Mitochondrial Function in Sepsis

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Sepsis syndrome represents a leading cause of intensive care unit (ICU) admission, morbidity and mortality. Several areas within the pathophysiology of sepsis remain controversial despite extensive pre-clinical and clinical research. It has been postulated that mitochondrial dysfunction may contribute to organ dysfunction and failure in sepsis. Nevertheless, many aspects of mitochondrial malfunction and the exact mechanisms as to how it may be linked to organ dysfunction remain unknown. Here, we briefly review some basic concepts of mitochondrial function in sepsis. The main available methods to assess mitochondrial function are presented and pre-clinical and clinical work summarized to show and explain some of the main controversies surrounding the role of mitochondrial function in sepsis. Finally, we propose future directions for new research in the field.

Keywords: Sepsis; Septic shock; Mitochondria; Multiple organ failure; Animal model; Resuscitation

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Introduction

Despite being one of the oldest syndromes in critical care medicine, sepsis still represents a leading cause of morbidity and mortality in the intensive care unit (ICU) [1–3].

In sepsis, activation of immune and endothelial cells leads to a profound release of pro-inflammatory and anti-inflammatory mediators, increases adhesion molecule expression and upregulates complement and coagulation system activation. This leads to increased vascular permeability, fluid loss to the extravascular compartment, systemic vasodilatation, impaired myocardial function and derangements of microcirculatory blood flow, which impair tissue perfusion and oxygen delivery to the cells [3].

The decreased availability of oxygen to the tissues secondary to various combinations of low arterial oxygen tension (hypoxic hypoxia), decreased hemoglobin levels (anaemic hypoxia), and/or hypoperfusion (stagnant hypoxia), inhibit cellular aerobic production of adenosine triphosphate (ATP) [4].

Nevertheless, findings of decreased ATP production in sepsis [5–10] in the face of maintained or even increased tissue pO_2 levels [2, 11–14], coupled with a variable pattern of systemic oxygen consumption [15], have given rise to the controversial theory of cytopathic hypoxia [16, 17]. This theory suggests an acquired defect in cellular oxidative phosphorylation (i.e. mitochondrial dysfunction) prevents cells from different organs and tissues from using molecular oxygen for ATP production. Cytopathic hypoxia has been put forward as an explanation for sepsis-induced organ dysfunction and failure [16]. Several underlying mechanisms have been proposed to explain cytopathic hypoxia in sepsis. These include:

- ultrastructural damage to the mitochondrial membranes, allowing a proton leak across the inner mitochondrial membrane [18–20];
- derangements of the pyruvate dehydrogenase complex, which oxidatively decarboxylates pyruvate to acetyl-CoA [21];
- inhibition of key mitochondrial enzymes of the tricarboxylic acid cycle (Citric Acid cycle or Krebs cycle) and electron transport chain by nitric oxide (NO) [6];
- inhibition of mitochondrial enzyme complexes by peroxynitrite [22];
- damage caused by reactive oxygen species (ROS) [23]; and
- poly(ADP-ribose) polymerase (PARP-1) activation [24].

The aim of this review is to outline some basic concepts of mitochondrial function in the normal state and in sepsis, to present the main available methods to assess mitochondrial function, and to summarize pre-clinical and clinical work in an attempt to explain some of the main controversies surrounding

the role of mitochondrial function in sepsis. Finally, we propose future directions for new research in the field.

Mitochondrial structure and function

A mitochondrion is surrounded by a double layer of membranes, which form an inter-membrane space (Figure 1) [25]. The outer mitochondrial membrane is composed of an equal proportion of protein and lipids and is permeable to most metabolites. In contrast, the inner mitochondrial membrane is composed mainly of proteins (80%) with a small proportion of lipids (20%), and is highly selectively permeable. The inner membrane is folded inwards to form cristae, projecting into the mitochondrial matrix, and concentrates the respiratory chain enzymes. The mitochondrial matrix contains the enzymes of the tricarboxylic acid cycle (TCA), either free or attached to the inner mitochondrial membrane, ß-oxidation enzymes, and the pyruvate dehydrogenase complex (Figure 1) [25]. The mitochondrion is the main structure responsible for energy production in animal cells [26]. Energy production, i.e. ATP production, occurs in a three-step process which is intrinsically interconnected: glycolysis, TCA cycle and electron transport chain (oxidative phosphorylation) [26, 27]. Glycolysis occurs in the cytosol of all cells and represents the major pathway for glucose, galactose and fructose metabolism [26, 27].

Glycolysis

Glycolysis is an oxygen-independent process. Under anaerobic conditions, the resulting end product, pyruvate, is reduced by lactate dehydrogenase into lactate, generating two molecules of ATP per molecule of glucose. More often, under aerobic conditions, pyruvate is transported into the mitochondria matrix, where it is oxidatively decarboxylated to Acetyl-coenzyme A (Acetyl-CoA) by the pyruvate dehydrogenase complex [26, 27]. This Acetyl-CoA then enters the TCA cycle (Figure 1).

The TCA Cycle

The TCA cycle is a sequence of reactions that occur in the mitochondrial matrix, which is always followed by oxidative phosphorylation, the most important mechanism of ATP production (Figures 1 and 2) [26, 27]. In the TCA cycle, one molecule of Acetyl-CoA is oxidized and three molecules of nicotinamide adenine dinucleotide (NAD^+) and one molecule of flavin adenine dinucleotide (FAD) are reduced, respectively, to three molecules of NADH-H^+ and one molecule of $FADH_2$ [28]. These coenzymes (or carriers) are subsequently utilized (reoxidized) in the respiratory chain in the key process of ATP production.

The respiratory chain and oxidative phosphorylation

The flow of electrons through the complexes of the respiratory chain generates ATP by a process named oxidative phosphorylation (chemiosmotic theory) (Figure 2) [26]. Oxidative phosphorylation occurs on the inner mitochondrial membrane. Electrons from the NADH-H^+ and $FADH_2$ produced in the TCA cycle are transferred through the mitochondrial complexes (electron transport chain), re-oxidazing the carriers and generating ATP, a high-energy phosphate. Although oxygen is not necessary for the TCA cycle, it is crucial for oxidative phosphorylation [26].

The respiratory chain comprises five protein complexes embedded on the inner mitochondrial membrane (complexes I to V) (Figure 2). Electrons are transferred from NADH-H^+ to complex I, also known as NADH-Q oxidoreductase, coupled with the transfer of four H^+ to the intermembrane space. Electrons are also transferred from $FADH_2$ to complex II (succinate-Q reductase) [26]. The electrons coming from both complexes are then transferred to coenzyme Q (ubiquinone), which is reduced to ubiquinol (QH_2, dihydroquinone). Then, ubiquinol donates its electrons to complex III (Q-cytochrome c oxidoreductase) in a process called Q cycle, in which an additional four H^+ are transferred to the intermembrane space. The reduced cytochrome c shuttles electrons from complex III to complex IV (cytochrome c oxidase) simultaneously, with a reduction of molecular oxygen to water and the transfer of $2H^+$ to the intermembrane space. Therefore, oxygen is the final electron acceptor of the respiratory chain and water is the final product of oxygen reduction (Figure 2) [26]. While electrons are being carried through the complexes, the released energy in the process is used to pump H^+ to the intermembrane space. This is a key process. This electro-chemical gradient of protons, concentrated in the intermembrane space, is then used by the complex V (ATP synthase or F1FO ATPase), located on the inner mitochondrial membrane, to produce ATP, coupled with the release of energy by the flux of H^+ coming back to the mitochondrial matrix. In non-damaged mitochondria, protons return to the mitochondrial matrix from the intermembrane space through the complex V. The inner mitochondrial membrane must be physically intact to be able to create this proton gradient. Thus, the mitochondrion can control the re-entrance of protons into the mitochondrial matrix.

ATP synthase drives the synthesis of ATP from adenosine diphosphate (ADP) and inorganic phosphate, coupled with the release of energy from the passage of H^+ back to the mitochondrial matrix. ATP synthase is composed of two subunits: F1, which projects into the matrix and contains the phosphorylation mechanism, and FO, which spans the inner mitochondrial membrane creating a proton channel. The flow of H^+ through FO causes it to rotate, driving the production of ATP by the F1 subunit.

Impact of mitochondrial damage on function

In damaged mitochondria, in which an inner membrane is permeable to protons, ATP synthesis is not only reduced due to the reduced proton gradient, but it is also affected by the reverse action of ATP synthase. Under such circumstances, ATP synthase takes ATP from the mitochondrial matrix and works in a counterproductive way as an ATP hydrolase, reducing the ATP levels [18].

How is mitochondrial function assessed?

Over the years, the available methods to address mitochondrial function have evolved, becoming more user-friendly and robust. Mitochondrial function can be evaluated in-vivo or in-vitro, by spectrophotometric assays (enzymatic activity) or by polarography (Clark-type electrode or high-resolution respirometry) [29]. The technical aspects of each method are beyond the scope of this paper and have been described in detail elsewhere [30, 31].

Briefly, the spectrophotometric assay allows the assessment of the activity of respiratory chain complexes in human and animal tissues and cells. In a spectrophotometric assay, a tissue or cell homogenate is supplemented with different electron donors or acceptors, and the enzymatic activity of each complex is expressed as nanomole (nmol) cytochrome c reduced per minute per milligram protein (nmol cytochrome c·min-1·mg-1) [30, 31].

The high-resolution respirometry (oxygraph) technique (Oxygraph-2k; Oroboros Instruments, Innsbruck, Austria) has

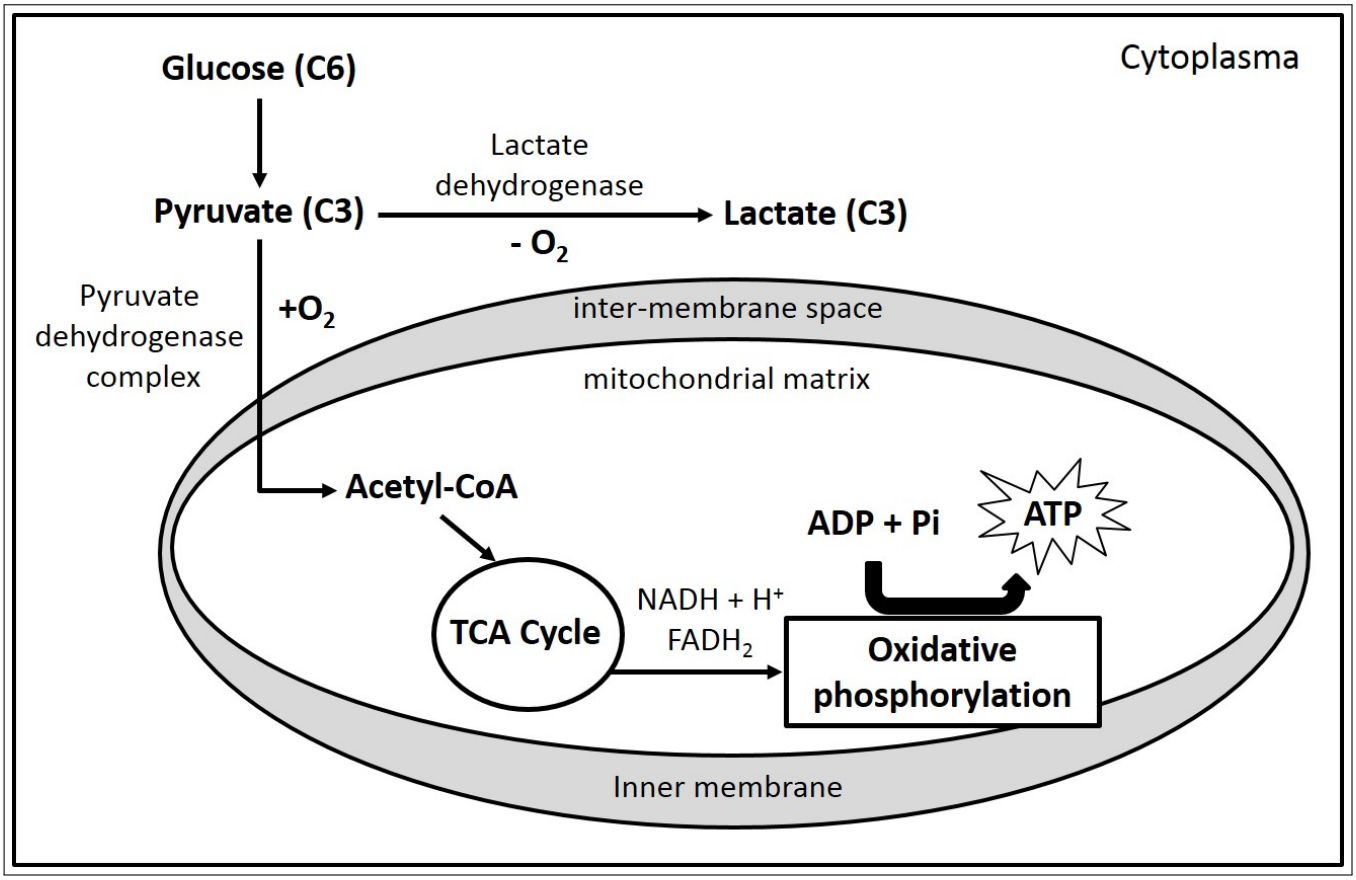

Figure 1. Energy production pathways in animal cells.

Under anaerobic conditions (-O_2), pyruvate is reduced by lactate dehydrogenase into lactate, generating two molecules of adenosine triphosphate (ATP) per molecule of glucose. More often, under aerobic conditions (+O_2), pyruvate is transported into the mitochondria matrix, where it is oxidatively decarboxylated to Acetyl-CoA by pyruvate dehydrogenase complex. The tricarboxylic acid (TCA) cycle is a sequence of reactions that occur in the mitochondrial matrix and is always followed by oxidative phosphorylation. In the TCA cycle, Acetyl-CoA is oxidized and nicotinamide adenine dinucleotide (NAD) and flavin adenine dinucleotide (FAD) reduced, respectively, to NADH-H^+ and $FADH_2$. These coenzymes are subsequently reoxidized in the respiratory chain coupled with ATP production.

been used in many studies (Figure 3) [10, 32–38]. Through this method, intact cells, permeabilized cells, or isolated mitochondria sampled from different human or animal tissues are processed, immersed into a medium, and placed inside a sealed chamber containing a known oxygen concentration. The high-resolution respirometry analysis is based on a continuous measurement by polarography of oxygen concentration inside the sealed chamber. As mitochondria consume oxygen inside the chamber, the oxygen concentration declines, and a plot of oxygen concentration by time is provided (Figure 3) [31]. As part of this process, various respiratory states of mitochondria are referred to. A basic understanding of these becomes important when reviewing results of the studies on mitochondrial dysfunction in sepsis. The states include [39]:

- State 1: The first state in an oxygraph protocol, where mitochondria are present in a medium with oxygen and inorganic phosphate, but there is no ADP or respiratory substrate present as yet;
- State 2: This refers to a substrate limited state of residual oxygen consumption (ADP has been added but respiratory substrates have not);
- State 3: ADP stimulated respiration after addition of respiratory substrates - this represents the mazimum capacity of the respiratory chain;
- State 4: Following on from state 3, when the available ADP has been completely converted to ATP - this represents the resting level of respiration; and
- State 5: Following on from state 4, when the available oxygen has been completely depleted i.e. anaerobic.

The ratio of state 3 to state 4 is called the respiratory control ration (RCR), and is an index of how efficiently coupled phsophorylation is to oxidation.

The amount of tissue available, and whether fresh or frozen samples will be analysed, are critical aspects for the choice of the method used to address mitochondrial function [30, 31]. While spectrophotometric analysis can be performed using either fresh or frozen tissue samples, polarographic analysis requires a prompt analysis of fresh samples [30, 31]. The polarographic analysis has the advantage of allowing simultaneously evaluation of the electron transport chain and the TCA cycle, which more closely resembles the cell metabolism [31].

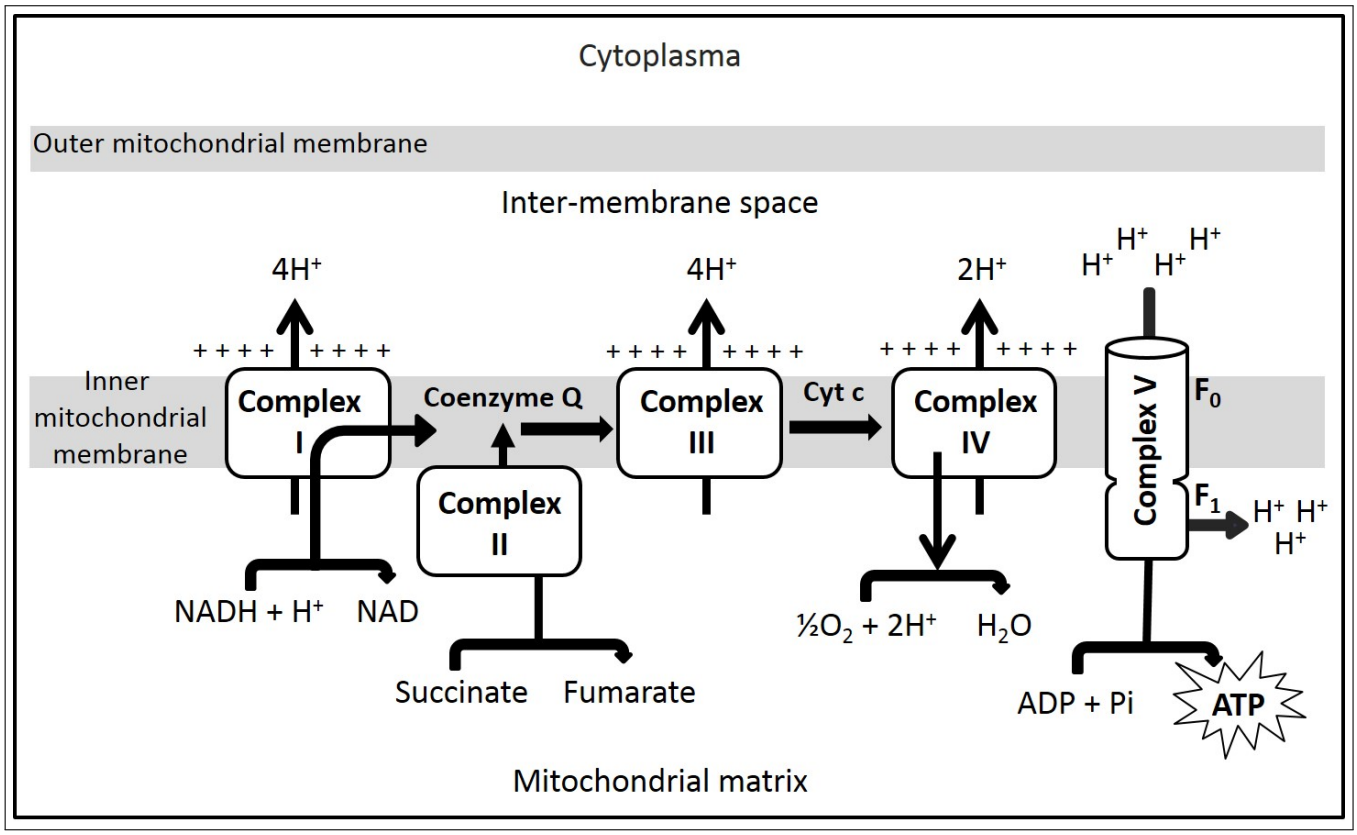

Figure 2. Summary of oxidative phosphorylation.

The electrons flowing through the respiratory chain complexes generate adenosine triphosphate (ATP) in a process named oxidative phosphorylation. The respiratory chain comprises five protein complexes embedded on the inner mitochondrial membrane (complexes I to V). Electrons are transferred from NADH-H^+ to complex I, also known as NADH-Q oxidoreductase, coupled with the transfer of $4H^+$ to intermembrane space. Electrons are also transferred from $FADH_2$ to complex II (succinate-Q reductase). The electrons coming from both complexes are then transferred to coenzyme Q (ubiquinone), which is reduced to ubiquinol. Then, ubiquinol donates its electrons to complex III (Q-cytochrome c oxidoreductase), in which additional $4H^+$ are transferred to the intermembrane space. The reduced cytochrome c (Cyt c) shuttles electrons from complex III to complex IV (cytochrome c oxidase) simultaneously, with a reduction of molecular oxygen to water and the transfer of $2H^+$ to the intermembrane space. Finally, ATP synthase (complex V) drives the synthesis of ATP from adenosine diphosphate (ADP) and inorganic phosphate coupled with the release of energy from the passage of H^+ back to the mitochondrial matrix. ATP synthase is composed of two subunits: F1, which projects into the matrix and contains the phosphorylation mechanism, and FO, which spans the inner mitochondrial membrane creating a proton channel. The flow of H^+ through FO causes it to rotate, driving the production of ATP by the F1 subunit.

Mitochondrial function in sepsis

Even though it has been over fifty years since the first studies about mitochondrial function were published [40–42], the role of mitochondrial dysfunction in sepsis remains controversial, and its contribution to the development of organ dysfunction is unknown [29, 43, 44]. Controversies exist given the observations that mitochondrial function might be depressed, improved, or unchanged in sepsis [29]. The putative mechanisms of mitochondrial dysfunction, the variable pattern of complexes involved, and the temporal sequence after the initial insult, i.e., early vs. late sepsis, are examples of the intriguing questions that remain to be answered [29]. Some authors have argued that the decreased mitochondrial function observed in experimental and/or clinical studies might represent an adaptive mechanism, occurring in response to varying degrees of tissue hypoperfusion and hypoxia [45]. This state has been termed "mitochondrial hibernation", which can be characterized by a cellular down-regulation of all non-essential functions, followed by a decreased global rate of oxygen and ATP consumption [45]. The hibernation phenomenon may explain the observations that organ dysfunction and failure in sepsis were seldom associated with histopathological damage [46].

Inhibitory effects of intravenously administered endotoxin on mitochondrial respiration and/or enzymatic activity in different animal species have been described [29]. Similar findings have also been reported when intact live bacteria are administrated through different routes, and in different species [29]. Moreover, in the few studies which addressed mitochondrial function in septic patients, isolated mitochondria from skeletal muscle and blood cells were assessed [6, 42, 47–55]. Thus, it is important to emphasize that most of what is known about mitochondrial dysfunction in sepsis has been provided by experimental animal models [29].

For instance, decreased complex I dependent respiration was reported in skeletal muscle and hepatocytes after twenty-four hours of abdominal sepsis in rats [8]. Moreover, hepatic ATP content was shown to be lower in the most severe animals in comparison to the sham controls or mildly affected rats [8]. A decreased complex II-III activity was reported in the diaphragm of rats after twelve hours of faecal

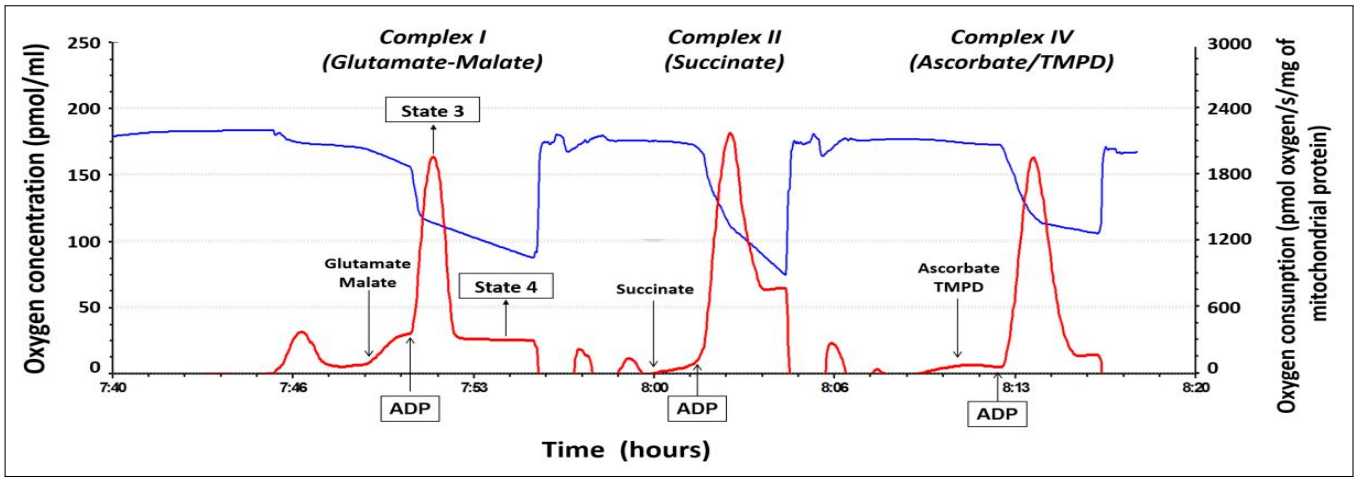

Figure 3. Representative diagram of measurement of respiration rates in isolated liver mitochondria from the left liver lobe of a healthy pig assessed by high-resolution respirometry.

The high-resolution respirometry (Oxygraph-2k; Oroboros Instruments, Innsbruck, Austria) allows the evaluation of different states of respiratory control. By applying specific substrates, different complexes of the electron transport chain can be studied. Complex-I dependent respiration can be evaluated by adding glutamate and malate as substrate, which provides NADH-H+ to complex I. Complex-II-dependent respiration can be evaluated after inhibition of complex I by rotenone, by adding succinate as substrate, which provides FADH2 to complex II. Finally, ascorbate plus TMPD (N,N,N′,N′-Tetramethyl-p-phenylenediamine dihydrochloride) can be used to address complex-IV dependent respiration. The state 3 represents the maximal capacity of the respiratory chain itself when saturating concentrations of ADP and substrates are provided. State 4 represents the resting respiration, when ADP is depleted by its phosphorylation to ATP (ADP-limited resting state). The respiratory control ratio (RCR) can be obtained by dividing the rate of oxygen consumption at state 3 by the rate of oxygen consumption at state 4. The RCR represents a marker of oxidative phosphorylation efficiency (the coupling of phosphorylation to oxidation). The red line represents the oxygen consumption by liver mitochondria expressed as pmol oxygen per second per mg of mitochondrial protein and the blue line represents the oxygen concentration inside the sealed chamber expressed as pmol oxygen per ml.

peritonitis, while a decreased activity of all mitochondrial complexes was demonstrated after forty-eight hours of sepsis [56]. It has been demonstrated that isolated liver complex I and II dependent respiration, but not isolated skeletal muscle nor kidney mitochondrial respiration, were impaired when pigs were rendered septic by intravenous endotoxin (Escherichia coli lipopolysaccharide B0111:B4) infusion during a 24 hours period [57]. On the other hand, Kozlov and colleagues demonstrated impaired kidney complex II dependent mitochondrial respiration after twelve hours of faecal peritonitis in pigs [34]. Impaired brain complex I dependent respiration after twenty-four hours of faecal peritonitis was described [19], and decreased cerebellum, hippocampus, striatum and cortex mitochondrial complex I dependent respiration demonstrated after 24, 48 and 96 hours of faecal peritonitis in rats [58].

Evidence of mitochondrial dysfunction has also been reported in humans. Brealey and colleagues demonstrated that complex I activity was lower in patients with septic shock who died in the ICU, in comparison to non-septic patients [6]. In another study, a 60% reduction in complex I activity of intercostal muscle, but not of vastus lateralis muscle, of ten mechanically ventilated septic patients was reported [47]. Nevertheless, decreased ATP content was observed in vastus lateralis muscle while intercostal muscle ATP content was unchanged [47].

Conversely, an increased skeletal muscle (vastus lateralis) complex I activity was demonstrated in seven human healthy volunteers two hours after intravenous endotoxin infusion [49]. Moreover, Sjövall and colleagues demonstrated an increased complex I and II state 3 dependent respiration in permeabilized platelets isolated from eighteen patients with severe sepsis/septic shock during the first seven days of the disease, both in comparison to days 1/2 and to non-septic (healthy) controls [50]. The same group also demonstrated increased complex I, II and IV state 3 dependent mitochondrial respiration in permeabilized peripheral blood immune cells obtained from patients with severe sepsis/septic shock, in comparison to healthy controls [53].

Are the experimental models used to address mitochondrial function appropriate?

The results of studies across rodent and pig models of sepsis are summarised in Tables 1 and 2. It is important to highlight that experimental models used to study mitochondrial function in sepsis differ in many respects from clinical sepsis in ICU patients [87, 88]. Therefore, the reliability of experimental models has been questioned [89]. Septic patients admitted to the ICU are often elderly, and exhibit multiple comorbidities [90], while experimental animals are usually young, of a single gender, with no comorbidities and from a similar genetic background [87, 88, 90]. Moreover, due to feasibility and costs, researchers very often set up short-term (shorter than 24 hours) models of sepsis, while the clinical course of human sepsis usually develops over several hours or days [91].

Small rodents are the most common animals used in experimental sepsis [29]. Nevertheless, they have significant physiological and pharmacological differences in comparison to humans. Indeed, mice are more resistant to endotoxin infusion than humans, with a different pattern of inflammatory response to an infectious insult [92]. Porcine models of sepsis, using medium-size pigs (30-40 kg), have been used to better reproduce the clinical aspects of sepsis and its treatment [93]. Pigs share many aspects of human cardiovascular anatomy

Table 1. The effect of sepsis on mitochondrial function in rodents addressed by polarography (Clark-type electrode or high-resolution respirometry).

Author	Reference	Year	Animal	Model	Time (h)	RS	Tissue	Results
Fry	[59]	1981	rats	i.v. LPS	6	No	Liver	C-I and C-II state 3 and RCR increased
Tavakoli	[60]	1982	rats	CLP	144	No	Liver	RCR decreased
							SM	RCR decreased
Garrison	[61]	1982	rats	CLP	2,4 or 6	No	Kidney	Unchanged
Geller	[62]	1986	rats	i.p. LPS	18	No	SM	Unchanged
Dawson	[63]	1988	rats	i.v. LPS	4	Yes#	Heart	C-I RCR increased
							SM	C-I RCR increased
Kopprasch	[64]	1989	rats	i.p. LPS	6	No	Liver	C-I and C-II state 4 increased
Takayama	[65]	1990	rats	i.p. LPS	24	No	Liver	C-I and C-II state 3 and RCR increased
Llesuy	[66]	1994	rats	CLP	6, 12, 24	Yes	Liver, SM	Liver unchanged, SM C-I and C-II State 3 and RCR decreased
Taylor	[67]	1995	rats	CLP	16	No	Liver	Unchanged
Malaisse	[68]	1997	rats	i.p. LPS	24	No	Liver	C-II state 3, state 4 and RCR increased
Kantrow	[69]	1997	rats	CLP	16	No	Liver	C-I and C-II state 3 increased
Markley	[70]	2002	rats	i.p. LPS	2	No	Liver	Unchanged
Fukumoto	[71]	2003	rats	i.p. LPS	2 or 6	No	Heart	C-I RCR unchanged at 2 hrs but decreased at 6 hrs
							Kidney	Unchanged
Suliman	[72]	2004	rats	i.v. LPS	6, 24 or 48	No	Heart	C-I State 3 decreased at 6 hrs and unchanged at 24 and 48 hrs
Nin	[73]	2004	rats	CLP	48	No	Heart	RCR decreased
							Diaphragm	RCR decreased
Kozlov	[74]	2006	rats	i.p. LPS	16	No	Heart	C-I decreased
							Liver	C-I and C-II increased
Larche	[75]	2006	mice	CLP	Up to 96	No	Heart	C-I state 3 and RCR decreased, C-IV unchanged
Mason	[76]	2007	rats	i.p. LPS	6, 12 or 24	No	Heart	C-I state 3 unchanged at 6 or 12 but decreased at 24 hrs
Protti	[77]	2007	rats	FP	48	No	SM	C-I decreased, C-II unchanged
Kozlov	[78]	2007	rats	i.p. LPS	16	No	Liver	C-I and C-II state 3 increased
Duvigneau	[79]	2008	rats	i.v. LPS	2, 4, 8, 12	No	Liver	C-I RCR increased at 2 hrs and 12 hrs, decreased between 4 and 8 hrs
Hassoun	[80]	2008	rats	i.v. LPS	4	No	Heart	Decreased C-1 state 3 and RCR. Increased state 4
Vanasco	[81]	2008	rats	i.p. LPS	6	No	Liver	C-I State 3 decreased at 6 hrs and unchanged at 24 and 48 hrs
							Heart	C-I State 3 decreased
							Diaphragm	C-I State 3 decreased
Kozlov	[33]	2009	rats	i.v. LPS	16	No	Liver	C-I and C-II RCR increased
Reynolds	[82]	2009	mice	i.p. LPS	6, 24, 48, 72	No	Heart	C-I and C-II State 3 decreased at 24 hrs and increased at 72 hrs
Aguirre	[38]	2012	mice	i.p. LPS	24	No	SM	C-II decreased
Vanasco	[83]	2012	rats	i.p. LPS	6	No	Heart	C-I and C-II State 3 decreased

RS = repeated samples, i.v. = intravenous, i.p. = intraperitoneal, SM = skeletal muscle, LPS = lipopolysaccharide, CLP = caecal ligation and puncture, FP = faecal peritonitis, # = postmortem period, C-I = Complex-I dependent respiration, C-II = Complex-II dependent respiration, C-IV = Complex-IV dependent respiration and RCR = respiratory control ratio (state 3 / state 4).

and physiology, and allow the reproduction of the clinical management of sepsis with full hemodynamic monitoring, fluid resuscitation, antibiotics and support with vasoactive drugs. In addition, repeated tissue samples for mitochondrial analysis are possible. Such interventions are usually not feasible in small-size experimental animals.

Another important aspect of sepsis models that may affect mitochondrial function is the severity of the disease. Three methods are commonly used to produce experimental sepsis: exogenous administration of a toxin (lipopolysaccharide; LPS), methods that alter the endogenous protective barrier [caecal ligation and puncture (CLP) and colon ascendens stent peritonitis (CASP)], and exogenous administration of viable pathogens in the lungs, peritoneal cavity, subcutaneously or intravenously [94].

Endotoxin infusion models accounted for approximately 40% of studies of mitochondrial function in sepsis [29]. However, it is well known that the immune, inflammatory, and cardiovascular responses triggered by LPS infusion are completely different from those induced by a living pathogen [92]. This different pathophysiology may affect mitochondrial function [94].

Models of faecal peritonitis were developed to overcome the pitfalls of the endotoxin models. Those models more closely resemble human sepsis, demonstrating several advantages in comparison to LPS models, including a polymicrobial infection caused by living pathogens and a well-defined focus of infection, which triggers an immune, inflammatory and cardiovascular response more comparable to human sepsis [94]. The severity of experimental sepsis, which may have important implications on mitochondrial function, can be adjusted by varying the amount of LPS infused, the size of the caecal punctures and/or the distance of cecum ligated, the diameter

Table 2. The effect of sepsis on mitochondrial function in pigs addressed by polarography (Clark-type electrode or high-resolution respirometry).

Author	Ref	Year	Model	Time	RS	Resuscitation	Tissue	Results
Hirai	[5]	1984	CLP	0,2,4,7,12-14 days	No	Fluids	Liver	C-I state 3 and RCR decreased by days 12-14
Porta	[57]	2006	i.v. LPS	24h	No	Fluids	SM	Unchanged
							Liver	C-I state 4 increased and RCR decreased
							Kidney	C-I, C-II and C-IV unchanged
Li $	[84]	2007	FP	12h	No	Fluids	Heart	C-I activity decreased
Regueira	[85]	2008	i.v. LPS	10h	No	Fluids, vasopressors	Liver	C-I and C-II RCR increased
							SM	C-I state 3 increased
Brandt	[32]	2009	FP	24h	No	Fluids	SM	Unchanged in FP
			i.v. LPS				Liver	Unchanged in FP. C-I state IV decreased in i.v. LPS group
Kozlov	[34]	2010	FP	12h	No	Fluids	Liver	C-I and C-II state 3, state 4 and RCR decreased
							Kidney	C-I states 3 & 4 increased, RCR unchanged.
								C-II states 3 & 4 increased, RCR decreased
Corrêa	[35]	2012	FP	6, 12 or 24h sepsis no therapy	Yes*	Fluids, vasopressors,	SM	C-I RCR increased after 12 hrs of PI
				+ 48h of resuscitation		inotropes, antibiotics	Brain	C-II state 3 decreased after 72 hrs of PI
							Liver	Unchanged
							Heart	Unchanged
Regueira	[10]	2012	FP	0, 6 and end (max 24h)	Yes*	Fluids	Liver	C-I, C-II unchanged
							SM	C-I RCR decreased
Vuda	[86]	2012	FP	27h	Yes*	Fluids	Liver	C-I, C-II and C-IV unchanged
						Vasopressors	SM	C-I, C-II and C-IV unchanged
Corrêa	[36]	2013	FP	12h sepsis no therapy	Yes*	Fluids, vasopressors,	SM	Unchanged
				+ 48h of resuscitation		inotropes, antibiotics	Liver	Unchanged
Corrêa	[36]	2013	FP	22h #	Yes	No	SM	C-I state increased

RS = repeated samples, i.v. = intravenous, SM = skeletal muscle, LPS = lipopolysaccharide, CLP = cecal ligation and puncture, FP = fecal peritonitis, PI = peritonitis induction, # = postmortem period, C-I = Complex-I dependent respiration, C-II = Complex-II dependent respiration, C-IV = Complex-IV dependent respiration, RCR = respiratory control ratio (state 3 / state 4), * = for skeletal muscle analysis, $ = spectrophotometric analysis and # = median (range) survival time of 22 (16 to 28) hours.

of the inserted stent into the ascending colon or the bacterial load infused into the abdominal cavity [95].

The effect of time on mitochondrial assessment

Sepsis is characterized by a biphasic inflammatory, immune, hormonal, and metabolic response triggered by an infection [3]. While early sepsis is characterized by a pronounced release of inflammatory mediators, increased release of stress hormones, metabolic activity and mitochondrial function, late sepsis is characterized by an anti-inflammatory immunosuppressive state, and impaired energy production secondary to mitochondrial inhibition and/or damage [96].

Thus, it is been postulated that a severe inflammation, endocrine and metabolic shutdown leads to a decreased energy production, i.e. cell "hibernation" or "stunning", which may be a protective mechanism. This reduced cellular metabolism might boost the chances of cellular survival after an overwhelming insult [46]. Such different stages of the inflammatory, immune, hormonal, and metabolic response in septic animals and patients might explain why several groups have reported conflicting results (unchanged, impaired or improved) regarding mitochondrial function in sepsis, with marked organ-specific differences (Tables 1 and 2)[29].

Impaired respiration in the heart but not in the kidney of endotoxemic rats [71], in the hepatocytes but not in the heart of pigs rendered septic by intravenous infusion of Pseudomonas aeruginosa [7], in the liver but neither in the kidney nor in skeletal muscle of endotoxemic pigs [57], in small bowel mucosa but not in the muscular layer of challenged pigs with continuous infusion of endotoxin [97] have all been reported.

We recently evaluated the impact of treatment delay on the development of sepsis-associated mitochondrial dysfunction in skeletal muscle, liver, heart and brain using a swine model of faecal peritonitis (peritoneal instillation of autologous faeces) [35]. After 6, 12 or 24 hours of untreated sepsis, all animals received 48 hours of protocolized resuscitation consisting of fluids, vasopressors and broad spectrum antibiotics. An increased skeletal muscle Complex I dependent respiration after 12 hours of untreated sepsis was the only sepsis-associated alteration observed before the beginning of resuscitation. At the end of study (i.e. 72 hours after peritonitis induction), a decreased maximal brain mitochondrial Complex II respiration was found in the animals resuscitated after 24 hours of untreated sepsis, while hepatic and myocardial mitochondrial respiration were not affected).

Are the most appropriate organs being evaluated?

The contribution of each organ and system to the outcomes of critically ill patients is variable [98, 99]. Although the liver, heart, kidneys, brain and bowel are easily assessed experimentally, most of the studies addressing the mitochondrial function in humans are limited to skeletal muscle [100], peripheral blood immune cells [53], isolated platelets [55, 101] monocytes [102] and cultured human hepatocytes [103, 104], which may have limited clinical significance. Therefore, one can argue that the lack of association between mitochondrial dysfunction and organ dysfunction or failure in septic patients occurs because only

organs with a non-critical impact on prognosis have been properly addressed in humans.

Additionally, the time necessary for different organs and systems to reach the maximum degree of dysfunction is variable. Therefore, serial sampling in such organs would be necessary to allow for early detection of mitochondrial dysfunction. Currently, such analysis is neither feasible nor ethically acceptable. Thus, most of our knowledge on the role of mitochondrial dysfunction in sepsis will continue to be provided by experimental studies. This fact highlights the importance of a considered approach to selection of the experimental model.

Future directions

The development of easy to handle, non-invasive, devices to address mitochondrial function at the bedside may improve knowledge about the contribution of mitochondrial dysfunction to sepsis pathophysiology. Ideally, a better understanding of the mechanisms of mitochondrial damage and dysfunction in sepsis would be accompanied by new therapies aimed at decreasing the progression to organ dysfunction and failure, and ultimately decreasing sepsis mortality. Furthermore, there are currently no available therapies to treat mitochondrial dysfunction in sepsis [105]. Nevertheless, it has been assumed that appropriate early management of sepsis might prevent the development of mitochondrial dysfunction in such a population of critically ill patients [35]. Finally, the development of standards for the performance and reporting of mitochondrial function analysis would help researchers and clinicians to more readily compare the results obtained by different investigators.

Conclusion

Despite decades of research, the pathophysiology of sepsis has not been completely elucidated. It seems that mitochondrial dysfunction may play a role in organ dysfunction and failure. Nevertheless, the clinical significance of mitochondrial dysfunction, and its association with organ failure, remain unclear. The development of user-friendly, non-invasive devices might allow us to address the role of mitochondrial dysfunction on vital organs in humans, and over appropriate time-scales. Lastly, it may also allow us to develop and study new therapies to improve the outcomes of septic patients.

References

1. Funk DJ, Parrillo JE, Kumar A. Sepsis and septic shock: a history. Crit Care Clin. 2009;25(1):83–101, viii. Available from: http://dx.doi.org/10.1016/j.ccc.2008.12.003.
2. Hotchkiss RS, Rust RS, Dence CS, Wasserman TH, Song SK, Hwang DR, et al. Evaluation of the role of cellular hypoxia in sepsis by the hypoxic marker [18F]fluoromisonidazole. Am J Physiol. 1991;261(4 Pt 2):R965–R972.
3. Angus DC, van der Poll T. Severe sepsis and septic shock. N Engl J Med. 2013;369(9):840–851. Available from: http://dx.doi.org/10.1056/NEJMra1208623.
4. Fink MP. Bench-to-bedside review: Cytopathic hypoxia. Crit Care. 2002;6(6):491–499.
5. Hirai F, Aoyama H, Ohtoshi M, Kawashima S, Ozawa K, Tobe T. Significance of mitochondrial enhancement in hepatic energy metabolism in relation to alterations in hemodynamics in septic pigs with severe peritonitis. Eur Surg Res. 1984;16(3):148–155.
6. Brealey D, Brand M, Hargreaves I, Heales S, Land J, Smolenski R, et al. Association between mitochondrial dysfunction and severity and outcome of septic shock. Lancet. 2002;360(9328):219–223. Available from: http://dx.doi.org/10.1016/S0140-6736(02)09459-X.
7. Hart DW, Gore DC, Rinehart AJ, Asimakis GK, Chinkes DL. Sepsis-induced failure of hepatic energy metabolism. J Surg Res. 2003;115(1):139–147.
8. Brealey D, Karyampudi S, Jacques TS, Novelli M, Stidwill R, Taylor V, et al. Mitochondrial dysfunction in a long-term rodent model of sepsis and organ failure. Am J Physiol Regul Integr Comp Physiol. 2004;286(3):R491–R497. Available from: http://dx.doi.org/10.1152/ajpregu.00432.2003.
9. Huang LJ, Hsu C, Tsai TN, Wang SJ, Yang RC. Suppression of mitochondrial ATPase inhibitor protein (IF1) in the liver of late septic rats. Biochim Biophys Acta. 2007;1767(7):888–896. Available from: http://dx.doi.org/10.1016/j.bbabio.2007.03.009.
10. Regueira T, Djafarzadeh S, Brandt S, Gorrasi J, Borotto E, Porta F, et al. Oxygen transport and mitochondrial function in porcine septic shock, cardiogenic shock, and hypoxaemia. Acta Anaesthesiol Scand. 2012;56(7):846–859. Available from: http://dx.doi.org/10.1111/j.1399-6576.2012.02706.x.
11. Boekstegers P, Weidenhöfer S, Pilz G, Werdan K. Peripheral oxygen availability within skeletal muscle in sepsis and septic shock: comparison to limited infection and cardiogenic shock. Infection. 1991;19(5):317–323.
12. VanderMeer TJ, Wang H, Fink MP. Endotoxemia causes ileal mucosal acidosis in the absence of mucosal hypoxia in a normodynamic porcine model of septic shock. Crit Care Med. 1995;23(7):1217–1226.
13. Rosser DM, Stidwill RP, Jacobson D, Singer M. Oxygen tension in the bladder epithelium rises in both high and low cardiac output endotoxemic sepsis. J Appl Physiol. 1995;79(6):1878–1882.
14. Sair M, Etherington PJ, Peter Winlove C, Evans TW. Tissue oxygenation and perfusion in patients with systemic sepsis. Crit Care Med. 2001;29(7):1343–1349.
15. Friedman G, De Backer D, Shahla M, Vincent JL. Oxygen supply dependency can characterize septic shock. Intensive Care Med. 1998;24(2):118–123.
16. Fink MP. Cytopathic hypoxia. Mitochondrial dysfunction as mechanism contributing to organ dysfunction in sepsis. Crit Care Clin. 2001;17(1):219–237.
17. Fink MP. Cytopathic hypoxia. Is oxygen use impaired in sepsis as a result of an acquired intrinsic derangement in cellular respiration? Crit Care Clin. 2002;18(1):165–175.
18. Crouser ED, Julian MW, Huff JE, Joshi MS, Bauer JA, Gadd ME, et al. Abnormal permeability of inner and outer mitochondrial membranes contributes independently to mitochondrial dysfunction in the liver during acute endotoxemia. Crit Care Med. 2004;32(2):478–488. Available from: http://dx.doi.org/10.1097/01.CCM.0000109449.99160.81.
19. d'Avila JdCP, Santiago APSA, Amâncio RT, Galina A, Oliveira MF, Bozza FA. Sepsis induces brain mitochondrial dysfunction. Crit Care Med. 2008;36(6):1925–1932. Available from: http://dx.doi.org/10.1097/CCM.0b013e3181760c4b.
20. Takasu O, Gaut JP, Watanabe E, To K, Fagley RE, Sato B, et al. Mechanisms of cardiac and renal dysfunction in patients dying of sepsis. Am J Respir Crit Care Med. 2013;187(5):509–517. Available from: http://dx.doi.org/10.1164/rccm.201211-1983OC.

21. Vary TC. Sepsis-induced alterations in pyruvate dehydrogenase complex activity in rat skeletal muscle: effects on plasma lactate. Shock. 1996;6(2):89–94.
22. Radi R, Rodriguez M, Castro L, Telleri R. Inhibition of mitochondrial electron transport by peroxynitrite. Arch Biochem Biophys. 1994;308(1):89–95. Available from: http://dx.doi.org/10.1006/abbi.1994.1013.
23. Galley HF. Oxidative stress and mitochondrial dysfunction in sepsis. Br J Anaesth. 2011;107(1):57–64. Available from: http://dx.doi.org/10.1093/bja/aer093.
24. Goldfarb RD, Marton A, Szabó E, Virág L, Salzman AL, Glock D, et al. Protective effect of a novel, potent inhibitor of poly(adenosine 5′-diphosphate-ribose) synthetase in a porcine model of severe bacterial sepsis. Crit Care Med. 2002;30(5):974–980.
25. Logan DC. The mitochondrial compartment. J Exp Bot. 2006;57(6):1225–1243. Available from: http://dx.doi.org/10.1093/jxb/erj151.
26. Saraste M. Oxidative phosphorylation at the fin de siècle. Science. 1999;283(5407):1488–1493.
27. Adeva-Andany M, López-Ojén M, Funcasta-Calderón R, Ameneiros-Rodríguez E, Donapetry-García C, Vila-Altesor M, et al. Comprehensive review on lactate metabolism in human health. Mitochondrion. 2014;17:76–100. Available from: http://dx.doi.org/10.1016/j.mito.2014.05.007.
28. Brière JJ, Favier J, Gimenez-Roqueplo AP, Rustin P. Tricarboxylic acid cycle dysfunction as a cause of human diseases and tumor formation. Am J Physiol Cell Physiol. 2006;291(6):C1114–C1120. Available from: http://dx.doi.org/10.1152/ajpcell.00216.2006.
29. Jeger V, Djafarzadeh S, Jakob SM, Takala J. Mitochondrial function in sepsis. Eur J Clin Invest. 2013;43(5):532–542. Available from: http://dx.doi.org/10.1111/eci.12069.
30. Chretien D, Rustin P. Mitochondrial oxidative phosphorylation: pitfalls and tips in measuring and interpreting enzyme activities. J Inherit Metab Dis. 2003;26(2-3):189–198.
31. Barrientos A, Fontanesi F, Díaz F. Evaluation of the mitochondrial respiratory chain and oxidative phosphorylation system using polarography and spectrophotometric enzyme assays. Curr Protoc Hum Genet. 2009;Chapter 19:Unit19.3. Available from: http://dx.doi.org/10.1002/0471142905.hg1903s63.
32. Brandt S, Regueira T, Bracht H, Porta F, Djafarzadeh S, Takala J, et al. Effect of fluid resuscitation on mortality and organ function in experimental sepsis models. Crit Care. 2009;13(6):R186. Available from: http://dx.doi.org/10.1186/cc8179.
33. Kozlov AV, Duvigneau JC, Miller I, Nürnberger S, Gesslbauer B, Kungl A, et al. Endotoxin causes functional endoplasmic reticulum failure, possibly mediated by mitochondria. Biochim Biophys Acta. 2009;1792(6):521–530. Available from: http://dx.doi.org/10.1016/j.bbadis.2009.03.004.
34. Kozlov AV, van Griensven M, Haindl S, Kehrer I, Duvigneau JC, Hartl RT, et al. Peritoneal inflammation in pigs is associated with early mitochondrial dysfunction in liver and kidney. Inflammation. 2010;33(5):295–305. Available from: http://dx.doi.org/10.1007/s10753-010-9185-4.
35. Corrêa TD, Vuda M, Blaser AR, Takala J, Djafarzadeh S, Dünser MW, et al. Effect of treatment delay on disease severity and need for resuscitation in porcine fecal peritonitis. Crit Care Med. 2012;40(10):2841–2849. Available from: http://dx.doi.org/10.1097/CCM.0b013e31825b916b.
36. Corrêa TD, Vuda M, Takala J, Djafarzadeh S, Silva E, Jakob SM. Increasing mean arterial blood pressure in sepsis: effects on fluid balance, vasopressor load and renal function. Crit Care. 2013;17(1):R21. Available from: http://dx.doi.org/10.1186/cc12495.
37. Corrêa TD, Jeger V, Pereira AJ, Takala J, Djafarzadeh S, Jakob SM. Angiotensin II in septic shock: effects on tissue perfusion, organ function, and mitochondrial respiration in a porcine model of fecal peritonitis. Crit Care Med. 2014;42(8):e550–e559. Available from: http://dx.doi.org/10.1097/CCM.0000000000000397.
38. Aguirre E, López-Bernardo E, Cadenas S. Functional evidence for nitric oxide production by skeletal-muscle mitochondria from lipopolysaccharide-treated mice. Mitochondrion. 2012;12(1):126–131. Available from: http://dx.doi.org/10.1016/j.mito.2011.05.010.
39. Chance B, Willaims GR. Respiratory enzymes in oxidative phosphorylation: III. The steady state. J Biol Chem. 1955;217:409–427.
40. DePalma RG, Harano Y, Robinson AV, Holden WD. Structure and function of hepatic mitochondria in hemorrhage and endotoxemia. Surg Forum. 1970;21:3–6.
41. Mela L, Bacalzo L Jr, White R 4th, Miller LD. Shock induced alterations of mitochondrial energy-linked functions. Surg Forum. 1970;21:6–8.
42. Schumer W, Das Gupta TK, Moss GS, Nyhus LM. Effect of endotoxemia on liver cell mitochondria in man. Ann Surg. 1970;171(6):875–882.
43. Duran-Bedolla J, Montes de Oca-Sandoval MA, Saldaña-Navor V, Villalobos-Silva JA, Rodriguez MC, Rivas-Arancibia S. Sepsis, mitochondrial failure and multiple organ dysfunction. Clin Invest Med. 2014;37(2):E58–E69.
44. Singer M. The role of mitochondrial dysfunction in sepsis-induced multi-organ failure. Virulence. 2014;5(1):66–72. Available from: http://dx.doi.org/10.4161/viru.26907.
45. Levy RJ. Mitochondrial dysfunction, bioenergetic impairment, and metabolic down-regulation in sepsis. Shock. 2007;28(1):24–28. Available from: http://dx.doi.org/10.1097/01.shk.0000235089.30550.2d.
46. Hotchkiss RS, Karl IE. The pathophysiology and treatment of sepsis. N Engl J Med. 2003;348(2):138–150. Available from: http://dx.doi.org/10.1056/NEJMra021333.
47. Fredriksson K, Hammarqvist F, Strigård K, Hultenby K, Ljungqvist O, Wernerman J, et al. Derangements in mitochondrial metabolism in intercostal and leg muscle of critically ill patients with sepsis-induced multiple organ failure. Am J Physiol Endocrinol Metab. 2006;291(5):E1044–E1050. Available from: http://dx.doi.org/10.1152/ajpendo.00218.2006.
48. Belikova I, Lukaszewicz AC, Faivre V, Damoisel C, Singer M, Payen D. Oxygen consumption of human peripheral blood mononuclear cells in severe human sepsis. Crit Care Med. 2007;35(12):2702–2708.
49. Fredriksson K, Fläring U, Guillet C, Wernerman J, Rooyackers O. Muscle mitochondrial activity increases rapidly after an endotoxin challenge in human volunteers. Acta Anaesthesiol Scand. 2009;53(3):299–304. Available from: http://dx.doi.org/10.1111/j.1399-6576.2008.01851.x.
50. Sjövall F, Morota S, Hansson MJ, Friberg H, Gnaiger E, Elmér E. Temporal increase of platelet mitochondrial respiration is negatively associated with clinical outcome in patients with sepsis. Crit Care. 2010;14(6):R214. Available from: http://dx.doi.org/10.1186/cc9337.
51. Japiassú AM, Santiago APSA, d'Avila JdCP, Garcia-Souza LF, Galina A, Castro Faria-Neto HC, et al. Bioenergetic

failure of human peripheral blood monocytes in patients with septic shock is mediated by reduced F1Fo adenosine-5′-triphosphate synthase activity. Crit Care Med. 2011;39(5):1056–1063. Available from: http://dx.doi.org/10.1097/CCM.0b013e31820eda5c.
52. Garrabou G, Morén C, López S, Tobías E, Cardellach F, Miró O, et al. The effects of sepsis on mitochondria. J Infect Dis. 2012;205(3):392–400. Available from: http://dx.doi.org/10.1093/infdis/jir764.
53. Sjövall F, Morota S, Persson J, Hansson MJ, Elmér E. Patients with sepsis exhibit increased mitochondrial respiratory capacity in peripheral blood immune cells. Crit Care. 2013;17(4):R152. Available from: http://dx.doi.org/10.1186/cc12831.
54. Quoilin C, Mouithys-Mickalad A, Lécart S, Fontaine-Aupart MP, Hoebeke M. Evidence of oxidative stress and mitochondrial respiratory chain dysfunction in an in vitro model of sepsis-induced kidney injury. Biochim Biophys Acta. 2014;1837(10):1790–1800. Available from: http://dx.doi.org/10.1016/j.bbabio.2014.07.005.
55. Protti A, Fortunato F, Artoni A, Lecchi A, Motta G, Mistraletti G, et al. Platelet mitochondrial dysfunction in critically ill patients: comparison between sepsis and cardiogenic shock. Crit Care. 2015;19:39. Available from: http://dx.doi.org/10.1186/s13054-015-0762-7.
56. Peruchi BB, Petronilho F, Rojas HA, Constantino L, Mina F, Vuolo F, et al. Skeletal muscle electron transport chain dysfunction after sepsis in rats. J Surg Res. 2011;167(2):e333–e338. Available from: http://dx.doi.org/10.1016/j.jss.2010.11.893.
57. Porta F, Takala J, Weikert C, Bracht H, Kolarova A, Lauterburg BH, et al. Effects of prolonged endotoxemia on liver, skeletal muscle and kidney mitochondrial function. Crit Care. 2006;10(4):R118. Available from: http://dx.doi.org/10.1186/cc5013.
58. Comim CM, Rezin GT, Scaini G, Di-Pietro PB, Cardoso MR, Petronilho FC, et al. Mitochondrial respiratory chain and creatine kinase activities in rat brain after sepsis induced by cecal ligation and perforation. Mitochondrion. 2008;8(4):313–318. Available from: http://dx.doi.org/10.1016/j.mito.2008.07.002.
59. Fry DE, Kaelin CR, Giammara BL, Rink RD. Alterations of oxygen metabolism in experimental bacteremia. Adv Shock Res. 1981;6:45–54.
60. Tavakoli H, Mela L. Alterations of mitochondrial metabolism and protein concentrations in subacute septicemia. Infect Immun. 1982;38(2):536–541.
61. Garrison RN, Ratcliffe DJ, Fry DE. The effects of peritonitis on murine renal mitochondria. Adv Shock Res. 1982;7:71–76.
62. Geller ER, Jankauskas S, Kirkpatrick J. Mitochondrial death in sepsis: a failed concept. J Surg Res. 1986;40(5):514–517.
63. Dawson KL, Geller ER, Kirkpatrick JR. Enhancement of mitochondrial function in sepsis. Arch Surg. 1988;123(2):241–244.
64. Kopprasch S, Hörkner U, Orlik H, Kemmer C, Scheuch DW. Energy state, glycolytic intermediates and mitochondrial function in the liver during reversible and irreversible endotoxin shock. Biomed Biochim Acta. 1989;48(9):653–659.
65. Takeyama N, Itoh Y, Kitazawa Y, Tanaka T. Altered hepatic mitochondrial fatty acid oxidation and ketogenesis in endotoxic rats. Am J Physiol. 1990;259(4 Pt 1):E498–E505.
66. Llesuy S, Evelson P, González-Flecha B, Peralta J, Carreras MC, Poderoso JJ, et al. Oxidative stress in muscle and liver of rats with septic syndrome. Free Radic Biol Med. 1994;16(4):445–451.
67. Taylor DE, Ghio AJ, Piantadosi CA. Reactive oxygen species produced by liver mitochondria of rats in sepsis. Arch Biochem Biophys. 1995;316(1):70–76. Available from: http://dx.doi.org/10.1006/abbi.1995.1011.
68. Malaisse WJ, Nadi AB, Ladriere L, Zhang TM. Protective effects of succinic acid dimethyl ester infusion in experimental endotoxemia. Nutrition. 1997;13(4):330–341.
69. Kantrow SP, Taylor DE, Carraway MS, Piantadosi CA. Oxidative metabolism in rat hepatocytes and mitochondria during sepsis. Arch Biochem Biophys. 1997;345(2):278–288. Available from: http://dx.doi.org/10.1006/abbi.1997.0264.
70. Markley MA, Pierro A, Eaton S. Hepatocyte mitochondrial metabolism is inhibited in neonatal rat endotoxaemia: effects of glutamine. Clin Sci (Lond). 2002;102(3):337–344.
71. Fukumoto K, Pierro A, Spitz L, Eaton S. Neonatal endotoxemia affects heart but not kidney bioenergetics. J Pediatr Surg. 2003;38(5):690–693. Available from: http://dx.doi.org/10.1016/jpsu.2003.50184.
72. Suliman HB, Welty-Wolf KE, Carraway M, Tatro L, Piantadosi CA. Lipopolysaccharide induces oxidative cardiac mitochondrial damage and biogenesis. Cardiovasc Res. 2004;64(2):279–288. Available from: http://dx.doi.org/10.1016/j.cardiores.2004.07.005.
73. Nin N, Cassina A, Boggia J, Alfonso E, Botti H, Peluffo G, et al. Septic diaphragmatic dysfunction is prevented by Mn(III)porphyrin therapy and inducible nitric oxide synthase inhibition. Intensive Care Med. 2004;30(12):2271–2278. Available from: http://dx.doi.org/10.1007/s00134-004-2427-x.
74. Kozlov AV, Staniek K, Haindl S, Piskernik C, Ohlinger W, Gille L, et al. Different effects of endotoxic shock on the respiratory function of liver and heart mitochondria in rats. Am J Physiol Gastrointest Liver Physiol. 2006;290(3):G543–G549. Available from: http://dx.doi.org/10.1152/ajpgi.00331.2005.
75. Larche J, Lancel S, Hassoun SM, Favory R, Decoster B, Marchetti P, et al. Inhibition of mitochondrial permeability transition prevents sepsis-induced myocardial dysfunction and mortality. J Am Coll Cardiol. 2006;48(2):377–385. Available from: http://dx.doi.org/10.1016/j.jacc.2006.02.069.
76. Mason KE, Stofan DA. Endotoxin challenge reduces aconitase activity in myocardial tissue. Arch Biochem Biophys. 2008;469(2):151–156. Available from: http://dx.doi.org/10.1016/j.abb.2007.10.018.
77. Protti A, Carré J, Frost MT, Taylor V, Stidwill R, Rudiger A, et al. Succinate recovers mitochondrial oxygen consumption in septic rat skeletal muscle. Crit Care Med. 2007;35(9):2150–2155.
78. Kozlov AV, Gille L, Miller I, Piskernik C, Haindl S, Staniek K, et al. Opposite effects of endotoxin on mitochondrial and endoplasmic reticulum functions. Biochem Biophys Res Commun. 2007;352(1):91–96. Available from: http://dx.doi.org/10.1016/j.bbrc.2006.10.180.
79. Duvigneau JC, Piskernik C, Haindl S, Kloesch B, Hartl RT, Hüttemann M, et al. A novel endotoxin-induced pathway: upregulation of heme oxygenase 1, accumulation of free iron, and free iron-mediated mitochondrial dysfunction. Lab Invest. 2008;88(1):70–77. Available from: http://dx.doi.org/10.1038/labinvest.

3700691.
80. Hassoun SM, Marechal X, Montaigne D, Bouazza Y, Decoster B, Lancel S, et al. Prevention of endotoxin-induced sarcoplasmic reticulum calcium leak improves mitochondrial and myocardial dysfunction. Crit Care Med. 2008;36(9):2590–2596. Available from: http://dx.doi.org/10.1097/CCM.0b013e3181844276.
81. Vanasco V, Cimolai MC, Evelson P, Alvarez S. The oxidative stress and the mitochondrial dysfunction caused by endotoxemia are prevented by alpha-lipoic acid. Free Radic Res. 2008;42(9):815–823. Available from: http://dx.doi.org/10.1080/10715760802438709.
82. Reynolds CM, Suliman HB, Hollingsworth JW, Welty-Wolf KE, Carraway MS, Piantadosi CA. Nitric oxide synthase-2 induction optimizes cardiac mitochondrial biogenesis after endotoxemia. Free Radic Biol Med. 2009;46(5):564–572. Available from: http://dx.doi.org/10.1016/j.freeradbiomed.2008.11.007.
83. Vanasco V, Magnani ND, Cimolai MC, Valdez LB, Evelson P, Boveris A, et al. Endotoxemia impairs heart mitochondrial function by decreasing electron transfer, ATP synthesis and ATP content without affecting membrane potential. J Bioenerg Biomembr. 2012;44(2):243–252. Available from: http://dx.doi.org/10.1007/s10863-012-9426-3.
84. Li CM, Chen JH, Zhang P, He Q, Yuan J, Chen RJ, et al. Continuous veno-venous haemofiltration attenuates myocardial mitochondrial respiratory chain complexes activity in porcine septic shock. Anaesth Intensive Care. 2007;35(6):911–919.
85. Regueira T, Bänziger B, Djafarzadeh S, Brandt S, Gorrasi J, Takala J, et al. Norepinephrine to increase blood pressure in endotoxaemic pigs is associated with improved hepatic mitochondrial respiration. Crit Care. 2008;12(4):R88. Available from: http://dx.doi.org/10.1186/cc6956.
86. Vuda M, Brander L, Schröder R, Jakob SM, Takala J, Djafarzadeh S. Effects of catecholamines on hepatic and skeletal muscle mitochondrial respiration after prolonged exposure to faecal peritonitis in pigs. Innate Immun. 2012;18(2):217–230. Available from: http://dx.doi.org/10.1177/1753425911398279.
87. Marshall JC, Deitch E, Moldawer LL, Opal S, Redl H, van der Poll T. Preclinical models of shock and sepsis: what can they tell us? Shock. 2005;24 Suppl 1:1–6.
88. Dyson A, Singer M. Animal models of sepsis: why does preclinical efficacy fail to translate to the clinical setting? Crit Care Med. 2009;37(1 Suppl):S30–S37. Available from: http://dx.doi.org/10.1097/CCM.0b013e3181922bd3.
89. Marshall JC. From the bedside back to the bench: the role of preclinical studies in understanding clinical therapies. Crit Care Med. 2010;38(1):329–330. Available from: http://dx.doi.org/10.1097/CCM.0b013e3181b9d4b4.
90. Mouncey PR, Osborn TM, Power GS, Harrison DA, Sadique MZ, Grieve RD, et al. Trial of early, goal-directed resuscitation for septic shock. N Engl J Med. 2015;372(14):1301–1311. Available from: http://dx.doi.org/10.1056/NEJMoa1500896.
91. Russell JA, Singer J, Bernard GR, Wheeler A, Fulkerson W, Hudson L, et al. Changing pattern of organ dysfunction in early human sepsis is related to mortality. Crit Care Med. 2000;28(10):3405–3411.
92. Copeland S, Warren HS, Lowry SF, Calvano SE, Remick D, Inflammation and the Host Response to Injury Investigators. Acute inflammatory response to endotoxin in mice and humans. Clin Diagn Lab Immunol. 2005;12(1):60–67.
93. Goldfarb RD, Dellinger RP, Parrillo JE. Porcine models of severe sepsis: emphasis on porcine peritonitis. Shock. 2005;24 Suppl 1:75–81.
94. Buras JA, Holzmann B, Sitkovsky M. Animal models of sepsis: setting the stage. Nat Rev Drug Discov. 2005;4(10):854–865. Available from: http://dx.doi.org/10.1038/nrd1854.
95. Singleton KD, Wischmeyer PE. Distance of cecum ligated influences mortality, tumor necrosis factor-alpha and interleukin-6 expression following cecal ligation and puncture in the rat. Eur Surg Res. 2003;(6):486–491. Available from: http://dx.doi.org/10.1159/000073387.
96. Cuesta JM, Singer M. The stress response and critical illness: a review. Crit Care Med. 2012;40(12):3283–3289. Available from: http://dx.doi.org/10.1097/CCM.0b013e31826567eb.
97. Revelly JP, Liaudet L, Frascarolo P, Joseph JM, Martinet O, Markert M. Effects of norepinephrine on the distribution of intestinal blood flow and tissue adenosine triphosphate content in endotoxic shock. Crit Care Med. 2000;28(7):2500–2506.
98. Le Gall JR, Klar J, Lemeshow S, Saulnier F, Alberti C, Artigas A, et al. The Logistic Organ Dysfunction system. A new way to assess organ dysfunction in the intensive care unit. ICU Scoring Group. JAMA. 1996;276(10):802–810.
99. Moreno R, Vincent JL, Matos R, Mendonça A, Cantraine F, Thijs L, et al. The use of maximum SOFA score to quantify organ dysfunction/failure in intensive care. Results of a prospective, multicentre study. Working Group on Sepsis related Problems of the ESICM. Intensive Care Med. 1999;25(7):686–696.
100. Fredriksson K, Rooyackers O. Mitochondrial function in sepsis: respiratory versus leg muscle. Crit Care Med. 2007;35(9 Suppl):S449–S453. Available from: http://dx.doi.org/10.1097/01.CCM.0000278048.00896.4B.
101. Sjövall F, Morota S, Frostner EÅ, Hansson MJ, Elmér E. Cytokine and nitric oxide levels in patients with sepsis–temporal evolvement and relation to platelet mitochondrial respiratory function. PLoS One. 2014;9(7):e103756. Available from: http://dx.doi.org/10.1371/journal.pone.0103756.
102. Merz TM, Hefti JP, Hefti U, Huber A, Jakob SM, Takala J, et al. Changes in mitochondrial enzymatic activities of monocytes during prolonged hypobaric hypoxia and influence of antioxidants: A randomized controlled study. Redox Rep. 2015;Available from: http://dx.doi.org/10.1179/1351000215Y.0000000007.
103. Djafarzadeh S, Vuda M, Takala J, Ochs M, Jakob SM. Toll-like receptor-3-induced mitochondrial dysfunction in cultured human hepatocytes. Mitochondrion. 2011;11(1):83–88. Available from: http://dx.doi.org/10.1016/j.mito.2010.07.010.
104. Djafarzadeh S, Vuda M, Takala J, Jakob SM. Effect of remifentanil on mitochondrial oxygen consumption of cultured human hepatocytes. PLoS One. 2012;7(9):e45195. Available from: http://dx.doi.org/10.1371/journal.pone.0045195.
105. Zheng G, Lyu J, Huang J, Xiang D, Xie M, Zeng Q. Experimental treatments for mitochondrial dysfunction in sepsis: A narrative review. J Res Med Sci. 2015;20(2):185–195.