Mitochondrial Fusion, Fission, and Biogenesis in Prolonged Critically Ill Patients

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Context: Critical illness induces swelling, enlargement, and dysfunction of mitochondria, which in liver, but not in muscle, is aggravated by excessive hyperglycemia. We previously demonstrated impaired autophagic clearance of damaged mitochondria in fed prolonged critically ill patients. Impaired fusion/fission-mediated repair and/or renewal through biogenesis may further accentuate mitochondrial abnormalities.

Objective: We studied mitochondrial fusion/fission and biogenesis and how these are affected by preventing hyperglycemia with insulin during critical illness.

Design and Setting: Patients admitted to a university hospital surgical/medical intensive-care unit participated in a randomized study.

Patients: We studied adult prolonged critically ill patients vs. controls.

Intervention: Tolerating hyperglycemia up to 215 mg/dl was compared with intensive insulin therapy targeting normoglycemia (80–110 mg/dl).

Main Outcome Measures: In liver and skeletal muscle, we quantified levels of several proteins involved in mitochondrial fusion/fission and biogenesis.

Results: Key players in mitochondrial fusion/fission and biogenesis were up-regulated in postmortem liver (1.4- to 3.7-fold) and rectus abdominis (1.2- to 4.2-fold) but not in in vivo or postmortem vastus lateralis biopsies of critically ill patients. Maintaining normoglycemia with insulin attenuated the hepatic response in the mitochondrial fusion/fission process but did not affect the markers of mitochondrial biogenesis in liver or muscle.

Conclusions: Our observations suggest tissue-dependent attempts of compensatory activation of mitochondrial repair mechanisms during critical illness. Considering the previously observed persistent mitochondrial damage, this activation may be insufficient and contribute to mitochondrial dysfunction. Suppressed activation of fusion/fission when excessive hyperglycemia is prevented with insulin may reflect reduced need for diluting (less) damage during normoglycemia or, alternatively, a suppressive effect of insulin on repair. (J Clin Endocrinol Metab 97: E59–E64, 2012)

Critically ill patients face a high risk of death, which is usually the consequence of nonresolving multiple organ failure, irrespective of intensive care unit (ICU) admission diagnosis. Patient and animal studies found an association of mitochondrial dysfunction with severity of organ dysfunction and risk of adverse outcome of critical illness (1, 2). Pronounced hyperglycemia, which develops in response to illness or trauma and is worsened by artificial (parenteral) nutrition, aggravates such mitochondrial damage. This is supported by the observation that insulin-titrated normoglycemia protects mitochondrial morphology and function (3), which appears explained by avoidance of damage by cellular glucose overload rather than direct protection by insulin (4, 5).

Abbreviations: Drp1, Dynamin-related protein-1; Fis1, fusion-1; ICU, intensive care unit; mtDNA, mitochondrial DNA; nDNA, nuclear DNA; OPA1, optic atrophy-1; Pol-γ, mitochondrial DNA polymerase-γ; RIP-140, receptor-inhibitory protein-140; TFAM, mitochondrial transcription factor-A.
We recently demonstrated that activation of autophagy, the pathway responsible for clearance of damaged organelles and potentially toxic protein aggregates, is impaired in liver and muscle of fed prolonged critically ill patients (6). This may have contributed to the persistent mitochondrial structural damage and dysfunction. However, disturbances in two other major mechanisms that repair or compensate for mitochondrial damage may have played a role as well. In this regard, mitochondrial fusion and fission allow exchange of damaged components leading to dilution of molecular damage or bundling of dysfunctional structures in a single, irreversibly damaged organelle that is subsequently targeted for removal by autophagy (7, 8). Mitochondrial biogenesis generates new mitochondria when needed, such as in response to mitochondrial damage and increased energy demand (9). Figure 1 shows a simplified schematic overview of the mitochondrial repair mechanisms.

We hypothesized that impaired mitochondrial fusion or fission and/or compromised activation of mitochondrial biogenesis could contribute to mitochondrial dysfunction in critical illness. We also investigated whether the partial alleviation of such damage by preventing hyperglycemia could in part be mediated via these pathways. We therefore studied several key readouts of mitochondrial dynamics in liver and skeletal muscle biopsies obtained from critically ill patients.

**Patients and Methods**

**Patients**

We studied liver and rectus abdominis biopsies taken within 30 ± 20 min after death from 36 randomly selected surgical ICU patients and Methods

![FIG. 1. Simplified scheme of the mitochondrial repair mechanisms and their interaction. Mitochondria are subjected to frequent cycles of fusion and fission. Mitochondrial fusion requires participation of the mitofusins on the outer mitochondrial membrane and OPA1 in the intermembrane space, whereas recruitment of Drp1 from the cytosol to the mitochondria by the outer membrane protein Fis1 is necessary for mitochondrial fission. Fusion and fission events are important in determining the overall shape of the mitochondria and potentiate dilution of damage over different organelles. They are also involved in mitochondrial biogenesis as well as autophagy of these organelles (mitophagy), which normally balances biogenesis. Generation of uneven daughter organelles by asymmetric fission segregates the daughter unit that contains most of the defects, has an impaired fusion capacity (due to low membrane potential Δψm and OPA1 content), and is predisposed to removal by autophagy. Autophagy is an inducible, complex process that starts with the formation of a phagophore or isolation membrane, which is elongated to surround portions of cytoplasm and/or organelles with the formation of an autophagosome. The mature autophagosome fuses with a lysosome to form an autolysosome, containing all enzymes for degradation of the invaginated content. Multiple transcriptional pathways operate in the control of both nuclear and mitochondrial gene expression to regulate mitochondrial biogenesis. RIP-140 inhibits mitochondrial biogenesis. Peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) promotes mitochondrial biogenesis through regulation of nuclear respiratory factor-1 (NRF-1) and NRF-2. Nuclear respiratory factors regulate TFAM, Pol-γ and single-strand binding protein (SSB), which in turn direct mtDNA replication and transcription of several mtDNA-encoded respiratory chain complex subunits. NRF-1 and NRF-2 also directly regulate other proteins with the corresponding response elements in their promoter, including nDNA-encoded respiratory chain complex subunits.](https://academic.oup.com/jcem/article-abstract/97/1/E59/2833702)
Statistical analyses

Differences analyzed by Kruskal-Wallis and Mann-Whitney U test were considered statistically significant when two-sided P values were <0.05 (StatView version 5.0.1; SAS Institute, Cary, NC).

Results

Blood glucose and insulin infusion

Mean morning blood glucose levels during the ICU stay of surgical critically ill patients were 178 ± 16 mg/dl (mean ± SD) in the conventional and 101 ± 7 mg/dl in the intensive insulin therapy group (P < 0.0001). Patients received a median (interquartile range) insulin dose of 14 (2–34) and 44 (23–87) IU/d, respectively (P = 0.005). The mean morning blood glucose levels of medical critically ill patients were 156 ± 26 mg/dl in the conventional and 99 ± 12 mg/dl in the intensive insulin therapy group (P < 0.0001), with insulin doses of 12 (3–32) and 77 (57–98) IU/d, respectively (P = 0.0002).

Mitochondrial fusion and fission

Protein levels of the mitochondrial fusion mediators mitofusin-2 and OPA1 and the mitochondrial fission mediator Drp1, but not Fis1, were increased above controls in liver of conventionally treated patients (Fig. 2A). Patients who received intensive insulin therapy had comparable levels of mitofusin-2, Drp1, and Fis1 as controls, whereas OPA1 remained elevated. In postmortem rectus abdominis biopsies, critical illness had up-regulated the four proteins, irrespective of insulin treatment group (Fig. 2A). In contrast, vastus lateralis showed no up-regulation, whether taken in vivo (Fig. 2A, without a difference between eventual survivors or nonsurvivors, data not shown) or postmortem (data not shown).

Mitochondrial biogenesis

Protein levels of nDNA-encoded (NDUFA9) and mtDNA-encoded (ND6) complex I subunits were up-regulated in liver and rectus abdominis, but not in vivo (Fig. 2B) or postmortem (data not shown) vastus lateralis biopsies, of conventionally treated critically ill patients. Similar results, including also an increase in in vivo vastus lateralis muscle, were obtained for TFAM. Pol-γ was up-regulated only in liver. Protein levels of the mitochondrial biogenesis inhibitor RIP-140 were always comparable to controls. All tissues failed to up-regulate mtDNA levels. For all tissues, the patients in the normoglycemic, intensive insulin therapy group responded similarly as the hyperglycemic conventional group. Markers in in vivo vastus lateralis were comparable for eventual survivors and nonsurvivors (data not shown).

Discussion

Critical illness is associated with mitochondrial damage and impaired cellular energy metabolism, which have been implicated in organ dysfunction and adverse outcome. We demonstrated an up-regulation of several key mediators of mitochondrial fusion/fission and biogenesis in postmortem liver and rectus abdominis, but not in vivo or postmortem vastus lateralis of the critically ill. Overall, maintenance of normoglycemia with insulin did not affect this...
response, except for a partial attenuation of mitochondrial fusion and fission markers in liver. We previously observed mitochondrial abnormalities, more pronounced in hyperglycemic than in normoglycemic livers (3) and, regardless of glycemic control, also impaired autophagy (6). Together, our observations suggest that mitochondrial protection brought about by glycemic control is mediated by prevention of direct glucose toxicity, rather than via an effect on mitochondrial repair.

Mitochondria constantly undergo regulated fusion and fission, which determine mitochondrial morphology, organelle number, shape, size, content, distribution, and function (13, 14). Fusion of nonfunctional, damaged mitochondria with healthy, fully functional mitochondria provides a mechanism to regain essential components and dilute molecular damage after subsequent fission (13). However, giant mitochondria that accumulate within aged or diseased cells are unable to fuse and exchange contents with other mitochondria (7). Recent findings put forward fusion followed by selective fusion as quality control mechanism to segregate irreparable, dysfunctional mitochondria and target them for removal by autophagy.

**FIG. 2.** Mitochondrial fusion/fission and biogenesis in critically ill patients. Relative protein expression levels of several key mediators of mitochondrial fusion and fission (A) and mitochondrial biogenesis (B) were normalized to levels of cytokeratin-18 (liver) or to actin (skeletal muscle) and to levels of controls. These data as well as mtDNA levels (normalized to levels of controls, B) are shown as box plots, with medians, interquartile ranges and 10th and 90th percentiles. *, P ≤ 0.05; (*), 0.05 < P < 0.1 vs. healthy reference; #, P ≤ 0.05; (#), 0.05 < P < 0.1 between critically ill patients receiving conventional insulin therapy (CIT) or intensive insulin therapy (IIT). Representative blots are shown of, from left to right, three samples from controls and CIT and IIT patients each for liver and rectus abdominis and two controls followed by three to four CIT and IIT patients for vastus lateralis.
Mitochondrial fission thus can generate two asymmetrical daughter organelles, one of which contains the defects. These fusion-incompetent mitochondria are then eliminated by autophagy. Importantly, increased fusion or reduced fission compromises autophagy (8) and may spare mitochondria from degradation (15). Data on impact of critical injury on these processes are scarce and available only from experimental models. These suggest protection by mitofusin-2, whereas extensive fission may be detrimental by triggering apoptosis (16, 17). Importantly, the two processes need to be appropriately balanced, because both unopposed fusion and unopposed fission are detrimental for cellular function. Proteins involved in fusion and fission were up-regulated in liver and rectus abdominis of our patients, but not in vastus lateralis. In liver, however, Fis1 was not increased. Although relative protein levels as such preclude conclusions on the balance between the processes, it could be speculated that fusion would prevail over fission, because Fis1 may be rate limiting (14). This could further contribute to impaired targeting for mitophagy (8, 15). The hepatic responses were partially attenuated with insulin therapy, either suggesting a reduced need for repair of mitochondrial damage in line with better-preserved structure and function (3) or, alternatively, a negative effect of insulin on mitochondrial repair.

In animal models, severe sepsis induces oxidative mitochondrial damage, with decreased mtDNA copy number, mitochondrial transcription, and oxidative phosphorylation. Mitochondrial biogenesis is activated to restore the damage (9, 18, 19). This response allows metabolic recovery and was put forward as a powerful pro-survival mechanism (9). Indeed, skeletal muscle taken on d 1–2 in the ICU from critically ill patients showed a mitochondrial biogenesis response in survivors but not in vastus lateralis. In liver, however, Fis1 was not increased. Although relative protein levels as such preclude conclusions on the balance between the processes, it could be speculated that fusion would prevail over fission, because Fis1 may be rate limiting (14). This could further contribute to impaired targeting for mitophagy (8, 15). The hepatic responses were partially attenuated with insulin therapy, either suggesting a reduced need for repair of mitochondrial damage in line with better-preserved structure and function (3) or, alternatively, a negative effect of insulin on mitochondrial repair.

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Mitochondrial dysfunction and severity and outcome of septic shock.
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