Mitochondrial Dysfunction and Critical Illness Myopathy

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Introduction

Generalized muscle weakness is increasingly recognized to be a common and serious complication after prolonged intensive care treatment. This form of muscle weakness and paralysis was initially described by Bolton as a critical illness neuropathy [1]. However, research has demonstrated that critical illness polyneuropathy is frequently associated with critical illness myopathy, as well as existing as the sole pathology [2, 3]. This chapter will focus primarily on the pathophysiology of critical illness myopathy, and the possible role of mitochondrial dysfunction as a contributing factor.

Critical Illness Myopathy

Critical illness myopathy, a primary myopathy, is a term that encompasses a number of different types of myopathy. This includes a spectrum from myopathies with a pure functional impairment with normal histology, to those with atrophy and necrosis on histology [4]. There are also electrophysiological and biochemical criteria to support the diagnosis. The true occurrence of critical illness myopathy is unknown, as different incidences are reported depending on the case-mix, the diagnostic criteria used and the timing of the evaluation. However, in one multicenter study, 25% of all patients ventilated for 7 or more days had severe weakness during recovery [3]. Of those who underwent muscle biopsy, all had some element of myopathy as judged by histology.

Critical illness myopathy is probably under recognized because it has a similar clinical appearance to critical illness polyneuropathy and muscle biopsy is usually necessary to firmly establish the diagnosis [5]. Long-term follow-up of survivors with the acute respiratory distress syndrome (ARDS) suggest that critical illness myopathy may be more common than initial reports indicated. Prolonged (>1 year) muscle weakness was reported by the majority of patients at follow-up [6]. In addition, severe weakness and functional impairment were universally reported in intensive care unit (ICU) survivors in another long-term study [7]. Both of these reports suggest that critical illness myopathy may be an almost universal occurrence in the critically ill. Only the more extreme cases are currently identified clinically, but subtle weakness may be present in most survivors of severe illness.

Normal Skeletal Muscle Physiology

Skeletal muscle is composed of a large number of individual muscle fibers (Fig. 1). These cells lie parallel to one another and are separated by connective tissues, which contain blood vessels and nerves. There are two distinct types of muscle cells, type I and type II, which appear in a random mix. Each muscle cell is made up of myofibrils, which lie parallel to one another. Each myofibril contains many shorter components called sarcomeres, which are also arranged in parallel. The sarcomeres are composed of myofilaments. A myofilament consists of two parts: a thick filament composed of myosin molecules and a thin filament composed mainly of actin molecules together with troponin and tropomysin. The thick and the thin filaments interact to produce muscle contractions. Another important feature of skeletal muscle is the mitochondria [8], which lie next to the muscle filaments and produce the energy required for muscle contraction. These mitochondria are found in two distinct muscle compartments. Subsarcolemmal mitochondria are found close to the muscle periphery, under the sarcolemma. The mitochondria in the fiber centre, i.e., between the myofibrils, are called intermyofibrillar mitochondria. Different external stimuli, e.g., exercise, have selective effects on these populations.

The marked plasticity of human skeletal muscle is well documented [9]. Both atrophy and hypertrophy are under complex physiological control and a number of factors that influence muscle plasticity have clear relevance to the critically ill. These include [10]:

- Inactivity/bed rest/immobilization
- Denervation (neuromuscular blockade)
- Caloric restriction (malnutrition)
- Ageing
- Hyperglycemia
- Hormonal factors
- Pro-inflammatory mediators

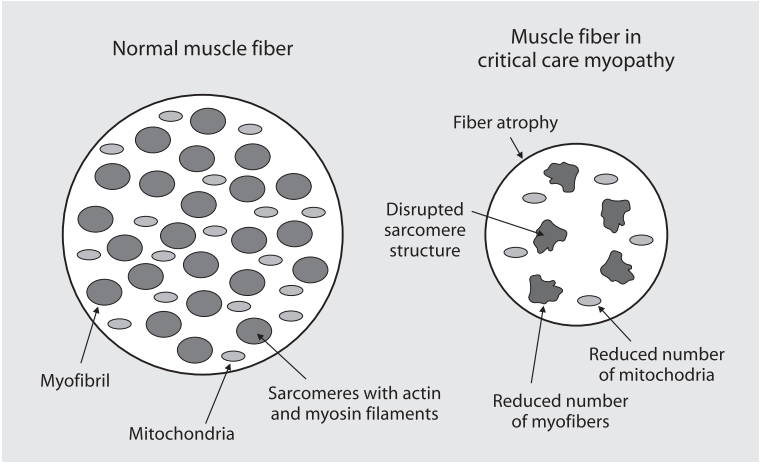

Fig. 1. Changes in skeletal muscle cell structure in critical illness myopathy. These include: 1) atrophy of myofibrils, 2) loss of sarcomere structure, 3) increase in space between myofibrils, and 4) reduction in mitochondria numbers. Selective loss of myosin filaments has also been reported

Structural Changes in the Muscle in Critical Illness Myopathy

There is a spectrum of histological changes that appear to be associated with critical illness myopathy, from normal histology to atrophy and necrosis (Fig. 1). The pathological features of myopathy are complex, but are classified into three main types:
1) atrophy of myofibers predominantly involving type 2 fibers;
2) degenerative-necrotic changes with signs of regeneration; and
3) selective loss of thick myosin myofilaments [11].

However, there is often significant overlapping of pathologies in the same patient.

Stibler and co-workers performed muscle biopsies on 11 patients with critical illness myopathy, as part of a study to develop a method to quantify myosin [12]. They found pathological changes in all muscle specimens. These were described as severe and extensive structural changes. On light microscopy, structural alterations included muscle fiber atrophy, degeneration, necrosis and regeneration. Loss of myosin ATPase activity was found to a varying degree in all the specimens, affecting both type I and II fibers. In severely affected muscles there was a loss of fiber differentiation. The most striking histological change was rounded and enlarged nuclei. On electron microscopy there was preferential loss of thick filaments, with a resulting loss of normal sarcometric structure. The loss of myosin filaments was seen even in the early stage of critical illness myopathy. In more severe disease stages there was increased space between the myofibrils, and complete loss of the sarcomeric pattern.

Bednarik and colleagues reported similar myopathic changes in muscle biopsies from patients with critical illness myopathy [11]. Ultra-structural examination showed myofibrilar disarray with loss of myosin filaments. Loss of myosin was considered to be a pathognomonic change with atrophy of myofibers being less specific.

Helliwell and co-workers examined 98 muscle biopsies from 57 critically ill patients, using histochemical staining for myosin ATPase and cytochrome c oxidase [13]. These authors found that fiber atrophy was a common observation and was related to the loss of the filamentous structure of myosin. The loss of structure occurred before there was substantial degradation of actin or cytoskeletal protein and was associated with increased expression of lysosomal enzymes and ubiquitin. Degenerative changes in the fibers were often associated with fiber atrophy, but both appeared to occur independently of muscle fiber necrosis [13].

In summary, there is only a limited amount of histological information on critical illness myopathy. Most studies have, of necessity, focused on severely weak patients. A broad range of changes are reported. While there is a consensus that severe inflammatory changes are lacking, there do not appear to be any clear defining histological features consistently present.

Causes of Critical Illness Myopathy

Critical illness myopathy was initially reported in patients following a prolonged ICU stay, with a higher incidence in patients diagnosed with sepsis, septic shock, and multiple organ failure (MOF) [14]. For example, de Letter and colleagues re-

ported that the APACHE III score, a quantitative index of disease severity based on clinical and laboratory physiologic data, was a valuable predictor of development of critical illness myopathy [15]. The association with severe sepsis led to the hypothesis that microcirculatory dysfunction or hyperinflammation may be involved in the damage of the motor neuron integrity [16]. In recent years, further research has shown that other factors contribute to critical illness myopathy. These include the use of glucocorticoids and muscle relaxants and the presence of hyperglycemia.

High-dose glucocorticoids may induce significant myopathy, with loss of the thick myofilaments. However, no simple relationship between the duration or dose and occurrence of myopathy has been observed. Short-term, high-dose steroid administration as used in acute exacerbations of asthma may lead to myopathy [17]. Steroids may cause weakness by inducing a form of functional denervation. Rich and Pinter have demonstrated a hyperpolarizing shift of voltage-dependence of sodium channel fast inactivation in rat muscle fibers exposed to steroids, which caused reduced excitability [18].

Patients receiving prolonged muscle relaxants have a higher incidence of critical illness myopathy in some studies. The mechanism is likely to be similar to that observed in denervation, both experimental and following spinal cord injury.

Hyperglycemia also appears to be a risk factor for critical illness myoneuropathy [16]. In a study of patients with ARDS, 60% had electromyographic evidence of myoneuropathy and this was associated with poor glycemic control. Insulin resistance and hyperglycemia accompany critical illness. It has recently been shown that preventing hyperglycemia with insulin improves outcomes. The Leuven study showed that the benefit of intensive insulin therapy in the ICU was particularly apparent among patients with prolonged critical illness [19]. In addition to decreasing mortality, intensive insulin therapy also prevented complications such as critical illness polyneuropathy and muscle weakness.

The Role of Mitochondria in Muscles

Mitochondria are a subcomponent of muscle cells that are bound by a double membrane [20]. They are involved in cellular homeostasis, intracellular signaling, apoptosis, intermediary metabolism, and in the metabolism of amino acids, lipids and nucleotides. They play a central role in cellular energy metabolism, including fatty acid oxidation, the urea cycle and the final pathway for ATP production, which involves the respiratory chain. The respiratory chain is a group of five enzyme complexes, which are situated on the inner mitochondrial membrane. Each complex is composed of multiple subunits. Electrons are donated to complex 1 and 2 from reduced cofactors (NADH and FADH). These electrons flow between the complexes, down an electrochemical gradient. They are transported via complexes 3 and 4 and by two-electron carriers, ubiquinone and cytochrome c. The liberated energy is ultimately used by complex 5 to synthesize adenosine triphosphate (ATP) from adenosine diphosphate and an inorganic phosphate. The overall process is called oxidative phosphorylation. ATP is the high-energy source, which is used for all active metabolic processes within the cells. It is released from the mitochondrion in exchange for cytosolic ADP. In muscle, mitochondria are strategically placed next to the muscle filaments to enable energy produced by the mitochondria to be transported easily to the site where it will be used by the muscle filaments.

Mitochondrial proteins are encoded for by two distinct genetic systems: mitochondrial DNA (mtDNA) and nuclear DNA [21].

Mitochondria and Critical Illness Myopathy

The existence of several, inherited conditions, known as mitochondrial myopathies, demonstrates that mitochondrial dysfunction can cause severe muscle weakness [20]. These illnesses are characterized by DNA mutations, either mitochondrial or nuclear, in the proteins of the mitochondrial respiratory chain. Mitochondria are very sensitive to damage by both intrinsic and extrinsic factors and it can be postulated that both short-term and longer-term damage to mitochondria could occur in the critically ill (Fig. 2).

Short-term changes could include:

- Direct damage to the mitochondria caused by both exogenous and endogenous toxins.
- A 'physiological' shutdown of mitochondrial energy generation. This postulated protective mechanism has been called the "hibernation hypothesis" of MOF [22].
- A physiological loss of mitochondrial numbers driven by disuse atrophy and prolonged bed rest.

Longer-term problems could involve illness-induced mutations in mitochondrial DNA which could impair skeletal muscle recovery and regeneration. These changes could be accentuations of those reported during normal human ageing [23].

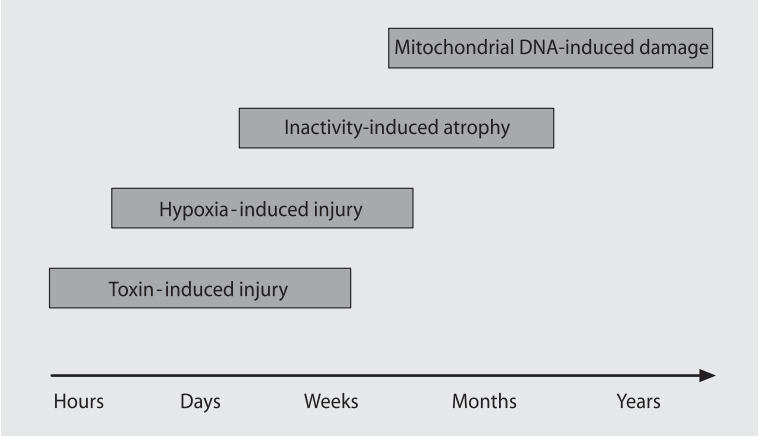

Fig. 2. Proposed time course of onset of various processes that may damage or disrupt skeletal muscle mitochondria in the critically ill

Short-term Damage to Mitochondria

Toxin-induced Injury

There is a substantial body of evidence that pro-inflammatory toxins cause mitochondrial dysfunction and damage [reviewed in [24]]. This evidence includes studies using separated mitochondria, whole cell preparations, isolated organs (including skeletal muscle), and animals exposed to experimental sepsis and endotoxins. A variety of molecular mechanisms has been proposed, including physical injury to the mitochondrial membrane and inhibition of respiratory chain proteins. In man, the data are less substantial. One of the few studies in human sepsis was conducted by Brealey and colleagues who performed skeletal muscle biopsies on 28 critically ill septic patients on the ICU [25]. The biopsy samples were analyzed for respiratory-chain activity, ATP concentration, reduced glutathione concentration, and nitrite/nitrate concentrations and were compared with control specimens. These researchers found that skeletal muscle ATP concentrations and ATP:ADP ratios were significantly lower in the septic patients who died, compared with survivors. Complex 1 activity appeared to be inversely related to illness severity. Damage or inhibition of complex 1 could decrease mitochondrial capability to generate ATP. Sepsis, therefore, appeared to cause mitochondrial dysfunction and decreased ATP concentrations that related to organ failure. Their data suggest bioenergetic failure as a pathophysiological mechanism underlying MOF [25]. Although the study did not directly investigate skeletal muscle function, it seems likely that mitochondrial energy failure in skeletal muscle would lead to significant muscle failure which would be clinically apparent as weakness.

Hypoxemia-induced Injury

Oxygen delivery is frequently impaired in the severely ill and could result in mitochondrial dysfunction. Hoppeler and co-workers reviewed the response of skeletal muscle mitochondria to hypoxia [26]. They examined the effects of short-term hypoxia (minutes to hours) and long-term hypoxia (weeks), both situations that are applicable to critically ill patients. Muscle biopsies from healthy volunteers exposed to long-term hypoxia (simulated Everest ascent) showed loss of muscle mitochondrial volume of approximately 30%, with a net loss of muscle oxidative capacity. Muscle biopsies showed considerable signs of muscle wasting and an accumulation of lipofuscin granules in muscle cells. Hypoxemia may induce mitochondrial damage by causing the release of reactive oxygen species (ROS) from mitochondria [27]. This could lead to a self-perpetuating situation where mitochondria exposed to hypoxia produce increased ROS which further damage the mitochondria.

Dietary/glucose-induced Injury

Dietary composition and caloric restriction can influence mitochondria function. It is, therefore, interesting to note the presence of ultra-structural abnormalities in mitochondria in a recent study of patients who died following a critical illness [28]. Post-mortem studies reported hypertrophic mitochondria in the liver in the majority of patients not treated with intensive insulin therapy to maintain blood glucose between 4.4 mmol/l and 6.1 mmol/l. Increased numbers of abnormal and

irregular cristae and reduced matrix density were also seen. In contrast, skeletal muscle obtained from patients in both groups had normal ultrastructure. The striated muscle cells contained mainly normal sarcomeres, and the mitochondrial arrangement appeared normal. Enzyme activities of the mitochondria in skeletal muscle were not significantly affected by intensive insulin therapy. However, skeletal muscle total protein content was higher in the intensive insulin group. In a subanalysis of septic versus non-septic patients, complex I activity was reduced in the septic group. This seems to confirm the existing evidence that sepsis is a contributing factor to mitochondrial dysfunction in skeletal muscle. The difference in the effects of insulin on the mitochondria in the liver and skeletal muscle remains unexplained but different mechanisms of glucose uptake may be important.

Inactivity-induced Injury

Critically ill patients have prolonged periods of inactivity due to the severity of the illness as well as through enforced sedation and muscle relaxation to facilitate appropriate treatment. Substantial evidence exists indicating that mitochondrial biogenesis is stimulated by aerobic exercise and reduced by inactivity [29]. Muscle mitochondrial oxidative enzymes, cytochrome-c oxidase and citrate synthase, and mRNA concentrations of several genes encoding mitochondrial proteins are enhanced by aerobic exercise. Mitochondrial biogenesis and mitochondrial DNA replication are stimulated by the expression of a constitutively active form of calcium/calmodulin-dependent protein kinase IV. Chronic electrical stimulation of skeletal muscle causes a sustained rise in intracellular calcium and activates calcium-regulated enzymes such as calcineurin and calcium/calmodulin-dependent protein kinase, both of which have been shown to activate slow and oxidative fiber gene expression in muscle cells. Muscle contractile activity though this mechanism enhances mitochondrial biogenesis and oxidative phenotype of skeletal muscle [23].

Longer-term Damage to Mitochondria

Age and Reduction in Mitochondrial Function

The population currently admitted to critical care units is increasingly elderly with median ages of over 70 years reported by some groups. Skeletal muscle function falls with age with a concomitant reduction in mitochondrial function [23]. Oxidant-induced mitochondrial damage, resulting in progressive loss of cellular energy resources is believed to play a key role in aging [30]. Mitochondria suffer more damage than other cellular components as they are the primary site of ROS formation.

The underlying cause of the reduction in mitochondrial biogenesis and ATP production seem to be decreases in mitochondrial DNA and messenger RNA content. Increased mitochondrial DNA oxidative damage with aging and cumulative DNA damage could explain the overall reduction in mitochondrial DNA in skeletal muscle. This reduction may contribute to reduced mRNA which results in reduced mitochondrial protein synthesis and enzyme activity. The overall effect is a reduced capacity for oxidative phosphorylation.

Mitochondrial alteration in ageing myocytes has been described extensively in skeletal muscles [31]. Aged myocyte mitochondria are more variable in size; some mitochondria enlarge enormously. Swelling, loss of cristae, and even complete destruction of inner membranes result in the formation of electron dense material. There is a drop in the inner membrane potential and a decrease in energy production.

A small number of experimental studies suggest that sepsis could produce persistent structural damage to mitochondrial DNA. In one study, liver mitochondrial DNA was examined following intra-peritoneal injection of lipopolysaccharide (LPS) in rats [32]. An oxidant-dependent mitochondrial DNA deletion was reported in the region encoding NADH dehydrogenase sub-units 1 and 2 and cytochrome oxidase sub-unit 1. The total liver mitochondrial DNA copy number also fell. The study did not examine changes in skeletal muscle. Similar, short-term falls in mitochondrial DNA copy number were reported in hearts from rats exposed to LPS [33]. This was reflected in a substantial fall in many respiratory chain proteins coded by mitochondrial DNA. In both studies, mitochondria recovery occurred by biogenesis. Whether similar damage to mitochondrial DNA occurs in human sepsis is unknown but the similarity of sepsis-induced mitochondrial DNA damage to that observed in normal human ageing is interesting.

Conclusion

Muscle weakness following critical illness is a significant clinical problem. It is likely to contribute to the continuing morbidity and mortality of prolonged ICU stay. However, its pathophysiology remains poorly understood. Several major issues remain unresolved. These include the question of whether critical illness myopathy is a distinct illness or represents a continuum of skeletal MOF.

Also unresolved is the differentiation of a postulated specific, sepsis-related muscle failure from other causes of muscle dysfunction in the critically ill. In particular, differences between diffuse atrophy, the normal ageing process, and sepsis-induced injury need to be explored. Finally, the question of possible mitochondrial DNA damage in human sepsis should be addressed. Normal human ageing is characterized by gradual damage to mitochondrial DNA. Could the long-term adverse consequences of prolonged critical care be partially a result of an increase in this process – a sort of 'ageing in the fast lane'?

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