SUMMARY

BACKGROUND: ICU-acquired weakness impairs functional outcome in survivors of critical illness. Therefore, deepening our understanding of its pathogenesis is an important goal as muscle-specific therapeutic targets are urgently needed. Systemic inflammation and sepsis are the main risk factors of ICUAW, and these syndromes are associated with mitochondrial dysfunction. The aim of our study was to collect reliable information on the mitochondrial function of human skeletal muscle in the protracted phase of critical illness. Additionally, we explored mitochondrial respiratory parameters following experimentally induced changes in the availability or composition of selected nutrients (fatty acids and glutamine).

MATERIALS and METHODS: Vastus lateralis muscle biopsy samples from patients with ICU-acquired weakness and age-matched healthy controls were obtained. In human skeletal muscle tissue homogenates mitochondrial functional indices were assessed by high-resolution respirometry, individual functional capacities of respiratory complexes were measured by spectrophotometry and correlated with concentrations of electron transport chain key subunits measured by western blot. Additionally, using human myoblasts and myotubes we studied the influence of extracellular environment manipulations by extracellular flux analysis.

RESULTS: The ability of aerobic ATP synthesis was reduced to ~54 % in ICU patients ($p<0.01$), in correlation with the depletion of complexes III (~38 % of controls, $p = 0.02$) and IV (~26 % of controls, $p<0.01$) and without signs of mitochondrial uncoupling. When mitochondrial functional indices were adjusted to citrate synthase activity, the activities of complexes II and III were increased in ICU patients 3-fold ($p<0.01$) respectively 2-fold ($p<0.01$). In myotubes form ICU patients the mitochondrial density was 69% of healthy controls ($p=0.051$). Fatty acid oxidation (FAO) capacity in these patients was 157% of FAO capacity in controls ($p=0.015$). Moreover, exposure of ICU myotubes to FFA significantly ($p=0.009$) increased maximum respiratory chain capacity. Of note, glutamine concentrations, consistent with moderate clinical hypoglutaminemia (300µM), bring about an optimal condition of myoblast proliferation and for efficiency of aerobic phosphorylation in an in vitro model of human skeletal muscle.

CONCLUSION: We first adopted high resolution respirometry to homogenates of human skeletal muscle and validated this method against isolated mitochondria and we adopted the protocol of extracellular flux analysis for the use in human myotubes. To our knowledge, this is the first study to demonstrate mitochondrial dysfunction in the skeletal muscle of patients with protracted critical illness. Importantly, functional mitochondria are depleted, but remaining mitochondria have a relative increase of fatty acid oxidation capacity and a long-term exposure to free fatty acids of these myotubes in turn leads to an increase in the capacity of the respiratory chain. Further studies are needed to evaluate whether similar changes are achievable by nutritional manipulations in vivo and whether improved mitochondrial function would translate to improved functional outcomes in ICU survivors.