, Germany). Although sufficient induction of PpIX by this plaster takes several hours, it provides a practical way of applying ALA to the skin in a clinical setting. The COMET system has by now been tested in several

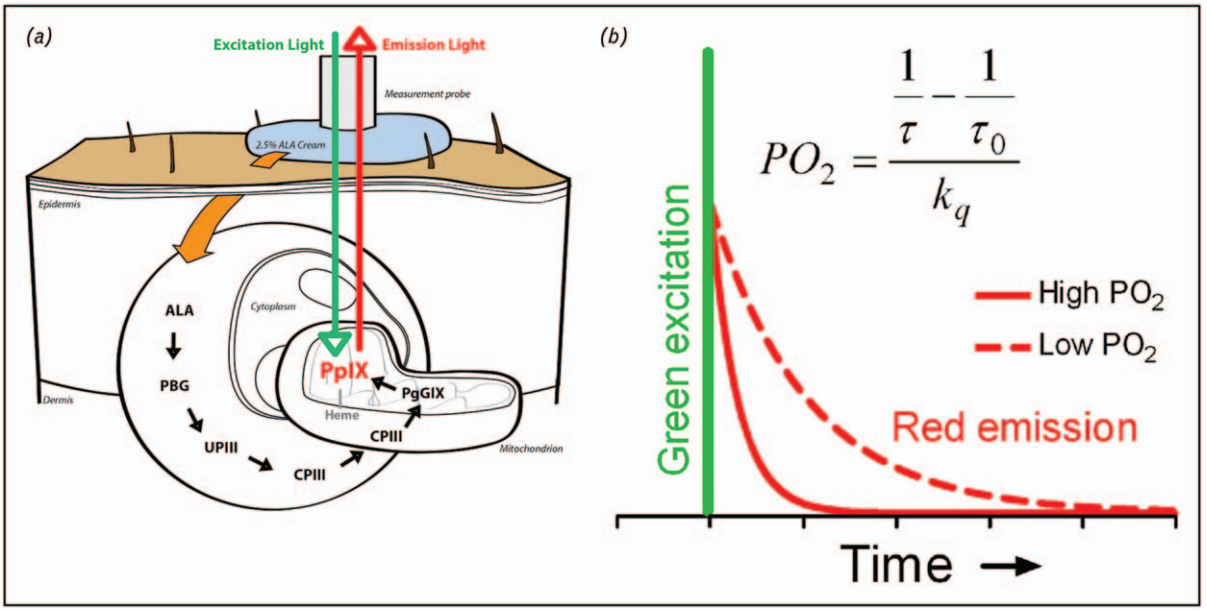

FIGURE 2. (a) Principle of protoporphyrin IX-Triplet State Lifetime Technique. The pathway by which topical ALA administration enhances mitochondrial PpIX levels and the principle of delayed fluorescence detection after an excitation pulse with green (510 nm) light. Emission light is the delayed fluorescence (red light, 630–700 nm) and its lifetime is oxygen-dependent. (b) PpIX emits delayed fluorescence after excitation by a pulse of green (510 nm) light. The delayed fluorescence lifetime is oxygen-dependent according to the Stern–Volmer equation (inset), in which k_q is the quenching constant and τ_0 is the lifetime at zero oxygen. ALA, 5-aminolevulinic acid; CPIII, coporporphyrinogen III; PBG, porphobilinogen; PO_2, oxygen tension; PpIX, protoporphyrin IX; UPIII, urporphyrinogen II. Reproduced with permission from Harms *et al.* [60].

healthy volunteer studies [41,42▪] and is currently being evaluated in clinical studies, both in perioperative and intensive care setting [22,40,43].

Importantly, the use of COMET is not limited to mitoPO$_2$ measurements in skin. The system has been used to demonstrate the feasibility of assessing the mucosal oxygenation in the gastrointestinal system via endoscopy [44▪]. To this end, the ALA was administered systemically, via the oral route, and oxygen-dependent delayed fluorescence was measured via an optical fiber through the working channel of an endoscope. The authors propose to use mitoPO$_2$ measurements as a functional test in the workup for the diagnosis of chronic mesenteric ischemia, but since the gut is very sensitive for shock [45], such an approach might ultimately also be of benefit for resuscitation purposes in the intensive care.

THE MYTH OF LOW MITOPO$_2$

As oxygen transport from microcirculation into the tissue cells is driven by diffusion, it is generally anticipated, according to the classical oxygen cascade that mitochondrial oxygen tension should be very low (several mmHgs) to create a big enough oxygen gradient [46,47]. However, average mitoPO$_2$ measured with the PpIX-TSLT technique appears to be, depending on the specific tissue, close to microvascular oxygen tension [33,48] and known values for tissue and/or interstitial oxygen levels [49,50▪▪]. In fact, mitoPO$_2$ is unlikely to be an order of magnitude lower than microvascular and interstitial oxygen tension. First, oxygen does not disappear stepwise so several mitochondria will see a PO$_2$ close to intravascular values. Second, larger vessels (not only capillaries) also contribute to diffusional oxygen delivery [51] so some mitochondria might see a PO$_2$ higher than the oxygen tension in the capillaries. Third, the oxygen gradient over the cell membrane is small [28] and will not cause mitoPO$_2$ to be substantially lower than interstitial PO$_2$. Typically reported cutaneous mitoPO$_2$ values under baseline circumstances are 40–70 mmHg and considered to be matching well with other measurements in skin [50▪▪]. Importantly, we demonstrated in both a preclinical [34] and clinical setting [40] that mitoPO$_2$ provides different information than hemoglobin saturation-based techniques like near-infrared spectroscopy. In situations, where visible light spectroscopy and near-infrared spectroscopy failed to show any response on a perturbation, mitoPO$_2$ clearly dropped to indicate cellular distress.

A POTENTIAL NEW TRANSFUSION TRIGGER

In current clinical practice, optimization of hemodynamics and tissue oxygen delivery in perioperative and intensive care patients is focusing on the administration of fluids, blood transfusion and vasoactive medication, targeting normal systemic hemodynamic parameters such as blood pressure, cardiac output, hemoglobin levels and venous saturation. For example, the management of acute anemia is mainly focused on the use of allogeneic blood transfusion guided on specific hemoglobin levels instead of a patient's personal need. Allogeneic blood transfusion itself is not without risks and has been shown to be an independent factor for an increased mortality and morbidity [52,53].

Transfusion guidelines use hemoglobin levels to indicate the need for blood transfusion. Such guidelines are based on data of large groups and incorporate a safety margin that might lead to unnecessary transfusion in individual cases. As ultimately the mitochondria are the target for oxygen delivery, it seems reasonable to use $mitoPO_2$ as a measure for an individual's transfusion need. This presupposition was fostered by the finding that in hemodiluted pigs $mitoPO_2$ dropped as a result of ongoing hemodilution. Reaching the physiological limit of an individual pig, $mitoPO_2$ acutely dropped and this drop preceded other signs of inadequate oxygen delivery, like a rise in serum lactate. Thus, $mitoPO_2$ measurements can be useful as a novel transfusion trigger for personalized transfusion medicine. Studies that show that this drop in $mitoPO_2$ can be reversed by transfusion of autologous blood and that $mitoPO_2$ could indeed be a potential physiological transfusion trigger are under way.

UNRAVELING THE OXYGEN BALANCE

Fluid resuscitation, based on systemic hemodynamic parameters remains key in the treatment of sepsis shock. The substantiation for this type of treatment is based on the hypothesis that the development of septic shock and multiorgan failure is caused by tissue hypoxia because of a higher metabolic rate together with impaired diffusion processes in the microcirculation [54]. However, many clinical trials have failed to demonstrate benefits of resuscitation on hemodynamic parameters, such as blood pressure, central venous pressure, cardiac output and central venous saturation [3,4,55,56]. This suggests that other mechanism, such as mitochondrial dysfunction, also play a role in the pathogenesis of sepsis shock. However, the literature about mitochondrial dysfunction in sepsis shows conflicting results [57▪▪], most likely because of the lack of a valid and reliable measurement method to monitor mitochondrial dysfunction [58].

Therefore, we suggested PpIX-TSLT as a possible noninvasive monitoring tool for measuring $mitoPO_2$ and mitochondrial oxygen consumption ($mitoVO_2$) *in vivo*. Oxygen consumption is determined by a dynamic $mitoPO_2$ measurement, measuring $mitoPO_2$ every second for approximately 90 s, while microvascular oxygen supply is blocked by applying pressure on the skin with the measuring probe. $mitoVO_2$ can then be derived from the resulting oxygen disappearance curve [59]. We demonstrated the feasibility to measure the $mitoPO_2$ and $mitoVO_2$ in an endotoxemic model of acute critical illness [60]. In this study, we observed a decreased mitochondrial oxygen consumption in endotoxemic rats independently of the fact whether $mitoPO_2$ was reduced or restored by fluid resuscitation, suggesting that endotoxemia had a lasting effect on mitochondrial function, even in the absence of evident hemodynamic shock.

Another recent study compared the PpIX-TSLT measurements with a widely used 'ex vivo' mitochondrial respirometry technique. The same decrease in $mitoPO_2$ and mitochondrial oxygen consumption were measured with the PpIX-TSLT after the induction of sepsis, but 'ex vivo' mitochondrial function measurements remained unchanged before and after induction of sepsis. This results are probably caused by a higher sensitivity of the 'in vivo' PpIX-TSLT measurements compared with the classic 'ex vivo' measurements.

After demonstrating the feasibility of cutaneous $mitoVO_2$ measurements, it remained important to demonstrate that cutaneous $mitoPO_2$ and $mitoVO_2$, at least to some extent, reflect such mitochondrial parameters in other vital organs. Therefore, we conducted a study that compared the values and responses of cutaneous $mitoPO_2$ and $mitoVO_2$ with liver and gastrointestinal tract [61]. The results showed that the absolute value of $mitoPO_2$ and $mitoVO_2$ in the skin may differ from other organs, but that the trend of a decreased $mitoPO_2$ and $mitoVO_2$ was observed in all studied organs after the administration of endotoxin.

CONCLUSION

Mitochondria are the ultimate destination of oxygen delivery. Measurement of oxygen and oxygen utilization at the mitochondrial level is expected to be of benefit for guiding therapies aimed at restoring or optimizing tissue oxygenation and ultimately organ function. PpIX-TSLT is a noninvasive and well tolerated technique to measure $mitoPO_2$ and

mitoVO$_2$. The COMET system allows bedside use of this technique, providing a next step in monitoring.

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Conflicts of interest

E.G.M. is listed as inventor on patents related to mitochondrial oxygen measurements held by the Academic Medical Center Amsterdam and the Erasmus Medical Center Rotterdam, The Netherlands. E.G.M. is founder and shareholder of Photonics Healthcare, a company that holds exclusive licenses to these patents and that markets the COMET system. Other authors declare no conflict of interest.

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