Diaphragm and cardiac mitochondrial creatine kinases are impaired in sepsis

Leigh A. Callahan and Gerald S. Supinski
Pulmonary and Critical Care Division, Department of Medicine, Medical College of Georgia, Augusta, Georgia

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Callahan LA, Supinski GS. Diaphragm and cardiac mitochondrial creatine kinases are impaired in sepsis. J Appl Physiol 102: 44–53, 2007. First published August 17, 2006; doi:10.1152/japplphysiol.01204.2005.—Previous studies indicate that ATP formation by the electron transport chain is impaired in sepsis. However, it is not known whether sepsis affects the mitochondrial ATP transport system. We hypothesized that sepsis inactivates the mitochondrial creatine kinase (MtCK)-high-energy phosphate transport system. To examine this issue, we assessed the effects of endotoxin administration on mitochondrial membrane-bound creatine kinase, an important trans-mitochondrial ATP transport system. Diaphragms and hearts were isolated from control (n = 12) and endotoxin-treated (8 mg·kg⁻¹·day⁻¹; n = 13) rats after pentobarbital anesthesia. We isolated mitochondria using techniques that allow evaluation of the functional coupling of mitochondrial creatine kinase MtCK activity to oxidative phosphorylation. MtCK functional activity was established by 1) determining ATP/creatine-stimulated oxygen consumption and 2) assessing total creatine kinase activity in mitochondria using an enzyme-linked assay. We examined MtCK protein content using Western blots. Endotoxin markedly reduced diaphragm and cardiac MtCK activity, as determined both by ATP/creatine-stimulated oxygen consumption and by the enzyme-linked assay (e.g., ATP/creatine-stimulated mitochondrial respiration was 173.8 ± 7.3, 60.5 ± 9.3, 210.7 ± 18.9, was 67.9 ± 7.3 natsoms O·min⁻¹·mg⁻¹ in diaphragm control, diaphragm septic, cardiac control, and cardiac septic samples, respectively; P < 0.001 for each tissue comparison). Endotoxin also reduced diaphragm and cardiac MtCK protein levels (e.g., protein levels declined by 39.5% in diaphragm mitochondria and by 44.2% in cardiac mitochondria; P < 0.001 and P = 0.009, respectively, comparing sepsis to control conditions). Our data indicate that endotoxin markedly impairs the MtCK-ATP transporter system; this phenomenon may have significant effects on diaphragm and cardiac function.

ATP/creatine stimulated respiration; mitochondrial creatine kinase activity; endotoxin; heart; respiratory muscles

Despite the utilization of increasingly sophisticated and potent antibiotic and adjunctive therapies, sepsis remains a leading cause of death (20). In many cases, the organ failure that accompanies this syndrome is the result of the direct and indirect damage to parenchymal organs by host defenses rather than to the direct effects of the infecting infectious agents (11). Recent studies suggest, moreover, that sepsis-induced alterations in mitochondrial function play an important role in the genesis of the tissue injury seen in this syndrome (3–6, 8, 11, 16, 25, 29). There are several mechanisms by which mitochondria have been postulated to contribute to tissue dysfunction in sepsis, including 1) mitochondrial generation of toxic substances, i.e., free radical species, that can damage cell organelles directly (by reaction with cellular constituents) and indirectly (by activating signaling pathways) (3, 5, 29); 2) triggering of mitochondrial-dependent pathways of cellular apoptosis (2); and 3) reduction in cellular high-energy com-

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by the Institutional Animal Care and Use Committee. Rats were housed in the Animal Resource Center; food and water were allowed ad libitum. Animals were divided into two groups: 1) control animals injected intraperitoneally with saline (0.5 ml) at time 0 and again at 24 h and 2) animals injected intraperitoneally with endotoxin (8 mg·kg⁻¹·day⁻¹, Escherichia coli lipopolysaccharide; Sigma, St. Louis, MO) at time 0 and at 24 h. All animals were also given subcutaneous doses of saline (60 ml·kg⁻¹·day⁻¹) to prevent dehydration. At 48 h after the first injection of saline or endotoxin, animals were anesthetized with pentobarbital sodium (50 mg/kg ip), a midline abdominal incision was made, and the abdominal aorta was flushed with 60 ml of isolation buffer (0.3 M sucrose, 10 mM HEPES, 2 mM HEPES·KOH, 20% glycerol, 4% SDS, 1.0% 2-mercaptoethanol, 0.005% bromphenol blue, pH 6.8). Samples were then loaded onto tandem Tris-glycine polyacrylamide gels (12.5 μg of protein/lane), and proteins were separated by electrophoresis (Novex Minigel II, Carlsbad, CA). Proteins from one gel were visualized with Silver Stain Plus (Bio-Rad Laboratories, Hercules, CA), and approximate molecular weights were determined with scan software (SigmaScan Gel, Chicago, IL) and known standard molecular weight markers. Mitochondrial proteins from the second SDS-PAGE gel were then transferred to nitrocellulose membranes. After electroblotting was completed, membranes were washed twice in PBS, blocked for 1.5 h at room temperature in PBS containing 3% BSA and 0.05% Tween 20, and incubated over night at 4°C with a monoclonal anti-sarcomeric MtCK antibody (kindly provided as a gift by Dr. Z. Khudhia, St. Louis, MO) diluted to 1 μg/ml in PBS-BSA. Subsequently, membranes were incubated with anti-mouse horseradish peroxidase-conjugated IgG (1:2,000 for 1.5 h at room temperature). Antibody binding to proteins was detected by enhanced chemiluminescence (NEP Life Science Products, Boston, MA). Gel densitometry was performed with a Microtek scanner (Carson, CA) and Un-Scan-IT software (Silk Scientific, Orem, UT).

Additional Western blot analyses of mitochondrial protein and cytosolic protein content were performed to exclude the possibility that sepsis-induced alterations in MtCK were either caused by 1) a generalized reduction in mitochondrial protein content or 2) leakage of the protein from damaged or swollen mitochondria. To accomplish this, we utilized similar techniques as described above. Specifically, we evaluated

![Diagram](image)

**Fig. 1. Schematic of method to assay mitochondrial creatine kinase (MtCK) activity in isolated mitochondria. The assay is designed to test functional coupling of MtCK activity with oxidative phosphorylation. Mitochondria are stimulated with ATP in the presence or absence of creatine; when the MtCK enzyme system is functional, the reaction yields phosphocreatine and ADP (step 1). ADP generated from this reaction then drives mitochondrial oxygen consumption through the electron transport (ET) chain (step 2). Standard assays of oxygen consumption rates in isolated mitochondria routinely measure ADP-stimulated respiration (step 2) and thereby do not take into account the function of the mitochondrial ATP-creatine kinase shuttle (step 1).**

Morphological alterations were monitored in response to addition of increasing doses of ATP (0–1,000 μM) in the presence or absence of creatine (20 mM) (7). It is important to note that most standard assessments of mitochondrial oxidative phosphorylation routinely utilize ADP to stimulate maximal oxygen consumption; however, this method only assesses oxygen consumption as a function of the electron transport chain and does not consider the activity of the mitochondrial ATP transport system (see Fig. 1, step 2). On the other hand, the assay that we employed directly assesses the functional activity of MtCK and its coupling to oxidative phosphorylation. As such, in the absence of MtCK activity, addition of ATP alone or ATP and creatine will elicit no increase in mitochondrial oxygen consumption. However, in the presence of functionally active MtCK, addition of ATP will stimulate oxidative phosphorylation (because of the presence of endogenous levels of creatine within the mitochondrial intermembrane space) with subsequent generation of ADP; the ADP so generated then drives oxidative phosphorylation. Moreover, addition of both ATP and exogenous creatine will further stimulate oxidative phosphorylation because both are substrates for the enzyme (see Fig. 1). Therefore, the magnitude of the increment of oxygen consumption observed with ATP and creatine addition is a function of MtCK activity. For these determinations, mitochondrial samples (200 μg) in buffer (120 mM KCl, 5 mM KH₂PO₄, 5 mM MOPS, 1 mM EDTA, 10 mM pyruvate, 2.5 mM malate, pH 7.25 at 30°C) were placed in a closed chamber containing a Clark-type electrode to assess oxygen consumption rates. Resting oxygen consumption was measured, and oxygen consumption rates were reassessed after addition of various concentrations of ATP (0–1,000 μM). In separate determinations, in which we used additional aliquots of mitochondrial suspensions, oxygen consumption rates were measured in the presence of both ATP and creatine (20 mM).

We also determined the total MtCK activity for mitochondrial samples using an enzyme-linked assay as previously described (22). For this assay, mitochondrial samples were incubated with a mixture containing hexokinase, glucose, and glucose-6-phosphate dehydrogenase. Active creatine kinase will produce NADH, which can be assessed spectrophotometrically by measuring absorbance at 340 nm. We used a spectrophotometer (Shimadzu Scientific Instruments, Columbia, MD) to perform assays, with addition of 20 μl of mitochondria in a 1-ml reaction chamber at a temperature of 25°C. Change in absorbance over time at 340 nm was measured, and the NADH generation rate was determined by use of the millimolar absorbitivity of NADH (6, 22). Results were expressed in activity units, with one unit defined as production of one micromole of NADH per minute.

**Western blot analysis of MtCK protein levels.** Dual protein gels were used to perform Western blot analyses for determination of MtCK protein levels in diaphragm and cardiac mitochondrial isolates (12). For each determination, mitochondrial samples were diluted with an equal volume of loading buffer (126 mM Tris·HCl, 20% glycerol, 4% SDS, 1.0% 2-mercaptoethanol, 0.005% bromphenol blue, pH 6.8). Samples were then loaded onto tandem Tris-glycine polyacrylamide gels (12.5 μg of protein/lane), and proteins were separated by electrophoresis (Novex Minicell II, Carlsbad, CA). Proteins from one gel were visualized with Silver Stain Plus (Bio-Rad Laboratories, Hercules, CA), and approximate molecular weights were determined with scan software (SigmaScan Gel, Chicago, IL) and known standard molecular weight markers. Mitochondrial proteins from the second SDS-PAGE gel were then transferred to nitrocellulose membranes. After electroblotting was completed, membranes were washed twice in PBS, blocked for 1.5 h at room temperature in PBS containing 3% BSA and 0.05% Tween 20, and incubated over night at 4°C with a monoclonal anti-sarcomeric MtCK antibody (kindly provided as a gift by Dr. Z. Khudhia, St. Louis, MO) diluted to 1 μg/ml in PBS-BSA. Subsequently, membranes were incubated with anti-mouse horseradish peroxidase-conjugated IgG (1:2,000 for 1.5 h at room temperature). Antibody binding to proteins was detected by enhanced chemiluminescence (NEP Life Science Products, Boston, MA). Gel densitometry was performed with a Microtek scanner (Carson, CA) and Un-Scan-IT software (Silk Scientific, Orem, UT).
protein levels of complex I subunit NDUFS3 and complex IV subunit 1 (COX-1) in diaphragm and cardiac mitochondrial isolates using anti-mouse monoclonal antibodies (MitoScience, Eugene, OR). To determine the distribution of MtCK, we compared protein levels in total homogenates, mitochondrial fractions, and cytosolic fractions of diaphragm and cardiac samples from control and endotoxin-treated animals. We also assessed cytosolic MM creatine kinase protein content in cytosolic fractions of muscle homogenates from the diaphragms and hearts of control and septic animals to establish whether endotoxin administration produced alterations in this critical component of the phosphocreatine shuttle (actin levels served as a loading control).

**Statistical analyses.** Oxygen consumption for mitochondrial isolates was plotted as a function of the level of added ATP. Separate plots were made for samples assayed with and without added creatine (20 mM). Comparison of oxygen consumption values between control and septic (i.e., endotoxin-treated) animals was accomplished by ANOVA. Unpaired t-tests were used to compare total MtCK activity, mitochondrial protein levels of sarcomeric creatine kinase, complex I subunit NDUFS3, complex IV subunit 1 (COX-1), and cytosolic protein levels of MtCK, MM creatine kinase, and actin between control and endotoxin-treated groups (SigmaStat software, Chicago, IL). Data are expressed as means ± SE; a P value of <0.05 was taken as indicating statistical significance.

**RESULTS**

**Mitochondrial function.** Addition of ATP to diaphragm mitochondrial isolates from control animals resulted in a large increase in oxygen consumption (Fig. 2, top). Respiration rates increased to 133 ± 6 natsoms O\(_2\) min\(^{-1}\) mg\(^{-1}\) in response to high levels of ATP (i.e., ATP concentrations of 1,000 \(\mu\)M) in protein levels of complex I subunit NDUFS3 and complex IV subunit 1 (COX-1) in diaphragm and cardiac mitochondrial isolates using anti-mouse monoclonal antibodies (MitoScience, Eugene, OR). To determine the distribution of MtCK, we compared protein levels in total homogenates, mitochondrial fractions, and cytosolic fractions of diaphragm and cardiac samples from control and endotoxin-treated animals. We also assessed cytosolic MM creatine kinase protein content in cytosolic fractions of muscle homogenates from the diaphragms and hearts of control and septic animals to establish whether endotoxin administration produced alterations in this critical component of the phosphocreatine shuttle (actin levels served as a loading control).

**Electron microscopy.** Mitochondrial integrity was assessed by evaluation of tissue sections from the diaphragms and hearts of control and endotoxin-treated animals. Muscle samples were fixed in 2% paraformaldehyde-2% glutaraldehyde in 0.1 M cacodylate buffer in sucrose and postfixed for 1 h with osmium tetroxide. Processing and embedding of tissue in Epon 812 (EM-bed)-Araldite-502 (Electron Microscopy Sciences, Fort Washington, PA) were performed according to previously published techniques (17).
this group of experiments, a value nearly fivefold greater than rates observed before addition of ATP. In contrast, diaphragm mitochondria taken from endotoxin-treated animals demonstrated very little response to addition of ATP. Specifically, respiration rates increased to only 50 ± 6 n_atoms O·min⁻¹·mg⁻¹ in response to 1,000 μM ATP in this group, a value only slightly above that seen before ATP addition and one far lower than that achieved for isolates from control animals (P < 0.001; Fig. 2, bottom). In addition, diaphragm mitochondrial isolates from control animals demonstrated significant increases in respiration rates in response to addition of ATP + creatine, whereas addition of ATP + creatine to diaphragm mitochondria from endotoxin-treated animals produced virtually no increase in respiration.

Similar responses were noted with cardiac mitochondrial isolates from control and endotoxin-treated animals. Cardiac mitochondria isolated from control animals demonstrated a large increase in respiration rate in response to both ATP and creatine (Fig. 3, top), whereas isolates from endotoxin-treated animals demonstrated a much smaller response to ATP and virtually no increase in respiration rate in response to addition of ATP + creatine (Fig. 3, bottom). For example, respiration rate increased to 182 ± 19 n_atoms O·min⁻¹·mg⁻¹ in response to 1,000 μM ATP for cardiac mitochondrial samples from control animals and increased further to 211 ± 19 n_atoms O·min⁻¹·mg⁻¹ after addition of ATP + creatine. In contrast, oxygen consumption rates in cardiac mitochondrial samples from endotoxin-treated animals were 67 ± 8 n_atoms O·min⁻¹·mg⁻¹ in response to 1,000 μM ATP and 68 ± 7 n_atoms O·min⁻¹·mg⁻¹ after addition of ATP + creatine (P < 0.001 comparing levels for control animals to levels for samples from endotoxin-treated animals). Comparisons of maximal ATP + creatine-stimulated mitochondrial oxygen consumption rates for diaphragm and cardiac mitochondrial isolates from control and endotoxin-treated animals are shown in Fig. 4.

**MtCK activity assays.** We also assessed activity levels of MtCK for mitochondrial isolates using the commonly employed hexokinase/glucose-6-phosphate dehydrogenase-linked enzyme assay. For diaphragm mitochondrial samples, MtCK activity levels were significantly lower in the endotoxin-treated group (P < 0.025 for comparison, Fig. 5). Similar decrements in MtCK activity levels were found for cardiac mitochondrial isolates from endotoxin-treated animals compared with control animals (P = 0.003; Fig. 5).

**MtCK protein levels.** MtCK protein levels, assessed by Western blotting, were significantly lower for both diaphragm and cardiac mitochondrial samples taken from endotoxin-treated animals than in samples from control animals. A representative blot comparing samples taken from control and endotoxin-treated animals is shown in Fig. 6, top, whereas mean levels for protein concentrations for Western blots, assessed by densitometric analysis, are shown in Fig. 6, bottom. On average, densitometric values for MtCK levels in diaphragm mitochondrial samples from endotoxin-treated animals were 39.5% lower than those from controls (P < 0.001), whereas MtCK protein levels for cardiac mitochondria from endotoxin-treated animals were 44.2% lower than isolates from control animals (P = 0.009). Densitometric analysis of concomitantly performed silver-stained protein gels from dia-
phragm and cardiac mitochondrial samples indicated that average total protein loads were similar for paired diaphragm samples from control and endotoxin-treated animals and for paired cardiac samples from control and septic animals (Fig. 7). Additional Western blots were performed on diaphragm and cardiac mitochondrial isolates for complex I subunit NDUFS3 and complex IV subunit 1 (COX-1) to exclude the possibility that sepsis produces a widespread depletion of mitochondrial proteins (Fig. 8). We found that levels of NDUFS3 and COX-1 were similar in diaphragm and cardiac samples compared with controls ($P < 0.001$ and $P < 0.009$, respectively).

Fig. 7. Representative silver-stained protein gels of diaphragm (left) and cardiac (right) mitochondrial isolates from control and endotoxin-treated animals. Equal amounts of mitochondrial proteins were loaded in each lane. As shown, many mitochondrial proteins show no change in response to endotoxin administration, whereas several proteins appear to increase or decrease in sepsis. These data indicate that sepsis is not associated with generalized depletion of mitochondrial proteins in either the diaphragm or the heart.
mitochondrial isolates from control and endotoxin-treated animals. These data argue that sepsis does not result in depletion of all mitochondrial proteins; rather, it induces selective reduction of several mitochondrial proteins.

To determine whether sepsis induced changes in MtCK content by eliciting leakage from the mitochondrial membranes, we compared MtCK levels in total homogenates and in mitochondrial and cytosolic fractions from diaphragm and cardiac samples in control and septic animals. If MtCK leaked out of the mitochondria, one might expect that cytosolic levels of the protein would increase. As shown in Fig. 9, we found that the cytosolic levels of MtCK in both diaphragm and cardiac samples did not increase in response to endotoxin. These data do not support the possibility that the sepsis-induced alterations in MtCK activity or protein levels are because of leakage from structurally damaged mitochondria.

Finally, we determined whether endotoxin administration also produced alterations in cytosolic MM creatine kinase, the creatine kinase isoform in striated muscle that is responsible for delivery of high-energy phosphates to the myosin ATPase. As demonstrated in Fig. 10, we found no sepsis-induced alterations in cytosolic MM creatine kinase protein levels in diaphragm or cardiac tissues compared with tissues from control animals.

**Electron microscopy.** We also assessed mitochondrial integrity in diaphragm and cardiac muscle samples using electron microscopy. Representative electron micrographs from the diaphragms and hearts of control and septic animals are shown in Fig. 11. Our results demonstrate, in both diaphragm and cardiac mitochondria, that the cristae appear less densely packed. These findings are consistent with other reports of changes in mitochondrial morphology in sepsis (4, 8). In addition, a recent report by Speer et al. (26) indicates that MtCK is an important structural protein that stabilizes mitochondrial membrane architecture by cross-linking inner and outer mitochondrial membranes at contact sites.
which demonstrate that MtCK functional activity and protein content are severely altered in sepsis, potentially provide an explanation for the sepsis-induced alterations in mitochondrial ultrastructure, since lack of MtCK would be expected to result in disruption of inner and outer mitochondrial membrane contact sites and destabilization of membrane architecture.

**DISCUSSION**

We found a marked reduction in the ability of creatine to stimulate mitochondrial respiration for diaphragm and cardiac muscle samples taken from endotoxin-treated animals compared with samples from saline-treated controls. Similarly, ATP plus creatine administration to isolated mitochondria, which increases oxygen consumption by linkage to a MtCK-catalyzed reaction, had little stimulatory effect when added to mitochondrial samples from endotoxin-treated animals but produced a large increase in respiration when added to samples from control animals. These findings are consistent with an effect of endotoxin-induced sepsis to markedly reduce MtCK functional activity in the diaphragm and the heart. These sepsis-induced reductions in functional MtCK activity were paralleled by the concomitant finding of sepsis-induced reductions using a conventional enzyme-linked creatine kinase activity assay. We also observed a significant reduction in MtCK protein content in cardiac and diaphragm mitochondrial isolates from endotoxin-treated animals compared with samples from controls, suggesting that the reduced functional capacity of MtCK is due, in part, to a sepsis-induced reduction in protein content.

MtCK. The existence of MtCK was first discovered by Jacobus and Lehninger (15), who recognized that this enzyme was responsible for the often observed effect of creatine to stimulate respiration when added to suspensions of isolated mitochondria. MtCK catalyzes the reaction of creatine with mitochondrial ATP to form phosphocreatine and ADP. The ADP so generated diffuses back to the electron transport chain where this molecule stimulates oxygen consumption, whereas the phosphocreatine produced by this reaction is directed to the adjacent cytosol. MtCK is localized to the outer mitochondrial membrane, enabling this enzyme to effectively facilitate the conversion of mitochondrial ATP to cytosolic phosphocreatine (21, 23). In cardiac and skeletal muscle, this enzyme is situated so that the phosphocreatine produced need only diffuse a short distance to reach the contractile proteins, where another creatine kinase isoform (cytosolic MM creatine kinase) uses phosphocreatine to replenish ATP levels in the vicinity of the myosin ATPase (21, 23). Effectively, these two enzyme systems act together to provide a physiologically and structurally linked enzyme system that can rapidly move high-energy compounds from the site of generation (the mitochondria) to the site of major usage (the contractile proteins). An elegant review describing the functional importance of MtCK and the phosphocreatine shuttle in human health and disease has recently been published by Schlattner et al. (24).

Other work suggests that this enzyme plays a critical role in facilitating rapid restoration of cytosolic phosphocreatine lev-

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**Cytosolic Levels of Actin and MM-Creatine Kinase**

Fig. 10. Representative Western blots of MM creatine kinase (CK) in cytosolic fractions from diaphragm (left) and cardiac (right) samples from control and septic animals (actin was used as a loading control). Densitometric analyses of cytosolic levels of these proteins (data not shown) were not significantly different between the 2 groups.
els in highly metabolic tissues (i.e., the heart, skeletal muscle, and brain) during periods of intense activity. For example, ~90% of the transport of high-energy phosphate compounds out of mitochondria in the active heart is mediated by the activity of this transport system (27). As a result, an impairment of this transport system would be expected to reduce cytosolic phosphocreatine levels and increase free phosphate ion concentrations. Increasing phosphate concentrations, in turn, would be expected to have a direct effect to inhibit contractile protein interactions, reducing muscle force generation and muscle shortening capacity (19).

A number of recent studies have examined the biochemical properties of purified MtCK in vitro. This work shows that the enzyme consists of identical protein chains that associate to form either octamers or dimers; the octamer has been shown to be the most physiologically relevant form of MtCK (28, 32). Exposure of octameric creatine kinase to either nitric oxide or peroxynitrite results in a rapid breakdown of the octameric structure and subsequent inactivation of the enzyme (28, 32). Importantly, irreversible inactivation of this enzyme can occur at extremely low levels of nitric oxide or peroxynitrite exposure (i.e., a 10-fold lower level of peroxynitrite than required to alter electron transport chain function) (28, 32).

In keeping with these in vitro observations, two recent studies found a marked reduction in MtCK activity in the presence of disease processes associated with the generation of high levels of oxidants in tissues. In the first of these studies, MtCK and cytosolic MM creatine kinase activities were reported to decrease in cardiac tissue in an animal model of congestive heart failure (10). In another study, mitochondria isolated from neurons of animals with an experimental model of amyotrophic lateral sclerosis also demonstrated a marked impairment in ubiquitous and cytosolic BB creatine kinase functional activity compared with controls (31).

The present study represents the first evidence that this enzyme system can also be inactivated by an acute disease process, i.e., endotoxin-induced sepsis. Moreover, the magnitude of the reductions in MtCK function observed in the present study appears to be comparable to those observed previously in the heart during heart failure or in neurons after the development of amyotrophic lateral sclerosis (10, 31). Although it is impossible to directly compare the impact that these alterations in enzyme function and content may have on tissue function in these three very dissimilar disease processes, it is nevertheless impressive that sepsis evoked a very sizable reduction in MtCK activity in a very short period of time (48 h).

We should note that the magnitudes of the functional reductions that we found (65% reductions in ATP/creatine stimulation of respiration, 85% reductions in the direct assessment of enzyme function using an enzyme-linked activity assay) were greater than the observed reductions in MtCK content (39–44%), as assessed by Western blotting. This combination of findings would seem to suggest that some of the protein detected by Western blotting may be functionally inactive. Such a possibility would be consistent with the findings of previous in vitro studies (28, 32). We and

Fig. 11. Representative electron micrographs of diaphragm (top, original magnification = ×10,000) and cardiac samples from control (left) and endotoxin-treated (right, original magnification = ×5,000) animals. Mitochondrial density and size are well-preserved after 48 h in this model of endotoxin-induced sepsis, although the cristae appear less densely packed than those of control samples.
others have previously suggested that sepsis leads to enhanced generation of reactive oxygen species (e.g., peroxynitrite) by mitochondria (3, 5, 18), and reactive oxygen species have been shown to rapidly inactivate sarcomeric creatine kinase in vitro (28, 32). In addition, a recent study (1) has shown that, with endotoxin administration, a number of diaphragm proteins, including MtCK, undergo oxidative modification. It is known that oxidative modification of proteins can result in protein unfolding and enhanced susceptibility to proteolytic degradation (9). It is therefore possible that sepsis results in structural modification of MtCK, which first causes reduction in enzymatic activity and subsequently facilitates protein degradation and removal. This could explain the pattern of our results, as this mechanism of inactivation of MtCK during sepsis should theoretically result in large functional reductions but smaller reductions in protein content.

**Potential implications.** The sepsis syndrome is associated with significant derangements in tissue blood flow, oxygen delivery, oxygen extraction, and organ function (4, 11). The pathogenesis of these alterations has been the subject of intense study, and a variety of potential mechanisms have been postulated (i.e., alterations in mitochondrial function within tissues, alterations in blood flow due to changes in blood coagulation cascades, alterations in microvascular control) (3–6, 8, 14, 20, 25, 29). In most of these previous studies, examination of mitochondrial function was confined to assessment of conventional indexes of mitochondrial respiration, e.g., state 3 respiration rate, characterized by the amount of oxygen used and the amount of ATP generated when ADP is added to mitochondrial isolates (3, 5, 6, 8, 29). These conventional methods of assessing mitochondrial function do not measure or take into account MtCK activity.

The new finding of the present work is that MtCK activity is essentially lost in the heart and skeletal muscle during the development of sepsis. The negative effects resulting from loss of MtCK would be expected to interact with and potentiate the deleterious effects of the other abnormalities thought to occur in this syndrome. Reductions in tissue oxygen delivery, due to alterations in microvascular flow regulation or localized clotting, and inhibition of the electron transport chain could result in reduced mitochondrial ATP generation. In this setting, the superimposition of a sepsis-induced reduction in MtCK activity would be expected to result in a marked impairment in the ability of cardiac and skeletal muscles to maintain adequate high-energy phosphate stores (phosphocreatine) in the cellular spaces adjacent to the contractile proteins at times of heighten ed contractile activity.

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