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REVIEW

Variability of Mitochondrial Respiration in Relation to Sepsis-Induced Multiple Organ Dysfunction

M. KOHOUTOVÁ[1,2], J. DEJMEK[2,3], Z. TŮMA[2], J. KUNCOVÁ[1,2,3]

[1]Institute of Physiology, Faculty of Medicine in Plzeň, Charles University, Plzeň, Czech Republic, [2]Biomedical Centre, Faculty of Medicine in Plzeň, Charles University, Plzeň, Czech Republic, [3]Institute of Biophysics, Faculty of Medicine in Plzeň, Charles University, Plzeň, Czech Republic

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Summary

Ample experimental evidence suggests that sepsis could interfere with any mitochondrial function; however, the true role of mitochondrial dysfunction in the pathogenesis of sepsis-induced multiple organ dysfunction is still a matter of controversy. This review is primarily focused on mitochondrial oxygen consumption in various animal models of sepsis in relation to human disease and potential sources of variability in experimental results documenting decrease, increase or no change in mitochondrial respiration in various organs and species. To date, at least three possible explanations of sepsis-associated dysfunction of the mitochondrial respiratory system and consequently impaired energy production have been suggested: 1. Mitochondrial dysfunction is secondary to tissue hypoxia. 2. Mitochondria are challenged by various toxins or mediators of inflammation that impair oxygen utilization (cytopathic hypoxia). 3. Compromised mitochondrial respiration could be an active measure of survival strategy resembling stunning or hibernation. To reveal the true role of mitochondria in sepsis, sources of variability of experimental results based on animal species, models of sepsis, organs studied, or analytical approaches should be identified and minimized by the use of appropriate experimental models resembling human sepsis, wider use of larger animal species in preclinical studies, more detailed mapping of interspecies differences and organ-specific features of oxygen utilization in addition to use of complex and standardized protocols evaluating mitochondrial respiration.

Key words

Sepsis • Mitochondria • Oxygen consumption • Multiple organ dysfunction • Animal models

Corresponding author

J. Kuncová, Institute of Physiology, Faculty of Medicine in Plzeň, Charles University, Alej Svobody 76, 323 00, Plzeň, Czech Republic. E-mail: jitka.kuncova@lfp.cuni.cz

Introduction

Sepsis, life-threatening organ dysfunction caused by a dysregulated host response to infection, represents one of the most serious public health issues of the modern time. In the United States it affects more than 750,000 patients per year (Angus *et al.* 2001); its incidence is similar in Europe and probably even higher in low-income countries (Fleischmann *et al.* 2016). Mortality rate ranges between 30-70 % in relation to severity of the disease, particularly to the number of dysfunctional organs (da Silva *et al.* 2008).

Definition of sepsis

In the course of past few decades, definition of sepsis has substantially changed. Originally, it was based mainly on the evidence of bacteremia or toxemia. In 1991, sepsis-1 definition was established as an infection or suspected infection leading to the onset of systemic inflammatory response syndrome (SIRS). SIRS diagnostic criteria (Table 1) were based on the precisely defined changes in body temperature, heart rate, respiratory functions, and white blood cell count (Bone *et al.* 1992). In addition, severity of the disease was classified in three categories – sepsis (present/suspect

infection + at least 2 SIRS criteria), severe sepsis (disease complicated by organ dysfunction) and septic shock ("sepsis-induced hypotension persisting despite adequate fluid resuscitation"). Criteria of SIRS were further expanded in sepsis-2 definition in 2001 (Levy *et al.* 2003). More substantial modification of definition of sepsis was approved in 2016 at a consensus conference of the Society of Critical Care Medicine and the European Society of Intensive Care Medicine (Marik and Taeb 2017). This sepsis-3 definition is based on better understanding of the pathophysiology of the disease and emphasizes maladaptive host response to infection that results in organ dysfunction (Singer *et al.* 2016). Presence of SIRS is not further regarded as a crucial marker of sepsis as it could be associated with a desired response of the body to the infection challenge. Consequently, category of severe sepsis became needless.

Table 1. Definitions of sepsis.

SEPSIS-1 (1992) Suspected/documented infection + SIRS criteria	**SEPSIS-2** (2003) Suspected/documented infection + extended SIRS criteria	**SEPSIS-3** (2016) Suspected/documented infection + SOFA score
SIRS criteria • Temperature • Heart rate • WBCC/Bands • Respiratory rate/$PaCO_2$ **Organ dysfunction** SBP, MAP, coagulation, bilirubin concentration, urine output, creatinine concentration, oxygen saturation	**Extended SIRS criteria** • **General parameters** Temperature, heart rate, respiratory rate, hyperglycemia without DM, altered mental status, edema • **Inflammatory parameters** WBCC/Bands, CRP, procalcitonin • **Hemodynamic parameters** MAP, SBP, mixed venous oxygen saturation, cardiac index • **Organ dysfunction** PaO_2, urine output, creatinine concentration, coagulation, platelet count, liver function (bilirubin concentration), gastrointestinal motility • **Tissue perfusion** Lactate concentration, capillary refill	**SOFA** • PaO_2/FiO_2 ratio • Glasgow coma scale score • MAP • Vasopressors • Serum creatinine • Bilirubin • Platelet count
Staging **Sepsis** = infection + ≥ 2 SIRS **Severe sepsis** = sepsis + organ dysfunction, hypoperfusion, or hypotension **Septic shock** = sepsis + hypotension despite adequate fluid resuscitation + hypoperfusion or organ dysfunction	**Staging** **Sepsis** = infection + ≥ 1 extended SIRS **Severe sepsis** = sepsis + organ dysfunction **Septic shock** = sepsis + refractory hypotension unexplained by other causes	**Staging** **Sepsis** = life-threatening organ dysfunction caused by a dysregulated host response to infection **Septic shock** = a subset of sepsis in which profound circulatory, cellular and metabolic abnormalities are associated with a greater risk of mortality than with sepsis alone

Variables evaluated in diagnosis of sepsis. SIRS – systemic inflammatory response syndrome, WBCC – white blood cell count, $PaCO_2$ – arterial partial pressure of CO_2, SBP – systolic blood pressure, MAP – mean arterial pressure, DM – diabetes mellitus, CRP – C-reactive protein, PaO_2 – arterial partial pressure of O_2, SOFA – sepsis-related (sequential) organ failure assessment score, FiO_2 – fraction of inspired O_2.

Pathophysiology of sepsis

Sepsis is characterized by homeostatic dysbalance that could progress in multiple organ dysfunction (MOD), septic shock and death. Pathogen-associated molecular patterns (PAMPs) stimulate plasma complement and coagulation systems and bind to pattern recognition receptors on the cell membranes and/or intracellular organelles. The simultaneous attack on the multilevel body's defense mechanisms provokes both passive and active release of danger-associated molecular patterns (DAMPs) from dying cells or cells challenged by PAMPs, like heat shock proteins, genomic and mitochondrial DNA, or ATP (Sharma and Naidu 2016) and others. These molecules further invade the host's tissues causing increased expression and release of a number of inflammatory mediators and biomarkers including those that promote inflammatory response, those that fight against infection, membrane receptors and their downstream effectors, molecules released from damaged cells, chemicals associated with activation of the coagulation and complement systems, vasoactive substances, acute phase proteins, biomarkers of various organs dysfunction, and others (Angus and van der Poll 2013). To date, about 180 biomarkers of sepsis have been identified (Pierrakos and Vincent 2010). Dysregulated immune reaction along with hormonal dysbalance, disturbed activity of the autonomic nervous system and dysfunction of epithelial and endothelial cells lead to the loss of barriers tightness, changes in intermediary metabolism, and subsequently to organ dysfunction. MOD is frequently manifested by respiratory distress, myocardial depression, systemic vasodilatation, acute kidney injury, impaired liver function, disturbed gastrointestinal motility, and coagulopathy (Reinhart *et al.* 2012, Fig. 1).

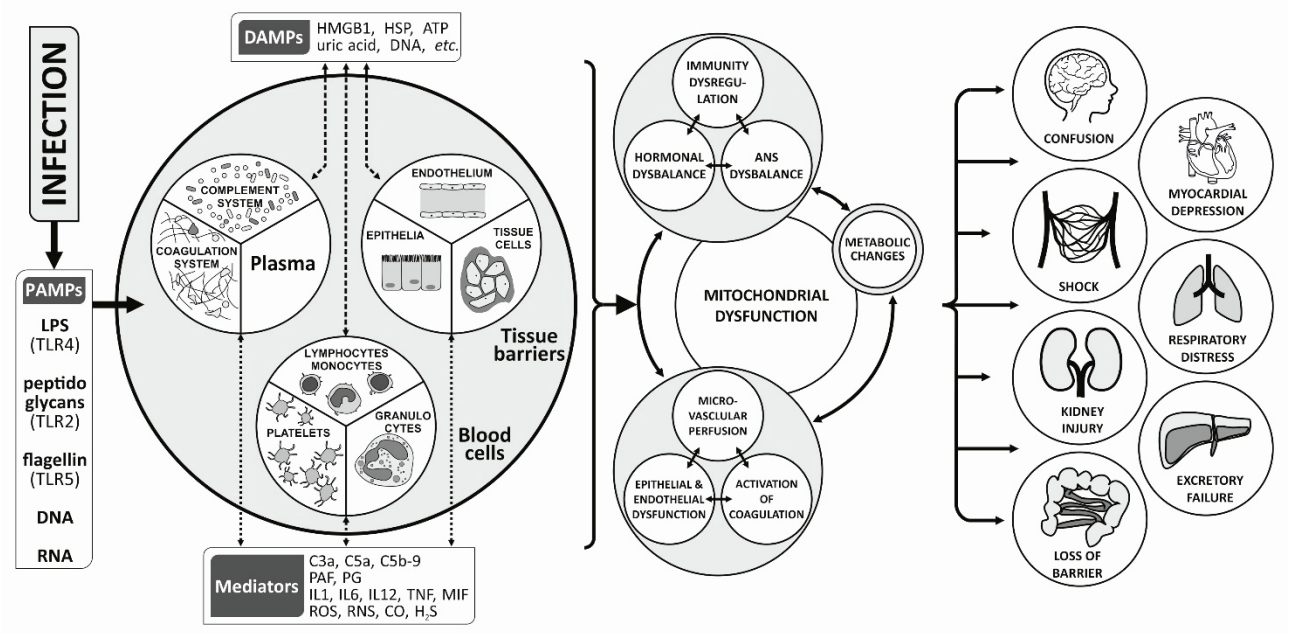

Fig. 1. Pathophysiology of sepsis. Pathogen-associated molecular patterns (PAMPs) bind to pattern recognition receptors in the cell membranes and intracellular organelles. Subsequent release of danger-associated molecular patterns (DAMPs) invading the host's tissues causes increased expression and release of inflammatory mediators and biomarkers. Dysregulated host response to the septic insult includes disturbed immune, endocrine, and autonomic nervous systems regulation, loss of barriers tightness, changes in intermediary metabolism, and subsequently multiple organ dysfunction. PAMPs – pathogen-associated molecular patterns, LPS – lipopolysaccharide, TLR – Toll-like receptor, DNA – deoxyribonucleic acid, RNA – ribonucleic acid, DAMPs – danger-associated molecular patterns, HMGB1 – high mobility group box 1, HSP – heat shock protein, ATP – adenosine triphosphate, PAF – platelet-activating factor, PG – prostaglandins, IL – interleukin, TNF – tumor necrosis factor, MIF – macrophage migration inhibitory factor, ROS – reactive oxygen species, RNS – reactive nitrogen species, CO – carbon monoxide, H_2S – hydrogen sulfide, ANS – autonomic nervous system.

Mitochondria in health

Mitochondria are semi-autonomous cell organelles that are primarily designed to produce biologically available energy in the form of adenosine triphosphate (ATP). The inner mitochondrial membrane houses a sophisticated electron-transport system (ETS) designed to transfer electrons from appropriate substrates sequentially across multiheteromeric respiratory complexes (I to IV) and two mobile elements (coenzyme Q and cytochrome c) to their final acceptor,

molecular oxygen (Fig. 2A). Gradually released energy is used to pump protons from the mitochondrial matrix into the intermembrane space thus generating a proton gradient driving ATP synthesis by complex V, ATP-synthase (Fig. 2). The substrates fueling directly or indirectly oxidative phosphorylation are mainly produced in the mitochondrial matrix in crucial metabolic processes, i.e. tricarboxylic acid (TCA) cycle and β-oxidation of fatty acids (Gnaiger 2014). Electrons for ETS are also provided by pyruvate dehydrogenase complex, glutamate dehydrogenase, sulfite oxidase (Velayutham *et al.* 2016), sulfide ubiquinone oxidoreductase, dihydroorotate dehydrogenase (Lemieux *et al.* 2017) or mitochondrial glycerol phosphate dehydrogenase (Mráček *et al.* 2013, Fig. 2B).

Besides their key role in energy production, mitochondria are also site of production of steroid hormones, heme, iron-sulfur clusters, and endogenous gases including reactive oxygen and nitrogen species that are required for cellular signaling. Mitochondria are also considered important regulators of intracellular calcium concentration; they generate heat thus contributing to temperature regulation and basal metabolic rate, initiate apoptotic cell death and may also have critical and multiple functions in the initiation of cell differentiation, cell-type determination, cell movement, and pattern formation (Maeda and Chida 2013).

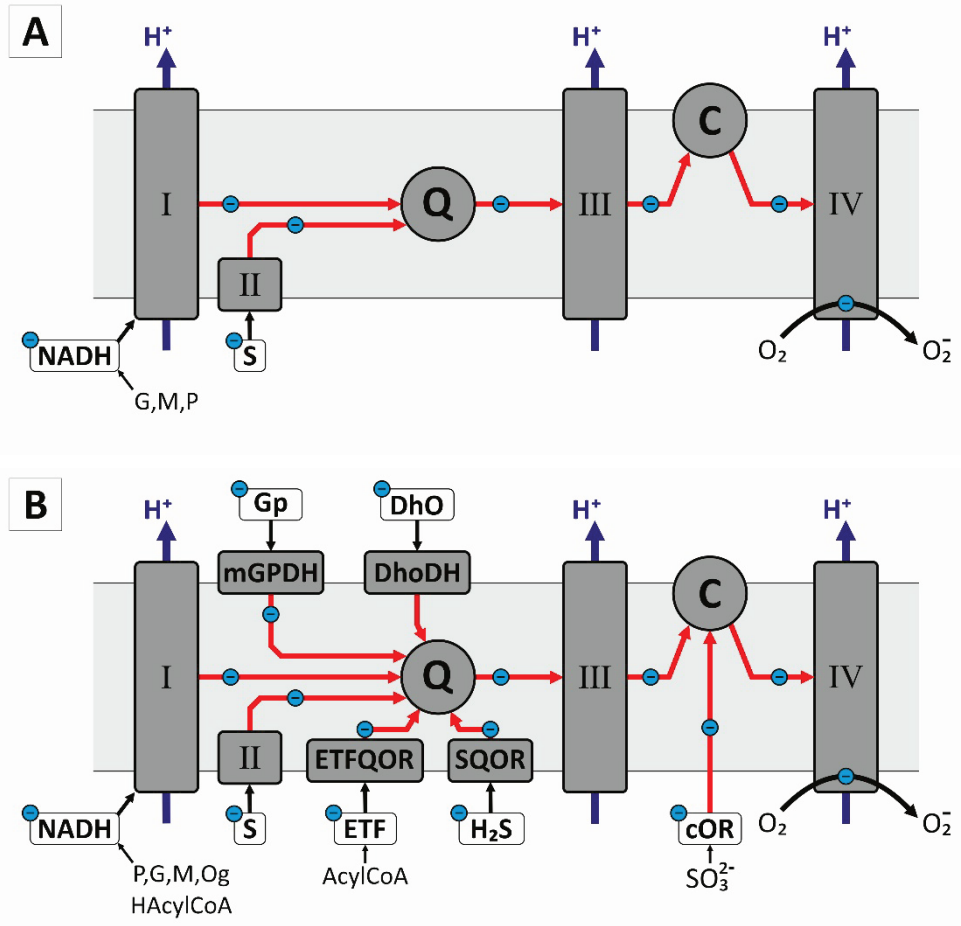

Fig. 2. Mitochondrial electron transport. Electron-transport system (ETS) serves to transfer electrons from substrates sequentially across four respiratory complexes (I to IV), two mobile carriers (coenzyme Q and cytochrome c) to final electron acceptor, molecular oxygen. (**A**) Simplified version of ETS reflecting widely used experimental protocols evaluating mitochondrial oxygen consumption. (**B**) More complex view on ETS including additional electron donors. Electron transport is coupled with proton pumping from the mitochondrial matrix into the intermembrane space thus generating a proton gradient driving ATP synthesis by complex V, ATP-synthase. Electrons fueling ETS come from substrates produced in tricarboxylic acid cycle and β-oxidation of fatty acids, from pyruvate dehydrogenase complex, glutamate dehydrogenase, mitochondrial glycerol phosphate dehydrogenase, sulfite oxidase, dihydro-orotate dehydrogenase, sulfide-ubiquinone oxidoreductase, and choline dehydrogenase (not shown). II, III, IV – complexes of electron-transport system, Θ – electron, H^+ – proton, Q – coenzyme Q, c – cytochrome c, O_2 – oxygen, NADH – nicotinamide adenine dinucleotide, G – glutamate, M – malate, P – pyruvate, Og – oxoglutarate, HAcylCoa – hydroxyacyl-coenzyme A, S – succinate, Gp – glycerol phosphate, DhO – hydroxyorotate, H_2S – hydrogen sulfide, mGPDH – mitochondrial glycerol phosphate dehydrogenase, AcylCoA – acyl coenzyme A, ETF – electron transferring flavoprotein, SQOR – sulfide:quinone oxidoreductase, ETFQOR – electron-transfer flavoprotein:ubiquinone oxidoreductase, cOR – cytochrome c oxidoreductase, SO_3^{2-} – sulfite anion.

Mitochondria in sepsis

Multiple experimental data on animals as well as humans suggest that exaggerated inflammatory response could interfere with any mitochondrial function; i.e. appropriate production of reactive oxygen and nitrogen species (recently reviewed by Duvigneau and Kozlov 2017), intracellular calcium homeostasis (Pinto *et al.* 2017), mitochondrial biogenesis and turnover (Inata *et al.* 2018), or regulation of apoptosis (Chen *et al.* 2017). This review is primarily focused on mitochondrial oxygen consumption in various animal models of sepsis in relation to human disease. Despite intense research in this field, the precise role of mitochondria in the chain of events leading to MOD is still a matter of controversy. So far reported experimental data on changes in mitochondrial respiration are far from being uniform and seem to depend on a number of factors including the organ studied, selected model of sepsis, animal species, severity and phase of the disease, and the experimental set-up (Singer 2014). Interestingly, despite frequently suggested key role of mitochondrial bioenergetics in the development and progression of MOD, the most frequent finding in experimental studies dealing with mitochondrial respiration or respiratory enzymes activities in various rodent models of sepsis was unchanged oxygen consumption by the heart, liver and skeletal muscle mitochondria (Jeger *et al.* 2013). However, it should be noted that similar number of studies reported sepsis-associated decrease or even increase in mitochondrial oxygen consumption in above mentioned organs (Jeger *et al.* 2013) and that unchanged respiration does not necessarily mean that the energy production is appropriately adjusted to the tissue needs (Dyson and Singer 2011). In general, there are at least three possible explanations of sepsis-associated dysfunction of the mitochondrial respiratory system and consequently impaired energy production (Fig. 3):

1. Increased oxygen demand associated with immune system activation, elevated body temperature, and increased metabolic rate together with impaired diffusion processes in microcirculation lead to tissue hypoxia that is reflected by decreased oxygen consumption in the mitochondria and impaired ATP generation (Kozlov *et al.* 2017). In such view, mitochondria would be victims of pathological processes initiated elsewhere and would only follow inadequate oxygen delivery with insufficient energy production. As reviewed by Chioléro *et al.* (1997), sepsis is frequently associated with increased metabolic rate, protein and fat catabolism, negative nitrogen balance, hyperglycemia, and insulin resistance. However, these metabolic changes are not correlated with the body temperature and severity of the disease, nor they seem to be in causal relationship to hyperdynamic pattern of circulation, typical for SIRS in humans (Kreymann *et al.* 1993). Although alterations in microcirculatory blood flow have been frequently identified in sepsis (De Backer *et al.* 2002), true tissue hypoxia manifested by decreased tissue pO_2 was regularly reported mainly in hypodynamic stages of sepsis or short-term rodent models of endotoxemia (Dyson *et al.* 2011), where decline in tissue perfusion resulting in decreased tissue oxygen tension could be regarded as a predictable consequence (Matejovic *et al.* 2011). In addition, tissue pO_2 does not reflect only oxygen delivery to tissues, but rather a balance between oxygen supply and its cellular consumption. Hypoxic theory of mitochondrial dysfunction in sepsis was challenged by studies demonstrating no effect of improved tissue perfusion on the recovery from sepsis (Gattinoni *et al.* 1995, Hayes *et al.* 1997). Taken together, despite an ample evidence of individual factors contributing to the aforementioned chain of events leading to an energy crisis during sepsis, their poor correlation argues against the simple passive role of mitochondria in the progression of the disease.

2. Mitochondria could be directly challenged by DAMPs and mediators of inflammation that cause decrease in oxygen consumption in conditions of preserved tissue blood flow and oxygen tension. The putative inability of mitochondria to consume oxygen as a mechanism contributing to organ dysfunction in sepsis was suggested by Fink and termed cytopathic hypoxia (Fink 1997).

As mentioned above, unchanged or even increased tissue oxygen tension was repeatedly reported in the skeletal muscles of septic patients (Boekstegers *et al.* 1991, Boekstegers *et al.* 1994) as well as decreased oxygen consumption and ATP generation by skeletal muscle mitochondria (Brealy *et al.* 2002, Fredriksson *et al.* 2006), findings that would fit the theory of cytopathic hypoxia. However, data obtained from critical tissues in animal resuscitated models are less convincing. For example, conflicting results were obtained from the liver, intestinal mucosa or kidneys where both decreased and unchanged tissue pO_2 were documented (Lund *et al.* 1995, Dyson *et al.* 2011). In addition, results of studies on mitochondrial respiration in these organs display

strong variation thus not providing compelling evidence of impaired oxygen utilization preceding overt organ dysfunction (Patil *et al.* 2014, Porta *et al.* 2006). Nevertheless, numerous data suggest that mitochondrial oxygen processing could be significantly affected in response to various mediators of inflammation. Among them, reactive oxygen and nitrogen species, carbon monoxide and hydrogen sulfide have drawn more attention due to their site of origin and quite consistently reported dual effect on mitochondrial electron transport (Duvigneau and Kozlov 2017, Kozlov *et al.* 2017, Módis *et al.* 2014).

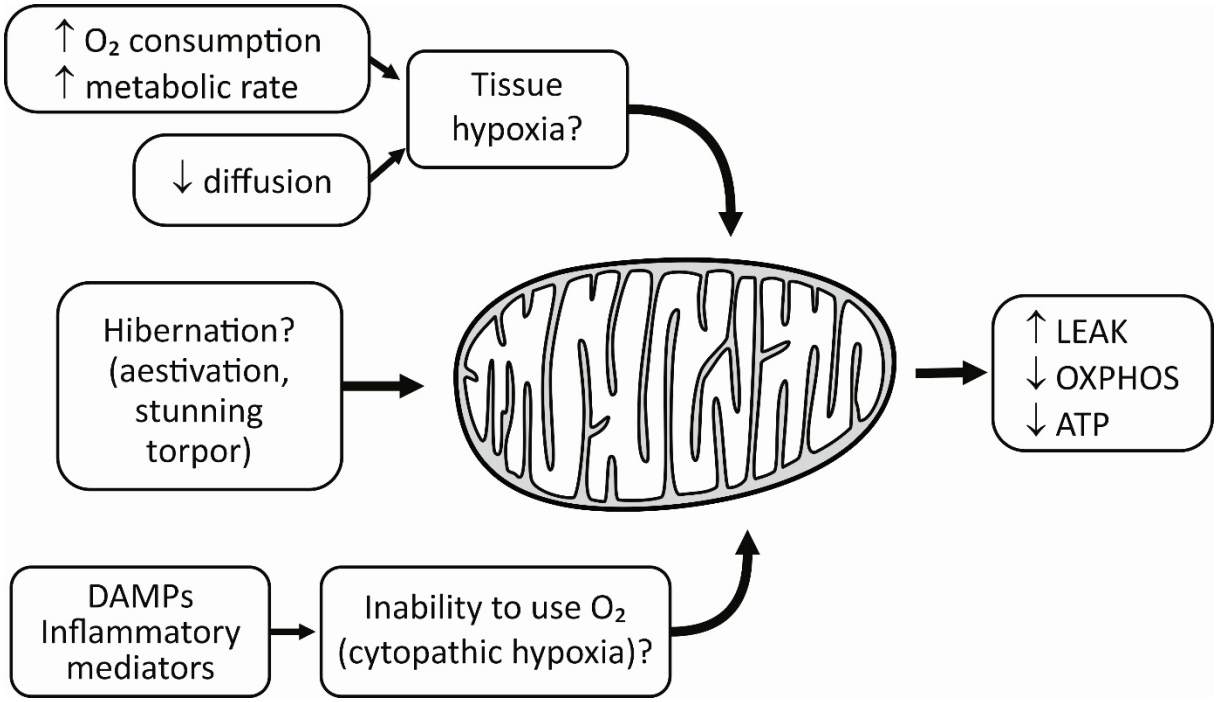

Fig. 3. The role of mitochondria in sepsis – 3 possible explanations of sepsis-associated dysfunction of the mitochondrial respiratory system and energy production. 1. Increased oxygen demand together with impaired diffusion processes in microcirculation leads to tissue hypoxia manifested by decreased oxygen consumption and impaired ATP generation. 2. Mitochondria could be directly challenged by DAMPs and mediators of inflammation that cause decrease in oxygen consumption in conditions of preserved tissue blood flow and oxygen tension. 3. Reduced oxygen consumption could be related to the active role of mitochondria in orchestration of survival strategy resembling stunning or hibernation. These processes could lead to an increase in the mitochondrial respiratory state LEAK, decrease in oxidative phosphorylation (OXPHOS) and limited ATP production. DAMPs – danger-associated molecular patterns, O_2 – oxygen, ATP – adenosine triphosphate).

3. Reduced oxygen consumption could be related to the active role of mitochondria in orchestration of survival strategy resembling stunning or hibernation, characterized by regional contractile dysfunction of myocardium due to compromised oxygen supply (Singer 2017). Both phenomena were originally described in the hearts subjected to ischemia. The term stunned myocardium was initially used to characterize an abnormal contractile function of the left ventricle persisting for hours or days after coronary occlusion lasting not more than 15 min and not being accompanied with cell death (Braunwald and Kloner 1982, Heyndrickx *et al.* 1975). Hibernation was related to chronically compromised coronary perfusion associated with impaired left ventricular function that could be completely restored if blood flow was returned to normal (Braunwald and Rutherford 1986, Rahimtoola 1993). Ischemia-induced hibernation is regarded as a regulatory measure that maintains myocardial integrity and viability (Heusch *et al.* 2005). At the cellular level, both mechanisms overlap being accompanied with metabolic adaptation characterized by shifting energy production away from oxidative phosphorylation and oxygen utilization. Many features of this metabolic adjustment have been identified also in human and mouse cardiomyocytes challenged by sepsis or endotoxemia including downregulation of enzymes in TCA cycle, suppression of catabolism of energy substrates including fatty acids, glucose and ketone bodies, accumulation of glycogen and triacylglycerols in the heart (Matkowich *et al.* 2017, Umbarawan *et al.* 2017), elevated pyruvate dehydrogenase kinase activity leading to suppression of

pyruvate dehydrogenase (Standage *et al.* 2017) or decreased mitochondrial biogenesis (Lancel *et al.* 2009). In contrast, no changes in the levels of TCA intermediates and rather increased than decreased mitochondrial biogenesis were detected in the hearts of septic rats (Hotchkiss *et al.* 1991, Vanasco *et al.* 2014). More importantly, studies dealing with recovery from sepsis suggested that better energy-producing fatty acid catabolism was associated with survival of the fittest in sepsis (Langley *et al.* 2013) and that PPARα expression (peroxisome proliferator-activated receptor alpha – marker of mitochondrial biogenesis) and increased fatty acid oxidation were associated with the hyperdynamic cardiac response early in the course of sepsis and decreased morbidity and mortality (Standage *et al.* 2017). Some clinical studies on sepsis-induced myocardial depression indicated that left ventricular systolic (but not diastolic) dysfunction could be associated with improved outcome of sepsis (Jardin *et al.* 1999, Landesberg *et al.* 2012, Parker *et al.* 1984), however, recently published systematic review and meta-analysis of the data on newly diagnosed left ventricular systolic dysfunction in critically ill patients admitted to the intensive care unit with severe sepsis or septic shock concluded that the presence of new left ventricular systolic dysfunction is neither a sensitive nor a specific predictor of mortality (Sevilla Berrios *et al.* 2014).

In conclusion, although numerous data indicate substantial role of mitochondria in sepsis-induced MOD, none of the proposed hypotheses seems to be supported by convincing experimental and clinical evidence.

Potential sources of variability

In the literature, dysfunctional mitochondrial respiration is usually reported as universal feature of sepsis-induced organ failure and conclusions are made across the animal species, models of sepsis, analyzed organs or cells, and methods used for analysis of mitochondrial oxygen consumption. For example, the classical study by Brealey *et al.* (2002) performed on skeletal muscle biopsies taken from 28 septic patients and 9 controls recruited from orthopedic department showed an association between nitric oxide overproduction, antioxidant depletion, mitochondrial dysfunction, and decreased ATP concentrations that related to severity of the disease and eventual outcome. However, results and conclusions from this important work (646 citations in WoS) were taken as applicable on mitochondrial function of organs crucial in the development of MOD, like kidneys or heart (Dennis and Wittig, 2017, Gomez *et al.* 2014, Martin *et al.* 2017). Experimental studies dealing with mitochondrial respiration in these organs are relatively numerous for heart, but rare for kidneys (Jeger *et al.* 2013) and report conflicting results. The putative sources of considerable variability of sepsis-related mitochondrial oxygen consumption are discussed below.

1. In humans, *sepsis per se* is an extremely variable entity due to heterogeneous genetic background, frequent co-morbidities (diabetes mellitus, chronic kidney injury, heart failure, tumors etc.), physical fitness, age, source and degree of the triggering insult and its type (Christaki and Giamarellos-Bourboulis 2014, Giraldo *et al.* 2009, Villar *et al.* 2004). In addition, therapeutic interventions could have dual effect on metabolic turnover and mitochondrial respiration (Chioléro *et al.* 1997).

2. *Species differences*. Experimental animals used in the research on sepsis include a number of mammalian species from small rodents to pigs and primates (Fink 2014). At the end of April 2018, a simple query "given species and sepsis" in PubMed provided 13,000/9,000/2,800/2,400/2,000/700/300 hits for mouse/ rat/dog/pig/rabbit/cat/baboon (reviews not excluded) with the highest counts for mouse (approx. 500-800 hits per year over the past 10 years). The use of laboratory rodents (namely mouse and rat) has been challenged by a number of studies pointing out substantial morphological and functional differences that could be related to discrepancies in the progression and outcome of sepsis between these species and humans. Besides well-known differences in the life-span, body weight, anatomy and functions of the organ systems (cardiovascular, urinary, gastrointestinal – Beuchat 1990, Kararli 1995, Milani-Nejad and Janssen 2014), species-dependent variations in the genetic background (Seok *et al.* 2013), immune system (Mestas and Hughes 2004), intermediary and drug metabolism (Fujiwara *et al.* 2018, MacDonald *et al.* 2011, Mathew *et al.* 2017), production of reactive oxygen species (Barja 2007), susceptibility to infection (Cross *et al.* 1993), or ability to enter the metabolic torpor response (Schubert *et al.* 2010) might be directly related to poor outcome of clinical trials documenting no benefit or even deleterious impact of agents that exerted promising effects in animal experiments (Deitch 2005, Fink 2014).

3. *Models of septic insult.* Sepsis is an enormously complex condition that is difficult to be

reproduced in experimental setting (Marshall *et al.* 2005). Selected aspects of processes associated with the impact of the disease on individual cell types can be studied *in vitro* on cultured tissue cells or freshly isolated blood cells challenged by septic plasma (Mariano *et al.* 2008, Sjövall *et al.* 2010). Blood cells isolated from septic patients (leukocytes and platelets) also represent the only easily available biological material that can be used to measure mitochondrial oxygen consumption in humans (Belikova *et al.* 2007, Puskarich *et al.* 2016). However, applicability of the results of these studies on mitochondrial functions of solid organs is limited as they are not in direct contact with blood. Leukocytes and platelets are also those cells that are considered major producers of many inflammatory mediators. More complex imitation of septic conditions can be achieved using *in vivo* animal models. Of note, it is extremely difficult to find more than two research groups using exactly the same animal model of sepsis. Besides aforementioned animal species used in sepsis research, the method of disease induction, performance and timing of resuscitation and sampling, age and sex of experimental animals, their genetic background and absence of complicating diseases represent factors contributing to the extreme variability of the research results in the field of sepsis-induced mitochondrial dysfunction (Singer 2007). Over the past two decades, various models of sepsis on experimental animals have been extensively reviewed (Deitch 1998, Freise *et al.* 2001, van der Poll 2012, Zanotti-Cavazzoni and Goldfarb 2009) with emphasis on their relevance to human disease (Dyson and Singer 2009, Esmon 2004, Poli-de-Figueiredo *et al.* 2008, Rittirsch *et al.* 2007), translational potential (Dejager *et al.* 2011, Fink 2014, Pitts and Simpson 2010), particular process to be studied (Doi *et al.* 2009, Fink 2008, Siempos *et al.* 2014) or animal species used (Lewis *et al.* 2016, Redl and Bahrami 2005, Stortz *et al.* 2017). The list of some biological models used in sepsis research is shown in Table 2.

4. *Mitochondrial diversity.* Mitochondrial DNA diversity is a well-known phenomenon used to make various inferences about the origins of modern humans (Cann *et al.* 1987, Stoneking and Soodyall 1996), phylogeny (Hurst and Jiggins 2005) or in forensic analysis (Melton and Nelson 2001). Tissue diversity of mitochondrial morphology, quantity and composition has been reported in mouse tissues (Mootha *et al.* 2003, Pagliarini *et al.* 2008). An excellent study by Benard *et al.* (2006) determined the composition and functional features of the respiratory chain in muscle, heart, liver, kidney, and brain of male Wistar rats and concluded that tissues could be categorized at least into three groups: muscle and heart, brain, and liver and kidney. Tissue-dependent values of mitochondrial oxygen consumption were also reported in porcine skeletal muscle, liver, and kidney (Porta *et al.* 2006) and rat heart, liver, brain and kidney (Pecinová *et al.* 2011). It is thus clear that conclusions about sepsis-driven changes in mitochondrial oxygen consumption made across different organs could be misleading.

5. *Analytical methods.* Although mitochondrial oxygen consumption would seem to be a robust and stable parameter if given experimental conditions are maintained, reproducibility and comparability of mitochondrial analyses depend on a number of factors that arise from the preparation of organelles and the methodological approach used. Oxygen consumption could be studied on isolated mitochondria, tissue homogenates, isolated cells or permeabilized tissue samples (Kuznetsov *et al.* 2008). Isolation of mitochondria by differential centrifugation could lead to loss of organelles that were damaged by pathological processes induced by sepsis and subsequent determination of oxygen consumption then could give misleading results (Piper *et al.* 1985). Measurement of mitochondrial respiration on the whole cells is suitable for blood cells, isolated hepatocytes or cultured tissue cells, quality of which can be dependent on isolation procedure (Frezza *et al.* 2007). In particular, platelets and white blood cells can be stimulated by mechanical manipulation and contact with media and laboratory plastics (Kramer *et al.* 2014). Mechanical and chemical permeabilization of the tissue is well standardized for skeletal and cardiac muscles, reported for the liver tissue, but not yet available for other organs. Tissue homogenates are widely used in the evaluation of mitochondrial respiration; however, the homogenization itself is difficult to be standardized and can damage intracellular organelles (Cantó and Garcia-Roves 2015).

Activity of individual respiratory complexes can be determined using methods based on analysis of oxygen consumption (using classical oxygraphy, high resolution respirometry, or fluorescent probes) in freshly dissected tissue or isolated mitochondria or by classical spectrophotometric methods determining their enzymatic activities (Perry *et al.* 2013). *In vivo*, mitochondrial respiration is substantially dependent not only on the ability of ETS to transport electrons and to couple it with

phosphorylating process, but also on the availability of substrates and presence of regulatory factors (Ozkok *et al.* 2016, Schöpf *et al.* 2016, Tantama *et al.* 2013). Such a situation is difficult to be reproduced in *ex vivo* conditions. In classical oxygraphy or more sophisticated high resolution respirometry, saturating concentrations of substrates are provided (Pesta and Gnaiger 2012), whereas in the living cells in tissues challenged by SIRS, availability of natural substrates could be challenged (Bar-Or *et al.* 2018, Waltz *et al.* 2016). In addition, various oxygraphic protocols only partly reproduce the situation *in vivo*, where the substrates providing electrons to ETS are more numerous. In the most frequent protocol used to evaluate activity of complex 1, only glutamate and malate are used as substrates providing NADH, although the real situation is much more variable (Fig. 2B). For example, combinations of substrates malate + glutamate could give different results than malate + pyruvate (Gnaiger 2009, Lemieux *et al.* 2017). The total oxygen consumption by mitochondria is not dependent only on convergent flow of electrons from complexes I and II on coenzyme Q, but could be also affected by functional integrity of electron transporting flavoprotein and other electron donors that are not regularly included in analyses of mitochondrial respiration, like mGPDH (Ramos-Filho *et al.* 2015, Rauchová *et al.* 2014). In addition, the parameter determined by oxygraphic techniques after addition of substrates and ADP might not be true oxygen consumption by in situ mitochondria, but capacity of ETS to consume oxygen under coupled conditions (i.e. OXPHOS capacity; Wüst *et al.* 2015).

Table 2. Experimental models of sepsis.

In vitro Models	
Cultured cells Blood cells Freshly isolated tissue cells from septic animals	Do not reproduce true sepsis (-) Suitable to study subcellular pathways in the particular cell types (+) Blood cells challenged by sepsis can be obtained from human patients (+)
In vivo Models	
Peritonitis models	
Cecal ligation and puncture Bacteria and fibrin clot implantation Colon ascendens stent peritonitis Cecal slurry injection Autologous feces inoculation	Polymicrobial (+) Presence of infection focus (+) Prolonged elevation of cytokines (+) Variable duration and severity (-) Approach true human sepsis (+) Poor control of bacterial load (-) Surgical training required (-) Resuscitation desirable (-)
Intravascular models	
Bacterial injection Endotoxin injection or infusion	Standardized, simple and reproducible (+) Dependent on the type of toxin, its dose, route of administration, host species (-) Different from peritonitis models (-) Do not mimic human sepsis (-) Strictly dependent on given PRR (-) High levels of inflammatory cytokines peak earlier (-) Usually fulminant disease (-) Suitable to study isolated effects of given endotoxin (+)

List of experimental models of sepsis with their advantages (+) and disadvantages (-). PRR – pattern recognition receptor.

Perspectives

Despite substantial progress in understanding of pathophysiology of sepsis and identification of a number of molecules that could be potentially beneficial in the treatment of sepsis, clinical trials designed to approve the use of these agents in the human medicine have been remarkably unsuccessful (Fink 2014). To improve the translational potential of animal experiments, several strategies have been suggested.

Modern omics technologies enable identification of multiple pathways potentially involved in the onset and progression of MOD in human septic patients (Evangelatos *et al.* 2018, Langley *et al.* 2013, Liu *et al.* 2014). Strategy of "reverse translation" based on identification of these pathways in humans and their subsequent verification on appropriate animal model would help to avoid analysis and fruitless translation of phenomena that are strictly species-specific (Efron *et al.* 2015).

The animal models are being continuously improved to better reproduce key features of human sepsis (Stortz *et al.* 2017). In rodents, use of cecal ligation and puncture (CLP) resuscitated model is preferred to endotoxin and peritonitis models without resuscitation (Dejager *et al.* 2011). Experiments on small laboratory rodents will also enable evaluation of impact of age and various comorbidities on the pathophysiology and outcome of sepsis (Loftus *et al.* 2018, Miyaji *et al.* 2003). In contrast to vast majority of sepsis models in rodents, porcine experimental peritonitis is regularly associated with hyperdynamic pattern of circulation, increased oxygen delivery and unchanged systemic oxygen uptake, findings that mimic the course of human disease (Benes *et al.* 2011, Chvojka *et al.* 2008). Wider use of larger animal species could be recommended in preclinical studies designed to approve the use of new pharmacological agents for the treatment of sepsis.

To answer the question if mitochondrial dysfunction is a real determinant of induction/progression of sepsis-related organ dysfunction further intense research work is still needed. Research in basic mitochondrial physiology requires more detailed mapping of interspecies differences and organ-specific features of oxygen utilization in addition to use of complex and standardized protocols evaluating mitochondrial respiration (Lemieux *et al.* 2017). Porcine models of sepsis allow repeated sampling that would help to answer many questions concerning progression of mitochondrial dysfunction and its impact on MOD (Matejovic *et al.* 2016).

Conflict of Interest

There is no conflict of interest.

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