Derangements in mitochondrial metabolism in intercostal and leg muscle of critically ill patients with sepsis-induced multiple organ failure

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Sepsis patients treated in the intensive care unit (ICU) frequently develop skeletal muscle dysfunction. This dysfunction can be because of muscle weakness (decreased muscle strength), muscle fatigability (reduced capacity to continue muscle contractions), or other causes such as acquired neuropathy (1, 6, 13, 25, 26).

This muscle dysfunction often persists after ICU discharge. For instance, patients treated in the ICU for acute respiratory distress syndrome showed a persistent functional limitation up to one year after discharge from the ICU, which was associated with muscle fatigue by most patients (22). Also, respiratory muscles are susceptible to muscle dysfunction in critically ill patients. The majority of critically ill patients treated in ICUs in Northern Europe suffer from respiratory dysfunction and need mechanical ventilation. As much as 20% of all mechanically ventilated patients have problems with weaning from the ventilator, prolonging their ICU treatment (27, 32, 33). Both the respiratory dysfunction and the prolonged weaning are thought to be related to respiratory muscle weakness and fatigue.

In several animal models for severe sepsis and septic shock, decreased mitochondrial function and a consequent decrease in energy supplies in skeletal muscle have been shown (4, 9, 12, 30, 31). Because mitochondria are the main producers of cellular energy, a decreased mitochondrial function will interfere with muscle performance. Studies on skeletal muscle of septic patients are, however, limited. Three studies have shown decreased mitochondrial function in skeletal muscle of patients with acute cardiogenic or septic shock (8, 11, 17). These studies show an acute effect of shock on the mitochondrial membrane-bound enzymes of the respiratory chain. As discussed by Brealey at al. (8), this decreased respiratory chain activity might be related to the high mortality seen with acute shock. This mortality is most likely not the result of muscle dysfunction but more the result of a bioenergetic failure in more vital organs. Whether a similar derangement of mitochondrial function is present during a later phase of sepsis, where muscle dysfunction is more apparent, is not known. Septic patients surviving the initial shock phase often require prolonged ICU treatment and develop multiple organ failure (MOF). It is during this prolonged ICU treatment that muscle dysfunction develops and interferes with respiration and mobility during recovery. In the present study, we hypothesize that patients treated in the ICU for sepsis-related MOF are characterized by mitochondrial derangements and bioenergetic failure in both leg and intercostal muscle.

In this descriptive pilot study, for the first time, we measured mitochondrial enzyme activities, energy-rich phosphates, and a marker of oxidative stress in leg and intercostal muscle of mechanically ventilated ICU patients with sepsis-induced MOF. Intercostal muscle was used as a representative of respiratory muscle. Metabolically healthy, age- and sex-matched patients undergoing elective surgery served as controls.

MATERIAL AND METHODS

Subjects. Ten mechanically ventilated patients with MOF admitted to the general ICU at Karolinska University Hospital Huddinge were included in the study. All patients had sepsis according to the criteria...
of Bone et al. (7) but were stabilized and had MOF with two or more organs failing. Patients with known preexisting neuromuscular disease, chronic obstructive pulmonary disorder (COPD), or severe coagulopathies not enabling muscle biopsies were excluded. Treatment and nutrition were according to the ICU routines and the discretion of the attending intensivist. None of the patients was treated with muscle relaxants. As a control group, 10 metabolically healthy age- and sex-matched patients undergoing elective surgery were included. Muscle biopsies were taken from leg (vastus lateralis) muscle using a Bergström biopsy needle and from the lateral external intercostal muscle between the fifth and sixth rib by open surgery. In the control patients, muscle biopsies were obtained just after induction of anesthesia but before surgery was started. Control patients were normoventilated. A small portion of the muscle biopsies was immediately fixed (2% glutaraldehyde and 0.5% paraformaldehyde in 0.1 M sodium cacodylate buffer containing 0.1 M sucrose and 3 mM MgCl₂, pH 7.4) for morphological examination by electron microscopy. The rest of the biopsy specimens were quickly weighed, frozen in liquid nitrogen, and stored at −80°C until analysis. All patients or close relatives gave informed consent to participate in the study. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and had received an a priori approval by the Ethical Committee of Karolinska Institutet, Stockholm, Sweden.

**Enzymatic measurements.** Muscle samples were homogenized in a Potter-Elvhem homogenizer in KCl buffer (100 mM KCl, 50 mM Tris·HCl, 5 mM MgCl₂, 1.8 mM ATP, and 1 mM EDTA) to obtain a 5% homogenate (16). Parts of the homogenate were frozen, and the rest was used to isolate mitochondria by subsequent centrifugation. The homogenate was centrifuged at 700 g at 4°C for 10 min. The supernatant was decanted and centrifuged at 15,000 g at 4°C for another 10 min. Thereafter, the mitochondrial pellet was washed two times in the KCl buffer. The final mitochondrial pellet was suspended in 0.25 M sucrose, 2 mM EDTA, and 10 mM Tris (pH 7.4) and stored frozen at −80°C until analysis (16). Mitochondrial enzyme activities (citrate synthase, complex I and IV of the mitochondrial respiratory chain) were measured in both total muscle homogenate and isolated mitochondria. All enzymes were analyzed using spectrophotometric assays (16) adapted for analyses on a Konelab 20 Analyzer (Thermo Clinical Labsystems, Vantaa, Finland). The limit of detection in the Konelab 20 analyzer is 0.0005 absorbance units.

Total muscle and mitochondrial superoxide dismutase (SOD) activities were also measured in both muscle homogenate and isolated mitochondria using a Ransod kit (Randox Laboratories) on a Konelab 20 spectrophotometer analyzer.

**Energy-rich phosphates and lactate.** Muscle samples were freeze-dried. The dried muscle pieces were dissected free from visible fat, blood, and connective tissue and pulverized in a mortar. The sample was subsequently suspended in 0.5 M perchloric acid, kept on ice for 10 min, and neutralized using 2 M KHCO₃. A portion of the neutralized samples was frozen for later lactate analysis, and the other part was used for immediate analysis of ATP and creatine phosphate.

ATP and creatine phosphate concentrations were measured enzymatically, as has been described previously (21), and adapted for analysis on a Konelab 20 Analyzer. The neutral samples were mixed with a reagent (89 mM imidazole buffer, 1.4 mM EDTA, 13.4 mM MgCl₂, 6H₂O, 1.4 mM dithiothreitol, 0.5 mM ADP, 1.3 mM NADP, and 3.3 mM glucose, pH 7.2). The samples were incubated for 10 min with glucose-6-phosphate dehydrogenase at 37°C. The absorbance was measured at 340 nm, and hexokinase was added. After another 10 min, the absorbance was measured again at 340 nm, and creatine phosphokinase was added. After another 10 min, the absorbance was again measured at 340 nm. The concentration of ATP was calculated using the difference in absorbance between the first and second measurement, and creatine phosphate was calculated using the difference in absorbance between the second and third measurement.

To measure concentrations of lactate, an EnzyPlus i-lactic acid kit (Diffchamb, Gothenburg, Sweden) was used.

**Morphological evaluation.** Morphological evaluation of the subsarcolemmal mitochondria (i.e., the mitochondria that are located just beneath the muscular cell membrane) and the intermyofibrillar mitochondria (located in between the myofibrils of the muscle cell) was performed blindly by a trained pathologist, using randomized muscle samples on a Tecnai 10 electron microscope. The fixed muscle samples were rinsed (0.15 M sodium cacodylate buffer containing 3 mM CaCl₂, pH 7.4), postfixed for 2 h (2% osmium tetroxide in 0.07 mM sodium cacodylate buffer containing 1.5 M CaCl₂, pH 7.4, 4°C), dehydrated (ethanol followed by acetone), and embedded in LX-112 (Ladd, Burlington, VT). Semithin sections were cut and stained with toluidine blue and used for light microscopic analysis. Ultrathin sections (~40–50 nm) were cut and contrasted with uranyl acetate followed by lead citrate and examined blindly in a Tecnai 10 electron microscope at 80 kV.

A score from two to zero was given, where two was normal/not influenced, one was influenced, and zero was bad. Both the matrix and the membrane cristae were evaluated. Mitochondria displaying a homogenous density in the matrix were scored as two. Well-defined, tightly connected membrane cristae of mitochondria were also scored as two. Thus a maximum score of four could be obtained for the subsarcolemmal and the intermyofibrillar mitochondria, respectively (Fig. 1).

**Statistical analysis.** Results are presented as median and range. All data were analyzed using nonparametric Mann-Whitney U-test (Sta-
results

Patients. Characteristics of the ICU patients are given in Table 1. All ICU patients suffered from respiratory dysfunction as indicated by the PaO2/fraction of inspired oxygen ratio and MOF as indicated by the Sepsis-related Organ Failure Assessment score (37) scores on the day of the study. All ICU patients had sepsis according to the criteria of Bone et al. (7). At the time of the study, all patients were stable and were given no or small doses of vasoactive drugs. Control subjects underwent elective surgery for hernia repair, ileostomy closure, recurrent diverticulitis, or colorectal resection. One control patient had a malignant disease. Three women and seven men were included as controls with a median age of 67 (range 45–87) yr. All control patients were sedated using propofol, and they were all given small doses of rocuronium, a nonpolarizing muscle relaxant, just before the muscle biopsies were obtained.

Intercostal muscle. In muscle homogenate, the activity of citrate synthase was 53% lower in the ICU patients compared with controls (P = 0.003; Fig. 2). The activities of complexes I and IV expressed per dry weight of muscle showed a 60% lower complex I activity (P = 0.03), but no difference was found in the activity of complex IV (P = 0.06; Fig. 2). The activities of complex I and IV were not significantly different from controls when expressed per citrate synthase activity (i.e., mitochondrial content; complex I: patient median 0.12, range 0.03–0.31, control median 0.16, range 0.01–0.30, P = 0.25; complex IV: patient median 0.26, range 0.09–0.44, control median 0.31, range 0.01–0.41, P = 0.62). In the isolated mitochondria from intercostals muscle, activities of complex I and IV (expressed per citrate synthase activity) were not significantly different from control values (complex I: patient median 0.59, range 0.34–0.82, control median 0.51, range 0.33–0.86, P = 0.55; complex IV: patient median 0.76, range 0.11–1.09, control median 0.69, range 0.59–0.99, P = 0.45).

The concentrations of ATP, creatine phosphate, and lactate were not significantly different from controls in intercostal muscle of the ICU patients (ATP: P = 0.096; creatine phosphate: P = 0.50; lactate: P = 0.41; Fig. 3).

In muscle homogenates, there were no significant differences in the activity of SOD (P = 0.82), but when the same activity was measured in isolated mitochondria a 230% higher activity was detected in the patients (P = 0.0004; Table 2). No significant difference in the morphology of the mitochondrial was seen in intercostal muscle. The subsarcolemmal mitochondria had a scoring ranging from zero to four with a median value of three in both ICU patients and controls. The intermyofibrillar mitochondria scored from two to four with a median value of three in the controls.

Leg (vastus lateralis) muscle. In leg muscle homogenates, there was no significant difference in citrate synthase activity between the ICU patients and controls (P = 0.11; Fig. 2). When the activities of the mitochondrial complexes I and IV were expressed per dry weight of leg muscle, complex I activity was not significantly different from controls (P = 0.45), but complex IV activity was 38% lower in the ICU patients (P = 0.05; Fig. 2). Complexes I and IV in leg muscle were not significantly different in patients compared with controls when expressed per citrate synthase activity (i.e., mitochondrial content; complex I: patients median 0.2, range 0.01–0.80, controls median 0.18, range 0.02–0.27, P = 0.70; complex IV: patients median 0.34, range 0.01–0.51, controls median 0.46, range 0.10–0.88, P = 0.06).

In the isolated mitochondria, the activities of complexes I and IV (expressed per citrate synthase) in the ICU patients were not significantly different from those in the control subjects (complex I: patients median 0.55, range 0.14–1.93; controls median 0.56, range 0.29–0.67, P = 0.76; complex IV: patients median 0.72, range 0.06–1.30, controls median 0.70, range 0.25–0.85, P = 0.82).

Compared with controls, the ICU patients had a 40% lower ATP concentration (P < 0.01), a 34% lower creatine phosphate concentration (P < 0.01), and a 43% higher lactate concentration (P < 0.01) in leg muscle (Fig. 3)

There was no significant difference in the activity of SOD in leg muscle homogenate (P = 0.11), but when the activity was measured in isolated mitochondria, a 411% higher activity was detected in patients compared with controls (P = 0.004; Table 2).

Table 1. ICU patient characteristics at the time of biopsy

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Days in ICU</th>
<th>Survival</th>
<th>SOFA</th>
<th>PF Ratio</th>
<th>Glucocorticoid</th>
<th>Mechanical Ventilation</th>
<th>Nutrition†</th>
<th>Sedation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Throat abscess/pneumonia/readmission to ICU</td>
<td>67/F</td>
<td>2</td>
<td>Yes</td>
<td>7</td>
<td>70</td>
<td>None</td>
<td>Pressure support EN</td>
<td>Propofol</td>
</tr>
<tr>
<td>Liver transplant/quadriplegia</td>
<td>62/F</td>
<td>9</td>
<td>Yes</td>
<td>11</td>
<td>260</td>
<td>Treatment</td>
<td>Pressure support PN + EN</td>
<td>Propofol</td>
</tr>
<tr>
<td>Bacterial meningitis</td>
<td>51/F</td>
<td>7</td>
<td>Yes</td>
<td>8</td>
<td>99</td>
<td>Replacement</td>
<td>Pressure support PN + EN</td>
<td>Propofol</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>51/M</td>
<td>9</td>
<td>Died after 2 mo</td>
<td>14</td>
<td>136</td>
<td>Replacement</td>
<td>Pressure control PN + EN</td>
<td>Propofol</td>
</tr>
<tr>
<td>Surgical complications</td>
<td>74/M</td>
<td>22</td>
<td>Died day 30</td>
<td>7</td>
<td>260</td>
<td>None</td>
<td>Pressure support PN + EN</td>
<td>Propofol</td>
</tr>
<tr>
<td>Surgical complications/pneumonia</td>
<td>76/M</td>
<td>4</td>
<td>Yes</td>
<td>5</td>
<td>160</td>
<td>Treatment</td>
<td>Pressure support EN</td>
<td>Propofol</td>
</tr>
<tr>
<td>Ketoacidosis/pneumonia/quadriplegia</td>
<td>40/M</td>
<td>8</td>
<td>Yes</td>
<td>5</td>
<td>148</td>
<td>None</td>
<td>Pressure support EN</td>
<td>Propofol</td>
</tr>
<tr>
<td>Peritoneal abscess</td>
<td>78/M</td>
<td>6</td>
<td>Died after 2 mo</td>
<td>5</td>
<td>274</td>
<td>Replacement</td>
<td>Pressure support PN + EN</td>
<td>Unconscious/Propofol</td>
</tr>
<tr>
<td>ARDS</td>
<td>80/M</td>
<td>2</td>
<td>Yes</td>
<td>6</td>
<td>167</td>
<td>Treatment</td>
<td>Pressure control PN</td>
<td>Propofol</td>
</tr>
<tr>
<td>Surgical complications/ARDS</td>
<td>67/M</td>
<td>5</td>
<td>Yes</td>
<td>10</td>
<td>141</td>
<td>Replacement</td>
<td>Pressure support PN + EN</td>
<td>Propofol/Midasolan</td>
</tr>
<tr>
<td>Median</td>
<td>68</td>
<td>8</td>
<td>6</td>
<td>160</td>
<td>160</td>
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</table>

ICU, intensive care unit; F, female; M, male; SOFA, Sepsis-Related Organ Failure Assessment score; (PaO2/fraction of inspired oxygen) ratio, arterial oxygen concentration divided by the amount of inhaled oxygen; ARDS, acute respiratory distress syndrome; PN, parenteral nutrition; EN, enteral nutrition. †Nutrition was given at 20–25 kcal·kg⁻¹·day⁻¹, including supplementation of glutamine.
No significant differences in the morphology of the mitochondria were seen in leg muscle. The subsarcolemmal mitochondria in both groups had a score ranging from one to four with a median value of three for the ICU patients and of two for the controls. The intermyofibrillar mitochondria scored from zero to four with a median value of three in ICU patients and from zero to four with a median value of two in controls.

**DISCUSSION**

In this study, we found twofold decreases in mitochondrial enzyme activities in both intercostal and leg muscle of ICU patients suffering from sepsis-induced MOF compared with a group of metabolically healthy control patients. In leg muscle, the lower enzyme activities were accompanied by a lower concentration of ATP and creatine phosphate and by a higher lactate concentration, indicating a compromised energy production. A high activity of mitochondrial SOD in both intercostal and leg muscle of ICU patients suggests an increase in reactive oxygen species production. These results show, for the first time, a compromised mitochondrial content in both respiratory and leg muscle of critically ill patients with MOF.

In intercostal muscle of ICU patients, both citrate synthase and complex I activities were lower than in controls. In leg muscle...
Mitochondria produce energy for basal metabolism in all tissues. In skeletal muscle, the mitochondrial energy production is the rate-limiting step during endurance activity. It is therefore likely that the decreased mitochondrial content could lead to the muscle fatigue observed in ICU patients. Both during the recovery phase and during weaning of the ventilator, decreased endurance capacity is causing problems. However, it is not certain whether the observed decrease in mitochondrial enzyme activities in intercostal and leg muscle of the critically ill patients is sufficient to cause bioenergetic problems during rest. Two studies performed in rats have shown that a 25–40% decrease of muscle mitochondrial enzyme activity does not influence ATP and creatine phosphate levels at rest (14, 29). However, when the muscles were activated by electrical stimulation, ATP levels decreased much faster when the rats had decreased mitochondrial enzyme activities. In leg muscle of the critically ill patients in our study, ATP and creatine phosphate concentrations were already low and lactate levels high at rest, indicating that these patients probably will

Table 2. Markers of oxidative stress in different muscle groups in ICU patients and matched controls

<table>
<thead>
<tr>
<th></th>
<th>Intercostal ICU</th>
<th>Intercostal Control</th>
<th>Vastus Lateralis ICU</th>
<th>Vastus Lateralis Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD, U/g dry wt</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>53.2</td>
<td>50.4</td>
<td>53.8</td>
<td>44.9</td>
</tr>
<tr>
<td>Range</td>
<td>30.5–85.8</td>
<td>47.2–88.7</td>
<td>25.6–74.3</td>
<td>37.9–58.2</td>
</tr>
<tr>
<td>Mitochondrial SOD,</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>U/U CS</td>
<td>2.6*</td>
<td>1.1</td>
<td>2.1*</td>
<td>0.5</td>
</tr>
<tr>
<td>Median</td>
<td>1.6–3.9</td>
<td>0.4–2.0</td>
<td>0.4–5.7</td>
<td>0.3–1.5</td>
</tr>
</tbody>
</table>

SOD, superoxide dismutase; CS, citrate synthase. *P < 0.05.

Mitochondria are the major site of energy production in the cell; therefore, the lower mitochondrial enzyme activities are the likely cause of the changes in the energy-rich phosphates. However, a decreased blood flow and thereby oxygen and substrate supply also will potentially decrease energy-rich phosphates and increase lactate contents. A decreased blood flow in these patients could be the result of their severe insulin resistance or an ongoing inflammatory response (38, 40). However, previous studies have shown that skeletal muscle oxygen tension is not decreased in patients similar to ours (5). In leg muscle, we found lower concentrations of ATP and creatine phosphate and higher lactate levels in the ICU patients compared with controls. However, in intercostal muscle, concentrations of these energy metabolites were not different between ICU patients and controls. Similar results have been obtained in a study on energy metabolism in intercostal and leg muscle in COPD patients suffering from acute respiratory distress syndrome (19). Also in that study, ATP and creatine levels were lower in intercostal muscle compared with leg muscle in healthy controls. The COPD patients had lower concentrations of both ATP and creatine in leg muscle compared with controls, but the concentrations in intercostal muscle did not change. The reason for the generally lower concentrations of energy-rich phosphates and the lack of depletion in intercostal muscle is not known. However, one explanation could be that the intercostal muscle is passively stretched during mechanical ventilation, while leg muscle is not activated at all. This stretching of the intercostal muscle may increase blood flow, and that in turn could preserve the levels of energy-rich phosphates. Another explanation may be that respiratory muscles are more protected than other muscle groups. Our results show that different skeletal muscles do not respond to metabolic stress in the same way and that care should be taken to extrapolate results obtained in one muscle to all skeletal muscles in the body.

No difference in mitochondrial morphology was observed in our study, mainly because of the fact that the control subjects had more morphological changes than expected. These analyses were performed blindly by a trained pathologist and are therefore most likely not the result of the evaluation procedure itself. The most likely explanation is that these are the signs of aging. It is well established that aging has a negative effect on mitochondrial density, oxidative capacity, and morphology (2, 10, 18, 34–36). We therefore included control subjects carefully matched for age.

One of the main determinant factors of muscle mitochondrial density is physical activity (24). Critically ill patients lying in the ICU are always bed bound and often sedated, which most likely will influence mitochondrial density. Several human studies have shown that immobilization for 4–7 wk decreases mitochondrial enzyme activity in various muscle types by ~20% (3, 15, 23, 20, 28). The ICU patients included in the present study were immobilized for a shorter period of time (medium ICU stay of 7.5 days) and still had more pronounced mitochondrial derangements in skeletal muscle. Even though immobilization surely affects mitochondrial enzyme activity in critically ill patients, disease itself most likely plays a larger role.

Mitochondria produce energy for basal metabolism in all tissues. In skeletal muscle, the mitochondrial energy production is the rate-limiting step during endurance activity. It is therefore likely that the decreased mitochondrial content could lead to the muscle fatigue observed in ICU patients. Both during the recovery phase and during weaning of the ventilator, decreased endurance capacity is causing problems. However, it is not certain whether the observed decrease in mitochondrial enzyme activities in intercostal and leg muscle of the critically ill patients is sufficient to cause bioenergetic problems during rest. Two studies performed in rats have shown that a 25–40% decrease of muscle mitochondrial enzyme activity does not influence ATP and creatine phosphate levels at rest (14, 29). However, when the muscles were activated by electrical stimulation, ATP levels decreased much faster when the rats had decreased mitochondrial enzyme activities. In leg muscle of the critically ill patients in our study, ATP and creatine phosphate concentrations were already low and lactate levels high at rest, indicating that these patients probably will
face great problems coping with the increased energy demand during muscle activation. No human data are, however, available to confirm this hypothesis, and more studies are needed.

In summary, in ICU patients suffering from sepsis-induced MOF, there was a twofold decrease in mitochondrial content in both leg and intercostal muscle. It is likely that these changes will lead to problems with muscle weakness and fatigue when the patients are activated again after ICU discharge. The decreased mitochondrial content without an accompanying effect on energy substrates in intercostal muscle might suggest that respiratory muscles are more protected.

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GRANTS

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