European Journal of Anaesthesiology 2008; **25 (Suppl 42):** 73–82

doi: 10.1017/S0265021507003262

Original Article

Critical illness myopathy: sepsis-mediated failure of the peripheral nervous system

O. Friedrich

Ruprecht-Karls-University, Institute of Physiology & Pathophysiology, Department of System Physiology, Medical Biophysics, Heidelberg, Germany

Summary

With better survival of critically ill patients, '*de novo*' arising neuromuscular complications like critical illness myopathy or polyneuropathy have been increasingly observed. Prolonged hospitalization not only imposes risks like pneumonia or thrombosis on patients but also represents a real budget threat to modern intensive-care medicine. Clinical symptoms like muscle weakness and weaning failure are common to critical illness myopathy and critical illness polyneuropathy and do not allow for distinction. Specific therapies are not yet available, and the quest for the pathomechanisms has proved more complicated than anticipated. Especially for critical illness myopathy, multiple sites of disturbances to the excitation–contraction coupling cascade are possible causes of muscle weakness. The present review summarizes the epidemiological, clinical and diagnostic features of critical illness myopathy and then focuses on current concepts of the presumed pathomechanisms of critical illness myopathy. Sepsis was shown to be a major cause of critical illness myopathy and special emphasis will be placed on how sepsis and inflammatory mediators influence (i) the membrane excitability at the level of voltage-gated ion channels and (ii) the intracellular protein signalling that results in selective loss of myosin protein content and muscle wasting. For (i), critical illness myopathy represents a new type of acquired channelopathy affecting the inactivation properties of Na^+ channels. For (ii), both protein proteolysis and protein build up at the transcriptional level seem to be involved. Findings from different studies are put into a common context to propose a model for cytokine-mediated failure of muscle in severe sepsis. This can open a series of new possible trials to test specific therapeutic strategies in the future.

Keywords: POLYNEUROPATHY; CRITICAL CARE; MUCULAR DISEASES; NEUROMUSCULAR DISEASES; SEPSIS; MYOPATHY.

Introduction

What is bad? – Everything that arises from weakness.
(Friedrich Nietzsche, 1844–1900)

Critical illness is one of the major causes of hospital admissions and referral to ICUs world-wide with incidences of at least ~300/100 000 for Northern America although precise figures are difficult to assess [1]. About 5–10% of ICU admissions may represent prolonged-stay patients (e.g. >10 to >21 days [2]). However, this group may use up to ~40% of ICU beds and account for at least one third of overall hospital mortality [2].

Despite the progress made in intensive care medicine for treating the underlying conditions of critical illness (e.g. myocardial infarction), sepsis has become a major limiting complication over the years with ~750 000 cases of severe sepsis (sepsis plus organ dysfunction) per year in the USA and approximately 18 Mio. Worldwide [3]. Sepsis and severe sepsis together account for up to one fourth of

Correspondence to: Oliver Friedrich, Medical Biophysics, Department of System Physiology, Institute of Physiology & Pathophysiology, Ruprecht-Karls-University, Im Neuenheimer Feld 326, 69120 Heidelberg, Germany. E-mail: oliver.friedrich@physiologie.uni-heidelberg.de; Tel: +49-6221-54-4143; Fax: +49-6221-54-4123

ICU admissions and result in average mortalities of at least 50%. The incidence of severe sepsis/septic shock in Germany (110 per 100 000 residents) is now comparable to myocardial infarction and represents the third most frequent cause of death with ~160 patients dying each day [4]. Similar values might apply for other European countries. Apart from killing humans, sepsis represents a budget-killer with six times greater costs compared to ICU patients without sepsis. It is mostly because of sepsis-related organ dysfunction that intensive care costs are so high. Among these, '*de novo*' arising neuromuscular abnormalities have been more frequently observed in the last two decades [5–8]. These are represented by primarily neuropathic (*critical illness polyneuropathy*, CIP [9]) and primarily myopathic (*critical illness myopathy*, CIM [10]) disorders. Although not life-threatening, these disorders of the peripheral nervous system must now be regarded as part of the sepsis-associated multi-organ failure (MOF). Depending on the severity of sepsis and MOF, 60–80% of patients develop CIP and presumably a similar number CIM [6,11,12]. However, recent studies suggested that probably a majority of patients develop a mixture of both forms, sometimes termed critical illness polyneuromyopathy (CIPNM) [12–14]. The jury is still out on whether CIP and CIM are distinct disorders with a separate pathophysiology, or just different manifestations of a common pathomechanism applied to nerve or muscle tissue. Nonetheless, they are both triggered by sepsis [12] and other factors [6,15].

The present review gives an overview of current concepts regarding the pathomechanisms of CIM. After a short summary of the clinical features and recent approaches for early diagnosis, pathomechanisms and different trigger factors for CIM will be presented and discussed with a main focus on sepsis and inflammatory mediators. Finally, biophysical methods to further study the effects of sepsis in intact muscle cells using e.g. advanced high-resolution microscopy techniques will be introduced as an outline.

Clinical features of CIM

Muscle weakness

The clinical features and diagnostic signs of CIM are summarized in Table 1. Along with CIP, CIM is often anticipated in patients who experience difficulties with weaning or voluntary movements when sedation is ceased. Muscle weakness involves pronounced paresis of proximal and distal muscle groups and can be as severe as quadriplegia. Muscle atrophy may develop and muscle reflexes may vanish [5]. Upon extubation, patients with neuromuscular disorders develop drops in oxygen saturation and hyercapnia. Re-intubation rates are twice as high as in patients without neuromuscular disorders [16]. The quick onset of myopathy following sepsis supports an active process in muscle tissue that cannot be explained by disuse atrophy alone. Surprisingly, although weakness is the leading symptom, there have only been a few attempts to quantify both the time-course and the extent of force parameters in CIM as compared to immobilization itself [17,18]. In a prospective clinical trial, contractile parameters of foot and ankle muscles were measured in 19 critically ill patients who were ventilated and completely immobilized for 1 week and compared to healthy controls [19]. Involuntary isometric peak force upon triple pulse nerve stimulation was markedly reduced (~40%) and relaxation prolonged.

Table 1. Clinical symptoms and diagnostic signs of critical illness myopathy.

Clinical symptoms	• Weaning failure (re-intubation rate ↑, hypoxia, hypercapnia)
	• Muscle paresis or plegia (proximal and distal muscles), flaccid limbs
	• Hypo-/areflexia depending on degree of myopathy and co-existing CIP
	• Muscle atrophy may develop (>1 week)
	• Positive correlation with sepsis, steroids, NMBAs
	• Sensibility unaffected
Electrophysiology	• CMAP ↓, fibrillation potentials → common with CIP, CIPNM
	• Negative recruitment of motor unit potentials (not feasible in sedated or encephalopathic patients)
	• DMS: dmCMAP↓, neCMAP↔, ne/dmCMAP >0.5
Histopathology	• Minimal change myopathy: calibre variations, internalized nuclei, atrophy
	• Hyperproteolytic 'thick-filament myopathy': selective myosin loss
	• Necrotizing myopathy
	• Apoptotic myopathy

CIP: critical illness polyneuropathy; NMBA: neuro-muscular blocking agent; DMS: direct muscle stimulation; CMAP: compound muscle action potential; neCMAP: nervestimulated CMAP; dmCMAP: direct musclestimulated CMAP.

The patient cohort, however, was quite diverse with 6/19 septic patients and varying degrees of steroid use. Very recently, Eikermann and colleagues [20] followed the time-course of adductor pollicis force and fatigue pattern in patients with sepsis and MOF upon tetanic ulnar nerve stimulation and compared those to recordings in healthy volunteers immobilized for 2 weeks. Their results show that already 24 h after admission of septic patients, peak force is reduced by ~70% but fatigability is not increased compared to immobilized muscle. This can be explained by preferential atrophy of type-II fibres in immobilization and inflammation that will reduce the proportion of fatigable muscle [21,22]. However, more recent studies suggest a similar type-I and -II atrophy in immobilized animals [23]. CIM is a primary myopathy with selective proteolysis of muscle myosin. Therefore, weakness cannot be prevented or ameliorated by active muscle training [24].

Electrophysiological signs

In pure myopathic changes, nerve action potentials, nerve conduction and neuromuscular transmission appear normal after repeated stimulation, although the latter might be secondarily impaired by local inflammation within the muscle tissue [25]. Typically, compound muscle action potentials (CMAP) are markedly reduced [6]. Conventional needle electromyography (EMG) is complicated by the fact that (i) neuropathic signs such as fibrillation potentials and sharp waves do not exclude CIM as in most patients CIP and CIM might co-exist [12] and (ii) voluntary recruitment of motor unit potentials cannot be tested in sedated patients or in those with sepsis-related encephalopathy. However, early detection of CIM would be desirable as this might have an impact on therapeutic strategies. Based on probable EMG/electrophysiology criteria for CIM, the time-course of neuromuscular dysfunction in patients with severe sepsis was recently followed in a prospective study [12]. The authors found that 72 h after admission, 50% of patients had electrophysiological signs of neuromuscular dysfunction. Of those, 10% had an isolated CIM or CIP whereas 80% had CIPNM. With the introduction of 'direct muscle stimulation' techniques (DMS) [26], a more selective electrophysiological tool to differentiate between CIM and CIP has become available (Fig. 1). Briefly, nervestimulated CMAPs (neCMAP) are compared to CMAPs directly stimulated by an intramuscular electrode (dmCMAP, Fig. 1). In healthy situations the ratio ne/dmCMAP is close to unity, very small ratios indicate CIP and ratios >0.5 support the diagnosis of CIM or CIPNM (where dmCMAPs are strongly reduced and neCMAPs depending on

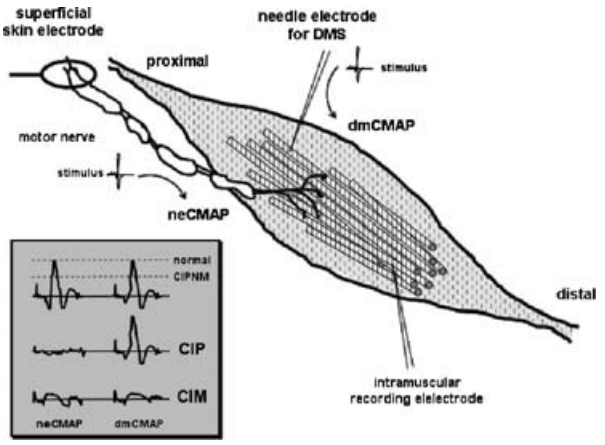

Figure 1.
Direct muscle stimulation (DMS) is suitable for distinguishing between CIP and CIM. Muscle compound action potentials (CMAP) are evoked either by a superficial electrode to stimulate the motor nerve (neCMAP) or directly via an intramuscular needle electrode (dmCMAP). In pure CIP, neCMAPs are diminished but dmCMAPs are normal. In pure CIM, both are diminished. In CIPNM, both signals may appear similar but with reduced amplitude depending on severity. However, if either CIP or CIM is predominant in CIPNM, the corresponding signals will be more CIP- or CIM-like.

whether there is a mixture of CIP and CIM and to what extent axons are affected) [11,13]. However, even in pure CIM there is no 100% proof from DMS as reduction in muscle membrane excitability is a prerequisite for its detection. Pure proteolytic or necrotic forms of CIM might be undetected as the remaining muscle fibres may present normal membrane excitability. In such cases, creatine phosphokinase levels may be of diagnostic relevance [27].

Histopathology

A muscle biopsy is suitable for proving CIM. However, given the high incidence of CIM, this invasive procedure is not feasible in all cases and often is not a guideline procedure in the diagnosis of ICU neuromuscular disorders. Different sub-types exist (Table 1). The most common feature in CIM is a selective loss of thick-myosin filaments [28–30].

Etiopathology and pathomechanisms for CIM: role of sepsis

Table 2 lists the major risk factors that have been associated with the etiopathology of CIM (for review see [5,6,11,15]). Female sex, duration of artificial ventilation and organ dysfunction are independent predictors of weakness in critically ill patients [31].

Muscle force production is the result of a complex cascade of signalling events that include binding of

Table 2. Etiopathological causes and their proposed mechanisms for critical illness myopathy.

Risk factor	Proposed mechanisms
Corticosteroids	• Activation of ubiquitin-proteasome
	• Reduced IGF-effects: less myosin synthesis, increased protein turnover
	• Fibre type-II atrophies
	• Apoptosis via Fas–Fas-ligand induction
	• Necrosis, proteolysis and apoptosis can all be induced in parallel in muscle by steroids.
Neuromuscular blockers (NMBA)	• Denervation
	• Up-regulation of juvenile AChR with lower responsivity
	• Hypoexcitability of muscle plasma membrane
	• Expression of embryonic Na^+-channel isoforms ($Na_v1.5$)
	• Increased steroid-sensitivity
	• Impaired presynaptic exocytosis and ACh release at neuromuscular junction
Sepsis/SIRS	• Cytokine-mediated activation of ubiquitin-proteasome (TNF-α, IL-1, IFN-γ
	• Catabolic metabolism: mobilization of muscle proteins for hepatic gluconeogenesis
	• Local insulin resistance and increased glucagon sensitivity
	• Impaired muscle repair mechanisms (e.g. MyoD-inhibition)
	• Bioenergetical breakdown (ATP-depletion)
	• Mitochondrial dysfunction (apoptose-trigger: cytochrome C)
	• Consumption of anti-oxidants, ROS production (peroxidative stress)
	• Acquired channelopathy (e.g. RYR, voltage-gated Na^+-channels)

IGF: insulin-like growth factor; MyoD: myogenic differentiation factor; TNF: tumour necrosis factor; IL: interleukin; IFN: interferon; ROS: reactive oxygen species; ACh: acetylcholine; RYR: ryanodine receptor.

acetylcholine (ACh) to the neuromuscular endplate, activation of voltage-gated Na^+ and K^+ channels to trigger action potentials, activation of dihydropyridine-receptor Ca^{2+} channels (DHPR) to release Ca^{2+} ions from the sarcoplasmic reticulum (SR) via a direct protein–protein coupling of the DHPR to ryanodine channels (RYR1), downstream to a rise in myoplasmic [Ca^{2+}] concentration and subsequent Ca^{2+} binding to troponin-C that in turn rotates the 'Ca^{2+} switch' of the troponin-tropomyosin complex to have myosin heads bind to actin filaments [32]. This triggers the cross-bridge cycle as long as [Ca^{2+}] is elevated and adenosine triphosphate (ATP) as fuel is present [33]. Therefore, Ca^{2+} is the main messenger of this system and has to be tightly regulated.

As disturbances at any stage will result in the common symptom 'muscle weakness,' a full spectra of pathomechanisms in CIM can be expected. The main clinical features of muscle inexcitability and proteolysis are also reflected in the primary targets at the cellular level: the membrane and the contractile proteins.

The role of neuromuscular blocking agents (NMBAs) in the development of CIM has recently been reviewed by Murray and colleagues [34]. Although the association of NMBAs and CIM has sometimes been controversial [35], metabolites of the nondepolarizing NMBAs are most likely responsible for persistent weakness seen in up to 70% of patients after prolonged administration [34]. The action of NMBAs seems mainly confined to the muscle membrane with the up-regulation of juvenile ACh-receptors that have a lower ACh-sensitivity and also impaired transmitter release into the neuromuscular cleft. NMBAs also sensitize muscle for glucocorticoids by increasing the amount of cytosolic glucocorticoid receptors [36], a mechanism that has also been confirmed in sepsis [37].

Mechanisms affecting membrane excitability

The hypo-/inexcitability of muscle in CIM originates from the molecular effects of sepsis and steroids on membrane ion channels. In the initial phase of sepsis where intense inflammatory activity predominates, high levels of the cytokines tumour necrosis factor-α (TNF-α), interleukin-1 (IL-1) and IL-6 stimulate the hypothalamic–pituitary axis. This results in an increase in circulating glucocorticoid levels to limit the immune response by the inhibition of further proinflammatory cytokine production [38]. In the course of severe sepsis or septic shock, however, adrenal insufficiency may develop reverting the cytokine–glucocorticoid ratio. Thus, there is an early emphasis on glucocorticoid action on muscle membrane followed, presumably, by a late dominant cytokine action. Some effects of

sepsis and steroids on the membrane are of surprising similarity while there are also distinct differences. For example, in a series of studies comparing membrane properties of rat *extensor digitorum longus* muscles 1 week following either surgical denervation or in combination with high-dose dexamethasone treatment (steroid-denervated, SD), Rich and colleagues [39] found that both denervation and steroid treatment induced a marked decrease in membrane excitability with the inability of fibres to produce action potentials. In this animal model of CIM, resting potentials were depolarized to similar extents in denervated and SD fibres, specific membrane resistances decreased and Na^+ current amplitudes cut down by 50%. This could be explained by an ~10 mV hyperpolarizing shift of the steadystate fast inactivation curves of adult skeletal muscle $Na_v1.4$ sodium channels reflecting reduced availabilities of Na^+ channels at the resting membrane potential. This explains the hypo-/inexcitability [40] (Fig. 2a). The reduced availability was not unique to steroid treatment but also occurred following denervation alone. However, steroids seemed to increase the percentage of fibres in which the fast inactivation of Na^+ channels was shifted, whereas denervation induced these shifts only in a minority of fibres. Slow inactivation of Na^+ channels that predominates the membrane excitability near the resting membrane potential on a longer time scale (~min) was also found to be an important contributor in SD inexcitable fibres [41]. However, some properties of slow inactivation, i.e. its very shallow sensitivity, also tended to decrease the percentage of slowly inactivated channels at the resting potential, representing a compensatory mechanism to increase excitability in chronically SD muscle [41]. Although denervation increases the percentage of embryonic $Na_v1.5$ channel isoforms (that have ~20 mV hyperpolarizing shifts in fast inactivation) in muscle by up to 30%, this relative decrease in Na^+ channel availability for action potential generation cannot fully explain fibre inexcitability [42]. Therefore, steroids and denervation seem to alter gating of channels directly via a yet unknown mechanism.

Sepsis and septic shock themselves have also been shown to alter the membrane properties of skeletal muscle, although there are only few studies in the literature. Early studies in the 80 s already found marked depolarizations, decreased action potential amplitudes and prolonged kinetics in mammals after septic shock [43,44]. TNF-α at plasma concentrations, found following lethal exposure to endotoxins, induced marked depolarizations by ~15 mV when acutely applied to healthy muscle cells [45]. Surprisingly, neither the exact underlying

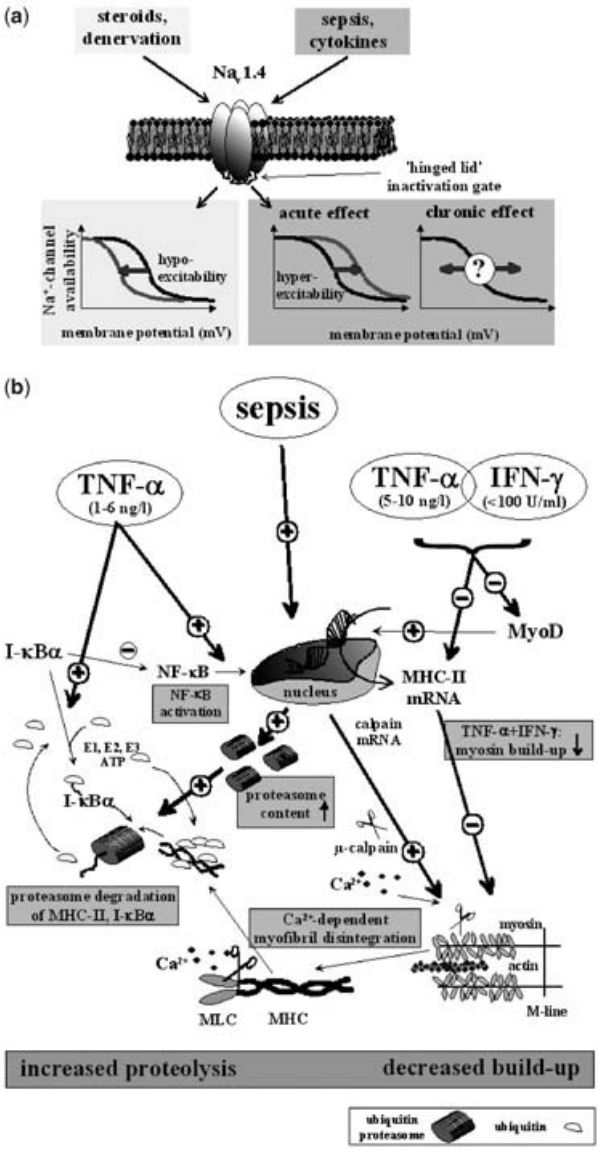

Figure 2.
*Pathomechanisms of CIM mediated by cytokines or steroids at the level of membrane excitability (**a**) or myofibrillar protein homeostasis (**b**). Steroids and sepsis both induce depolarized membrane resting potentials but act differerentially on the availability of voltage-gated $Na_v1.4$ channels. TNF-α and IFN-γ have been shown to differentially act either on selective myosin proteolysis or myosin build up, resulting in cytokine-mediated myosin loss. For details see text.*

mechanisms nor the isolated effects of other cytokines to the muscle membrane have been studied extensively to date.

In a top-down approach, we have tested the acute application of different serum fractions from CIM patients to healthy isolated murine toe muscle fibres and could confirm acute depolarizations of ~5–8 mV within several minutes [46]. However, a marked difference to the SD model (see above) was found. Whereas fast inactivation of Na^+ channels

was shifted towards hyperpolarized potentials in SD fibres, CIM sera sharply induced marked depolarization shifts (Fig. 2a), especially for serum fractions with molecular weight cut-offs >10 kDa. For 10 kDa cut-offs, the shift was slightly directed to hyperpolarized potentials [46]. This finding suggests that serum factors (>10 kDa) acutely induce an increase in Na^+ channel availability presumably to compensate for the depolarized resting membrane potentials. Therefore, in sepsis, there might be a sharp increase in muscle excitability. Indeed, very recently, acute sepsis induced by caecal ligation and puncture (CLP) in rats was found to attenuate the membrane effects of neuromuscular blockers and to increase the excitability of muscle membrane [47]. Further research is needed to clarify whether, in a long-term situation of sepsis in isolated muscle fibres, the hyperexcitability turns into hypoexcitability, as seen in patients. Also, bottom-up approaches testing single cytokine action profiles on muscle membrane excitability would be useful to reveal the differential action of cytokines in CIM.

Mechanisms of hyperproteolysis and muscle wasting in CIM

Among the pathophysiological aspects of CIM, hyperproteolysis induced by sepsis and steroids is probably the most extensively studied. Muscle wasting represents a loss of lean body mass as part of a common cachectic response seen in catabolic conditions such as tumour, chronic inflammatory diseases, AIDS, critical illness and sepsis that can achieve rates of up to 10% per week [48]. This depletion of muscle proteins was shown to be selectively targeted towards myosin heavy chains (MHCs) that make up to 40% of the myofibrillar protein content in adult muscle. Electromicrographs from septic muscle show a highly selective loss of thick filaments while other myofibrillar structure proteins, i.e. troponin-T, tropomyosin or actin, are unaffected [49]. Different mechanisms have been elucidated so far to explain myofibrillar proteolysis (Fig. 2b) including Ca^{2+}-dependent, lysosomal or ATP-dependent pathways (reviewed, e.g. in [15,50,51]). Release of myofilaments is enabled by sepsis-induced disintegration of sarcomer bands by the increased expression of calpains. The Ca^{2+} dependence of this proteolysis mechanism on Ca^{2+} release from intracellular stores is crucial as dantrolene was able to block this proteolytic response to sepsis in rats [49]. It seems well established that some proinflammatory cytokines, i.e. TNF-α, interferon-γ (IFN-γ) and IL-1 (Table 3), are able to increase the expression of the ubiquitin-proteasome. Protein degradation by the proteasome first requires conjugation of the protein substrate to ubiquitin in an ATP-dependent reaction involving different enzymes (E1, E2, ubiquitin-protein ligase E3). This conjugation cycle is repeated until the lysine residues of the protein are marked with a chain of several ubiquitins before it is transferred to the 26S proteasome. There, the proteins are cut down to small peptides in the 20S core proteasome and the ubiquitins are cut-off and recycled [50]. All steps herein require ATP. Although initially it has been reasoned that activation of the ubiquitin-proteasome was the primary cause of myofibrillar breakdown in sepsis this concept has been seen in a new light after finding that the proteasome does not degrade intact myofibrils probably due to some specific myofibrillar protein interactions that protect myosin from ubiquitination [52]. In their animal model of CLP-induced sepsis in rats, Williams and colleagues [49] found a predominant role of Ca^{2+}-dependent proteolysis 16 h after CLP that could be the initial step for degradation of intact myofibrils before they can be further processed by the proteasome. Increased activity of the latter would then just be the result of an increased amount of available substrate for ubiquitination and important for 'clean-up' in septic muscle [49]. TNF-α plays a key role in sepsis-mediated myosin II loss although it does not seem to stimulate muscle catabolism directly [53]. TNF-α induces a couple of transcription factors, among them nuclear factor-κB (NF-κB). During systemic inflammatory response, TNF-α stimulates ubiquitin-conjugation of the inhibitory protein I-κBα that is normally bound to NF-κB. This conjugation activates NF-κB, which is then transferred to the nucleus to induce the pleiotropic transcription of various proteins, i.e. inflammatory mediators, the ubiquitin-proteasome, Ca^{2+}-dependent proteases, etc. [54]. This is, e.g. reflected in the local inflammation found in septic muscle [25]. The increase in ubiquitin-proteasome activity in sepsis occurs at a very rapid speed. At concentrations of 3 ng mL^{-1} that are comparable to plasma concentrations found in inflammatory diseases and cancer patients [53], I-κBα levels markedly dropped already after 15 min in skeletal muscle myotubes. This was paralleled by a 100% increase in total cellular ubiquitin-conjugation after 30 min, resulting in a 30% loss of MHC protein content [53]. The activation of NF-κB and proteolysis is also enhanced by reactive oxygen species (ROS) as a result of mitochondrial dysfunction and also as a direct effect of TNF-α [3].

Muscle protein breakdown can not only be regulated by an increase in proteolysis but also by a reduction in protein build up induced by TNF-α. Interestingly, whether cytokines stimulate catabolism or impair anabolism in muscle seems to be

Table 3. Inflammatory mediators in sepsis and their putative role in development of critical illness myopathy.

Inflammatory mediator	Molecular weight (kDa)	Effects in muscle and other organs
TNF-α (cachectin)	26 (mature secreted form: 17)	• Depolarization of plasma membrane • Muscle proteolysis ↑ • Activates ubiquitin-proteasome pathway • Selective RNA-dependent down-regulation of MHC II (in combination with IFN-γ) • Activation of NF-κB • Down-regulation of MyoD inhibits myogenic differentiation • Insulin resistance, glucagon ↑
IL-1	13–17 (precursors: 33)	• Produced by activated macrophages • Muscle proteolysis ↑ • Peripheral protein wasting, liver protein content ↔ • Stimulates arachidonic acid metabolism • Ubiquitin gene expression ↑ or ↔
IL-6	23–30 (glycosylation diversity)	• Produced by activated macrophages and T-cells, tumour cells • No effect on ubiquitin expression • Up-regulation of complement factor receptors
IFN-γ	20–25	• Produced by activated T-cells and natural killer cells • Selective RNA-dependent down-regulation of MHC II (in combination with TNF-α) • Increases inducible NO-synthase activity in skeletal muscle endothelial cells • Induces cytokine receptor mRNA in muscle

TNF: tumour necrosis factor; MHC: myosin heavy chain; IFN: interferon; NF: nuclear factor; MyoD: myogenic differentiation factor; IL: interleukin.

regulated in a concentration-dependent manner. In a recent study, it was convincingly shown that in the same myotube preparation where TNF-α induced proteolysis at concentrations from 1 to $6\,ng\,mL^{-1}$ [53], TNF-α concentrations between 5 and $10\,ng\,mL^{-1}$ completely failed to promote muscle proteolysis [55], suggesting the concerted action of factors additional to TNF-α in sepsis/septic shock where TNF-α plasma concentrations can be as high as $20\,ng\,mL^{-1}$ [56]. However, the combination of TNF-α and IFN-γ potently reduced myosin expression in cell cultures and in mice through an RNA-dependent mechanism. Down-regulation of MHC IIa and IIb promoter activity required persistent TNF/IFN signalling and occurred as early as 6 h after cytokine treatment. Since the transcriptional activation of MHC II isoforms is under tight control of the myogenic-differentiation/myocyte-regulatory factor MyoD, the transcriptional block of MHC II was due to the cytokine-mediated inhibition of MyoD synthesis [55]. With the finding that TNF/IFN at higher concentrations did not alter the rate of MHC II turnover, it was concluded that proteolysis might not be the predominant mechanism in sepsis/severe sepsis, as compared to tumour cachexis, but a primarily impaired MHC II transcriptional build up [55] (Fig. 2b).

It is notable that the activation of the ubiquitin-proteasome is also under control of pH as experimentally induced metabolic acidosis causes an increase in the proteasome mRNA that can be reverted by the correction of acidosis [57]. Last but not least, steroids and denervation can also increase muscle protein turnover by the ubiquitin-proteasome [58]. Denervation sensitizes for steroids by the up-regulation of cytosolic glucocorticoid receptors [59]. Steroids themselves induce muscle wasting through a direct increase in ubiquitin-proteasome ATPase mRNA and activity [60], muscle necrosis and apoptosis [61].

Other targets for sepsis-induced failure of muscle

Besides muscle membrane and myofibrillar proteins, sepsis and systemic inflammation have been shown to have additional (sub)cellular targets: bioenergetic failure due to insulin resistance, cytopathic hypoxia, dyslipidemia, reduced anti-oxidative capacity due to increased ROS, mitochondrial dysfunction, hormonal derangement etc. These effects and their pathways have been reviewed recently elsewhere [6,11,15,62,63].

In our laboratory, we have studied the effects of acute 'septic load' on the muscle membrane (see

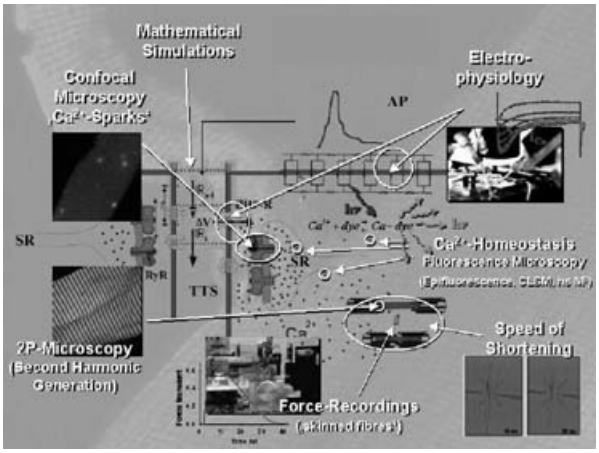

Figure 3.
Biophysical methods suitable to study the effects of inflammatory mediators in sepsis at various stages of the excitation–contraction coupling in intact single muscle fibres or functional subcellular extracts (e.g. actin–myosin extracts). Membrane excitability can be monitored using voltage- and patch-clamp techniques, Ca^{2+} homeostasis is resolved via different Ca^{2+}-fluorescence technqiues (epifluorescence, confocal, multifocal), myosin can be monitored using Second Harmonic Generation *and acto–myosin interaction is tested using either* 'skinned muscle fibre bundles', *speed of shortening imaging or* 'in vitro' *motility assays. Mathematical modelling is used at each instance. CLSM: confocal laser scanning microscopy. hsM^3: high-speed multi-focal-multi-photon-microscopy.*

above) and intracellular Ca^{2+} signalling homeostasis for muscle fibre activation. In permeabilized muscle fibres, caffeine-stimulated Ca^{2+}-activated force transients were depressed in amplitude by acute incubation with low-molecular weight serum fractions from septic patients who developed myopathy [46]. Molecular weights of actions were well below 30 kDa and may reflect the isolated or concerted action of various cytokines (Table 3) on the Ca^{2+} release at the level of the ryanodine receptors. Together with results from muscle membrane, CIM in sepsis may be regarded as an 'aquired channelopathy'.

Therapeutic concepts for treatment of CIM

There is no specific therapy yet for the treatment of CIM. As steroids and NMBAs may have a strong myotoxic potential, they should be used with caution. '*Stress steroid doses*' do not seem to induce myopathy. Although still a major risk factor, treatment of sepsis has the highest priority. Different established or experimentally promising treatment strategies, e.g. the use of activated protein C, nutritional support with substitution of glutamine, arginine, nucleotides or φ3-fatty acids, anti-oxidants, phosphodiesterase inhibitors etc. are reviewed in Riedemann and colleagues [64]. Intensified insulin therapy was successful in reducing the overall mortality in surgical ICU patients and has become part of many guidelines for ICU therapy. For medical patients the situation might be different and is still a matter of clinical trials [65].

Future directions for research on the pathomechanisms in CIM

As detailed in the previous sections, much of our current knowledge on the pathomechanisms of CIM has come from biochemical, histochemical and molecular biology '*in vitro*' and also from experimental animal studies. Single-cell physiological studies have been mainly used to elucidate effects of denervation and steroids on membrane ion channels. The effect of cytokines on the excitation–contraction coupling and the Ca^{2+} homeostasis has been mainly studied in cardiac muscles so far [66]. Results from our laboratory already point towards disturbances in the Ca^{2+} homeostasis in skeletal muscle related to sepsis [46]. Future challenges will be to resolve short- and long-term cytokine actions '*on-line*' in the living cell using high-resolution optical methods such as confocal Ca^{2+}-imaging or multi-photon microscopy of intrinsic myosin signals. Also, ion channel electrophysiology has not been studied in detail in septic muscle. Figure 3 gives an overview of methods that have been established in our laboratory in the last couple of years and can be used to resolve different aspects of muscle physiology and pathophysiology, i.e. to further elucidate the distinct subcellular mechanisms of CIM.

Grant support: Institutional funds.

References

1. Laupland KB. Population-based epidemiology of intensive care: critical importance of ascertainment of residency status. *Critical Care* 2004; **8**: R431–R436.
2. Martin CM, Hill AD, Burns K, Chen LM. Characteristics and outcomes for critically ill patients with prolonged intensive care unit stays. *Crit Care Med* 2005; **33**: 1922–1927.
3. Angus DC, Linde-Zwirble WT, Lidicker J, Clermont G, Garcillo J, Pinsky MR. Epidemiology of severe sepsis in the United States: analysis of incidence, outcome and associated costs of care. *Crit Care Med* 2001; **29**: 1303–1310.
4. Brunkhorst FM, Engel C, Reinhart K *et al*. Epidemiology of severe sepsis and septic shock in Germany: results from the German 'Prevalence' study. *Critical Care* 2005; **9**: P196.
5. Hund E. Neurological complications of sepsis: critical illness polyneuropathy and myopathy. *J Neurol* 2001; **248**: 929–934.
6. Bolton CF. Neuromuscular manifestations of critical illness. *Muscle Nerve* 2005; **32**: 140–163.

7. Bolton CF, Young GB, Zochodne DW. The neurological complications of sepsis. *Ann Neurol* 1993; **33**: 94–100.
8. Pastores SM. Critical illness polyneuropathy and myopathy in acute respiratory distress syndrome: more common than we realize! *Crit Care Med* 2005; **33**: 895–896.
9. Zochodne DW, Bolton CF, Wells GA *et al*. Critical illness polyneuropathy: a complication of sepsis and multiple organ failure. *Brain* 1987; **110**: 819–841.
10. Latronico N, Fenzi F, Recupero D *et al*. Critical illness myopathy and neuropathy. *Lancet* 1996; **347**: 1579–1582.
11. Latronico N, Peli E, Botteri M. Critical illness myopathy and neuropathy. *Curr Opin Crit Care* 2005; **11**: 126–132.
12. Khan J, Harrison TB, Rich MM, Moss M. Early development of critical illness myopathy and neuropathy in patients with severe sepsis. *Neurology* 2006; **67**: 1421–1425.
13. Bednarik J, Lukas Z, Vondracek P. Critical illness polyneuromyopathy: the electrophysiological components of a complex entity. *Crit Care Med* 2003; **29**: 1505–1514.
14. Latronico N. Neuromuscular alterations in critically ill patients: critical illness myopathy, critical illness neuropathy, or both? *Intensive Care Med* 2003; **29**: 1411–1413.
15. Friedrich O. Critical illness myopathy: what is happening? *Curr Opin Clin Nutr Metab Care* 2006; **9**: 403–409.
16. De Jonghe B, Bastuji-Garin S, Sharshar T, Outin H, Brochard L. Does ICU-acquired paresis lengthen weaning from mechanical ventilation? *Intensive Care Med* 2004; **30**: 1117–1121.
17. Zarzhevsky N, Menashe O, Carmeli E, Stein H, Reznick AZ. Capacity for recovery and possible mechanism in immobilisation atrophy of young and old animals. *Ann NY Acad Sci* 2001; **928**: 212–225.
18. Widrick JJ, Norenberg KM, Romantowski JG *et al*. Force velocity power and force-pCa relationships of human soleus fibres after 17 days of bed rest. *J Appl Physiol* 1998; **85**: 1949–1956.
19. Ginz HF, Iaizzo PA, Girard T, Urwyler A, Parrger H. Decreased isometric skeletal muscle force in critically ill patients. *Swiss Med Wkly* 2005; **135**: 555–561.
20. Eikermann M, Koch G, Gerwig M *et al*. Muscle force and fatigue in patients with sepsis and multiorgan failure. *Intensive Care Med* 2006; **32**: 251–259.
21. Diaz NL, Finol HJ, Torres SH, Zambrano CI, Adjounian H. Histochemical and ultrastructural study of skeletal muscle in patients with sepsis and multiple organ failure syndrome (MOFS). *Histol Histopathol* 1998; **13**: 121–128.
22. Reardon KA, Davis J, Kapsa RM, Choong P, Byrne E. Myostatin, insulin-like growth factor-1 and leukimia inhibitory factor mRNAs are upregulated in chronic human disuse muscle atrophy. *Muscle Nerve* 2001; **24**: 893–899.
23. Machida S, Booth FW. Changes in signalling molecule levels in 10-day hindlimb immobilized rat muscles. *Acta Physiol Scand* 2005; **183**: 171–179.
24. Caruso P, Denari SD, Ruiz SA *et al*. Inspiratory muscle training is ineffective in mechanically ventilated critically ill patients. *Clinics* 2005; **60**: 479–484.
25. De Letter MA, van Doorn PA, Savelkoul HF *et al*. Critical illness polyneuropathy and myopathy (CIPNM): evidence for local immune activation by cytokine-expression in the muscle tissue. *J Neuroimmunol* 2000; **106**: 206–213.
26. Rich MM, Bird SJ, Raps EC, McCluskey LF, Teener JW. Direct muscle stimulation in acute quadriplegic myopathy. *Muscle Nerve* 1997; **20**: 665–673.
27. Bolton CF. Sepsis and the systemic inflammatory response syndrome: neuromuscular manifestations. *Crit Care Med* 1996; **24**: 1408–1416.
28. Danon MJ, Carpenter S. Myopathy with thick filament (myosin) loss following prolonged paralysis with veuronium during steroid treatment. *Muscle Nerve* 1991; **14**: 1131–1139.
29. Hasselgren PO, Fischer JE. Sepsis: stimulation of energy-dependent protein breakdown resulting in protein loss in skeletal muscle. *World J Surg* 1998; **22**: 203–208.
30. Norman H, Kandala K, Kolluri R *et al*. A porcine model of acute quadriplegic myopathy: a feasibility study. *Acta Anaesthesiol Scand* 2006; **50**: 1058–1067.
31. De Jonghe B, Sharshar T, Lefaucheur JP *et al*. Paresis acquired in the intensive care unit: a prospective multicenter study. *JAMA* 2002; **288**: 2859–2867.
32. Berchtold MW, Brinkmeier H, Müntener M. Calcium ions in skeletal muscle: its crucial role for muscle function, plasticity and disease. *Physiol Rev* 2000; **80**: 1215–1265.
33. Gordon AM, Homsher E, Regnier M. Regulation of contraction in striated muscle. *Physiol Rev* 2000; **80**: 853–924.
34. Murray MJ, Brull SJ, Bolton CF. Brief review: non-depolarizing neuromuscular blocking drugs and critical illness myopathy. *Can J Anesth* 2006; **53**: 1148–1156.
35. De Letter MA, Schmitz PI, Visser LH *et al*. Risk factors for the development of polyneuropathy and myopathy in critically ill patients. *Crit Care Med* 2001; **29**: 2281–2286.
36. Hughes BJ, Krieg M. Increased glucocorticoid/androgen receptor ratios in denervated striated muscle. *J Steroid Biochem* 1986; **25**: 695–699.
37. Rich MM, Kraner SD, Barchi SL. Altered gene expression in steroid-treated denervated muscle. *Neurobiol Dis* 1999; **6**: 515–522.
38. Prigent H, Maxime V, Annane D. Clinical review: corticotherapy in sepsis. *Crit Care* 2004; **8**: 122–129.
39. Rich MM, Pinter MJ, Kraner SD, Barchi RL. Loss of electrical excitability in an animal model of acute quadriplegic myopathy. *Ann Neurol* 1998; **43**: 171–179.
40. Rich MM, Pinter MJ. Sodium channel inactivation in an animal model of acute quadriplegic myopathy. *Ann Neurol* 2001; **50**: 26–33.
41. Rich MM, Pinter MJ. Crucial role of sodium channel fast inactivation in muscle fibre inexcitability in a rat model of critical illness myopathy. *J Physiol* 2003; **547.2**: 555–566.
42. Filatov GN, Rich MM. Hyperpolarized shifts in the voltage dependence of fast inactivation of $Na_v1.4$ and $Na_v1.5$ in a rat model of critical illness myopathy. *J Physiol* 2004; **559.3**: 813–820.
43. Trunkey DD, Illner H, Wagner IY, Shires GT. The effect of septic shock on skeletal muscle action potentials in the primate. *Surgery* 1979; **85**: 638–643.
44. Gibson WH, Cook JJ, Gatipon G, Moses ME. Effect of endotoxin shock on skeletal muscle cell membrane potential. *Surgery* 1977; **81**: 571–577.

45. Tracey KJ, Lowry SF, Beutler B, Cerami A, Albert JD, Shires GT. Cachectin/tumor necrosis factor mediates changes of skeletal muscle plasma membrane potential. *J Exp Med* 1986; **164**: 1368–1373.
46. Friedrich O, Hund E, Weber C, Hacke W, Fink RHA. Critical illness myopathy serum fractions affect membrane excitability and intracellular calcium release in mammalian skeletal muscle. *J Neurol* 2004; **251**: 53–65.
47. Niiya T, Narimatsu E, Namiki A. Acute late sepsis attenuates effects of a non-depolarizing neuromuscular blocker, Rocuronium, by facilitation of endplate potential and enhancement of membrane excitability *in vitro*. *Anesthesiology* 2006; **105**: 968–975.
48. Reid CL, Campbell IT, Little RA. Muscle wasting and energy balance in critical illness. *Clin Nutr* 2004; **23**: 273–280.
49. Williams AB, Decourten-Myers GM, Fischer JE, Luo G, Sun X, Hasselgren PO. Sepsis stimulates release of myofilaments in skeletal muscle by a calcium-dependent mechanism. *FASEB J* 1999; **13**: 1435–1443.
50. Mitch WE, Goldberg AL. Mechanisms of muscle wasting: the role of the ubiquitin-proteasome pathway. *N Engl J Med* 1996; **335**: 1897–1905.
51. Laghi F, Tobin J. Disorders of the respiratory muscle. *Am J Respir Crit Care Med* 2003; **168**: 10–48.
52. Solomon V, Goldberg AL. Importance of the ATP-ubiquitin-proteasome pathway in the degradation of soluble and myofibrillar proteins in rabbit muscle extracts. *J Biol Chem* 1996; **271**: 26690–26697.
53. Li YP, Schwartz RJ, Waddell ID, Holloway BR, Reid MB. Skeletal muscle myocytes undergo protein loss and reactive oxygen-mediated NF-κB activation in response to tumor necrosis factor α. *FASEB J* 1998; **12**: 871–880.
54. Li YP, Reid MB. NF-κB mediates the protein loss induced by TNF-α in differentiated skeletal muscle myotubes. *Am J Physiol Regul Integr Comp Physiol* 2000; **279**: R1165–R1170.
55. Acharrya S, Ladner KJ, Nelson LL *et al*. Cancer cachexia is regulated by selective targeting of skeletal muscle gene products. *J Clin Invest* 2004; **114**: 70–378.
56. Martin C, Boisson C, Haccoun M, Thomachot L, Mege JL. Patterns of cytokine evolution (tumor necrosis factor-alpha and interleukin-6) after septic shock, hemorrhagic shock and severe trauma. *Crit Care Med* 1997; **25**: 1813–1819.
57. Price SR, England BK, Bailey JL, Van Vreede K, Mitch WE. Acidosis and glucocorticoids concomitantly increase ubiquitin and proteasome subunit mRNAs in rat muscle. *Am J Physiol* 1994; **267**: C955–C960.
58. Horinouchi H, Kumamoto T, Kimura N, Ueyama H, Tsuda T. Myosin loss in denervated rat soleus muscle after dexamethasone treatment. *Pathobiology* 2005; **72**: 108–116.
59. Fischer DR, Sun X, Prittis TA, Hasselgren PO. The amount of the glucocorticoid receptor (GR) and its hormone binding activity are increased in skeletal muscle during sepsis. *Surg Forum* 1999; **51**: 214–216.
60. Combaret L, Taillandier D, Dardevet D *et al*. Glucocorticoids regulate mRNA levels for subunits of the 19S regulatory complex of the 26S proteasome in fast-twitch skeletal muscle. *Biochem J* 2004; **378**: 239–246.
61. Lee MC, Wee GR, Kim JH. Apoptosis of skeletal muscle on steroid-induced myopathy in rats. *J Nutr* 2005; **135**: 1806S–1808S.
62. Vanhorebeek I, Langouche L, van den Berghe G. Endocrine aspects of acute and prolonged critical illness. *Nat Clin Pract Endocrinol Metab* 2006; **2**: 20–31.
63. Vanhorebeek I, van den Berghe G. The neuroendocrine response to critical illness is a dynamic process. *Crit Care Clin* 2006; **22**: 1–15.
64. Riedemann NC, Guo RF, Ward PA. Novel strategies fort he treatment of sepsis. *Nat Med* 2003; **9**: 517–524.
65. Brunkhorst FM, Kuhnt E, Engel C *et al*. Intensive insulin therapy in patients with severe sepsis and septic shock is associated with an increased rate of hypoglycemia – results from a randomized multicenter study (VISEP). *Infection* 2005; **33**: 19–20.
66. Aghajani E, Nordhaug D, Korvald C *et al*. Mechanoenergetic inefficiency in the septic left ventricle is due to enhanced oxygen requirements for excitation–contraction coupling. *Cardiovas Res* 2004; **63**: 256–263.