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Mitochondrial Function in Sepsis

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Abstract

Mitochondria are an essential part of the cellular infrastructure, being the primary site for high energy adenosine triphosphate (ATP) production through oxidative phosphorylation. Clearly, in severe systemic inflammatory states, like sepsis, cellular metabolism is usually altered and end organ dysfunction not only common but predictive of long term morbidity and mortality. Clearly, interest is mitochondrial function both as a target for intracellular injury and response to extrinsic stress have been a major focus of basic science and clinical research into the pathophysiology of acute illness. However, mitochondria have multiple metabolic and signaling functions that may be central in both the expression of sepsis and its ultimate outcome. In this review, the authors address five primary questions centered on the role of mitochondria in sepsis. This review should be used as both a summary source in placing mitochondrial physiology within the context of acute

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Authors' contributions

JK, HG, AG and PM all contributed to the pre-conference and post-conference e-mail discussions on this review and reviewed and edited the final version of this manuscript. In addition, all contributed to the group breakout sessions during the ADQI XIV conference. NA, CD and MP created the first drafted of this manuscript, and NA, MP, CD, BZ and PS develop subsequent drafts and approved the final manuscript. All authors (appendix 1) contributed to group discussion and consensus.

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illness and as a focal point for addressing new research into diagnostic and treatment opportunities these insights provide.

Introduction

The authors were tacked with developing five specific questions regarding mitochondrial function in sepsis within the context of the Acute Dialysis Quality Initiative 14 (ADQI XIV) meeting held in Bogotá, Colombia in late 2014. The authors presented these questions to the res to the panel of participants and from group discussions focused these questions to address specific aspects of mitochondrial function. Then off-line reviewed the literature and complied the answers to these questions which were vetted by all authors prior to publication. What follows is the synthesis of this effort arranged under the heading of five key questions.

Methods

Complete methods are available in the companion article to this series. (ref) Briefly, we assembled a group of international experts with distinct clinical and scientific backgrounds; this group included physicians, specialists in critical care, anesthesiology, nephrology, surgery and emergency medicine, and basic scientists with expertise in biology and physiology, who were recruited based on their expertise in sepsis and organ dysfunction. The group consisted of 23 international experts from 5 continents. A set of questions was generated through mutual agreement and we sought evidence to answer each question by searching the Cochrane Controlled Trials Register, the Cochrane Library, MEDLINE, and EMBASE from 1966 to present. Search terms for question regarding epithelial dysfunction are provided in Appendix 2. Finally we reviewed the evidence with the group and used the Delphi method to achieve consensus.

Results

Based on literature review and consensus among the workgroup members, the following key questions were considered:

1. Are mitochondria initiators, amplifiers, victims or innocent bystanders in the organ dysfunction in sepsis?
2. To the extent that mitochondria are disrupted in sepsis, is the molecular mechanism related to bioenergetic function, oxygen-dependent oxidative phosphorylation, cell death regulatory functions, biosynthetic, regulatory or stress-related signaling (e.g. reactive oxygen species [ROS]) functions? What is the relationship between endothelial altered function and organ function?
3. To what extent does a disruption in mitochondrial dynamics and homeostasis contribute to cellular and organ system dysfunction in sepsis?
4. Is the trade-off between “cell-adaptive” and “organ-maladaptive” responses a driver of organ dysfunction and long-term recovery in sepsis? Do organ-specific differences in this dichotomy determine outcome?

5. *What are the Mitochondria-Based Therapeutic Targets and Opportunities for Intervention?*

Question 1. Are mitochondria the initiators, amplifiers, victims, or innocent bystanders in the organ dysfunction in sepsis?

Although a number of studies have assessed mitochondrial morphology and function in experimental models of sepsis and in critically ill patients, the relationship between mitochondrial function and organ system dysfunction is not fully elucidated. Figure 1 summarizes the known roles mitochondria play during sepsis. Furthermore, Table 1 groups the cited studies into groups based on their reporting on these various aspects of mitochondrial health and function. Stresses associated with the systemic response to sepsis, including oxidative and nitrosative stress, can contribute to mitochondrial dysfunction. Conversely, mitochondria can function as a source of oxidant stress. Studies have described evidence of mitochondrial damage in critically ill patients and in experimental models of sepsis, but it is not clear whether this association represents organelle damage as a consequence of inflammation arising from the response to infection, or whether the changes in mitochondria are etiologic in the development of cellular and organ dysfunction. Evidence suggests that signals from healthy mitochondria can activate stress responses in cells, they can activate transcription factors including (hypoxia-inducible factor-1α) HIF-1α, NFκB and p53, and they can initiate a suppression of metabolic activity mediated by activation of AMP-dependent protein kinase (AMPK). Pathology samples from patients with critical illness frequently reveal normal cellular morphology despite organ system dysfunction. This raises the question of whether signals from mitochondria could be responsible for suppressing cellular function, possibly as an adaptive mechanism to preserve cell survival. So it is possible that in some cases mitochondria are damaged during the response to sepsis, that in other cases the generation of oxidants by mitochondria could induce or amplify tissue dysfunction, and yet in other cases the changes in mitochondria may represent a downstream marker of tissue damage. Further studies are needed to determine the significance of each of these roles in the septic patient.

Question 2. To the extent that mitochondria are disrupted in sepsis, is the molecular mechanism related to bioenergetic function, oxygen-dependent oxidative phosphorylation, cell death regulatory functions, biosynthetic, regulatory or stress-related signaling (e.g. reactive oxygen species [ROS]) functions?

Bioenergetic function and Oxygen-dependent oxidative phosphorylation—A functional alteration in O_2 consumption (VO_2) related metabolism may occur in sepsis (i.e. dysoxia). In resuscitated septic patients who have an increase in global O_2 delivery and adequate tissue perfusion, “non-vital organ” (e.g. skeletal muscle) O_2 tension remains elevated, suggesting a decrease in local VO_2 even though global VO_2 may increase (1,2). The mechanism underlying this reduction in regional O_2 utilization may be a consequence of changes in the mitochondrial respiratory chain complexes.

Respiratory protein subunits and transcripts for complexes I and IV were down-regulated in critically ill patients, with a more prolonged recovery course and greater reduction in ATP levels in eventual non-survivors (3, 4). Acute endotoxemia decreased cardiac muscle

mitochondrial O_2 consumption and complex I activity, a change that was associated with decreased ATP synthesis and ATP content (5), whereas cecal ligation and puncture (CLP) models show decreased complex IV activity, a change associated with decreased contractility. During sepsis, there is a significant increase in nitric oxide (NO), mediated in part by increased inducible nitric oxide synthase (iNOS) activity. The reaction of NO with superoxide generates peroxynitrite, a 'reactive nitrogen species' (RNS) (6). Mitochondrial complex I and complex IV are susceptible to persistent inhibition from nitrosylation and perhaps nitration *ex vivo* (7), and inhibition of NO ameliorates the impaired mitochondrial respiration in endothelial cells exposed to serum from septic patients (8).

In addition to mitochondrial complex expression and activity, cytochrome c oxidase (CCO) was inhibited in a mouse model of sepsis induced by CLP. CCO inhibition was initially competitive, but after 48hrs became non-competitive (9). Exogenous cytochrome C administered at 24hr after induction of sepsis restored cardiac mitochondria activity, increased cytochrome oxidase kinetic activity and improved cardiac function (10). Exogenous cytochrome c 24h post-CLP repleted mitochondrial substrate levels for up to 72h, restored myocardial cytochrome oxidase activity, and improved both contractility and survival (11). Caffeine administration 24 or 48 hours following CLP also improved CCO activity, restored cardiac contractility and improved survival (12)

There are discrepancies in the literature with regards to changes in complex activity in different muscle groups and organs in sepsis (11,13) and endotoxemia (14–16). This discrepancy has been attributed to differences in the organs studied (16), the time point of measurement, species involved and severity of illness. Importantly, changes in endotoxemia differ from those observed following CLP. Indeed, much of the controversy surrounding the effects of sepsis on mitochondria may reflect the inherent differences between LPS administration, an acute inflammatory state, and more clinically-relevant models, such as CLP (17–19).

Biosynthetic functions—Hypoxemia, which alters mitochondrial function, may be associated with sepsis. The responses to hypoxia include the up-regulation of hypoxia-inducible factors, vascular endothelium growth factor (VEGF), and glycolytic enzymes to maintain ATP production.

During normoxic conditions, constitutively expressed prolyl hydrolyxase hydroxylates HIF-1α, which leads to proteosomal degradation of HIF-1α (20). Hypoxia-induced mitochondrial ROS are responsible for HIF-1α stabilization. Cells with functional electron transport chain deficiencies or cells treated with electron transport chain (ETC) inhibitors cannot produce ROS and failed to stabilize HIF-1α (21, 22). Cells lacking complex III subunit cytochrome b were able to produce ROS but could not carry out oxidative phosphorylation, implicating the former in HIF-1α stabilization (23). Hypoxia and the mitochondrial ROS increases also increased VEGF transcription (24) and the contractile response of pulmonary arterial smooth muscle cells (25).

Immune functions—Mitochondrial ROS (mtROS) are potent initiators of the innate immune system. Inhibition of ETC complex I or III provoked a dose-dependent increase in

mitochondrial ROS production and NLRP3 activation in a human THP1 macrophage cell line (25). NO down - regulated mtROS-induced NLRP3 inflammasome activity and was protective in endotoxemia (27).

Macrophage clearance of bacteria involves phagocysis and ROS-mediated degradation of the pathogen. ROS in phagosomes, in turn, are produced by NADPH oxidase (NOX) and by an increase in uncoupling protein 2 (UCP-2) expression (28). Mitochondrial ROS also modulate Toll-like receptor (TLR) pathways. Depletion of mitochondrial ROS by catalase overexpression impaired clearance of intracellular organisms (Salmonella typimurium) (29). mtROS also function downstream in TLR-activated signaling pathways such as TNF mediated activation of NF-κB. (30).

Oxidative stress—Superoxide is the primary oxidant produced by mitochondria respiratory complexes I, II and III. In health, levels of superoxide are contained by manganese superoxide dismutase (MnSOD), which is confined to mitochondria. NO reacts with superoxide to generate the peroxynitrite and other reactive nitrogen species (RNS), (6) which are potent oxidizing agents and have been implicated in protein nitration, DNA damage, and mitochondrial dysfunction in isolated mitochondria and in cells treated with serum from septic patients (8, 31). Diaphragmatic and cardiac mitochondrial O_2^- and H_2O_2 production were increased up to 3-fold during endotoxemia and Mn-SOD activity showed a 2-fold increase in LPS-treated animals (32).

Mitochondria exposed to non-mitochondrial ROS may become a source of ROS themselves. Renal tubular cells increase their expression of inducible nitric oxide synthase (iNOS) and NADPH oxidase 4 (NOX-4) in response to LPS. This process can culminate in the cytosolic overexpression of NO and superoxide anion, the primary RNS and ROS, respectively (33), a positive feedback loop that may result in dysregulation of mitochondrial function.

Treatment with antioxidants ameliorates organ injury in experimental models of sepsis. Therapeutic agents tested include N-acetyl cysteine (NAC) (34) in hepatic oxidative stress and MITO-Tempo in renal injury (35). Limiting the production of peroxinitrite abundance (with tetrakis-(1-methyl-4-pyridyl) porphyrin pentachloride, MnTMPyP, a peroxinitrite which prevents decomposition catalyst or with NO with aminoguanidine (AG), a NOS-2 inhibitor) (AG) prevented renal injury (36).

Cell death—ATP depletion, loss of mitochondrial membrane potential, release of cytochrome C and oxidative stress can lead to apoptosis by altering the mitochondrial permeability transition (MPT) (37). Upon depletion of ATP, Ca^{+2} homeostasis cannot be maintained, and the MPT is induced, followed by cell death (38). Drugs such as cyclosporine A or carnitine inhibit MPT opening (39). Lymphocyte apoptosis is associated with immuneparesis and increased mortality among septic patients. The mechanism behind increased lymphocyte apoptosis is multifactorial, though cell death via mitochondrial-mediated apoptosis has been implicated. Apoptotic lymphocytes derived from septic patients contained active caspase 8 and caspase 9, consistent with death occurring by both mitochondrial-mediated and receptor-mediated pathways (40).

Temporal changes in mitochondrial function—The relationship between sepsis-induced changes in mitochondrial function and organ dysfunction/recovery is time-dependent and has important therapeutic implications. Alterations to respiratory protein subunits and transcripts occur within the first 24hrs of ICU admission and correlate with eventual outcome (4). Skeletal muscle antioxidant reserves are reduced within 48hrs of ICU admission, and are associated with mortality (3). It remains unclear when these changes begin to normalize. The production of mitochondrial ROS probably occurs early in sepsis, as it has a key role in innate immunity. It is unclear when generation of mitochondrial ROS ceases to be adaptive and becomes damaging. Lymphocyte apoptosis, while multifactorial, is a late phenomenon (41).

Monitoring mitochondrial health—At present, monitoring of mitochondrial health is limited to experimental work. Promising real-time *in vivo* techniques include NADH fluorometry, magnetic resonance spectroscopy (MRS), and near infrared spectroscopy to measure COX redox state (42). Mitochondrial O_2 tension can be estimated by measurement of the phosphorescence decay time of sensors containing protoporphyrin IX (43). These techniques have shown promise in animal models of different shock states and warrant further investigations in sepsis.

Mitochondria in circulating cells may provide insight into sepsis-associated temporal changes and how these changes relate to recovery. Respiratory chain biochemistry in platelets is variably inhibited, with no convincing association with either severity of illness or mortality (16). Reduced complex activity in platelets is not a consistent finding (44), and may be time-dependent. Decreases in mitochondrial bioenergetic reserve and increased uncoupling have been observed in peripheral blood mononuclear cells obtained from septic children. A higher mononuclear mitochondrial membrane potential on days 1–2 was associated with reduced organ injury by day 7 (45). The application of mitochondrial bioenergetic assessment in circulating cells requires further validation, and holds promise for monitoring illness progression and therapeutic interventions.

Question 3. To what extent does a disruption in mitochondrial dynamics and homeostasis contribute to cellular and organ system dysfunction in sepsis?

Multiple stressors have been shown to influence all known mitochondrial functions, including oxidative phosphorylation, as well as biosynthetic, regulatory and signaling functions. In order to carry out this complex array of activities, it is critical to maintain a population of viable mitochondria, typically defined as maintenance of normal global mitochondrial membrane potential (46).

Several responses allow the mitochondrial network to adapt to stress and a loss of membrane potential. These include mitochondrial fission and fusion, mitophagy, and mitochondrial biogenesis (47–50).

Mitochondrial fission and fusion responses are dynamic morphological changes that occur in the mitochondrial network (51). Fission is recognized as a coordinated process whereby the mitochondrial network sequesters damaged elements of the mitochondria to a focal region, and this area is then ‘pinched off’ to maintain the overall health of the network.

Mitochondrial fusion can promote complementation where a mildly damaged mitochondrion is assimilated into the healthier network, resulting in an overall maintenance of membrane potential. These processes have been studied minimally in the setting of sepsis. In a preclinical animal model of sepsis (CLP) there was an abnormal balance of fission and fusion responses thought to contribute to cellular injury and apoptosis. The *in vivo* pretreatment with mdivi-1 (Drp1 inhibitor) significantly attenuated mitochondrial dysfunction and apoptosis in CLP (52).

Autophagy is a well-conserved, intracellular, catabolic process where proteins and organelles are isolated within a double membrane vesicle (autophagosome), targeted to the lysosome for degradation (53–55). Specifically, mitochondrial autophagy (or mitophagy) can consume damaged and dysfunctional mitochondria. Individual depolarized mitochondria, thought to be separated from the network through fission, are targeted for autophagosome formation and lysosomal degradation (56). This process serves to eliminate the depolarized and damaged mitochondria that may otherwise produce oxidant stress within the cell, as well as release mitochondrial contents into the cytosol or extracellular space, which can promote inflammatory and immune responses.

A number of clinical studies have illustrated increased autophagocytic signaling in multiple organs and tissues in sepsis (57). Additionally, autophagy has been demonstrated in numerous animal models of sepsis or endotoxemia. Inhibition of autophagy in a CLP model resulted in increased apoptosis and organ injury (58). Moreover, in a burn wound model in rabbits, insufficient autophagy was more pronounced in non-surviving than in surviving animals, a finding that correlated with impaired mitochondrial function and more severe organ dysfunction (59). In contrast, key substrates and controllers associated with mitochondrial fusion/fission or biogenesis were not significantly different regarding survival status. Multiple preclinical studies have utilized non-specific pharmacologic approaches that enhance autophagy and have demonstrated amelioration of organ injury (60–62).

Mitophagy/autophagy blockade results in the accumulation of depolarized mitochondria, and increased ROS generation with an associated activation of the NLRP3 inflammasome (62). Similarly, *in vitro* studies of macrophages stimulated with LPS and ATP led to ultrastructural damage of the mitochondria and increased cytosolic levels of mitochondrial DNA (mtDNA). Inhibition of autophagic signaling was manipulated and impaired, this process was exacerbated with augmented release of IL-1β and IL-18 (64).

Mitochondrial biogenesis refers to the process of the generation of new mitochondria, which is a coordinated effort of transcription and translation, involving both mitochondrial and nuclear genomes. The generation of new mitochondria is potentially critical to meet cellular metabolic energy demands and to fulfill other roles including calcium homeostasis, maintenance of cellular redox state, and cell signaling. In muscle biopsies of septic patients, the transcriptional co-activator of mitochondrial biogenesis, PGC-1α, was significantly elevated in survivors. Survivors also had higher muscle ATP levels and a decreased phosphocreatine/ATP ratio (65). In patients with acute illness, skeletal muscle biopsies harvested from intensive care unit patients with organ dysfunction demonstrated a twofold decrease in mitochondrial content (66). In preclinical models of sepsis, the onset of

mitochondrial biogenesis has been shown to correspond to the restoration of normal mitochondrial oxidative respiration (67). The course of sepsis and recovery is characterized by an increment in markers of mitochondrial biogenesis and mitochondrial number and density (68).

Biogenesis and autophagy have been linked in several preclinical models of sepsis. One study suggested that mitochondrial biogenesis was dependent on an autophagy and mitochondrial DNA/Toll-like receptor 9 (TLR9) signaling (68).

Another study using an endotoxemia model demonstrated a simultaneous increase in mitochondrial biogenesis and mitophagy through the actions of Sirt1, Pink1, and Parkin. This was associated with lower levels of lung and mitochondrial injury as well as reactive oxygen species and improved survival (69).

Additionally, it is important to consider the temporal aspects of mitochondrial functions and dynamics as they relate to the stage of the disease process, specifically infection and sepsis. The biology inciting sepsis involves a complex interplay of microorganisms and host/host responses. It has been hypothesized that a decrease in mitochondrial function, which may lead to or coincide with a loss of critical cell specific functions, may be an adaptive response that prevents cell death to allow for eventual recovery (70–73). Down-regulation of certain mitochondrial functions, including oxidative phosphorylation, may be aimed at limiting the production of ROS which would otherwise damage cells further. It has been hypothesized that these responses are akin to cellular estivation (i.e. hibernation), allowing for a “slowing down” of energy utilizing processes. However, temporally there must also be restitution processes (including biogenesis) to allow for eventual recovery.

The complex interplay of these mitochondrial dynamics and homeostasis responses are critical to cell biology in response to stress. Further studies in preclinical models and in patients highlighting the relationship and temporal aspects of these processes are necessary. This potentially will guide the development of targeted therapies to harness adaptive and minimize maladaptive responses.

Question 4

Diverse cells respond to stresses (e.g., hypoxia, cytokines, mechanical deformation) by activating mitochondria-dependent signals that trigger cellular protective responses. Ischemic preconditioning is one such example. However, responses that are adaptive for survival of the individual cell may be detrimental for tissue/organ function. Is the trade-off between “cell-adaptive” and “organ-maladaptive” responses a driver of organ dysfunction and long-term recovery in sepsis? Do organ-specific differences in this dichotomy determine outcome?

The triggering of “danger” signals cells leads to the activation of a number of protective responses (74). “Danger,” in turn, may arise from a number of different stimuli. When confronted with even relatively mild hypoxia, protective mechanisms decrease potential damage should the reduction in the oxygen supply become critical (75). Inflammation, sensed via the binding of Danger-Associated Molecular Patterns (DAMPS) or Pathogen-

associated molecular patterns (PAMPS) to TLR receptors, is known to initiate protective mechanisms via the MyD88/TRADD/NF-κB and JAK1/STAT3 pathways (76). Responses linked to mechanical deformation may be the result of nuclear compression (77). It appears that the cellular response to these "danger" signals is modulated by mitochondria.

Responses to hypoxia lie, in part, in the terminal cytochrome oxidase, complex IV, which binds oxygen and thus can respond to critical hypoxia (75). Interestingly, potentially protective responses are activated at O_2 concentrations that do not compromise the synthesis of ATP: Complex IV continues to consume oxygen at a constant rate even as the O_2 levels decrease. Therefore, diminished ATP levels cannot be responsible for triggering protective responses until the oxygen supply becomes critically low. Electron transport, however, is in flux even when oxygen consumption and ATP generation are maintained. Even mild hypoxia can limit the re-oxidation of cytochrome c. As the cytochrome c pool becomes progressively more reduced, the capacity to absorb electrons is limited and the transfer of electrons to Complex IV becomes impaired. Electron transfer from Complex III is limited with a resultant increase in the generation of ROS: limiting the activity of Complex IV increases electron density at Complex III and results in enhanced generation of O_2^- (75). The generation of ROS has also been implicated in cytokine - and deformation - mediated signaling. Pro-inflammatory cytokines such as TNF, IL-1 (via TLR-MyD88-mediated NF-κB activation) and IL-6 (via JAK-1/STAT3 activation) accelerate ROS production and augment the release of Ca^{+2} and pro-apoptotic proteins (76, 78, 79). These processes damage mitochondria, accelerating mitophagy and activating NRF-2, HO-1, AMPK and SIRT-1. In addition, these stimuli activate nuclear paradigms that promote biogenesis (76). A rising ratio of AMP to ATP indicates energy supply limitations, with AMPK - mediated blockade of ATP-consuming processes, especially mitogenic pathways controlled by mTOR (80). Perturbations of cell architecture, such as those accompanying mechanical deformation, affect membrane potential by altering mitochondrial volume (77), an effect that likely results in ROS liberation (81, 82).

In addition to direct damage to membranes via lipid peroxidation, enhanced ROS production activates HIF-1α (83, 84). HIF-1α is a heterodimeric protein transcription factor that, under unstressed conditions, is inactive. Cellular abundance of the active heterodimer is low because continuous hydroxylation of proline residues targets the HIF-1α subunit for proteosomal degradation (83, 85). ROS prevent degradation, stabilizing the heterodimer and facilitating HIF-1α-mediated transcription (84).

One consequence of the activation of a "danger response" pathway in cells is a reduction in cellular activity beyond the level of maintenance of basic cellular integrity. Under this paradigm, cells suppress some energy-dependent activities in favor of those that are essential for cell survival (86). Examples abound in patients with sepsis and under other circumstances. The most obvious example is the sepsis-induced loss of cardiomyocyte contractility. High levels of pharmacological support are required to support cardiac ejection and vascular tone yet cell death is rare. Cardiac performance is analogous to that seen following myocardial infarction, where the remaining cardiomyocytes "hibernate", presumably to allow recovery (73). A similar response in liver is reflected in low levels in the synthesis of excreted proteins and impaired transformation of both exogenous and

endogenous (i.e. bilirubin) toxins (87). In pulmonary cells, this phenomenon has been called "hypoxic conformance" and is characterized in part by internalization, and thus inactivation, of ATPase-linked trans-membrane pumps (88). The net result is a failure to clear fluid from the alveolar spaces, resulting in pulmonary edema.

A number of sepsis-induced processes have been attributed to "anti-inflammation" or "immunosuppression" (89). However, these changes might rather reflect "leukocyte hibernation". Similarly, muscle catabolism is part of the inflammatory process and, under balanced conditions, is followed by anabolism (90, 91). However, decreased catabolism accompanied by failed or insufficient anabolism might reflect "muscle hibernation."

Cellular hibernation in renal epithelial cells would be consistent with the low levels of cell death observed in septic patients with AKI (92). With rapid recovery, the net effect would be preservation of renal function for the recovery phase. However, prolonged inhibition of energy-requiring functions, such as reduced trans-cellular electrolyte transport, impaired secretion of potential toxins and limited generation of essential circulating ions such as bicarbonate and ammonium, would diminish survival. It is possible that early institution of current or future therapeutic approaches could prevent the systemic effects that would result from a reduction in available energy resources with a re-prioritization of renal function. Thus, it is essential that a method for determining when limited renal activity becomes maladaptive be developed.

Question 5. What are the Mitochondria-Based Therapeutic Targets and Opportunities for Intervention?

In patients with sepsis, alterations in cellular metabolism can develop as a consequence of inflammation, cytokine signaling, tissue hypoxia, catecholamine stimulation, altered insulin signaling, and other factors. Disordered metabolism in sepsis can disrupt glucose metabolism, resulting in augmented glycolytic flux and increased lactate production, even in the absence of cellular hypoxia. It can also disrupt lipid metabolism, resulting in the generation of inflammatory lipid mediators that contribute to organ dysfunction. Altered metabolism can also result in the generation of metabolic intermediates that affect intracellular signaling pathways and thereby alter cell function. Finally, metabolic reprogramming can alter the availability of cofactors involved in post-translational modifications of proteins. This can affect signaling pathways and also induce epigenetic changes by affecting the post-translational modification of histone proteins. Mitochondria are centrally involved in cellular metabolism, through their energy production, ROS generation/oxidant signaling, and their ability to interconvert biomolecules through the Tricarboxylic Acid (TCA) cycle (93). The metabolic disruptions observed in tissues during sepsis show similarities to the patterns observed in other diseases, so it is possible that treatments for those conditions might also be useful in sepsis.

For example, increases in circulating lactate are common in septic patients, even in the apparent absence of tissue hypoxia (94). This may be the result of cytokine or catecholamine signaling, and some studies have observed a correlation between the degree of lactatemia and the severity of sepsis, the outcome, and the response to treatment. This shift to aerobic glycolysis resembles the Warburg effect seen in many cancers (95). The similarities in

altered metabolism in cancer and in sepsis raise the question of whether interventions that correct metabolism toward a normal phenotype could be useful therapeutically. Clearly, uncontrolled cell proliferation as seen in cancer is not evident during sepsis. Nevertheless, the increased glycolysis in cancer cells is important for channeling glycolytic intermediates into the pentose phosphate pathway, which generates NADPH needed for cellular antioxidant activity (96). Inhibition of NADPH synthesis causes lethal oxidant stress in tumor cells, and the augmented glucose utilization in sepsis may represent a physiological attempt to manage oxidant stress. Presently, it is not clear whether the increase in aerobic glycolysis in sepsis is a marker of inflammation or an upstream mediator of cellular dysfunction. More information is therefore needed to understand whether throttling the glycolytic flux would confer protection, or alternatively exacerbate the condition by undermining antioxidant capacity. In either case, limited therapeutic options for modifying aerobic glycolysis are currently available.

Septic patients frequently develop hyperglycemia. Insulin can be used to control blood glucose in septic patients although this carries the risk of inducing hypoglycemia (97). The antidiabetic drug metformin decreases gluconeogenesis in the liver and ameliorates hyperglycemia by lessening hepatic glucose release. This effect is mediated by its inhibition of mitochondrial Complex I, causing a decrease in ATP that activates AMP-dependent protein kinase (AMPK).

AMPK is a master regulator of cellular energy flux; it inhibits anabolic, energy-consuming processes while promoting catabolic, energy producing pathways. In sepsis, AMPK activation might confer protection by attenuating inflammation and inflammatory cytokine expression, by augmenting glucose uptake, or by suppressing the effects of inflammation on endothelium. AMPK is activated by hypoxia- or ischemia-induced bioenergetic crises that decrease ATP levels. However, it is also activated by mild hypoxia which causes release of mitochondrial ROS signals (98). AMPK has been shown to protect against organ failure and inflammation in mouse models of experimental sepsis (99). Therefore, manipulation of AMPK using available drugs might be therapeutic in sepsis.

As in cancer, metabolic reprogramming in sepsis may affect tissue function by promoting epigenetic changes that shape gene expression during acute illness and long after recovery. Key mechanisms of epigenetic change involve post-translational modifications to histone proteins in chromatin. The most common modifications involve the methylation or acetylation of lysine residues on histones. These modifications can increase or decrease expression of genes by altering the accessibility of specific sites to transcription factors, by creating DNA binding sites on chromatin, and by facilitating protein-protein interactions that modify transcriptional activation or repression. In sepsis, it is well established extracellular signals induced by inflammatory cytokines can alter cellular function. Similarly, intracellular metabolic reprogramming may lead to epigenetic changes through alterations in the availability of cofactors needed for the post-translational modification of histone proteins. In cancer and other diseases, epigenetic modifications to histones proteins can alter the cellular phenotype in terms of proliferation, survival and metastatic behavior. Conceivably, altered metabolic pathway functions in sepsis and critical illness could induce

epigenetic modifications and thereby shape organ system function during the disease and long after.

Lysine residues on histone tails are acetylated by histone acetyltransferases (HAT) that utilize acetyl CoA, an important metabolic intermediate, as a cofactor. In mitochondria, acetyl CoA generated by pyruvate dehydrogenase is condensed with oxaloacetate to generate citrate in the TCA cycle. Citrate can then be exported to the cytosol where acetyl CoA and oxaloacetate are released by ATP citrate lyase. Thus, the mitochondria function as an important source of acetyl CoA needed for HAT activity. Reversal of acetylation is achieved by histone deacetylases (HDAC), some of which (sirtuins and class III) utilize NAD^+ as a cofactor. Metabolic reprogramming in sepsis and in cancer can alter NAD^+ levels in the cytosol as a consequence of altered glycolytic and/or mitochondrial function, thereby altering the availability of this cofactor for HDAC activity. HDACs also regulate acetylation of cytosolic proteins, and can thereby affect cells signaling and protein trafficking. HDAC inhibitors have long been used as psychotropic medications, and recently have been utilized in the treatment of cancers and lymphoma. Recent emerging data suggests that HDAC inhibitors may be protective in animal models of sepsis (100). HDACs act on multiple targets, so it will be difficult to identify any protective mechanisms. Nevertheless, HDAC function remains an intriguing target of potential therapeutic interventions.

Lysines are also modified by DNA methyl transferases (DMAT) that utilize S-adenosyl methionine (SAM) as a methyl-donating cofactor, and by histone demethylases that utilize 2-oxoglutarate and O_2 as substrates. Sepsis-induced disruptions in signaling or metabolism may therefore modify chromatin remodeling by altering the availability of cofactors, and also by altering the expression, activity, or protein-protein interactions involved in the regulation of histone methylation. Demethylase activity could be inhibited by cellular hypoxia and/or alterations in mitochondrial production of 2-oxoglutarate in sepsis. The DMAT inhibitor, 5-aza 2-deoxycytidine, has been used for the treatment of myelodysplastic syndromes and leukemias. Interesting emerging data suggests that this drug may confer protection suggest that it may confer protection against acute lung injury in a rodent sepsis model.

Many cancer chemotherapeutic agents induce significant tissue injury in the heart, lungs, kidneys and the central nervous system, and would be unsuitable for the treatment of sepsis. However, the examples presented above suggest that some agents used for the treatment of diverse disorders might be useful in the treatment of acute sepsis. Clearly, additional studies are necessary to explore these possibilities.

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Appendix 1. ADQI XIV Workgroup

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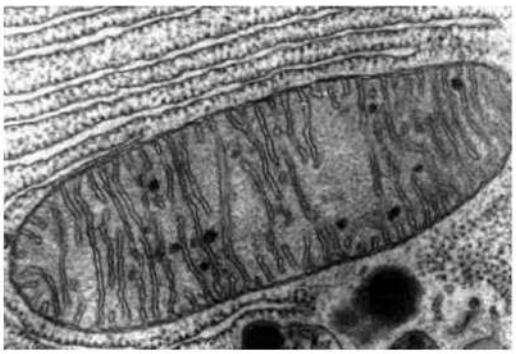
Appendix 2. Search Terms

Mitochondria, sepsis, inflammation, mitophagy, apoptosis, biogenesis.

Mitochondrial Function and Sepsis



Metabolism and Cell Signaling

- Oxidative phosphorylation and ATP production
  - Primary source of VO_2 and VCO_2
  - Energy use priority: Na^+ and Ca^{+2} transport, protein synthesis, RNA and DNA replication
- Intracellular Ca^{+2} homeostasis
- Reactive oxygen and nitrogen generation cell signaling
  - Essential for normal cell function
  - Potential for harm if excessive (e.g. reperfusion injury)

Mitochondrial Injury and Repair

- Apoptosis via intrinsic pathway: cytochrome c
  - Requires energy and is anti-inflammatory
- Necrosis via membrane rupture
  - Pro-inflammatory release of mtDNA as PAMPS
  - Damaged mitochondria internally cleared: Mitophagy
- Mitochondrial fusion, fission and biogensis
  - To maintain mitochondrial health quantified as membrane potential (Ψm) by fusing damaged mitochondria with healthy ones, pinching off damaged regions and synthesis of new mitochondria

Figure 1.

Table 1

Techniques to monitor mitochondrial health and function					
Reference	**Technique**	**Measurement**	**Clinical/Pre-clinical**	**In-vivo/ex-vivo**	**Comment**
Osbakken 1996 [1] Stidwill 1998 [2] Kraut 2004 [3] Clavijo 2008 [4]	NADH fluorometry	NADH autofluoresence (450 nm) NADH (but not NAD^-) autofluoresces in response to 340 nm excitation light	Pre-clinical	In-vivo	The emitted light relates to the concentration of the fluorescent compound and represents changes in mitochondrial NADH, assuming constant total $NADH/NAD^+$ pool.
From 2011 [5] Weiss 2005 [6]	Magnetic resonance spectroscopy (MRS)	^{31}P-nuclear magnetic resonance is primarily used, as it measures changes in phosphocreatine (PCr), ATP, Pi.	Pre-clinical	In-vivo	Ratio of PCr to ATP is used to determine level of metabolic stress
Kariman 1983 [7] Guery 1999 [8] Forget 2000 [9] Cairns 1997 [10]	Near infrared spectroscopy to measure COX redox state	The Cu A center in the oxidized form of COX strongly absorbs in the near-infrared spectrum with a characteristic shape and broad peak at 830 nm.	Pre-clinical	In-vivo	When oxygen availability falls, the Cu A center becomes more reduced
Mik [11]	Endogenous delayed fluoresce of protoporphyrin IX	Mitochondrial oxygen tension	Pre-clinical	Ex-vivo	Protoporphyrin IX is endogenously produced in mitochondria and reacts strongly with oxygen
Protti [12]	Spectrophotometry	Mitochondrial respiratory chain biochemistry (complex activity)	Clinical	Ex-vivo	Measured in homogenized in platelets and skeletal muscle
Sjovall [13] Weiss [14]	High-resolution oxygraph respirometry	Bioenergetic respiratory capacity, reserve and uncoupling	Clinical	Ex-vivo	Measured in isolated intact in peripheral blood cells

Mitochondrial functions in health and sepsis					
Mitochondrial Function	**Role in health**	**Change seen in sepsis**	**Time course**	**Organ/cell affected**	**Possible effects in sepsis**
Oxidative phosphoryaltion	ATP production to maintain cellular bioenergetics	Decreased	24–48hr	Skeletal muscle	Limitation of ATP, an extracellular DAMP Decreased cellular function, possibly conserving intra-cellular resources for future use.
Regulation of intracellular calcium levels	Intracellular signaling	Unknown	Unknown	Multiple	Upon depletion of ATP, Ca2+ homeostasis cannot be maintained, the MPT is induced, followed by cell death
ROS production	Signaling (e.g. HIF-1a upregulation in hypoxia)	Increased	Early-late	Multiple	Enhanced oxidative burst and pathogen clearance Toll-like receptor pathway activation TNF receptor signaling to promote NF-kB Protein nitration, DNA damage
Initiation of apoptosis	Mitochondria processes can activate apoptosis, an orderly, programmed form of cell elimination	Increased	Late	T Lymphocytes	Possible immunosuppression mtDNA, released via exocytosis or pyroptosis, is a potent DAMP

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Mitochondrial functions in health and sepsis					
Mitochondrial Function	**Role in health**	**Change seen in sepsis**	**Time course**	**Organ/cell affected**	**Possible effects in sepsis**
Mitophagy	Damaged mitochondria removed and replaced Limits the accumulation of depolarized mitochondria, ROS generation and activation of the NLRP3 inflammasome.	Unknown	-	-	-
Mitochondrial biogenesis	Creation of new and healthy mitochondria (biogenesis) to meet cellular metabolic energy demands	Decreased Increased	Early Late	Skeletal muscle	During acute illness, skeletal muscle shows a twofold decrease in mitochondrial content in intensive care unit patients with multiple organ failure. The course of sepsis and recovery is characterized by an increment in markers of mitochondrial biogenesis and mitochondrial number and density [15].

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