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

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Fredriksson, Katarina, Folke Hammarqvist, Karin Strigård, Kjell Hultenby, Olle Ljungqvist, Jan Wernerman, and Olav Rooyackers. Derangements in mitochondrial metabolism in intercostal and leg muscle of critically ill patients with sepsis-induced multiple organ failure. Am J Physiol Endocrinol Metab 291: E1044–E1050, 2006. First published June 27, 2006; doi:10.1152/ajpendo.00218.2006.—Critically ill patients treated for multiple organ failure often develop muscle dysfunction. Here we test the hypothesis that mitochondrial and energy metabolism are deranged in leg and intercostal muscle of critically ill patients with sepsis-induced multiple organ failure. Ten critically ill patients suffering from sepsis-induced multiple organ failure and requiring mechanical ventilation were included in the study. A group (n = 10) of metabolically healthy age- and sex-matched patients undergoing elective surgery were used as controls. Muscle biopsies were obtained from the vastus lateralis (leg) and intercostal muscle. The activities of citrate synthase and mitochondrial respiratory chain complexes I and IV and concentrations of ATP, creatine phosphate, and lactate were analyzed. Morphological evaluation of mitochondria was performed by electron microscopy. Activities of citrate synthase and complex I were 53 and 60% lower, respectively, in intercostal muscle of the patients but not in leg muscle compared with controls. The activity of complex IV was 30% lower in leg muscle but not in intercostal muscle. Concentrations of ATP and creatine phosphate were, respectively, 40 and 34% lower, and lactate concentrations were 43% higher in leg muscle but not in intercostal muscle. We conclude that both leg and intercostal muscle show a twofold decrease in mitochondrial content in intensive care unit patients with multiple organ failure, which is associated with lower concentrations of energy-rich phosphates and an increased anaerobic energy production in leg muscle but not in intercostal muscle.

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

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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 HCl, 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-Elvehjem 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 for 10 min. The supernatant was decanted and centrifuged at 15,000 g 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 the 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 spectrophotometric 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 KOAc. 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 L-lactic acid kit (Difffchamb, 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 M 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 (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.

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 intercostals 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 mitochondria 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</td>
<td>67/F</td>
<td>2</td>
<td>Yes</td>
<td>7</td>
<td>70</td>
<td>None</td>
<td>Pressure support</td>
<td>EN 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</td>
<td>PN + EN 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</td>
<td>PN + EN Propofol</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>51/M</td>
<td>Died after 2 mo</td>
<td>Yes</td>
<td>14</td>
<td>136</td>
<td>Replacement</td>
<td>Pressure control</td>
<td>PN + EN Propofol</td>
</tr>
<tr>
<td>Surgical complications</td>
<td>74/M</td>
<td>Died day 30</td>
<td>7</td>
<td>260</td>
<td>None</td>
<td>Pressure support</td>
<td>PN + EN Propofol</td>
<td></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</td>
<td>EN Propofol</td>
</tr>
<tr>
<td>Peritoneal abscess</td>
<td>78/M</td>
<td>Died after 2 mo</td>
<td>5</td>
<td>274</td>
<td>Replacement</td>
<td>Pressure support</td>
<td>PN + EN Unconscious/Propofol</td>
<td></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</td>
<td>PN 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</td>
<td>PN Propofol/Midasolan</td>
</tr>
<tr>
<td>Median values</td>
<td>67</td>
<td>6</td>
<td>8</td>
<td>160</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</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.

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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

Fig. 2. Activities of mitochondrial enzyme citrate synthase and complexes I and IV in human intercostal and leg muscle of intensive care unit (ICU) patients and controls, expressed per muscle weight. The long bar represents the median value. dw, Dry weight. * \( P < 0.05 \).

Fig. 3. Concentrations of creatine phosphate, ATP, and lactate in human intercostal and leg muscle from ICU patients and controls. The long bar represents the median value. * \( P < 0.05 \).
and controls. Similar results have been obtained in a study where energy metabolites were not different between ICU patients and controls. However, in intercostal muscle, concentrations of these energy-rich phosphates and increase lactate contents. A 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).

<table>
<thead>
<tr>
<th>SOD, U/g dry wt</th>
<th>Intercostal ICU</th>
<th>Intercostal Control</th>
<th>Vastus Lateralis ICU</th>
<th>Vastus Lateralis Control</th>
</tr>
</thead>
<tbody>
<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, U/U CS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>2.6*</td>
<td>1.1</td>
<td>2.1*</td>
<td>0.5</td>
</tr>
<tr>
<td>Range</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.
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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