From the Department of Physiology and Pharmacology
Section of Anesthesiology and Intensive Care Medicine
Karolinska Institutet, Stockholm, Sweden

Muscle function in the critically ill – clinical and experimental investigations

Karsten Ahlbeck
M.D.

Karolinska Institutet

Stockholm 2011

Printed by Larserics Digital Print AB

Layout Ringvor Hägglöf
ISBN 978-91-7457-416-6

To my surprise

There are 10 types of people -
those who understand binary, and those who don´t

Abstract

It is common that critically ill patients develop muscle weakness in the intensive care unit (ICU), not only delaying mobilisation and increasing the risk of co-morbidities, but also prolonging rehabilitation after hospital care. The aim of this thesis was to describe the diagnosis, time course and possible risk factors for this weakness.
When specific diseases such as CNS lesions, intoxication or other nerve and muscle disorders have been excluded in the ICU, a ”critical illness polyneuropathy and myopathy” (CIPNM) should be considered. The pathology behind this entity is unclear; among possible etiologic factors sepsis, corticosteroids and neuromuscular blocking agents (NMBAs) have been suggested.

CIPNM consists of a nerve pathology (neuropathy) and/or a muscle pathology (myopathy) and is diagnosed by a clinical assessment in combination with neurophysiological examination. The latter can be cumbersome due to the challenging environment in the ICU and is in itself not a definitive method of differentiating between a polyneuropathy and a myopathy.
We demonstrate a rapid method of electrophoresis, using an ultra-thin gel to evaluate the myosin to actin (M/A) ratio as a means of diagnosing critical illness myopathy (CIM). Using this diagnostic tool, there was a significant difference in M/A ratio between the patients having CIM, a control group, and patients having axonal neuropathies.
To evaluate the prevalence of CIPNM and the temporal pattern of its two major components critical illness polyneuropathy (CIP) and CIM, a prospective study was conducted including ICU patients who had been mechanically ventilated for at least 72 hours. The eventual prevalence of CIPNM was investigated, including neurophysiological and clinical examination. Muscle biopsies were obtained, in order to study the myosin to actin ratio and mitochondrial function. All septic patients, who were also receiving corticosteroid treatment, had a CIPNM diagnosis, whereas none of the non-septic patients fulfilled the necessary criteria. As a marker of oxidative stress, mitochondrial superoxide dismutase was increased in all patients, with a marked elevation in the CIPNM group.

To examine possible predisposing risk factors and mechanisms behind CIPNM in an experimental porcine ICU model over 5 days, groups were separated by interventions including corticosteroids, neuromuscular blocking agents and endotoxin, during mechanical ventilation. No group had a pathologic M/A ratio. All groups had significant changes in compound muscle action potential amplitude, including the inactivity/mechanical ventilation only group. The groups including corticosteroid treatment, endotoxin and the combination of all interventions had decreased muscle specific force and mitochondrial complex I activity, which were not seen in the mechanical ventilation group.

In conclusion, this thesis demonstrates an alternative method of diagnosing a critical illness myopathy, which could prove to be both time-efficient and reliable. In ICU patients there was a high prevalence of CIPNM in patients mechanically ventilated for more than 72 hours. An experimental model showed both decreased specific muscle force and mitochondrial complex I activity in intervention groups receiving corticosteroids, endotoxin or a combination, for both respiratory and non-respiratory muscles.

Key words: myopathy, polyneuropathy, critical illness, myosin to actin ratio, single muscle fibre force, mitochondrial dysfunction

List of Original Papers

This thesis is based on the following papers, which are referred to in the text by their roman numerals:

I. Stibler H, Edström L, Ahlbeck K, Remahl S, Ansved T.
Electrophoretic determination of the myosin/actin ratio in the diagnosis of critical illness myopathy.
Intensive Care Med. 2003; 29: 1515-1528

II. Ahlbeck K, Fredriksson K, Rooyackers O, Måbäck G, Remahl S, Ansved T, Eriksson L, Radell P.
Signs of critical illness polyneuropathy and myopathy can be seen early in the ICU course.
Acta Anaesthesiol Scand. 2009; 53: 717-723

III. Ochala J, Ahlbeck K, Radell P, Eriksson LI, Larsson L.
Factors underlying the early limb muscle weakness in acute quadriplegic myopathy using an experimental ICU porcine model.
PLoS ONE. 2011; 6: e20876

IV. Ahlbeck K, Fredriksson K, Rooyackers O, Remahl S, Ansved T, Eriksson LI, Radell P.
Mitochondrial enzyme activity in respiratory and non-respiratory muscles in a 5-day porcine ICU model.
Manuscript.

Contents

ABBREVIATIONS

ACh	Acetylcholine
ADP	Adenosine diphosphate
APACHE	Acute Physiology and Chronic Health Evaluation
ARDS	Acute Respiratory Distress Syndrome
ATP	Adenosine triphosphate
AQM	Acute Quadriplegic Myopathy
CIM	Critical Illness Myopathy
CIP	Critical Illness Polyneuropathy
CIPNM	Critical Illness Polyneuropathy and Myopathy
CMAP	Compound Muscle Action Potential
CNS	Central Nervous System
CoS	Corticosteroids (paper IV)
CP	Creatine phosphate
CS	Citrate synthase (paper IV), corticosteroids (paper III)
CSA	Cross Sectional Area
CV	Conduction Velocity
dl	Decilitre
EMG	Electromyography
ENeG	Electroneurography
ICU	Intensive Care Unit
LPS	Lipoprotein Polysaccharide
min	Minute
ml	Millilitre
mSOD	Mitochondrial Superoxide Dismutase
MyHC, MHC	Myosin Heavy Chains
NADH	Nicotinamide Adenine Dinucleotide
nm	Nanometre, $1x10^{-9}$ m
NMBA	Neuromuscular Blocking Agent
$PaCO_2$	Arterial carbon dioxide tension
SDS-PAGE	Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis
SIRS	Systemic Inflammatory Response Syndrome
SNAP	Sensory Nerve Action Potential
SR	Sarcoplasmic Reticulum
TG	Triglycerides
TnC	Troponin C
TnI	Troponin I
TnT	Troponin T
WBC	White Blood Cell

INTRODUCTION

Background

Early in my career as specialist in anaesthesia and intensive care medicine, I was intrigued by the fact that some patients in the Intensive Care Unit (ICU) were apparently awake and in some cases could only communicate with the help of their facial muscles, but were otherwise completely or partially paralysed. When trying to find diagnostic guidance for these patients in addition to regular bedside evaluation, there often seemed to be a practical problem, with neurophysiological examinations taking both time and space in the equipment-filled area around the ICU patient. At that time, corticosteroids were used in sepsis and other disease states in the ICU, and although rarely used in Sweden, neuromuscular blocking agents were often a part of regular ICU treatment in other parts of the world. Both these classes of agent were suspected of being part of the problem[1-3], but without a clear understanding of the mechanism(s) involved, it seemed attractive to use an established animal ICU model in order to examine these issues and agents.

It was brought to my attention during discussions with physiotherapists and occupational therapists at the hospital that the problem with skeletal muscular weakness involved not only the period of ICU care, but rather the whole period of in-hospital care after the initial period of critical illness. Indeed, several studies have focused on this issue, showing prolonged periods of neuromuscular dysfunction after hospital discharge with a need for rehabilitation, which clearly consumes both time and money. My thesis is based on these reflections.

The muscle

Muscle is classified into three subtypes: skeletal, smooth and cardiac. Skeletal muscle is voluntary and striated, cardiac muscle is striated and involuntary while smooth muscle (internal organ walls, blood vessels) is non-striated and involuntary. This thesis exclusively involves skeletal muscle.

Skeletal muscle can be categorised by means of physiology, biochemistry and histochemistry. Different subtypes of muscle fibres have been described when combining these techniques. Human muscle fibres usually are classified into three categories based on ATPase staining – type I, IIA and IIB. Using other techniques like electrophoresis show additional myosin isoforms. Some mammals, like pig, exhibit an intermediate form, MHCIIb, not seen in humans [4, 5] (table 1).

Skeletal muscle not only serves as support for body posture and a force generator. For instance, some interleukins - IL-6, IL-8 and IL-15 – are produced, expressed and released in muscle fibres, and have been termed myokines, together with other peptides that possess the same characteristics [6]. IL-6 is considered a pro-inflammatory cytokine but has anti-inflammatory properties as well [7]. It is released during muscle exercise and may protect against insulin resistance[8]. IL-8 is also induced by exercise, and may be involved in angiogenesis [9] and seems to exert its effect locally in the muscle[10]. IL-15 is suggested being a factor involved in muscle growth[11] and also in the interaction between muscle and adipose tissue[12]. Although these cytokines are not evaluated within this thesis, it certainly is

an example of additional important roles of muscle tissue.

Skeletal muscle is formed by cylinder-shaped units containing fibres made of actin and myosin, giving the muscle its characteristic striated appearance (figure 1).

Actin (”thin filament”) is an important component in the cytoskeleton, where it gives mechanical support to the cell and also promotes signal transduction. Together with troponin and tropomyosin it forms thin filaments in skeletal muscle. Troponin (Tn)

Table 1. Adult skeletal muscle myosin heavy chain (MHC) isoforms and their characteristics

Fibre type (mATPase)	**Type I**	**Type IIA**	**Type IIB**	**Not in humans**
MHC isoform	**MHCI**	**MHCIIa**	**MHCIIx**	**MHCIIb**
Contraction time	slow	medium fast	fast	very fast
Motor neuron size	small	medium	large	very large
Fatigue resistance	high	intermediate	intermediate	low
Activity	aerobic	long term anaerobic	short term anaerobic	short term anaerobic
Force production	low	medium high	high	very high
Mitochondrial density	high	high	medium	low
Capillary density	high	medium	low	low
Oxidative capacity	high	high	medium	low
Glycolytic capacity	low	high	high	high

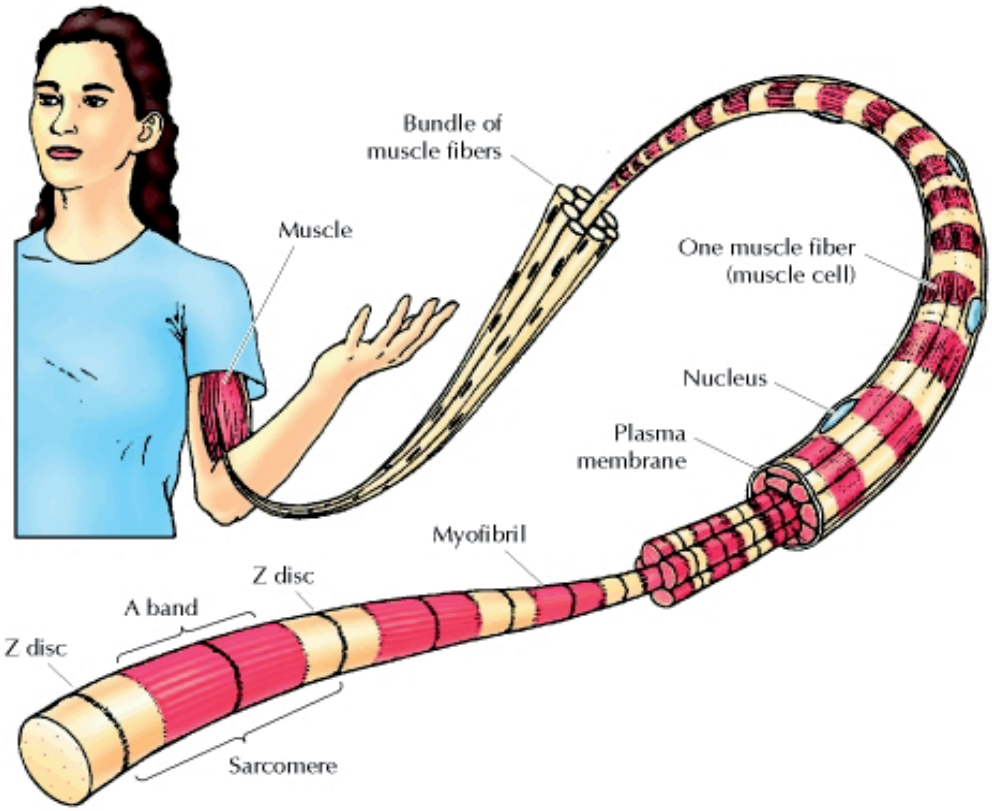

Figure 1. Schematic drawing of the muscle fibre. From Cooper: The Cell: A Molecular Approach, Second Edition, Sinauer Associates, Inc., Sunderland MA, USA, 2000

consists of three subunits: TnT which interacts with tropomyosin; TnC binds calcium and TnI binds to actin.
In relaxed muscle, tropomyosin situated on actin filaments blocks sites that bind myosin.

Myosin ("thick filament") is a motor protein which accounts for approximately 50% of total muscle protein and is dependent on ATP for its function. It moves along actin while hydrolysing ATP. There are at least 30 different genes encoding for myosin in the human genome; there are certainly many other, non-muscular functions which are not yet fully known [13].
Myosin and actin form a contractile complex, also called actomyosin, in skeletal muscle. The myosin class II – the actomyosin component in human skeletal muscle - was the first to be discovered, and up to date there are 35 known classes in eukaryotes[14].The ratio between myosin and actin has been shown to be a good marker of critical illness myopathy [15-16].

The muscle contraction (figure 2)
In brief, a skeletal muscle contraction starts with either conscious brain-initiated or reflex-initiated spinal cord activity. An electrical action potential propagates along the myelinated nerve axon and finally arrives at the nerve ending which forms the so-called motor end plate on a number of muscle fibres. One axon can connect to several muscle fibres, but each muscle fibre only has one axon connected to it. Triggered by the arrival of an action potential, acetylcholine (ACh) is released from the nerve ending into the synaptic cleft. Typically, this massive release of ACh results in activation of pre- and postsynaptic ACh receptors, causing a net influx of cations that ultimately changes the resting potential under the motor end plate. This change in end plate potential initiates a muscle action potential that spreads inside the muscle fibre through its T-tubules, and releases calcium from the sarcoplasmic reticulum (SR). Calcium binds to TnC which moves tropomyosin, allowing myosin to bind, and the muscle contracts. By cleaving ATP to ADP + phosphate, myosin produces mechanical force. When a new ATP molecule binds on the myosin head the actin/myosin bond is released and the muscle relaxes. Calcium is transported back to

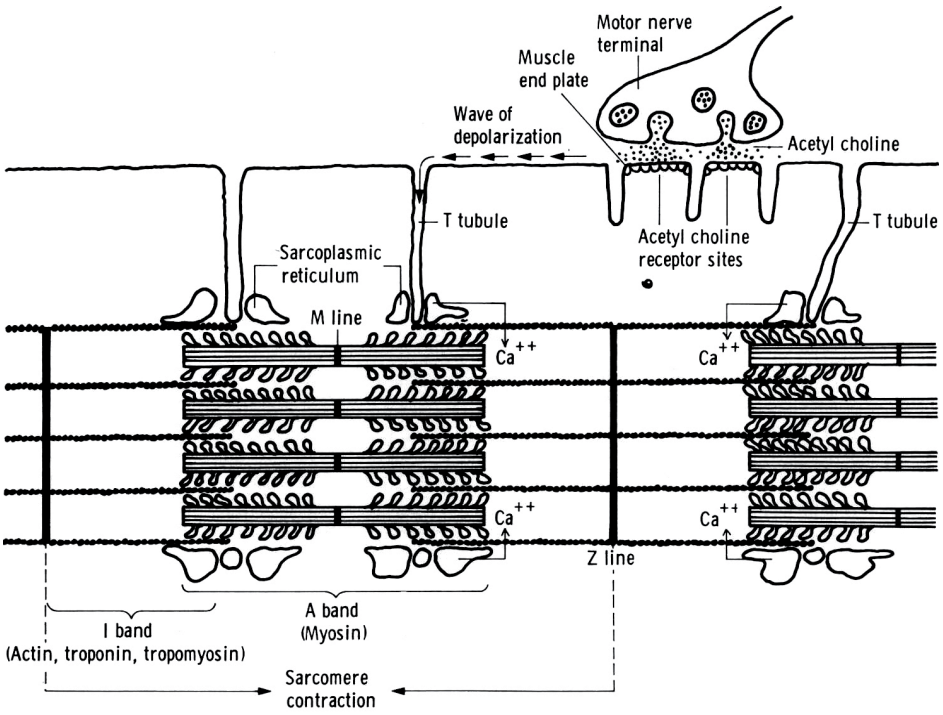

Figure 2. Schematic drawing of the muscle contraction.
Adapted from The muscle cell, J.C Sloper et al, J. Clin Pathol.Suppl. (Roy Coll. Path.), 1978;12, 25-43

the SR. For a more complete description, see Huxley [17].

The motor unit

A motor unit is a single nerve and the corresponding muscle fibres it supplies. The size of a motor unit depends on which type of muscle is involved; i.e. a muscle involved in very precise movements has fewer motor fibres per nerve fibre than a large muscle, like the quadriceps femoris. The more pronounced the muscle contraction, the more and larger motor units are recruited [18]. In a nerve injury (neuropathy), the connection beween the nerve and muscle fibres is initially lost. This results in fewer working motor units being activated in a muscle contraction, called ”reduced recruitment”. If, on the other hand, the muscle fibre itself is damaged (myopathy), the number of motor units is unaffected, but the generated force is lower than normal. To compensate for this, more motor units are fired at the same time to achieve the desired muscle force. This phenomenon is called ”early recruitment”. The force generated depends on how many motor units are activated, and the frequency with which they are activated.

Neurophysiology in clinical use

Neurophysiological investigations are used to assess neuromuscular function, e.g. when a patient in the intensive care unit (ICU) presents with muscular weakness without obvious cause. In addition to a clinical evaluation by a neurologist, a neurophysiological examination most often including electroneurography (ENeG) and electromyography (EMG) is performed. Together, these investigations usually can determine if there is an isolated, or combined, pathology in the nervous system; in the muscle, peripheral nerve, or the neuromuscular junction.

ENeG includes the investigation of nerve conduction velocity (CV), calculated from the time from a nerve stimulation to a compound muscle action potential (CMAP), or a sensory nerve action potential (SNAP) generation. This velocity is dependent on the diameter of the nerve fibre (the larger the faster) and if it is myelinated or not. A myelinated nerve fibre generally has a higher CV than an unmyelinated nerve fibre. In general, in an axonal degeneration disorder, CV is normal or near normal; in a demyelinating disease like demyelinating forms of the Guillain-Barré syndrome, the CV is decreased.

CMAP is the sum of the response of action potentials from the muscle fibres when the supplying motor nerve is stimulated. This amplitude is dependent on the number of axons stimulated, the neuromuscular transmission, and the size of the muscle fibres. CMAP is decreased in a myopathy and in an axonal degeneration disorder, but is usually normal or near normal in pure demyelinating diseases. The SNAP is recorded from superficial, sensory nerves. The SNAP is normal in pure motor disorders such as spinal muscular atrophy, whereas it is usually low or absent in sensory and sensorimotor polyneuropathies.

EMG can be conducted with either surface electrodes or a needle. Needle EMG is used clinically in the evaluation of a neuropathy or myopathy, since it provides detailed configuration information of the motor unit. In contrast, surface EMG is a quantitative method, giving little or no information of the motor unit complex.

The EMG needle can be of single-fibre type (recording of a single muscle fibre), concentric (recording of approximately 2-15 fibres at a time), or a macro-EMG (recording of an entire motor unit). Denervation activity, recruitment pattern and the configuration of the motor unit – amplitude, duration, area and complexity – can be investigated and is generally useful in differentiating between a neuropathy and a myopathy. Also, a differentiation between acute and chronic conditions can be distinguished with EMG[19].

Muscle weakness in the Intensive Care Unit

The history of specialised intensive care units (ICUs) started in 1953 when the first ICU was established in Copenhagen during a polio epidemic [20]. Patients in need of treatment and care in such units have frequently been referred to as being critically ill. More precisely, the term "critically ill", meaning a condition which acutely impairs one or more vital organ systems, such that there is a high probability of imminent or life-threatening deterioration in the patient's condition, was initially coined for ICU patients. Notably, improved care on regular wards and high demand for ICU care may nowadays render quite ill patients being treated outside the ICU on other hospital wards. Therefore it is important to remember that the muscular weakness described within this thesis is relevant not only for ICU patients, but rather for all patients suffering from multiple organ failure, regardless of the hospital location in which their care is provided.

Although new, specific ICU treatments might be causative, muscle weakness during intensive care has most probably always affected critically ill patients, but has generated increasing interest due to improved survival rates, leading to a focus on post-ICU outcomes and problems - including neuromuscular sequelae which had previously gone undetected.

A large proportion of ICU patients may either have sepsis on arrival, or will later develop sepsis during the intensive care period [21]. According to the definition proposed by Bone et al [22], sepsis "represents the systematic inflammatory response to the presence of infection". Importantly, this response is often associated with neuromuscular sequelae. Within this thesis, stated sepsis in the studies means a SIRS caused by a confirmed infection. The SIRS definition is stated in table 1.

Underlying disease and/or the treatment involved rendering the patient immobilised by muscular weakness is a well-known sequela of intensive care. When a patient exhibits clinical signs of muscular weakness, and other possible causes have been excluded (for differential diagnoses see Table 2), an intensive care-acquired weakness should be considered.

Table 1. SIRS definition.

SIRS (systemic inflammatory response syndrome) as manifested by two or more of the following conditions:			
Temperature	>38° or <36° Celsius		
Heart rate	> 90 beats · min^{-1}		
Respiration rate	> 20 breaths · min^{-1}	or	$PaCO_2$ < 4.2 kPa
WBC	>12 or <4 x 10^3 · ml^{-1}	or	> 10% immature (band) forms

Table 2. ICU weakness: differential diagnoses.

Causes of muscular weakness in the ICU	
Residual ICU drug effect	Pre-existing peripheral nerve disorders
Intoxication	Pre-existing muscle disorders
Guillain-Barré syndrome	Significant electrolyte imbalance
Myasthenia gravis	Disorders of the neuromuscular junction
CNS injury/lesion	Psychiatric disorders
Spinal cord injury	

Critical Illness Polyneuropathy and Myopathy (CIPNM)

Initially brought to our attention by two frequently cited case reports some 30 years ago [23-24], muscle weakness in the critically ill acquired during intensive care was later found to be both common and sometimes severe[25-27]. Steroids and neuromuscular blocking agents were soon considered to be possible pharmacological etiologies, but results in this area have been inconclusive [28-29].

A patient with muscular weakness, e.g. evaluated by using a motor sum score [30] (table 3), and by evidence of polyneuropathy and/or myopathy, is by definition suffering from CIPNM if the causes in table 2 have been excluded.
Nomenclature has been diverse for this condition, sometimes describing a pure myopathy or a polyneuropathy, and sometimes being a heterogeneous term describing the occurrence of one or both [31-36] exemplified below: CIPNM was coined by deLetter et al in 2000 [37], a term which includes a muscle weakness consisting of a polyneuropathy and/or a myopathy. Previous studies have described the occurrence of CIPNM, which is important, but have not shown the temporal changes in CIPNM during the course of intensive care.

Table 3. MRC muscle power scale.

Medical Research Council Scale for assessment of muscle power	
0	No movement
1	Perceptible muscle flicker
2	Movement if gravity is eliminated
3	Can move limb against gravity
4	Some examiner resistance
5	Normal power

Polyneuropathy (PNP) and myopathy (MP) diagnosis

Critical illness polyneuropathy (CIP), usually has both an axonal motor and a sensory origin. The motor deficit might be revealed by difficulties in weaning from mechanical ventilation, in physiotherapy mobilization [38], or by respiratory weakness forcing a patient from non-invasive ventilation to becoming mechanically ventilated (e.g. phrenic nerve neuropathy). There is no agreement upon an exact definition of a PNP, although clinical recommendations exist [39]. The following are usually considered diagnostic: a) a significant decrease in CMAP and/or SNAP amplitudes b) a normal, or near normal, nerve conduction velocity (in axonal forms; in the case of a demyelinating disease, CV is significantly decreased) c) a typical EMG pattern including reduced recruitment and possibly also spontaneous activity d) a predominantly distal muscular weakness, and/or distal sensory changes. A myopathy is most often diagnosed by muscular weakness, normal SNAP amplitudes, and myopathy-specific EMG findings such as short-duration, small-amplitude motor unit potentials with early recruitment. It is important to remember that voluntary effort by the patient often is required to make a diagnostic EMG.

Table 4. Examples of terms for intensive care-acquired weakness.

Critical illness myopathy	Intensive Care Unit Acquired Weakness (ICUAW)
Thick filament myopathy	Critical Illness neuromuscular abnormalities
Acute quadriplegic myopathy	ICU-acquired paresis
Acute necrotising myopathy	Critical Illness neuromyopathy
Acute corticosteroid myopathy	Critical Illness neuromuscular syndromes
Critical Illness Polyneuropathy and Myopathy (CIPNM)	Critical Illness myopathy and neuropathy (CRIMYNE)

CIPNM is thus a heterogeneous diagnosis, including either, or both, a polyneuropathy and a myopathy, which presents with a clinically reduced ability to contract muscles on command or to painful stimuli, usually located to distal, lower limbs. Facial muscle weakness in this condition is rare[40]. Because it can be difficult to differentiate between CIP and CIM [41], the term CIPNM has often been used.
Investigation of these entities can be cumbersome, due to the limitations which result from patients being confined to the ICU. Both the space available for additional equipment and undisturbed time for examination can be limited. We therefore wanted to present an efficient method as an additional tool for diagnosing CIM as an alternative to today's methods.

Based on the above, ICU-acquired muscular weakness is either a myopathy, a polyneuropathy or a combination of both. In this thesis, the confirmed diagnosis by neurophysiology and clinical examination is called CIPNM (paper II). In paper I and III the focus is on myopathy and therefore the term CIM or AQM is used. The term "muscular weakness" is a more general description of the muscular condition of the patient at the ICU.

Many mechanisms have been suggested for the development of CIPNM [42-48]. These include pathophysiological alterations in muscle and nerve axon circulation leading to local nerve/muscle ischemia, as typically seen in situations of circulatory instability, including sepsis-induced pro-inflammatory effects on nerve and muscle tissue with oedema and local hypoxia [25]. Other mechanisms involving metabolic derangement such as hyperglycemia, prolonged disuse and direct toxic pharmacological effects of medication used to treat the critically ill, have been suggested. Hence, by the time this thesis was launched, there was a controversy regarding the role of immobilisation and the systemic administration of corticosteroids and/or NMBAs in the development of CIPNM. We believe there are reasons to suggest that corticosteroids, without or in combination with prolonged pharmacological paralysis, play a key role. Based on previous findings in the ICU patient [49-50] we also wanted to explain whether there was an association between skeletal muscle mitochondrial dysfunction and skeletal muscle weakness in critical illness.

Immobilisation

Separating the effects of bed rest in the ICU from the effects of disease and pharmacological effects of medication on the muscle function is not feasible. After discharge from hospital, many patients with CIPNM still report muscle weakness up to years after hospitalisation and objective tests confirm this weakness[44,51]. Experimental inactivity models show that muscle mass decreases by around 2% daily the first two to three weeks of inactivity, which mainly is due to decreased muscle fibre size including the loss of contractile proteins[52]. Also, early in the immobilisation phase, an upregulation of ACh receptors takes place[53].

Corticosteroids

The use of steroids in the ICU has been debated for decades, balancing positive effects during sepsis, airway oedema, and autoimmune disorders against effects such as increased rates of infection, duration of ventilator support and ICU stay [54].
Several mechanisms for the pathogenesis responsible for CIPNM development have been suggested; for instance, activation of the ubiquitin-proteasome pathway, reduced IGF-effects and apoptosis [55]. In an animal model, corticosteroid administration led to changes similar to critical illness myopathy; changes also worsened with limb denervation before administration [56]. Chronic use of corticosteroids can result in a myopathy, clinically seen as a muscle weakness and an atrophy, which primarily affects type II fibres. The mechanisms behind this are not fully clarified, but decreased protein synthesis, increased myofibrillar proteolysis and apoptosis induction have been suggested[57].

Neuromuscular blocking agents (NMBAs)

As ICU care trends toward lighter sedation and earlier mobilisation, the use of NMBAs has decreased in many ICUs. Nonetheless, reports show that almost 15% of patients in the ICU receive NMBAs for at least one day, which is associated with a longer stay in the ICU and higher mortality [58], and in two recent ARDS studies a large proportion of the patients were treated with NMBAs [59-60]. In contrast to the proposed deleterious effects on neuromuscular function, there are also results which suggest that NMBAs specifically increase survival and decrease time on the ventilator in these patients[61]. Apart from effects on neuromuscular transmission and cholinergic signalling[62], pharmacological denervation per se is a mechanism by which NMBAs most likely contribute to CIPNM development, but changes in muscle plasma proteins have also been suggested [63].

The mitochondrion

Mitochondria (figure 3) are considered the ”power plant” of the cell, providing energy. Like the nucleus, the mitochondrion has a double membrane composed of proteins and phospholipids. The outer membrane is smooth, and allows the passage of nutrients, ATP, ADP and different ions. The inner membrane is only permeable to oxygen, carbon dioxide and water, but contains transport proteins which carry compounds across the membrane[64]. It is convoluted into folds called cristae, which greatly increase the surface area. On these cristae, enzymes and electron carriers (cytochromes) responsible for yielding energy from glucose are housed (figure 4). The enzyme complexes are usually named by roman numerals I-V (table 5). The two compartments formed by the membranes are the intermembrane space, which is important for oxidative phosphorylation, and the matrix, which contains hundreds of enzymes and mitochondrial DNA.

Mitochondrial dysfunction is involved in a large and increasing number of known diseases, including several that affect muscle, such as Kearns-Sayres syndrome, MELAS (mitochondrial encephalomyopathy with lactate acidosis and stroke-like episodes), and MNGIE (mitochondrial neurogastrointestinal encephalomyopathy). Changes in mitochondrial function have been observed in sepsis[65]. Muscular weakness is a known sequela of sepsis in the ICU, and there are some novel approaches to examining the specific mitochondrial changes in regard to muscular dysfunction in the ICU[49-50].
As already mentioned, corticosteroid and NMBA treatment are thought to contribute to this muscular weakness, and specific research on mitochondrial function in regard to these interventions has to my knowledge not been done before.

Table 5. Mitochondrial enzyme complexes. CoQ: coenzyme Q.

Complex I	NADH dehydrogenase
Complex II	Succinate-coQ-reductase
Complex III	CoQ-cytochrome c reductase
Complex IV	Cytochrome oxidase
Complex V	ATPsynthase

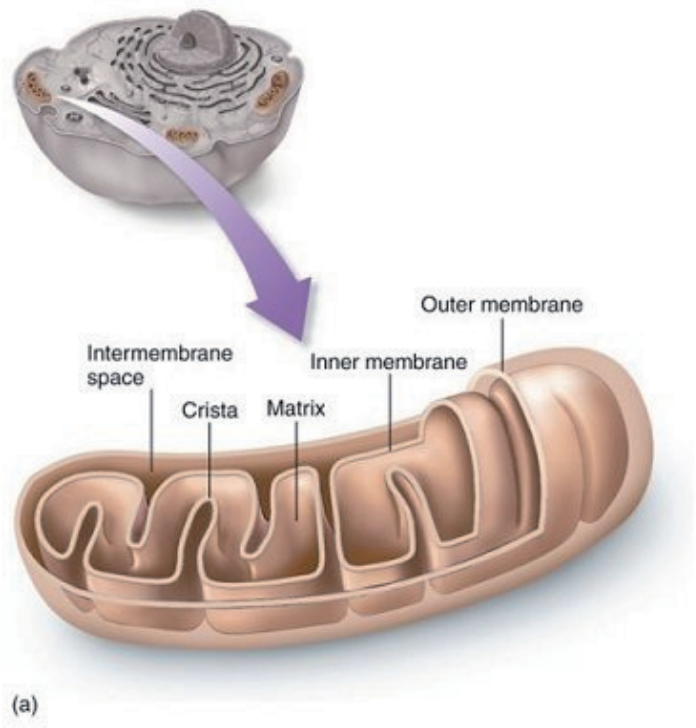

Figure 3. The mitochondrion as a) a schematic drawing, and b) in a transmission electron micrograph. From Johnson, Losos: Living World, 5ed 2008. McGraw-Hill Education, NY, USA

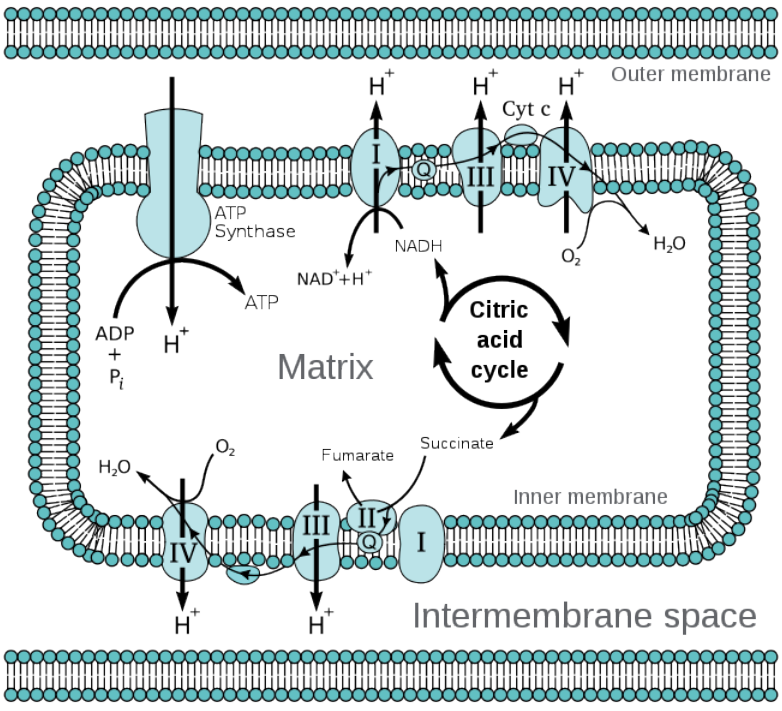

Figure 4. Schematic drawing of the respiratory chain in the mitochondrion. Courtesy of Fvasconcellos, Wikimedia commons.

Clinical and experimental studies

It is generally accepted that clinical studies in septic patients must often overcome multiple difficulties.[66]. As the ICU population is very heterogeneous, the challenge of separating and accounting for different factors, such as underlying disease and ICU complications (e.g. infection and administration of numerous different groups of medications) in a prospective study can be insurmountable. Animal models designed to account for various individual variables are therefore often used. Because there is a lack of ICU animal models of longer duration than a few days, we developed a 5-day porcine sepsis model [67] which combines pharmacological interventions with variables such as immobilisation and sepsis, that are suspected to have a causative role.

Aims

The overall aim of this thesis was to evaluate muscle function in the critically ill, with reference to the muscular weakness acquired in the intensive care unit.

The specific aims were:

I. To develop and evaluate a rapid method of quantifying myosin in patients with critical illness myopathy

II. To describe the time course of changes in muscle and nerve neurophysiology, histology and mitochondrial function in critically ill patients requiring mechanical ventilation

III. To explore the relative importance of immobilisation during mechanical ventilation, endotoxemia, and treatment with neuromuscular blocking agents and corticosteroids, on intensive care-acquired muscular weakness, using a five day porcine intensive care model

IV. To investigate the effect of immobilisation during mechanical ventilation, and of combinations of endotoxemia and pharmacological interventions on muscle function, as assessed by mitochondrial enzyme changes in a five-day porcine intensive care model.

Material and Methods

Patients

Paper I

Eleven patients, who all developed multiple organ failure in an intensive care unit at the Karolinska University Hospital, were enrolled. During the course of routine intensive care, the patients exhibited clinical signs of muscular weakness, and after a neurophysiological examination, consultation by a neurologist and a histological examination of muscle biopsies, either a pure CIM or a combination with a neuropathy was diagnosed. 42 controls had muscle biopsies taken as part of a routine investigation for various muscular symptoms. Twelve of these were considered healthy. In addition, five patients with an axonal neuropathy were included to study the effect of axonal injury upon the myosin/actin ratio.

Paper II

Ten patients admitted to one of the adult intensive care units at the Karolinska University Hospital were enrolled after approval by next-of-kin. Inclusion criteria were an age between 18-80 years and mechanical ventilation for at least 72 hours, plus the absence of a) any previous neuromuscular disorder; b) the use of neurotoxic drugs; and c) any condition precluding a muscle sample being taken. If participation of both the including ICU and neurophysiology physician was assured for all investigational days (4,14 and 28), the patient was included.

Animals

Paper III

22 female piglets, of a commercial crossbreed between Yorkshire and Swedish Landrace, entered this 5-day experimental study. They originated from the same farm and had free access to food, water and environmental enrichment before entering the study. Food, but not water, was withheld for 12 hours before scheduled anaesthesia. Two piglets died before the study protocol was finished, and in another two piglets the unexpected low quality of muscle specimen did not allow them to be included in the single muscle fibre examination. Therefore, 18 animals remained available for this study.

Paper IV

The same animals as in paper III were used for muscle mitochondrial analyses. The two piglets unsuitable for single-fibre analyses, and the two that did not survive for neurophysiological examinations in paper III but could be used for muscle biopsy, were analysed in this paper. Four sham surgery animals were also included, since muscle biopsies from the diaphragm and intercostal muscles could not otherwise be obtained before day 5, i.e. the last study day.

Neurophysiological examination (papers I-III)

In paper I and II, EMGs in the distal and proximal muscles of the upper and lower extremities, and ENeGs including the upper (median/ulnar nerves) and lower (peroneal/tibial/sural nerves) extremities were performed on all enrolled patients, including measurements of motor and sensory CVs, CMAPs and SNAPs, according to the standard clinical polyneuropathy protocol at the Neurophysiology Laboratory of the Karolinska University Hospital.

In paper III, the electroneurography (ENeG) analysis included peroneal motor nerve conduction velocities on days 1 and 5 of the experiment. The tibialis anterior muscle compound muscle action potential (CMAP) amplitudes were recorded upon supramaximal stimulation of the motor nerve. The EMG analysis included bilateral concentric needle EMG examination of proximal hind limb.

Muscle biopsy examination (papers I-IV)

Muscle biopsies from the tibial anterior (paper I), vastus lateralis (papers I+II) or the biceps femoris (paper III+IV) muscles were obtained under local anaesthesia. Cross sections were stained as per standard methods [68]. SDS-PAGE electrophoresis was carried out on homogenised and centrifuged muscle specimens and the gels were scanned to determine the myosin to actin ratio (papers I-III).

Mitochondrial analyses on citrate synthase, complexes I, IV and mSOD were performed using spectrophotometric assays, after homogenisation and centrifugation[49] (papers II+IV)

Single muscle fibre analyses regarding specific force and shortening velocity were conducted. MHC isoform expression was also performed (paper III). For more specific information, see Ochala et al [69]

Interventions (papers III+IV)

All animals were mechanically normoventilated using volume controlled ventilation using a Siemens 900A ventilator (Siemens-Elema, Solna, Sweden) at arterial normoxemia and normocapnemia. Arterial and central venous catheters, including a Swan-Ganz thermodilution pulmonary artery catheter, were positioned via the carotid artery and internal jugular vein, respectively. Continuous intravenous infusion of Ringer's acetate and glucose were administered to carefully maintain normoglycemia, because enteral feeding was not considered practical, and additional parenteral nutrition has been shown to result in fat vacuolisation in muscle cells [15]. Including a pure mechanical ventilation group, the animals were divided into different intervention groups: sepsis, NMBA, CS (corticosteroid) and ALL (all interventions combined).

Sepsis (endotoxin) and ALL groups

The group named "sepsis" in paper III is called "endotoxin" in paper IV, in order to reflect intervention rather than result. 10 ug LPS/kg (Sigma Chemical, St. Louis, Missouri, USA) was diluted in 20ml sodium chloride to 0.5 ug $\cdot$ kg^{-1} $\cdot$ ml^{-1}. The infusion was started at 2 ml $\cdot$ h^{-1}(1ug $\cdot$ kg^{-1} $\cdot$ h^{-1}) and titrated until a hemodynamic response occurred, consisting of a fall in arterial mean blood pressure >30% from baseline, with an increase of >50% in pulmonary artery systolic pressure from baseline. The infusion was paused if the animals required fluid resuscitation or administration of adrenalin for bradycardia, and was then restarted at a lower dose. If the animal required repeated interventions the infusion could be terminated earlier than one hour, and if tolerated could run for up to four hours.

NMBA group (and ALL group)

An intravenous infusion of 25 mg/hour of rocuronium (Esmeron, Schering-Plough AB, Stockholm, Sweden) was administered over the whole experimental period. If a return of spontaneous movement was observed, the animals received additional bolus doses of rocuronium of 10-20 mg.

Corticosteroid group (and ALL group)

Animals received 50 mg of hydrocortisone (Solu-Cortef, Pfizer AB, Sollentuna, Sweden) intravenously every 8 hours throughout the experimental period.

Results

Paper I

Histological and neurophysiological changes

Using a light microscope, pathological changes were observed in all biopsies (table 6). These included fibre atrophy (figure 5), de/regeneration, necrosis and rounded enlarged nuclei which were centralised or in the subsarcolemmal regions (figure 6). Preferential loss of thick filaments was observed in electron-microscope evaluation. There was no relationship between the degree of change, age and APACHE II score or time of biopsy. Neurophysiological data showed that motor nerve CV was normal and CMAP decreased for all patients.

Method evaluation and myosin to actin ratio

Sample dilution of 1:2 yielded the best results, compared to 1:5 dilution and undiluted specimen. The within-gel variation was 4%. Dried or stored gel increased the M/A ratio. All patients with confirmed CIM had a mean M/A ratio of 0.37±0.17, while the mean value for controls was 1.37±0.21. A group with axonal neuropathies had a mean M/A ratio of 1.57±0.19.

Table 6. Histopathological changes and myosin to actin ratio (M/A).

Patient	M/A	Age	Nuclear changes	Regeneration	ATPase loss	Fibre atrophy
1	0.37	54	+	+	+	+
2	0.17	76	++	+	+	++
3	0.22	61	++	+	+	+
4	0.31	63	+++	+	+	++
5	0.69	68	+++	+++	+++	+++
6	0.63	36	++	+	+	+
7	0.26	65	+	+	+	+++
8	0.28	59	+++	+++	+++	+++
9	0.37	57	+++	+++	+++	+++
10	0.31	61	+++	+++	+++	+++
11	0.43	16	++	+	+	+

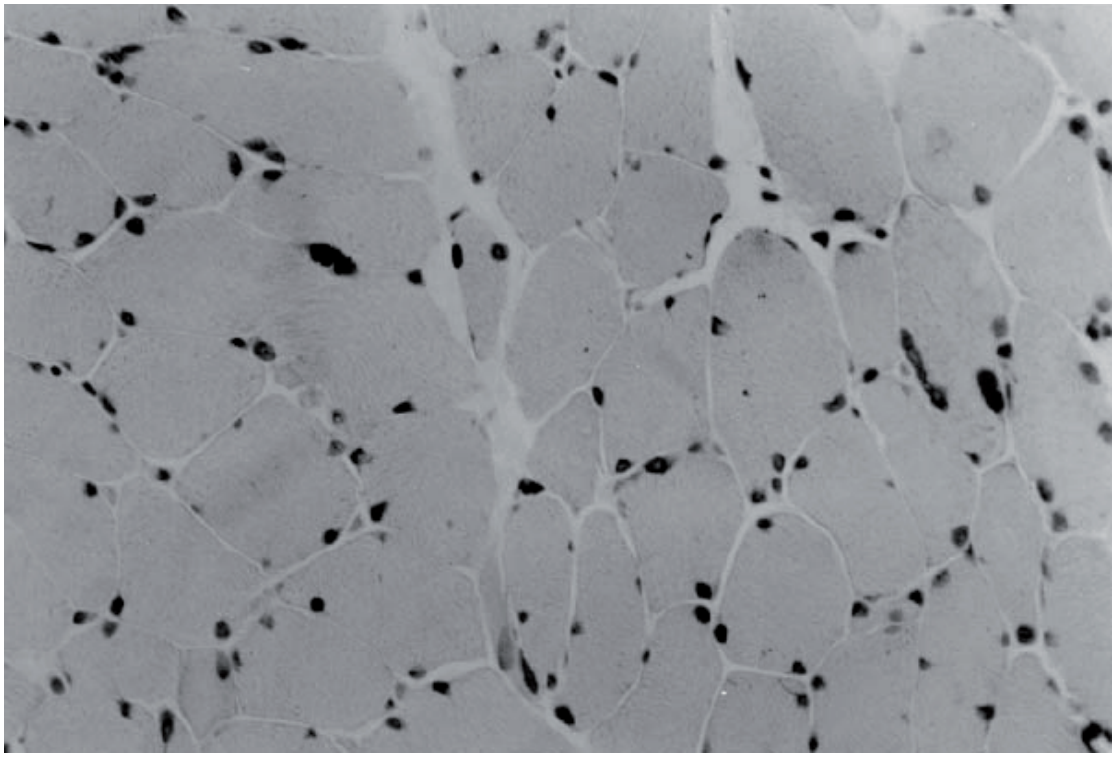

Figure 5. A muscle specimen from a patient with very mild histological changes.

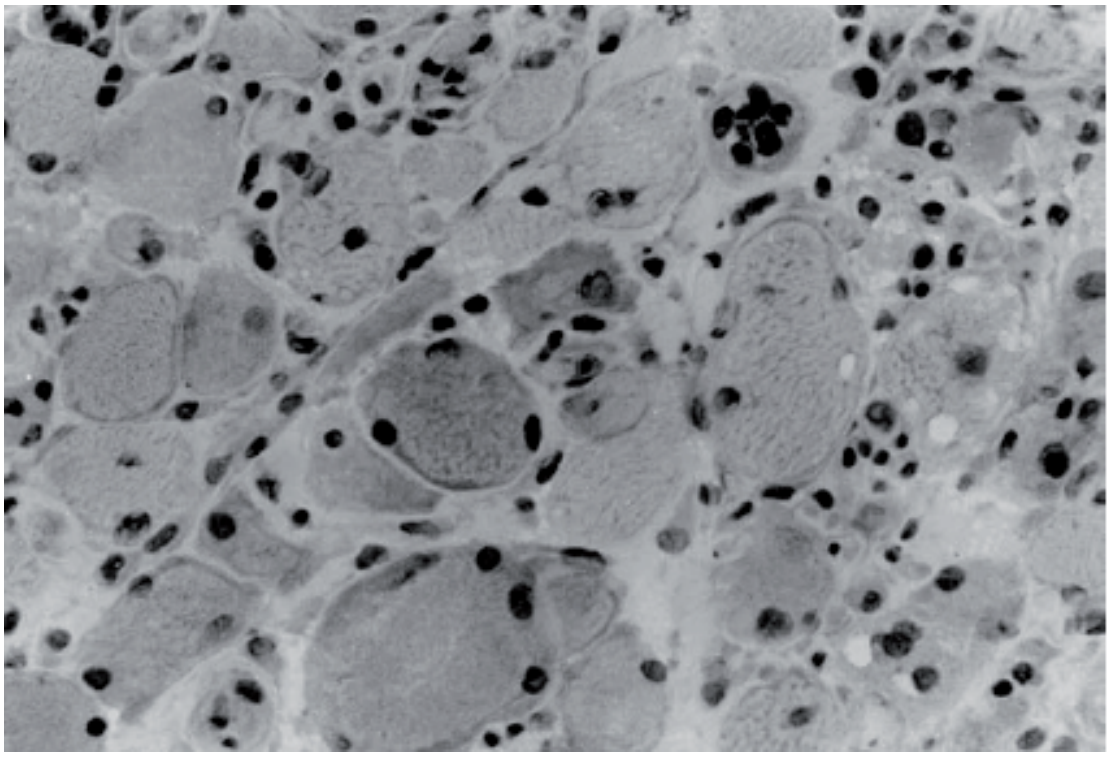

Figure 6. Severely affected muscle with obvious myopathic changes, including enlarged rounded nuclei.

Paper II

Apart from a single dose of succinylcholine before intubation, and a single dose of atracurium at day 15 for one patient, no NMBAs were given. All (5/10) septic patients received cortico-steroids (hydrocortisone 50-100mg three times daily for 5-10 days) and all fulfilled criteria for CIPNM. No non-septic patients fulfilled CIPNM criteria, including one with ongoing corticosteroid treatment for chronic obstructive pulmonary disease. Three patients had a M/A ratio <1.0, and all three were admitted to the ICU because of sepsis. The temporal changes are seen in table 7. Overall, septic patients showed more CMAP and SNAP pathology than non-septic patients (table 8).

Muscle biopsies and mitochondrial analyses

Discrete light microscopic changes were seen on day 14 for all but one patient. Citrate synthase decreased by 37% and mSOD increased by 61% in the whole group; for the septic patients mSOD increased by 116%. No significant changes were seen in mitochondrial complexes I and IV.

Table 7. Temporal changes in CIPNM, its components and clinical data.
M/A: myosin/actin ratio; n/a: not applicable;
CIP: critical illness polyneuropathy;
CIM: critical illness myopathy;
CIPNM: critical illness polyneuropathy and myopathy;
ICU: intensive care unit.

	Day 4	Day 14	Day 28
M/A < 1.0	0	3	n/a
CIP	5	3	2
CIM	0	2	1
CIPNM	5	5	2
Patients in the ICU	10	5	2
Mobilised patients	0	4	7
Clinical atrophy	0	3	2

Table 8. Overview of neurophysiological data. Pathological ENeG values divided into septic (left) and non-septic (right) patients.
Black boxes represent pathological values on day 4, 14 or 28; white boxes within normal ranges all three days. Med: median; per: peroneal.

Patient (septic)	Pathologic CMAP med+per nerve	Pathologic SNAP median nerve	Patient (non-septic)	Pathologic CMAP med+per nerve	Pathologic SNAP median nerve
1			5		
2			6		
3			8		
4			9		
7			10		

Paper III

Neurophysiological changes

No significant change in motor nerve CV was observed. CMAP was significantly reduced in all intervention groups between day 1 and 5 (figure 7).

Single-fibre muscle force

Muscle specific force (maximum muscle force normalised to cross-sectional area, CSA) was significantly decreased for the corticosteroid, sepsis and ALL groups (figure 8).

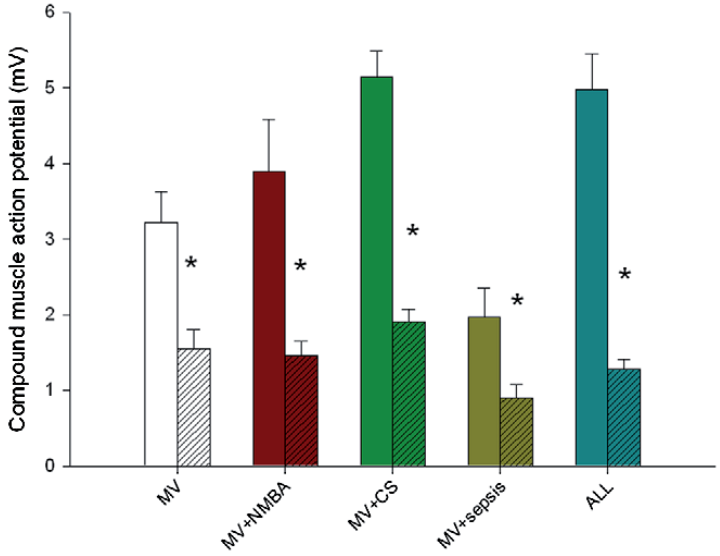

Figure 7. CMAP for the tibialis anterior muscle. Left bar: day 1, right bar: day 5. Asterisk denotes a statistical significance between day 1 and 5 ($p<0.05$). ALL: MV+sepsis+CS+NMBA.

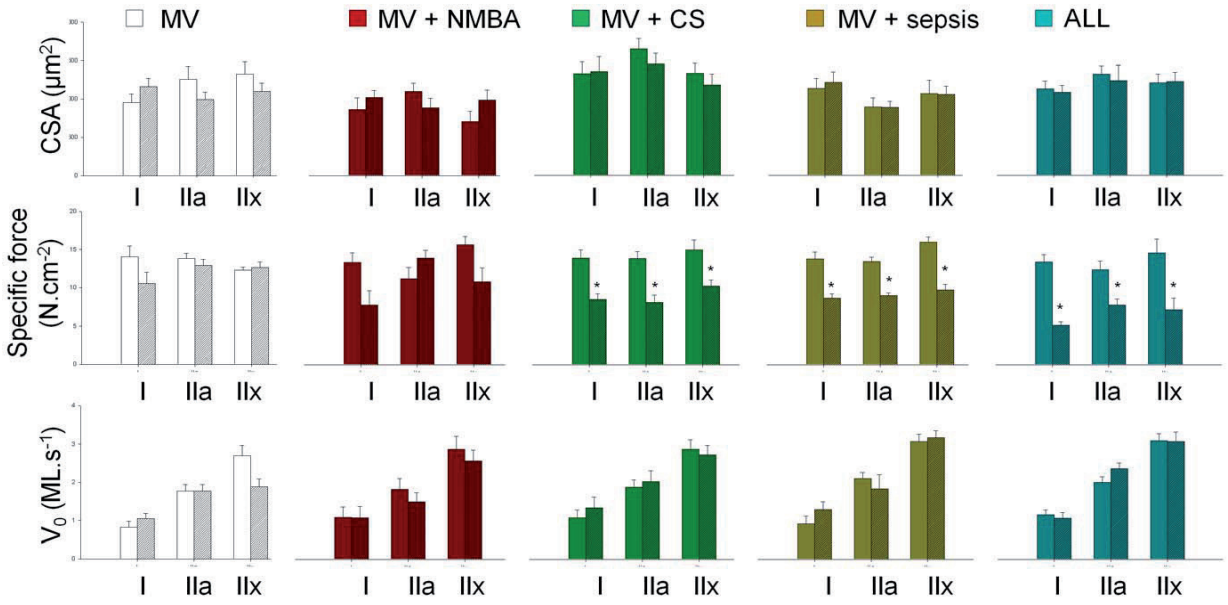

Figure 8. Single muscle fibre size and contractile function. Values for day 1 (left, brighter bars) and day 5 (right, darker bars). The asterisk denotes a statistically significant difference between days 1 and 5 ($p<0.05$).

Paper IV

Muscle biopsies and mitochondrial analyses
Light microscopy did not show any differences between the study groups; there were no signs of fibre atrophy. The myosin to actin ratio did not differ significantly between the MV and the ALL group.
In intercostal muscle, no differences were seen between the different intervention groups. For the biceps femoris and diaphragm muscles, complex I was decreased between the ALL and CoS groups compared with the control and MV groups (figures 9 and 10). If the ALL and CoS groups are combined, the changes were statistically significant versus controls (figure 11).

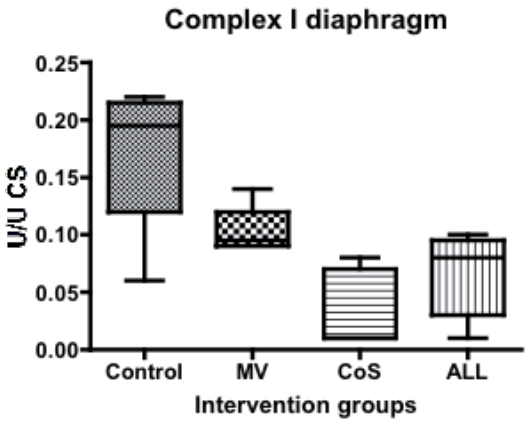

Figure 9. Mitochondrial enzyme changes at day 5 between intervention groups for the diaphragm muscle. ALL: MV+CoS+NMBA+Endotoxin.

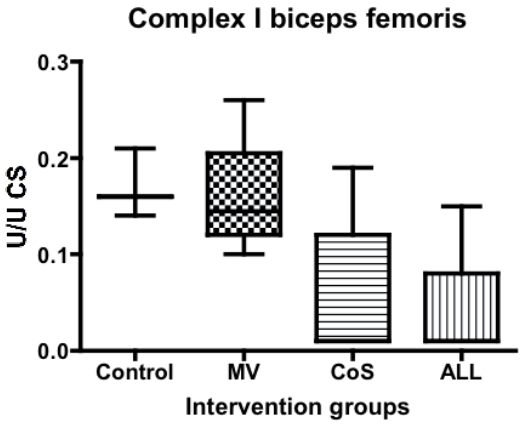

Figure 10. Mitochondrial enzyme changes at day 5 between intervention groups for the biceps femoris muscle. ALL: MV+CoS+NMBA+Endotoxin.

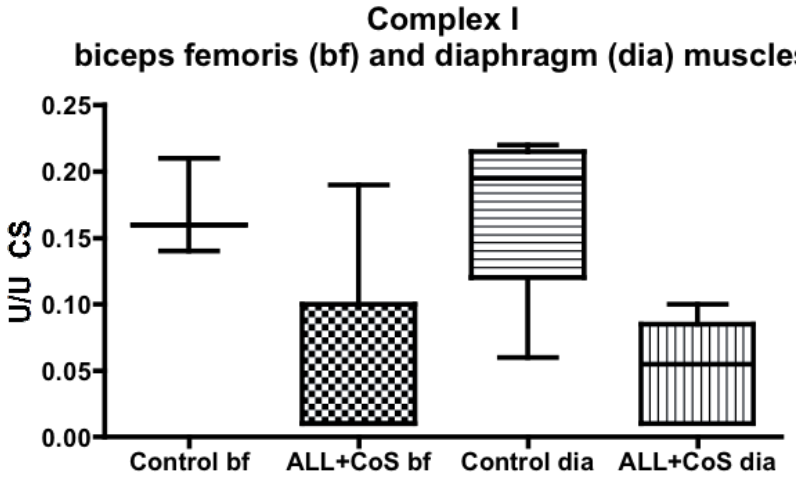

Figure 11. Mitochondrial changes in combined intervention groups. ALL: MV+CoS+NMBA+Endotoxin. $p<0.05$ for combined groups vs. controls.

Discussion

Why do some patients treated in the ICU become so weak that they can not move their extremities and are completely bedridden, while other patients with seemingly the same type of situation and disease are not affected? What can we learn from standard neurophysiological and bedside evaluation of neuromuscular function in the critical care setting, and can we obtain additional information from muscle samples? If the muscle weakness were a simple matter of inactivity, would we not see these symptoms in almost all patients? Obviously, this is not the case. Moreover, if sepsis were the single factor underlying CIPNM, one would expect the incidence to mirror that of sepsis in the critically ill. However, this is obviously not the case either. Hence, other factors may be anticipated to serve as the initiators of this condition.

The CIPNM diagnosis is challenging and is usually based on a neurophysiological examination fraught with difficulties in the ICU. The neurophysiological examination must often take place in a limited patient space which is occupied by necessary technical equipment, creating a possible risk of electrical disturbances. Wound dressings, casts and other obstacles to successful investigation are not uncommon, and the critically ill patient is often confined to certain positions, to avoid unwanted circulatory or respiratory effects if moved. Finally, ensuring that both patient and examiner are undisturbed during the whole examination can be a challenge. CIP and CIM often coexist, but even if they occur in isolation, the diagnosis can be difficult to make: even a pure myopathy cannot be distinguished from a pure axonal neuropathy if the patient cannot activate muscles voluntarily. In response to these problems, a shorter neurophysiological examination has been suggested to identify CIPNM patients, but includes the reservation that ”these [patients] should have full neurological and electrophysiological evaluations” [70]. A muscle biopsy might therefore be necessary to diagnose a myopathy. That said, there are descriptions of direct muscle stimulation (dmCMAP) examination, differentiating CIP and CIM, which could be valuable [71]. However, the technique is technically demanding and might be even more uncomfortable for the patient than obtaining a muscle biopsy under local anaesthesia. Patients relate discouraging stories of their traumatic perception of care-related procedures carried out during an often clouded mental state [72]. In view of this, we showed in paper I a rapid, alternative method of diagnosing CIM.

Using muscle samples followed by electrophoresis, like the SDS-PAGE, is an established method of calculating protein content, including myosin, actin and the myosin/actin ratio. The method described in paper I uses a horizontal, ultra-thin gel, working faster than other gels, with a between-gel variation of 5%. This could therefore be an attractive alternative to current clinical diagnostic tools. An obvious limitation is the method being invasive.

Studies show that CIPNM is common, with a prevalence often exceeding 50% [29, 73-74]. In prospective prevalence studies, the timing of serial examinations has varied. deLetter et al[45] used an interval between investigations (4, 11 and 25 days after the start of mechanical ventilation) similar to that used in paper II (4, 14 and 28 days) – and showed that SIRS was

present in 48%, and CIPNM in 33% of the patients.

Here (paper II), we prospectively included ICU patients who had been mechanically ventilated for 72 hours. Although a small number of patients were included, CIPNM was diagnosed in patients with sepsis during their ICU stay, and represented 50% of the study population. Importantly, the septic patients all received corticosteroids, so separating sepsis from corticosteroid administration was not possible. At day 4, all CIPNM patients had an isolated CIP, and at day 14, two of these also had a myopathy. At day 28, only one patient from day 4 still had an isolated CIP, and one patient had a combination of CIP and CIM. From these data, we suggest the diagnosis of CIP, CIM and/or CIPNM is dependent upon the time of the examination during the ICU course, owing to the variable temporal pattern. In order for results from clinical studies to be reproducible, or at least fairly comparable, examination dates should therefore be standardised. On the other hand, specific dates cannot be arbitrarily chosen for clinical diagnosis. Many factors can contribute to the patient being unable to comprehend commands for voluntary muscle activation, and a large number of ICU patients seem to meet neurophysiological criteria for CIPNM early in the ICU course. Our findings do not warrant a recommendation for performing prospective, comprehensive neurophysiological examinations for all ICU patients. Considering the examination difficulties described previously, an initial exam as a "starting point" at ICU admission does not seem feasible either.

Having completed a methodological study and an attempt to examine CIPNM prevalence, our attention turned to possible risk factors and mechanisms. In the literature, several factors have been suggested as being involved in the development of CIPNM, such as inactivity, NMBAs, corticosteroids and sepsis. In designing our study, no previous studies suggested how to construct intervention groups, nor how large the expected changes in neurophysiological examinations and mitochondrial enzyme analyses were likely to be. For 5-day studies, not only are there substantial costs for materials, but significant time and other resources are needed to make the study possible. Also, ethical standards dictate that the number of animals used for studies be kept to a minimum. We were therefore aware that the small size of the study groups was a limitation, but hoped that the study would provide initial information, and possibly enable us to use data from these studies to be able to design future studies with correctly powered groups, should these be warranted. In spite of this, there were significant changes.

From the earliest cases describing near paralysis related to intensive care [23-24], there is substantial evidence that high-dose cortisone is related to CIPNM development, but studies have so far been inconclusive. In paper II, we could not distinguish between sepsis and corticosteroids as risk factors, since patients with CIPNM had both sepsis and received corticosteroids. Therefore we wanted to include a CoS intervention group as a separate challenge in our animal model. We chose the dose of 50 mg, which corresponds to approximately 2 mg/kg, three times daily. This dose might seem high, but patients in study II received up to 100 mg three times daily. Therefore, we judged that the study dosage was justified.

In examining possible mechanisms for muscle changes, we examined neurophysiological variables, muscle fibre specific force and mitochondrial enzyme changes. Both for muscle fibre specific force (paper III) and mitochondrial enzyme I decrease (paper IV), there was a significant change in the intervention groups receiving CoS. These findings indirectly imply that corticosteroids have a deleterious effect on muscle function. The Cochrane Collaboration published a report

in 2009 [75], stating that no significant effect of corticosteroids was seen on the development of CIP/CIM. The one study [76] that this was based on included 180 ARDS patients, with only 93 having prospective CIP/CIM data. In this study, the authors stated they did not systematically assess nerve conduction or muscle function. Interestingly, there were nine reports of severe adverse events related to neuro/myopathy and all nine patients were treated with methylprednisolone. In contrast to the conclusions drawn by The Cochrane Collaboration, the authors wrote "methyl-prednisolone did not increase infectious complications but may have increased the risk of neuromyopathy associated with critical illness".

It has also been shown earlier that sepsis as well as corticosteroid treatment are associated with increased circulating levels of glucocorticoids[55], which are known to worsen the effect of immobilisation on muscle function[77]. Reactive oxygen species can increase due to higher levels of glucocorticoids[78], which may lead to changes in contractile proteins[79]. Many different mediators are involved intracellulary[56, 80], which can decrease protein synthesis as well as increased breakdown[56, 80-81]. Hence, these results combined with our present findings suggest a role for corticosteroids in the development of impaired nerve/muscle function in the critically ill.

Muscle contraction has earlier been shown to be pathologic in ICU patients with CIM[15, 82]. The neurophysiological criteria for CIPNM do not include assessment of muscle force, but need an additional clinical evaluation to evaluate muscular weakness which might then complete the diagnosis. In paper III, we wanted to examine neurophysiological findings together with muscle force experimentally, in intervention groups combining pharmacological agents with immobilisation and mechanical ventilation in respect to acquired myopathy.

In paper III, the motor nerve conduction velocity did not change significantly in any of the intervention groups between days 1 and 5, making myelin loss seem unlikely. The CMAP decreased significantly in all groups, with the largest decrease in the ALL group. In this context, inactivity and mechanical ventilation alone seem sufficient to induce changes consistent with the neurophysiological criteria for CIPNM. The CMAP decrease is considered to be due to defective sodium channel regulation which causes a decrease in muscle membrane excitability[83-85]. The single muscle fibre maximum force was normalised to CSA, and was found to decrease significantly in the intervention groups with either CoS or endotoxin, and in the ALL group. This was also regardless of MHC isoforms I, IIa or IIx. Nor was a difference in maximum shortening velocity seen. The myosin/actin ratio was normal. The contractile changes were thus seen before any myosin loss or fibre atrophy, suggesting that sepsis and CoS could induce an early loss of contractile proteins.

In paper II, we noted a difference between the patients with and without sepsis with regard to mitochondrial citrate synthase and mSOD. A somewhat unexpected finding was the lack of change, in either direction, in complexes I and IV, which could of course be due to the small number of patients.

Three of the septic/CIPNM patients (and none of the non-septic) had pathological myosin to actin ratios. Their Complex IV was unchanged, but Complex I was lower than that of the other patients (figure 12). This is without statistical significance, but the finding is interesting in the light of our findings in paper IV.

Morphologic changes seen in mitochondria after sepsis have been documented earlier [86] which suggest impaired function. There is currently a controversy regarding the impact of sepsis on mitochondrial function. Recent reports have shown divergent results (i.e. both increased and decreased function), but with longer duration studies there seems to be an

initial increase in activity that later during the course of sepsis turns into decreased activity [87]. Various theories behind mitochondrial dysfunction have been put forward, such as impaired microcirculation resulting in an oxygen shortage to the cell and mitochondrion[88]. However, studies have shown that oxygen delivery in multiple organ failure is within normal limits. For instance, intestinal oxygen consumption remained unchanged as impaired oxidative phosphorylation occurred [89]. Thus, although the time course of mitochondrial impairment is still only partly understood, overall mitochondrial dysfunction is well documented in sepsis.

Considering that mitochondria are the body's energy producer, that skeletal muscle contains vast amounts of mitochondria, that mitochondria are known to be affected in sepsis and that a large proportion of critically ill patients become weak, the mental leap to examining mitochondrial function in this animal model is not that great.

The results from study IV show a decreased complex I activity in the animals receiving corticosteroids, or belonging to the ALL group. The decrease was seen in the biceps femoris and diaphragm muscles, but not in intercostal muscle.

However, there was no change in myosin/actin ratio. As commented on paper III, there seem to be early changes that are not reflected by decreased myosin content. These early contractile changes might therefore be associated with a decreased mitochondrial complex I activity.

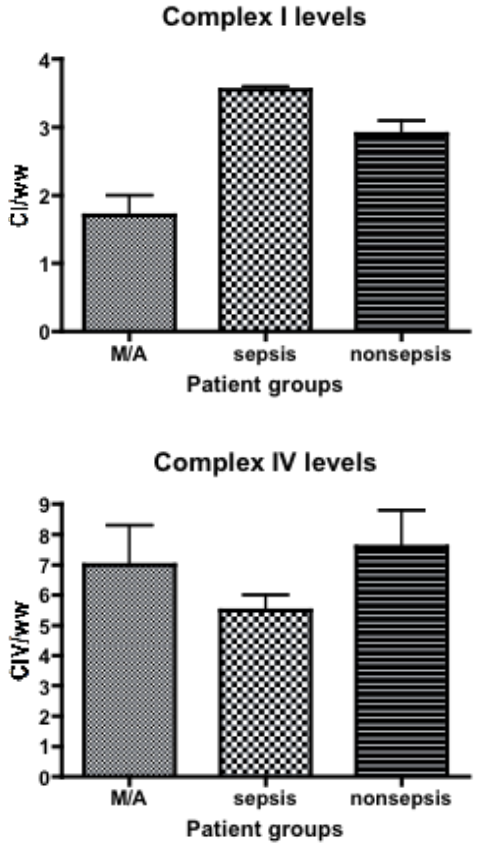

Figure 12. Mitochondrial complexes I and IV in different patient groups from paper II. M/A: group where myosin to actin ratio is less than 1.0.

In a study by Brealey et al [90], decreased mitochondrial function in skeletal muscle was associated with septic shock, and markers of oxidative stress correlated with Complex I decrease. Carré et al [91] likewise showed mitochondrial dysfunction in critically ill patients, with respiratory protein subunits depleted to a greater extent in non-surviving patients. The authors suggested that survival was associated with mitochondrial biogenesis activation early in the course of disease and that failure to activate this impaired recovery, hoping for new treatments to support biogenesis[92].

The studies included in this thesis did not examine nutrition as a factor in CIPNM, but obviously a catabolic patient with poor nutritional status is not well equipped to resist muscular weakness. In the Cochrane report on CIP/CIM [75] the authors stated that intensive insulin therapy reduced CIP/CIM (one heterogeneous group) incidence. The "conventional insulin therapy group" described in the Cochrane report was treated with insulin when blood glucose levels were over 215 mg/dL and stopped when below 180 mg/dL [48, 93]. In our practice, we believe these are very high glucose limits. The "intensive insulin therapy

group" we consider to have clinically normal limits. We did not see any significant difference in blood glucose level between the sepsis and the non-sepsis groups. We believe the reason for this is that none of the patients reached extremely high values other than occasionally in either the sepsis or the non-sepsis groups.

There are several different animal models for sepsis. Our aim was to simulate the ICU situation as closely as possible for up to 5 days. For this situation, the small animal gold standard model - the caecal ligation and puncture model - was judged to be unsuitable. Although the sepsis resemblance to endotoxin is debated, pigs are sensitive to endotoxin just like other primates but unlike rodents [94]. With regard to the potency of E.coli lipoprotein saccharide (LPS) infusion, the range of endotoxin in one mg of LPS can vary greatly between both strains and batches, and a more accurate way of describing the amount of LPS is to list the dosage in endotoxin units $\cdot$ mg^{-1} (EU $\cdot$ mg^{-1}). Since this is only occasionally mentioned in published articles, we had little prior information on which dose to use for these prolonged experiments. Therefore our endpoint was the clinical septic reaction instead of a specific dose. To avoid differences in potency between LPS batches, the same LPS batch was used for all animals.

We employed a porcine sepsis model due to the many similarities with human cardiovascular physiology, endotoxin sensitivity and antigenicity [95-97]. Animal size allows extensive instrumentation for serial and continuous haemodynamic monitoring. To our knowledge, there are few reports of prolonged sepsis models which have been maintained for as long as five days. Models of porcine sepsis have used endotoxin infusion, bacterial infusion, and peritonitis as infection stimuli [98] . Earlier porcine endotoxin infusion protocols have ranged from 0.25 ug $\cdot$ kg^{-1} $\cdot$ hr^{-1} for 3 hours up to 400 ug $\cdot$ kg^{-1} $\cdot$ hr^{-1} over 1.5 hours [99-103]. In this study the endotoxin dose was titrated to effect, an approach previously described [104-105] and which we found physiologically attractive and manageable over a five day experimental course. The endotoxin average dose of 8 ug^{-1} $\cdot$ kg^{-1} $\cdot$ hr^{-1} enabled the animals to survive a 5-day experiment after encountering a severe septic shock on day 1, that was defined by systemic hypotension and pulmonary hypertension.

Even when choosing a suitable animal model of ICU care that corresponds to interventions used for humans, there are limitations. First of all, we used young animals that do not correspond to the human age in our studies (56 years in paper I, 54 years in paper II). We only used female pigs and we cannot exclude the possibility of hormonal interference with our results. Finally, the concept of using otherwise healthy animals may be questioned, since patients in the clinical setting often have additional co-morbidities.

Since the earliest reports of critical illness polyneuropathy and myopathy, sepsis / SIRS and various medications have been suspected of contributing to the neuromuscular changes observed[42-48]. As a result, many institutions have revised routines for deep sedation during ICU care, opting instead for "lighter" sedation without paralysis, resulting in more conscious patients [106-107]. Lighter sedation, together with more active attempts at patient mobilisation, are thought to lead to shorter ICU stays and reduced risk of CIPNM [108-109]. As critical illness hampers patient mobilization in the ICU, it is very difficult to use retrospective data to separate and quantify how different aspects of ICU care contributed to complications such as CIPNM. Our data suggests, for example, that inactivity itself may contribute most to CIPNM. In addition to separate, unique pathologic mechanisms, an association between sepsis and CIPNM might also reflect the fact that sepsis prolongs ICU stay and thereby inhibits mobilisation. It may be suggested that sepsis is a surrogate marker for inactivity and delayed mobilisation. In support of this assertion, the duration of mechanical ventilation has been

associated with CIPNM in three studies, one of which included a multivariate analysis of independent risk factors [29, 44].

If a patient presents with symptoms of symmetrical extremity weakness, or signs of difficulty in weaning from mechanical ventilation, including having to change from non-invasive[110] to mechanical ventilation for no obvious reason, a thorough CIPNM investigation should be initiated. This should include EMG in addition to ENeG if the patient is cooperative, otherwise a muscle biopsy might be necessary. If there is reason to believe that the patient will encounter difficulties in rehabilitation after the ICU as a result of muscular weakness, the CIPNM diagnosis is also important. Not only is it important for the patient and relatives to comprehend why there is a pronounced muscle weakness [111], but also for future caregivers to evaluate the result of rehabilitation correctly and to adjust physiotherapy and training more closely to the individual patient's needs.

After World War II, there was already an insight into the importance of rehabilitation. Not only was the personal benefit of rehabilitation appreciated, but also the spectacular economical gain for society [112]. In 1949, when Rusk published this study, and for many years after, rehabilitation was considered "the third phase of medicine", applied after diagnosis and treatment. This view has now changed. Rehabilitation is so important that it should start as soon as the patient is sufficiently stable [108]. There is no doubt that physiotherapy is important in rehabilitation after ICU care [113-114] but there is a lack of sound scientific evidence as to which treatments are most effective, and this also holds true for CIPNM rehabilitation. In the NICE (National Institute for Health and Clinical Excellence) clinical guidelines entitled "rehabilitation after critical illness", it is stated that no evidence-based rehabilitation guideline for the management of critical care-associated morbidity exists. No clinical practice specifically targeted at physiotherapy and "muscle weakness" is suggested in the report [115].

Although physiotherapy probably does not enhance nerve reinnervation, a patient with polyneuropathy may experience problems both with balance and proprioception, and needs rehabilitation that targets these problems. If a pure myopathy exists, enhanced strength or endurance training might stimulate mRNA [116-117], thereby producing more myosin or other contractile proteins [118], which possibly could accelerate rehabilitation. If the early changes described in paper III and IV do occur, they probably do so before the patient has been diagnosed with CIPNM. More research into pharmacological intervention needs to be done to reverse these changes. As mentioned in the introduction, there are many more functions to the skeletal muscle fibre than being just a support and force generator. Intervening in highly specific pathways to enhance mitochondrial function in critical illness may involve many setbacks before we understand sufficiently.

There have been earlier interventions with both growth hormone and magnesium [119-121] which did not show benefit, and treatment with growth hormone actually increased the mortality [121]. Until further research has revealed more facts about muscular changes in critical illness, the usual ICU rule "make it normal" may apply. An individually targeted rehabilitation plan that includes physiotherapy for an ICU patient who has been correctly diagnosed with CIPNM is a good start.

Quoting Rusk on rehabilitation, in 1949 [112] :
"The .... physician who fails to see that those patients under his care receive the full benefits

of modern methods of medical rehabilitation and retraining, is in the same category as the physician who still persists in using dietary restriction alone in the management of diabetes, when insulin is available, for medical care is not complete until the patient has been trained to live and work with what he has left".

Conclusions

I. For rapid diagnosis, horizontal SDS-pore PAGE is suggested as a diagnostic tool to determine the myosin/actin ratio in patients with suspected critical illness myopathy.

II. CIPNM can be seen early in the ICU course and seems to be more likely to occur in patients with sepsis and corticosteroid treatment. The temporal pattern of CIPNM varies and does not seem to predict the clinical course.

III. Immobilisation appears to be a key element triggering the lowering of the CMAP amplitude. Sepsis and/or corticosteroid treatment decreased the specific force generation at the single muscle fibre level, independent of muscle fibre type.

IV. In an experimental ICU model, inactivity does not seem to affect mitochondrial function. During mechanical ventilation, interventions with either corticosteroids or endotoxin, or the combination of endotoxemia, corticosteroids and NMBA, decrease Complex I activity in both respiratory and non-respiratory muscle.

Acknowledgments

My sincere thanks go to many, many people. I provide a representative sample:

My wife Christine: this book is not thick enough to express my gratitude, love and everything else. You play in a different league. Or scientifically speaking: wow. Especially your love, but also your clear-sighted academic/common sense eyes made me come through some very difficult times. When this thesis is long forgotten (should not take too long) I still have you. What a blessing.

My (ok Christine, I mean our) children Tilde, Theodor and Edward. I love you so much, so much. The meaning of life is not 42, now I know. Ni äger!

Mats and Berit, for your continous support. This last summer was really extraordinary - thank you for locking me up in that little room of yours.

My supervisor Peter Radell. Being an excellent clinician, researcher and human being, I am really thankful I got to know you. Even after you fell asleep during my discussion of the results with you.

My co-supervisor, Lars I Eriksson. I would not have started this research project without your ideas and positive spirit. If I had a tenth of your energy I would fit in a Duracell commercial.

My co-supervisor Tor Ansved. I can not think of any better patient-oriented doctor than you. Also being an academic role model and king of puns (even surpassing me) you continue to amaze me.

Professor Sten Lindahl. Thanks for employing me in those golden days, and really showing what visions in research are.

Associate Professor Lars Irestedt. Thanks for supporting my work and being a good friend. I always enjoyed sharing those cinnamon rolls with you. Egengkligen.

Professor Claes Frostell. When I felt a bit down-hearted during research you gave me some excellent advice. Good luck in the future – I feel honoured I had the opportunity getting to know you.

Professor Eddie Weitzberg. I am sorry we had too few academic discussions, maybe because you tried to poison me with beetroots.

Professor Björn Biber. Thanks for introducing me to the field of research in 1996. I will never forget the ”gun ball” which ”screwed in”.

Kirsi, the one and only humanised schedule computer in the world. Thanks for listening to my wishes and making them come true (almost) every schedule period. Sorry I will not be on your roster anymore.

Pellina, for helping us out in papers III and IV. Always trying to help me when asking silly questions. And I never caught you in a bad mood. Remarkable.

My former roommates, Olof and Håkan. You added to my inferiority complex every day. And you filled the room with technical equipment all the time. Love you guys.

Everyone at the Pain Clinic at Karolinska Solna: Berit, Hélène, Carina, Lisa, Inger, Ylva, Birgitta, Barbro and Anders – thank you! Special thanks to Karl-Fredrik who made it possible to combine my clinical duties with research. Thanks Staffan Arnér for your clinical support when I was a newbie!

All my new colleagues and friends at the Pain Clinic at Capio St Görans Hospital. Thank you for your consideration when I just started working…..and tried to concentrate on my research. Thank you Gunilla for giving me research time.

Spotify and tyda.se, for offering a service which really helped my writing.

My friend Derrick Garwood, for reading and commenting on my so-called English.

All co-authors and all members of the research group. Keep up the good work, with sincere wishes from the fifth wheel.

Ringvor Hägglöf, for making this book look so good. Any errors are on me!

Riadh, for letting me use your picture on the front of this book. Good luck with the leukemia project!

All my colleagues and friends at the ANOPIVA clinic, setting the best clinical and academic standards. I always enjoyed working together with you, both day- and nighttime. I wish patients, hospital administrators and politicians knew the goldmine they have.

And of course, _____ (fill in blank). I almost forgot you. The only reason I did not mention you is my lack of memory. Please forgive me.

References

1. Bolton CF. Neuromuscular conditions in the intensive care unit. Intensive Care Med 1996;22:841-3.
2. Friedrich O. Critical illness myopathy: what is happening? Curr Opin Clin Nutr Metab Care 2006;9:403-9.
3. Hudson LD, Lee CM. Neuromuscular sequelae of critical illness. N Engl J Med 2003;348:745-7.
4. Brooke MH, Kaiser KK. Three "myosin adenosine triphosphatase" systems: the nature of their pH lability and sulfhydryl dependence. J Histochem Cytochem 1970;18:670-2.
5. Pette D, Peuker H, Staron RS. The impact of biochemical methods for single muscle fibre analysis. Acta Physiol Scand 1999;166:261-77.
6. Pedersen BK, Akerstrom TC, Nielsen AR, Fischer CP. Role of myokines in exercise and metabolism. J Appl Physiol 2007;103:1093-8.
7. Petersen AM, Pedersen BK. The anti-inflammatory effect of exercise. J Appl Physiol 2005;98:1154-62.
8. Starkie R, Ostrowski SR, Jauffred S, Febbraio M, Pedersen BK. Exercise and IL-6 infusion inhibit endotoxin-induced TNF-alpha production in humans. FASEB J 2003;17:884-6.
9. Koch AE, Polverini PJ, Kunkel SL, et al. Interleukin-8 as a macrophage-derived mediator of angiogenesis. Science 1992;258:1798-801.
10. Akerstrom T, Steensberg A, Keller P, Keller C, Penkowa M, Pedersen BK. Exercise induces interleukin-8 expression in human skeletal muscle. J Physiol 2005;563:507-16.
11. Furmanczyk PS, Quinn LS. Interleukin-15 increases myosin accretion in human skeletal myogenic cultures. Cell Biol Int 2003;27:845-51.
12. Argiles JM, Lopez-Soriano J, Almendro V, Busquets S, Lopez-Soriano FJ. Cross-talk between skeletal muscle and adipose tissue: a link with obesity? Med Res Rev 2005;25:49-65.
13. Sellers JR. Myosins: a diverse superfamily. Biochim Biophys Acta 2000;1496:3-22.
14. Odronitz F, Kollmar M. Drawing the tree of eukaryotic life based on the analysis of 2,269 manually annotated myosins from 328 species. Genome Biol 2007;8:R196.
15. Larsson L, Li X, Edstrom L, et al. Acute quadriplegia and loss of muscle myosin in patients treated with nondepolarizing neuromuscular blocking agents and corticosteroids: mechanisms at the cellular and molecular levels. Crit Care Med 2000;28:34-45.
16. Matsumoto N, Nakamura T, Yasui Y, Torii J. Analysis of muscle proteins in acute quadriplegic myopathy. Muscle Nerve 2000;23:1270-6.
17. Huxley HE. The mechanism of muscular contraction. Science 1969;164:1356-65.
18. Hodson-Tole EF, Wakeling JM. Motor unit recruitment for dynamic tasks: current understanding and future directions. J Comp Physiol B 2009;179:57-66.
19. Feinberg J. EMG: myths and facts. HSS J 2006;2:19-21.
20. Berthelsen PG, Cronqvist M. The first intensive care unit in the world: Copenhagen 1953. Acta Anaesthesiol Scand 2003;47:1190-5.
21. Weinstein RA. Epidemiology and control of nosocomial infections in adult intensive care units. Am J Med 1991;91:179S-84S.
22. Bone RC, Balk RA, Cerra FB, et al. Definitions for sepsis and organ failure

and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/ Society of Critical Care Medicine. Chest 1992;101:1644-55.
23. MacFarlane IA, Rosenthal FD. Severe myopathy after status asthmaticus. Lancet 1977;2:615.
24. Bolton CF, Gilbert JJ, Hahn AF, Sibbald WJ. Polyneuropathy in critically ill patients. J Neurol Neurosurg Psychiatry 1984;47:1223-31.
25. Bolton CF. Neuromuscular manifestations of critical illness. Muscle Nerve 2005;32:140-63.
26. Latronico N, Fenzi F, Recupero D, et al. Critical illness myopathy and neuropathy. Lancet 1996;347:1579-82.
27. Stevens RD, Dowdy DW, Michaels RK, Mendez-Tellez PA, Pronovost PJ, Needham DM. Neuromuscular dysfunction acquired in critical illness: a systematic review. Intensive Care Med 2007;33:1876-91.
28. Leijten FS, De Weerd AW, Poortvliet DC, De Ridder VA, Ulrich C, Harink-De Weerd JE. Critical illness polyneuropathy in multiple organ dysfunction syndrome and weaning from the ventilator. Intensive Care Med 1996;22:856-61.
29. Witt NJ, Zochodne DW, Bolton CF, et al. Peripheral nerve function in sepsis and multiple organ failure. Chest 1991;99:176-84.
30. Medical Research Council. Aids to the Investigation of peripheral nerve injuries. London, England: HMSO 1975.
31. Faragher MW, Day BJ, Dennett X. Critical care myopathy: an electrophysiological and histological study. Muscle Nerve 1996;19:516-8.
32. Gutmann L. Critical illness neuropathy and myopathy. Arch Neurol 1999;56:527-8.
33. Hirano M, Ott BR, Raps EC, et al. Acute quadriplegic myopathy: a complication of treatment with steroids, nondepolarizing blocking agents, or both. Neurology 1992;42:2082-7.
34. Sander HW, Golden M, Danon MJ. Quadriplegic areflexic ICU illness: selective thick filament loss and normal nerve histology. Muscle Nerve 2002;26:499-505.
35. Sher JH, Shafiq SA, Schutta HS. Acute myopathy with selective lysis of myosin filaments. Neurology 1979;29:100-6.
36. Zochodne DW, Ramsay DA, Saly V, Shelley S, Moffatt S. Acute necrotizing myopathy of intensive care: electrophysiological studies. Muscle Nerve 1994;17:285-92.
37. 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;106:206-13.
38. Schweickert WD, Hall J. ICU-acquired weakness. Chest 2007;131:1541-9.
39. England JD, Gronseth GS, Franklin G, et al. Distal symmetric polyneuropathy: a definition for clinical research: report of the American Academy of Neurology, the American Association of Electrodiagnostic Medicine, and the American Academy of Physical Medicine and Rehabilitation. Neurology 2005;64:199-207.
40. Coakley JH, Nagendran K, Yarwood GD, Honavar M, Hinds CJ. Patterns of neurophysiological abnormality in prolonged critical illness. Intensive Care Med 1998;24:801-7.
41. Latronico N. Neuromuscular alterations in the critically ill patient: critical illness myopathy, critical illness neuropathy, or both? Intensive Care Med 2003;29:1411-3.
42. Amaya-Villar R, Garnacho-Montero J, Garcia-Garmendia JL, et al. Steroid-induced myopathy in patients intubated due to exacerbation of chronic obstructive pulmonary disease. Intensive Care Med 2005;31:157-61.
43. Bednarik J, Vondracek P, Dusek L, Moravcova E, Cundrle I. Risk factors

for critical illness polyneuromyopathy. J Neurol 2005;252:343-51.
44. De Jonghe B, Sharshar T, Lefaucheur JP, et al. Paresis acquired in the intensive care unit: a prospective multicenter study. JAMA 2002;288:2859-67.
45. 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;29:2281-6.
46. Garnacho-Montero J, Madrazo-Osuna J, Garcia-Garmendia JL, et al. Critical illness polyneuropathy: risk factors and clinical consequences. A cohort study in septic patients. Intensive Care Med 2001;27:1288-96.
47. Thiele RI, Jakob H, Hund E, et al. Sepsis and catecholamine support are the major risk factors for critical illness polyneuropathy after open heart surgery. Thorac Cardiovasc Surg 2000;48:145-50.
48. Van den Berghe G, Schoonheydt K, Becx P, Bruyninckx F, Wouters PJ. Insulin therapy protects the central and peripheral nervous system of intensive care patients. Neurology 2005;64:1348-53.
49. Fredriksson K, Hammarqvist F, Strigard K, et al. Derangements in mitochondrial metabolism in intercostal and leg muscle of critically ill patients with sepsis-induced multiple organ failure. Am J Physiol Endocrinol Metab 2006;291:E1044-50.
50. Fredriksson K, Tjader I, Keller P, et al. Dysregulation of mitochondrial dynamics and the muscle transcriptome in ICU patients suffering from sepsis induced multiple organ failure. PLoS One 2008;3:e3686.
51. Herridge MS, Cheung AM, Tansey CM, et al. One-year outcomes in survivors of the acute respiratory distress syndrome. N Engl J Med 2003;348:683-93.
52. Adams GR, Caiozzo VJ, Baldwin KM. Skeletal muscle unweighting: spaceflight and ground-based models. J Appl Physiol 2003;95:2185-201.
53. Fink H, Yasuhara S, Blobner M, Martyn JA. Up-regulation of acetylcholine receptors during subchronic infusion of pancuronium is caused by a posttranscriptional mechanism related to disuse. Crit Care Med 2004;32:509-13.
54. Britt RC, Devine A, Swallen KC, et al. Corticosteroid use in the intensive care unit: at what cost? Arch Surg 2006;141:145-9; discussion 9.
55. Lecker SH, Solomon V, Mitch WE, Goldberg AL. Muscle protein breakdown and the critical role of the ubiquitin-proteasome pathway in normal and disease states. J Nutr 1999;129:227S-37S.
56. Schakman O, Gilson H, Thissen JP. Mechanisms of glucocorticoid-induced myopathy. J Endocrinol 2008;197:1-10.
57. Dirks-Naylor AJ, Griffiths CL. Glucocorticoid-induced apoptosis and cellular mechanisms of myopathy. J Steroid Biochem Mol Biol 2009;117:1-7.
58. Arroliga A, Frutos-Vivar F, Hall J, et al. Use of sedatives and neuromuscular blockers in a cohort of patients receiving mechanical ventilation. Chest 2005;128:496-506.
59. Meade MO, Cook DJ, Guyatt GH, et al. Ventilation strategy using low tidal volumes, recruitment maneuvers, and high positive end-expiratory pressure for acute lung injury and acute respiratory distress syndrome: a randomized controlled trial. JAMA 2008;299:637-45.
60. Mercat A, Richard JC, Vielle B, et al. Positive end-expiratory pressure setting in adults with acute lung injury and acute respiratory distress syndrome: a randomized controlled trial. JAMA 2008;299:646-55.
61. Papazian L, Forel JM, Gacouin A, et al. Neuromuscular blockers in early acute respiratory distress syndrome. N Engl J Med;363:1107-16.
62. Martyn JA, Richtsfeld M. Succinylcholine-induced hyperkalemia in acquired pathologic states: etiologic factors and molecular mechanisms.

Anesthesiology 2006;104:158-69.
63. Friedrich O, Fink RH, Hund E. Understanding critical illness myopathy: approaching the pathomechanism. J Nutr 2005;135:1813S-7S.
64. Harrois A, Huet O, Duranteau J. Alterations of mitochondrial function in sepsis and critical illness. Curr Opin Anaesthesiol 2009;22:143-9.
65. Singer M, Brealey D. Mitochondrial dysfunction in sepsis. Biochem Soc Symp 1999;66:149-66.
66. Cohen J, Guyatt G, Bernard GR, et al. New strategies for clinical trials in patients with sepsis and septic shock. Crit Care Med 2001;29:880-6.
67. Radell PJ, Eleff SM, Traystman RJ, Nichols DG. In vivo diaphragm metabolism: comparison of paced and inspiratory resistive loaded breathing in piglets. Crit Care Med 1997;25:339-45.
68. Lane R. Handbook of muscle disease. New York: Marcel Dekker. 1996.
69. Ochala J, Ahlbeck K, Radell PJ, Eriksson LI, Larsson L. Factors underlying the early limb muscle weakness in acute quadriplegic myopathy using an experimental ICU porcine model. PLoS One;6:e20876.
70. Latronico N, Bertolini G, Guarneri B, et al. Simplified electrophysiological evaluation of peripheral nerves in critically ill patients: the Italian multi-centre CRIMYNE study. Crit Care 2007;11:R11.
71. Rich MM, Bird SJ, Raps EC, McCluskey LF, Teener JW. Direct muscle stimulation in acute quadriplegic myopathy. Muscle Nerve 1997;20:665-73.
72. van de Leur JP, van der Schans CP, Loef BG, Deelman BG, Geertzen JH, Zwaveling JH. Discomfort and factual recollection in intensive care unit patients. Crit Care 2004;8:R467-73.
73. Berek K, Margreiter J, Willeit J, Berek A, Schmutzhard E, Mutz NJ. Polyneuropathies in critically ill patients: a prospective evaluation. Intensive Care Med 1996;22:849-55.
74. Hund E, Genzwurker H, Bohrer H, Jakob H, Thiele R, Hacke W. Predominant involvement of motor fibres in patients with critical illness polyneuropathy. Br J Anaesth 1997;78:274-8.
75. Hermans G, De Jonghe B, Bruyninckx F, Van den Berghe G. Interventions for preventing critical illness polyneuropathy and critical illness myopathy. Cochrane Database Syst Rev 2009:CD006832.
76. Steinberg KP, Hudson LD, Goodman RB, et al. Efficacy and safety of corticosteroids for persistent acute respiratory distress syndrome. N Engl J Med 2006;354:1671-84.
77. Fitts RH, Romatowski JG, Peters JR, Paddon-Jones D, Wolfe RR, Ferrando AA. The deleterious effects of bed rest on human skeletal muscle fibers are exacerbated by hypercortisolemia and ameliorated by dietary supplementation. Am J Physiol Cell Physiol 2007;293:C313-20.
78. Houstis N, Rosen ED, Lander ES. Reactive oxygen species have a causal role in multiple forms of insulin resistance. Nature 2006;440:944-8.
79. Reid MB. Nitric oxide, reactive oxygen species, and skeletal muscle contraction. Med Sci Sports Exerc 2001;33:371-6.
80. Schakman O, Gilson H, Kalista S, Thissen JP. Mechanisms of muscle atrophy induced by glucocorticoids. Horm Res 2009;72 Suppl 1:36-41.
81. Goldberg AL, Tischler M, DeMartino G, Griffin G. Hormonal regulation of protein degradation and synthesis in skeletal muscle. Fed Proc 1980;39:31-6.
82. Ochala J, Larsson L. Effects of a preferential myosin loss on Ca2+ activation of force generation in single human skeletal muscle fibres. Exp Physiol 2008;93:486-95.
83. Rich MM, Pinter MJ. Sodium channel inactivation in an animal model of acute quadriplegic myopathy. Ann Neurol 2001;50:26-33.
84. 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;547:555-66.
85. Rich MM, Pinter MJ, Kraner SD, Barchi RL. Loss of electrical excitability in an animal model of acute quadriplegic myopathy. Ann Neurol 1998;43:171-9.
86. Kilpatrich-Smith L, Erecinska M, Silver IA. Early cellular responses in vitro to endotoxin administration. Circ Shock 1981;8:585-600.
87. Hirai F, Aoyama H, Ohtoshi M, Kawashima S, Ozawa K, Tobe T. Significance of mitochondrial enhancement in hepatic energy metabolism in relation to alterations in hemodynamics in septic pigs with severe peritonitis. Eur Surg Res 1984;16:148-55.
88. Lam C, Tyml K, Martin C, Sibbald W. Microvascular perfusion is impaired in a rat model of normotensive sepsis. J Clin Invest 1994;94:2077-83.
89. Schaefer CF, Biber B, Lerner MR, Jobsis-VanderVliet FF, Fagraeus L. Rapid reduction of intestinal cytochrome a,a3 during lethal endotoxemia. J Surg Res 1991;51:382-91.
90. Brealey D, Brand M, Hargreaves I, et al. Association between mitochondrial dysfunction and severity and outcome of septic shock. Lancet 2002;360:219-23.
91. Carre JE, Orban JC, Re L, et al. Survival in critical illness is associated with early activation of mitochondrial biogenesis. Am J Respir Crit Care Med;182:745-51.
92. Piantadosi CA. Carbon monoxide, reactive oxygen signaling, and oxidative stress. Free Radic Biol Med 2008;45:562-9.
93. Hermans G, Wilmer A, Meersseman W, et al. Impact of intensive insulin therapy on neuromuscular complications and ventilator dependency in the medical intensive care unit. Am J Respir Crit Care Med 2007;175:480-9.
94. Michie HR. The value of animal models in the development of new drugs for the treatment of the sepsis syndrome. J Antimicrob Chemother 1998;41 Suppl A:47-9.
95. Bertoni G, Kuhnert P, Peterhans E, Pauli U. Improved bioassay for the detection of porcine tumor necrosis factor using a homologous cell line: PK(15). J Immunol Methods 1993;160:267-71.
96. Murtaugh MP. Porcine cytokines. Vet Immunol Immunopathol 1994;43:37-44.
97. Suzuki Y, Yeung AC, Ikeno F. The representative porcine model for human cardiovascular disease. J Biomed Biotechnol;2011:195483.
98. Zanotti-Cavazzoni SL, Goldfarb RD. Animal models of sepsis. Crit Care Clin 2009;25:703-19, vii-viii.
99. Abraham E, Reinhart K, Opal S, et al. Efficacy and safety of tifacogin (recombinant tissue factor pathway inhibitor) in severe sepsis: a randomized controlled trial. JAMA 2003;290:238-47.
100. Aranow JS, Wang H, Zhuang J, Fink MP. Effect of human hemoglobin on systemic and regional hemodynamics in a porcine model of endotoxemic shock. Crit Care Med 1996;24:807-14.
101. Haglund U, Hulten L, Ahren C, Lundgren O. Mucosal lesions in the human small intestine in shock. Gut 1975;16:979-84.
102. Herbertson MJ, Werner HA, Studer W, Russell JA, Walley KR. Decreased left ventricular contractility during porcine endotoxemia is not prevented by ibuprofen. Crit Care Med 1996;24:815-9.
103. Nieman GF, Gatto LA, Paskanik AM, Yang B, Fluck R, Picone A. Surfactant replacement in the treatment of sepsis-induced adult respiratory distress syndrome in pigs. Crit Care Med 1996;24:1025-33.
104. Murphey ED, Traber DL. Cardiopulmonary and splanchnic blood flow during 48 hours of a continuous infusion of endotoxin in conscious pigs: a model of hyperdynamic shock. Shock 2000;13:224-9.
105. Vassilev D, Hauser B, Bracht H, et al. Systemic, pulmonary,

and hepatosplanchnic effects of N-acetylcysteine during long-term porcine endotoxemia. Crit Care Med 2004;32:525-32.
106. Martin J, Heymann A, Basell K, et al. Evidence and consensus-based German guidelines for the management of analgesia, sedation and delirium in intensive care--short version. Ger Med Sci;8:Doc02.
107. Skrobik Y, Ahern S, Leblanc M, Marquis F, Awissi DK, Kavanagh BP. Protocolized intensive care unit management of analgesia, sedation, and delirium improves analgesia and subsyndromal delirium rates. Anesth Analg;111:451-63.
108. Bailey P, Thomsen GE, Spuhler VJ, et al. Early activity is feasible and safe in respiratory failure patients. Crit Care Med 2007;35:139-45.
109. Morris PE, Goad A, Thompson C, et al. Early intensive care unit mobility therapy in the treatment of acute respiratory failure. Crit Care Med 2008;36:2238-43.
110. Sancho J, Servera E. Noninvasive ventilation for patients with neuromuscular disease and acute respiratory failure. Chest 2008;133:314-5; author reply 5.
111. Misak CJ. ICU-acquired weakness: obstacles and interventions for rehabilitation. Am J Respir Crit Care Med;183:845-6.
112. Rusk HA. Rehabilitation; the third phase of medicine. Can Med Assoc J 1949;61:603-7.
113. Evans RL, Connis RT, Hendricks RD, Haselkorn JK. Multidisciplinary rehabilitation versus medical care: a meta-analysis. Soc Sci Med 1995;40:1699-706.
114. Johnston MV, Wood KD, Fiedler R. Characteristics of effective and efficient rehabilitation programs. Arch Phys Med Rehabil 2003;84:410-8.
115. Tan T, Brett SJ, Stokes T. Rehabilitation after critical illness: summary of NICE guidance. BMJ 2009;338:b822.
116. Akimoto T, Pohnert SC, Li P, et al. Exercise stimulates Pgc-1alpha transcription in skeletal muscle through activation of the p38 MAPK pathway. J Biol Chem 2005;280:19587-93.
117. Wright DC, Han DH, Garcia-Roves PM, Geiger PC, Jones TE, Holloszy JO. Exercise-induced mitochondrial biogenesis begins before the increase in muscle PGC-1alpha expression. J Biol Chem 2007;282:194-9.
118. Gelfi C, Vasso M, Cerretelli P. Diversity of human skeletal muscle in health and disease: contribution of proteomics. J Proteomics;74:774-95.
119. Johnson D, Gallagher C, Cavanaugh M, Yip R, Mayers I. The lack of effect of routine magnesium administration on respiratory function in mechanically ventilated patients. Chest 1993;104:536-41.
120. Pichard C, Kyle U, Chevrolet JC, et al. Lack of effects of recombinant growth hormone on muscle function in patients requiring prolonged mechanical ventilation: a prospective, randomized, controlled study. Crit Care Med 1996;24:403-13.
121. Takala J, Ruokonen E, Webster NR, et al. Increased mortality associated with growth hormone treatment in critically ill adults. N Engl J Med 1999;341:785-92.

This is not the beginning. It´s not even the end of the beginning. It´s the end.