Impaired physical function, loss of muscle mass and assessment of biomechanical properties in critical ill patients

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THE FOUR ORIGINAL PAPERS ARE

1. Poulsen JB, Møller K, Kehlet H and Perner A. Long-term physical outcome in patients with septic shock. Acta Anaesthesiol Scand. 2009; 53: 724-30

2. Poulsen JB, Møller K, Jensen CV, Weisdorf S, Kehlet H and Perner A. Effect of transcutaneous electrical muscle stimulation on muscle volume in patients with septic shock. Crit Care Med. 2011; 39: 456-61

3. Poulsen JB, Rose MH, Perner A, Møller K and Jensen BR. Fatigability of the quadriceps muscle in non-cooperating subjects: reliability of a novel non-invasive model. Submitted

4. Poulsen JB, Rose MH, Jensen BR, Møller K and Perner A. Biomechanical and non-functional assessment of physical capacity in male intensive care unit survivors. Accepted for publication in Crit Care Med

INTRODUCTION

The idea to conduct the studies in this dissertation originated from an article published by Herridge and colleagues [1] in 2003. The authors reported that one-year after being treated for acute respiratory distress syndrome survivors were burdened with severe impairment of physical function and not by, as expected, sequelae related to the respiratory failure they initially were treated for in the intensive care unit (ICU). We therefore set out to further elucidate the consequences that critical illness and intensive care have on muscle mass and physical function in ICU patients at different stages.

AIMS

The aims of the study was

To assess the physical function and socio-economical outcomes in survivors of septic shock (Study I)

To assess the effect of early transcutaneous electrical muscle stimulation (TEMS) on muscle volume in septic patients admitted to the ICU (Study II).

To develop a non-invasive method to evaluate fatigability of the quadriceps muscle in non-cooperating subjects and to assess the reliability of this method (Study III).

To investigated the biomechanical properties of the quadriceps muscle in ICU survivors 12 months after ICU discharge (Study IV).

BACKGROUND

ICU OUTCOME ASSESSMENT

During the last decades therapeutic and technologically advancements have significantly improved ICU survival rates [2-4]. This success has redirected ICU therapy assessment from traditional short-term outcomes, most frequently expressed in terms of mortality, toward an increased focus on long-term perspectives, where patient-centered outcomes such as morbidity and health related quality of life (HRQOL) are subjects for evaluation. Although reducing ICU mortality remains very important, it is becoming increasingly more evident that it cannot be the sole focus of ICU physicians. Short-term outcome measures fails to address the quality of those lives saved and the impact critical illness and intensive care has on ICU survivor's ongoing health [5]. The implementation of patient-centered outcomes has provided researchers and clinicians with valuable knowledge on physical recovery, HRQOL, socio-economic consequences and long-term prognosis of ICU admission. These outcome measures are essential to adequately advice patients and relatives and to clinical decision-making. In the pursuit of clinical improvement, the importance of appropriate outcome measures is hard to underestimate, as expressed in the report "Crossing the Quality Chasm: A New Health System for the 21st Century" by the US National Institute of Medicine:

"Finally, all organizations—whether or not health care related—can improve their performance only by incorporating care process and outcome measures into their daily work. Use of such measures makes it possible to understand the degree to which performance is consistent with best practices, and the extent to which patients are being helped."

Medical outcome survey Short Form-36 (SF-36)

The SF-36 is a generic measure designed to evaluate health-related quality of life (HRQOL) in general and specific populations. It consists of 36 items divided into eight domains. The eight domains are: physical functioning (PF), role physical (RP), bodily pain (BP), general health (GH), vitality (VT), social functioning (SF), role emotional (RE) and mental health (MH) [6]. Based on the response to several specific questions, each domain is linearly transformed into a subscale from 0 to 100 with a higher score reflecting a better quality of life and 0 implying the worst possible health status. Based upon the eight domains, two summary scales have been constructed for physical and mental health respectively. The domains mainly contributing to the physical component summary (PCS)-score are PF, RP, BP and GH. The values originating from the VT, SF, RE and MH domains dominate the mental component summary (MCS)-score [7].

Physical function

Decreased physical function is a commonly observed sequelae after critical illness. In the majority of ICU survivors physical recovery is slow and occurs predominantly within the first 6 month [1, 8]. Many patients never fully regain their preadmission functional status and residual physical limitations may persist for years after ICU discharge [9, 10]. Commonly used measures of physical function in ICU survivors include activities of daily living (ADL), HRQOL and performance based measurements (6-min walk test and strength test).

In the present study we focused our investigations on a single muscle group, musculus quadriceps femoris. We did so because the muscles of the lower leg, thigh and gluteal region are the main contributors to ADL and gait function and subject to a larger degree of muscle atrophy than muscles of the upper extremities after bedrest [11]. Accordingly, quadriceps weakness has been associated with decreased ADL performance, increased incidence of falls, loss of mobility and mortality in elderly subject [12-14]. Because old adults perform ADL near their maximal capabilities [15] even a moderate loss of muscle strength may reduce the ability to perform activities of daily living (Figure 1), degree of independency [16] and increased hospital readmission rates [17, 18] adding to the burden on ICU survivors and health care systems [19].

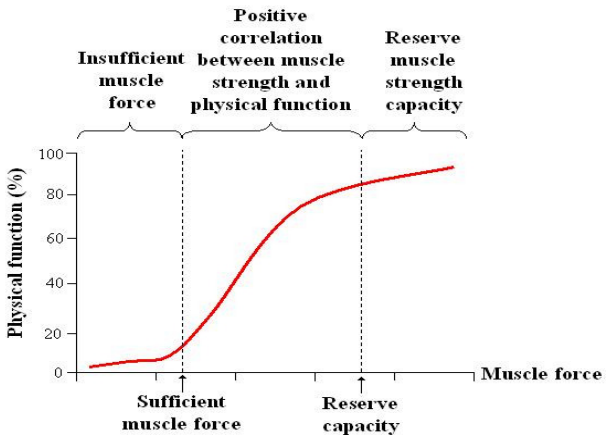

Figure 1: Schematic drawing of the muscle force/ physical function relationship. Modified from [20]

The abovementioned relations may explain why, twelve months after ICU discharge, 69 % of mechanically ventilated ICU patients were still restricted with regard to, and required additional home-based care to facilitate, activity of daily living [21]. It may also explain why, survivors of the acute respiratory distress syndrome (ARDS) exhibited reduced performance in a six-minute walking test, in conjunction with self-reported poor function, proximal weakness and fatigue, which was attributed to loss of muscle mass by the patients [22]. The prevalence and magnitude of the physical impairment reported in these studies were comparable to that of survivors of septic shock after ICU discharge [23] (Study I). Strikingly, despite the clinical significance of muscle atrophy for loss of function after ICU discharge, no preventive therapies have been identified as yet.

MUSCLE CATABOLISM INDUCED BY CRITICAL ILLNESS AND INTENSIVE CARE

Muscle wasting occurring from ICU treatment has become recognized as a common and likely important factor to the neuromuscular complications observed in ICU survivors [24-26]. As shown in Study II, the loss of muscle mass occurs rapidly, with a 20% reduction in thigh muscle volume in septic patients during the first week of ICU stay. Muscle loss in ICU patients follows a logarithmic curve26. As immobilization [27] and inflammation [28] are most pronounced in the early phase of ICU treatment, their contribution to the metabolic changes may explain why the rate of muscle loss is greatest during this period. Accordingly, septic patients showed an early massive loss of total body protein where approximately 70% of this protein originated from the hydrolysis of skeletal muscle protein. After 10 days the loss of protein was predominantly from the viscera [29]. Decreased muscle force as early as one week after onset of critical illness [30] may, in part, also be a consequence of these metabolic changes and their detrimental effect on skeletal muscle.

The cause of muscle atrophy in ICU patients is likely multifactorial, and inflammation, immobilization, insufficient nutrition and the administration of corticosteroids may all contribute [31-34]. ICU patients are potentially exposed to all these causes and may therefore experience a more profound impairment in physical function compared to non-critically ill patients. The role of inflammation and bed rest for skeletal muscle atrophy in critically ill patients is discussed in more detail below.

Inflammation

The metabolic response to critical illness is associated with global hyper-metabolism, insulin resistance and alterations in substrate utilization as well as hormonal and nutritional status. Various complex signaling pathways, triggered by pro-inflammatory cytokines and alterations in neuroendocrine hormones, may promote these changes. Thus, skeletal muscle homeostasis is characterized by the balance between protein synthesis and degradation. Cytokines such as interleukin-6 (IL-6) [35-38] and tumor necrosis factor-alpha (TNF-α) [39, 40] may influence this balance [41-50], both directly by modulating muscle protein turnover, and indirectly, by activation of the hypothalamic–pituitary–adrenal axis [51] (Figure 2).

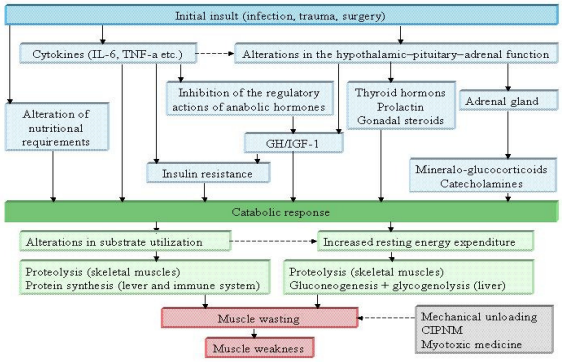

Figure 2: Schematic summary of the inflammation-induced catabolic response

Profound activation of the inflammatory cascade, in the form of the systemic inflammatory response syndrome (SIRS), is seen in conditions such as sepsis, trauma or after major surgery [52-54]. During SIRS, elevated TNF-a levels have been shown to be associated with loss of lean body mass [55, 56] and decreased muscle protein synthesis [57]. It is plausible that both these cytokines, as induced by SIRS, directly leads to disturbances in muscle cell homeostasis, altered protein synthesis rate and increased degradation [58-62]. Thus, as a consequence of their critical illness, the majority of ICU patients are in a hyper catabolic state with a high skeletal protein turnover [63, 64].

Bed rest

During bed rest, skeletal muscles, in particular those of the lower extremities, are exposed to mechanical unloading [65] and reduced neuromuscular activity [66, 67], which stimulates a complex adaptive response with slowing of protein synthesis [68, 69], increased protein degradation [70] and increased apoptosis [71] of the muscle cells. These mechanisms are the main contributors to the muscle atrophy [32, 72, 73] and differential loss of muscle strength [74] seen after prolonged bed rest (Figure 3). Accordingly, in health subject (assessed by magnetic resonance imaging) muscle mass decreases by approximately 14% during 8 weeks of bed rest, corresponding to 0.25% per day with a corresponding 16% loss of muscle strength [75]. The concurrent presence of inflammation synergistically promotes these detrimental effect on skeletal muscles [76], which is supported by the ten fold increased in muscle loss (2.3-2.9% per day) reported in bedridden patients with septic shock (Study II) [77].

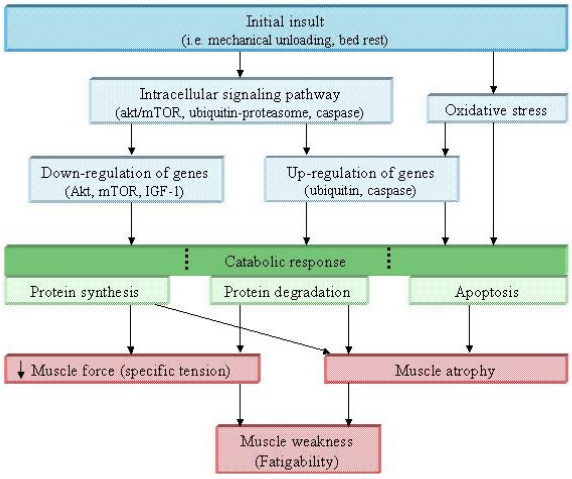

Figure 3: Schematic summary of the immobilization-induced catabolic response

Furthermore, as reported by Ferrando and colleagues [11, 78], approximately 70% of the total loss of lean muscle mass after bed rest occurred in the lower extremities which subsequently adversely affected the patients rehabilitation [79, 80]. Prolonged bed rest also causes comprehensive morphological changes such as altered fiber composition [81-83], reduced oxidative capacity84 and increased fatigability of the muscles [85-87]. These effects are particularly pronounced in ICU patients because of sedative treatment and the high incidence of concurrent inflammation. The diaphragm [88] and upper airway dilator muscles are also very susceptible to decreased muscle strength, which has apparent clinical consequences. Atrophy of the diaphragm may cause increased fatigue of the ventilator muscle with insufficient inspiratory muscle force and lack of upper airway patency due to dilator muscles weakness may cause collapse of upper airways leading to an increased incidence of extubation failures [89, 90]. Both these conditions are associated with prolonged mechanical ventilation [91], thereby further prolonging the period where patients are confined to bed rest – in theory a circulus vitiosus. Similar to muscle loss in critical ill humans, rats show a likewise exponentially decayed in immobilization induced muscle atrophy, with up to half of the total atrophy occurring within the first 4-6 days of immobilization [92].

COUNTERMEASURES

Basically, interventions to prevent loss of muscle mass in critically ill patients may be directed toward either reducing the impact of inflammation, countering the effect of mechanical unloading, or modulating the other major causes of skeletal muscle atrophy. Inflammation: Several generally accepted standard treatment regimes in sepsis might attenuate inflammatory signaling, thus limiting the impact on skeletal muscle tissue. This has been shown with early fluid resuscitation [93] and antibiotics [94, 95]; control of the source of infection and blood glucose levels [96, 97]; minimizing the use of myotoxic medical treatment i.e. corticosteroids [98]; administration of early enteral nutrition [99] and dietary supplements and correction of electrolytes and trace elements [100]. The preventive effect of immunonutrition on muscle wasting and weakness in ICU patients is yet to be fully clarified [101-103]. Antioxidants have in small studies so far presented inconclusive and somewhat conflicting results [104, 105], but are cur-

rently under investigation [106]. Finally, results from trials of growth hormone therapy in ICU patients indicate that this hormone may stimulate muscle protein synthesis [107] and improve nitrogen balance [108], but, on the other hand, may also negatively effect survival rates [109].
Countering mechanical unloading: Passive stretching reduces muscle atrophy in ICU patients [110]. The mechanism behind this effect is not completely elucidated. The effect may be caused by mechanical stress posed on mechanoreceptors in the skeletal muscle cells, inducing an anabolic response (most likely IGF-1) with subsequent adaptation of muscle tissue [111-113]. It seems plausible that electrical muscle stimulation may trigger a similar anabolic response. Recently, however, early mobilization has been shown to be feasible, safe and beneficial [114, 115] to important outcomes such as time to ambulation, ICU and hospital lengths of stay, and physical function before as well as after discharge [116-120]. Furthermore, minimizing the use of sedatives and neuromuscular blockers and optimizing pain treatment both facilitate early mobilization [121, 122]. It is important to recognize that electrical muscle stimulation cannot substitute early mobilization, but may bridge the gap from the early stages of ICU admission, where most patients are poor candidates for exercise therapy, to the time where resistance or weight bearing exercises can be initiated.

Transcutaneous electric muscle stimulation (TEMS)
TEMS is a non-invasive method directed at maintaining muscle properties through artificially induced contractions, independent of patient efforts. As a rehabilitation tool, TEMS is as effective at increasing skeletal muscle strength as is voluntary muscle contraction, when performed at the same intensity [123]. Accordingly, TEMS has been proven effective at preventing decreases in muscle strength and muscle volume of the thigh following immobilization in healthy subjects [124-127] and in different patient populations [128-137] i.e. functionally immobilized patients with chronic heart failure [138] and chronic obstructive pulmonary disease [139]. In these exercise-intolerant patients, TEMS enables passive stimulation of locomotor muscle groups, thus providing an alternative approach for improving physical capacity. Furthermore, in severely disabled COPD patients with incapacitating dyspnea, short term electrical stimulation of selected lower limb muscles involved in ambulation led to improved muscle strength and endurance, whole body exercise tolerance and increased HRQOL [140].
On the other hand, these studies are highly inhomogeneous and all assessed subjects with no or low-grade inflammation. In these individuals, muscle turnover happens at a slower rate, compared to the rapid muscle wasting that is seen in ICU patients. Evaluation of the TEMS in ICU patients is limited to one previous study, where a positive effect on quadriceps muscle thickness (absolute values) was shown [141]. Based on these considerations we found that TEMS was an interesting concept to investigate in a hypercatabolic bedridden ICU population.

NON-INVASIVE ASSESSMENT OF MUSCLE ATROPHY AND STRENGTH IN ICU PATIENTS

Radiological imaging
Due to the inherent difficulties in assessing muscle function in ICU patients, several studies have used radiological imaging to measured changes in muscle size (i.e. muscle cross sectional area, CSA) to evaluate treatment effects of muscle preserving interventions [77, 141-145]. However, changes in muscle volume or CSA in ICU patients assess by repeated radiological measurements are sensitive to a variety of factors including the technique used for making the measurements, the patient population, the study design and the timing of the measurements in relation to the onset of the critical illness (lead-time bias). Therefore, conclusions should be made only after careful consideration of these potential confounders.

Ultrasonography
Ultrasonography is, for pragmatic reasons, the preferred method of choice, and acceptable accuracy has been reported for a single muscle thickness measurement (the coefficient of variation (CV) close to 3% [146]). However, ultrasonography is very operator-dependent and, therefore, sensitive to intrinsic factors such as skill level and observer subjectivity, i.e. due to reestablishment of measuring location and angle during repeated measurements, but also to extrinsic factors such as subcutaneous edema141.

Computed tomography (CT)
Using CT scans to measure thigh muscle mass have also shown high accuracy [147, 148]; however, the sensitivity of the method is limited by the resolution of the CT scan. Mitsiopoulos and colleagues [149] evaluated CT for measuring muscle mass in cadavers and reported that skeletal muscle cross-sectional area was strongly correlated with values obtained directly ($r = 0.99$, $P < 0.001$) with a relative differences of 1.3%. Other studies using CT-scans have reported estimates of the coefficient of variation (CV) for repeat measurements of skeletal muscle area (cm^2) between 0.5-1.5% [150, 151], and 2.1% for muscle volume [152]. Interestingly, previous studies have compared measurements of muscle mass between MRI, CT and ultrasound and found no significant difference between these modalities [143-145]. In Study II we used a third generation CT-scanner (Toshiba Aquilion 64, Tokyo, Japan) with a slides thickness of 3 mm with a 512x512 matrix. The theoretical limit for detectable changes with this resolution fare exceeds any protocol used in the abovementioned studies.

Muscle force measurements
Assessment of muscle force and endurance is clinically important because these compound variables of neural and muscle functions are closely related to physical function and ADL [153], which, if compromised, may affect the quality of life in ICU survivors. Assessment of muscle force in ICU patients is, however, very complicated and several factors may greatly influence the results. These factors, including the choice of measuring tool, needs thorough consideration before conclusions can be drawn. Current methods available can roughly be divided into voluntary or non-voluntary assessment of muscle force.

Voluntary assessment of muscle force
The measurement of voluntary muscle force in ICU patients is susceptible to several limiting factors such as general fatigue, motivation, learning effects, pain during muscular contraction, and alteration of consciousness, effects that are probably particularly pronounced in the early stages of critical illness. This may explain the high variability of voluntary force measurements found in weak patients [154, 155]. Even when consciousness is regained reduced cognition are often pervasive throughout ICU admission [156], obviously hampering patient's cooperation and complicates voluntary assessment of muscle performance in the ICU setting. Even so, studies assessing objective force measure-

ments (handgrip strength in ICU patients using hand held dynanometer) have reported associations with global strength [157] and ADL impairments after ICU discharge [153]. Manual testing has also been used to assess voluntary muscle force in ICU patients [158] and, although crude, it provides some indication of muscle function. However, grading systems based on physical examinations are prone to subjective assessment by the clinician and are unfit to quantify small changes in muscle strength. Muscle weakness, if measured by voluntary methods, conceptually may refer to both a muscle function deficit, a reduction in nerve signaling, and a decline in mental function [159]. This emphasize the complexity in a manually distinction between a clinically significant physiological change in muscle force generating capacity and change due to mental changes. As manual testing and voluntary force measurements rely on patients' cooperation and cognitive function, any conclusions regarding specific contribution of muscle or nerve dysfunction in the development of muscle weakness cannot be justified.

Non-voluntary assessment of muscle force

There are several advantages of evoked force measurements over voluntary force measurements in an ICU setting. Among others, it is independent of subject's cooperation, motivation or cognitive function and, apart from the potentiation issues (relevant for twitch contractions), variability owing to learning effect is neglectable.

On the other hand, evoked force measurements also present some important limitations. In non-cooperating subjects intra-individual comparisons between testing days cannot, for obvious reasons, be based on maximal voluntary force. We therefore chose to calibrate baseline values to performed torque during the first contraction between test days and then evaluate the response of the subsequent sequence of 40 contractions, serving as an indicator of muscle function capacity or fatigability.

Furthermore, the mechanism behind TEMS-induced fatigue differs significantly from the normal physiological response to fatigue. During voluntary exercise, skeletal muscle tissue adapts progressively to fatigue by recruiting more motor units and increased firing frequencies, whereas electrical stimulation dictates a non-physiological fixed firing frequency and a uniform recruitment pattern. This static TEMS recruitment pattern may preferentially exhaust glycolytic pathways, which is mainly found in fast-twitch (type II) fibers [160]. The result is an anaerobic fatigue pattern with an early course of muscle fatigue. Thus, the torque response during fatigue test reflects the biochemical properties intrinsic to the muscle fibers [161] and, and therefore depends on the fiber type distribution of the stimulated muscle [162] and the stimulation protocol. Despite reports of a high correlation between electrical and voluntary torque responses using fatigue protocols [163, 164], this limits the generalizability of TEMS-associated skeletal muscle characteristics to other muscle groups and the comparability to physiological fatigue patterns. Another factor that limits the generalizability between muscle groups is that different muscle groups respond differently to inactivity. Muscles dominated by slow muscle fibers are more profoundly affected by inactivity than muscles with faster fiber compositions. This is probably due to the relatively large change in activity and mechanical stress experienced by postural muscles during general inactivity [165-168]. This is supported by studies reporting that thigh muscles are very sensitive to ICU-induced muscle atrophy [77] and are subject to a larger degree of muscle atrophy than muscles of the upper extremities after bed rest [11]. Quadriceps is the primary locomotor muscle for activities of daily living (ADL) [169] and is therefore likely to be closer related to ADL than force measurement from, for example, the often used thumb muscles [170]. This is also supported by two previous studies, first showing that quadriceps weakness is associated with decreased ADL performance [12-14] and secondly, that in COPD patients adductor pollicis strength can be preserved despite significant weakness in the quadriceps [171]. The choice of muscle group is therefore important in relation to the clinical relevance of the conclusion the force measurements. Accordingly, we chose the quadriceps as our target muscle.

There are two essentially different ways to electrically evoke muscle contractions, transcutaneous electrical muscle stimulation (TEMS) or by peripheral nerve stimulation. Each method has both pros and cons. Peripheral nerve stimulation may potentially recruit a larger portion of the muscle than TEMS, but may also exhibit increased activation of antagonist muscles. In contrast, TEMS has clear pragmatic advantages as motor points are easier identified compared to more profound located nerves, which may also explain why TEMS is perceived as less painful than nerve stimulation. Peripheral nerve stimulation is sensitive to even minor movements, rendering it unfit to use in a training protocol in an ICU We therefore chose TEMS as our preferred stimulation method.

Traditionally, two different test procedures have been used to characterize the contractile properties of muscle tissue, i.e., tetanic and twitch contractions. However, acquiring valid twitch contraction data require a rigid biomechanical experimental set-up, which is extremely difficult to obtain in a clinical environment. Combined with the increased requirement for measurement accuracy in weak patients, bedside measurements of twitch contractions in very weak patients may introduce severe interpretational difficulties. Our protocol therefore contained both testing paradigms, confirming that lack of rigidity in the mechanical set-up may have caused baseline drift, which hampers the data analysis.

GENERAL METHODOLOGICAL CONSIDERATIONS

MEDICAL OUTCOME STUDY SHORT FORM-36 (SF-36)

In the present study, we used the SF-36 questionnaire to evaluate HRQOL because compared with other generic health status instruments, the SF-36 has been shown to be easily applicable and have both a higher internal consistency and a better content validity [172, 173]. It is suitable for discrimination in health status between various groups and also is responsive to the effects of therapeutic interventions [172, 173]. The SF-36 is considered to be a reliable and valid measure of HRQOL in critically ill patients with a variety of different diseases [1, 174-180]. Although a Danish version of the SF-36 questionnaire has been transculturally validated and shown to have high internal consistency in various populations, this has never been done in critically ill patients [181-183]. However, measuring HRQOL in an ICU population has its limitations. Inherent in all generic HRQOL assessment tools is the problem associated with the assumption upon which it is based (i.e. the perception of HRQOL do not differ among different patients groups) and the attempt to convert patients' perception of own health into a score. Both factors may limit the comparability between different populations. Administration of a generic health-evaluating tool is undoubtedly more difficult in an ICU population than in the other populations as ICU survivors often suffer from general fatigue, confusion, attention deficits

and reduced recollection. Data acquisition may therefore introduce bias regardless of the source, patient or proxy [184]. Compared to an age- and sex-matched background population, ICU patients may be burdened with reduced physical function [185, 186] and a higher prevalence of co-morbidity [187] already before ICU admission. Because of this lack of baseline comparability, the contribution of intensive care to a poorer physical outcome among ICU survivors may be misleading, if pre-hospitalization status is not taken into account. Because of the unexpected nature of critical illness such baseline values is difficult to provide.
The magnitude of changes in the SF-36 scores that represents a meaningful change to the patients is difficult to establish188. However, studies of the score profiles of various patient groups have suggested that a 3–5 point change in scale scores may represent a clinically important difference [189, 190]. The heterogeneity of ICU patients and complexity of critical illness complicates acquisition and interpretation of the data; thus, the results should be interpreted with caution.

TRANSCUTANEOUS ELECTRICAL MUSCLE STIMULATION IN ICU PATIENTS

The choice of stimulation parameters is a key factor when using TEMS. Because of the complexity of muscle contraction and insufficient knowledge of how various stimulation parameters influences strength gain, no widely accepted stimulation protocol exists for TEMS treatment.
Intensity, pulse duration and frequency are important factors to consider as all contribute to the force development, fatigue and patient discomfort in relation to the stimulated muscle. Thus, if these factors are not chosen correctly, this may potentially limit the effect of TEMS treatment. Recruiting as much muscle mass as possible by increasing the duration, amplitude or frequency will increase the training effect. On the other hand, increasing these parameters also means increasing the discomfort of the patients [191] and the risk of muscle damage.

Intensity

The amplitude (intensity) of the current affects the muscle force - the higher the amplitude of the current the greater the force generated [192]. Concurrently, previous studies suggest that potential strength gain benefits are generally correlated to the intensity of the stimulation [193-195]. When the current intensity was increased twofold, the isometric torque was increased to a level 15–20 times higher than that at the threshold level [196]. Thus, high amplitude, within the clinical therapeutic range, seems to be the main parameter for enhancing the muscle strength [126, 197]. However, in the awake patient, the magnitude of the amplitude is generally limited by the tolerance of a subject (discomfort / pain) [198]. To activate as much muscle mass as possible, previous studies (exclusively done on awake and cooperating patients) adjusted the stimulation intensity to the maximally tolerated [126, 199-202]. This approach was not perceived to be feasible or safe in analgo-sedated ICU patients.

Pulse duration

Pulse duration recruit muscle fibers by way of spatial summation; thus, an increase in pulse duration recruits more motor units causing greater contractile force. This relationship is linear within the range of 100 to about 500 µs [203-206]. Gorgey et al. showed that increasing the pulse duration from 150 to 450 us increased torque by 55% and the amount of muscle activated by 40% [207, 208]. Because longer pulse durations are more likely to recruit nociceptors and therefore are painful [209] and sensory nerve fibers are recruited with pulse duration greater than 500 µs [210], a pulse duration of 300-400 µs is recommended for large muscle groups, such as the quadriceps and calf muscles [211]. The use of a medium pulse duration (200 µs) to produce maximum torque responses is further supported in a recent study by Scott et al. [212] and the five studies [139, 213-216] considered in a systematic review by Roig et al. [217] reporting pulse durations from 200 to 400 µs.

Frequency

Pulse frequency recruit muscle fibers by way of temporal summation; thus, an increase in the firing rate of the motor units enhances the torque during TEMS. This relation ship is linear within certain limits (up to about 50 Hz) [206, 218-220]. Thus, Han et al. [196] reported a 1.5–1.7 fold increase of the isometric torque of the wrist extensor when the frequency was doubled from 20 to 40 Hz. The firing rate of muscle fibers in the quadriceps muscle during normal voluntary contraction is between 15–30 Hz [221]. Low-frequency stimulation results in less muscle fatigue compared to higher frequencies [222]; and improves oxidative capacity (<50 Hz) [223, 224], which may explain the less fatigue. While throughout the literature a wide variety of protocols have been used (frequency of 10-100 Hz), a frequency <50 Hz is accepted as being well tolerated.

Position of electrodes

Position of electrodes in relation to the motor point of the muscle is important when considering force/ stimulation intensity relationship and patient comfort [225]. Prior to electrodes placement, we identified the muscle motor point, following standard procedures in line with most previous TEMS studies where stimulation was performed on the quadriceps muscle [126, 226].

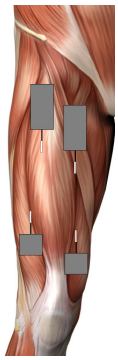

Figure 4: Placement of TEMS electrodes on the thigh. Two electrodes were placed distally over the medial and lateral heads of the quadriceps muscle, and another pair of electrodes were placed 5 cm distal to the inguinal fold.

Adverse events

TEMS may result in more extensive muscular damage compared to voluntary contractions [227, 228]. In healthy subjects, clinical signs such as muscle soreness, fatigue or discomfort are frequently used to monitor the effects of muscle strength training and to titrate training intensity level and duration accordingly. For obvious reasons, it is very difficult to apply this approach in order to monitor the effects of muscle strength training in analgo-sedated patients and patients with impaired consciousness. In

these patients, there is an increased risk of muscle injury and complications to exercise. Mackey et al. [229] found a trend toward a positive correlation between with the force produced by electrical muscle stimulation and signs of muscle damage (z-line disruption) and adverse events such as rhabdomyolysis [230] and compartment syndrome [231] has been reported.

Tissue conductivity

Adipose tissue and subcutaneous edema may significantly affect the response to TEMS. Subcutaneous fat has a high electrical resistance compared to the highly conductive muscle and dermal skin layers [232]. The high resistance fat layer separating skin from muscle may filter the electrical current [233, 234] and produce a weaker muscle contraction. Accordingly, Petrofsky et al. [235] found a positive correlation between subcutaneous fat thickness and the current required to stimulate the quadriceps muscle and Strasser et al.[236] found that the subsequent expression of muscle growth factor in response to TEMS was inversely correlated to the BMI.

COMPUTED TOMOGRAPHY (CT) BASED 3-D EVALUATION OF MUSCLE VOLUME

A common issue in clinical research in ICU patients is comparability. In Study II we tried to address this problem by using a single-leg exercise model where patients served as their own control. This eliminates the effect of inter-individual differences and allowed paired statistic, which improves comparability and statistical strength in the often very heterogeneous group of critically ill patients. The single-leg exercise model furthermore allowed scanning of the intervention and the control leg simultaneously with patients in a standard position during CT scans (using a standardized CT scanning protocol), which further adds to the comparability between legs and between initial and final scan. We chose to analyze multiple slices (n=20) using a 3D-model technique for evaluating muscle volume, as this technique has shown to be accurate [237, 238] and as a previous study reported that a single slice may underestimate the true CSA of the muscle [239]. A semiautomatic segmentation of CT slices with thresholds filters of 20 and 90 Hounsfield units (HU) (a measure of CT density) was performed prior to a manual segmentation. This procedure may be subject to assessor variability. However, to reduce inter-observer variability the manual segmentations of the CT scans of our study were completed independently by a single experienced user, who had received training and was familiar with the segmentation program (Mimics, Figure 5). The assessor was blinded in regards to intervention side (stimulated/ non-stimulated) and timing (baseline/post-intervention). Because radiological imaging modalities lack functional information about the nerve conducting system and the contractile apparatus of the muscle tissue, it may not be appropriate to use muscle thickness as a surrogate for more clinically relevant and patient-centered measures such as muscle strength and physical function. This is supported by the observation that strength, but not muscle mass, is associated with mortality in healthy elderly subjects [12].

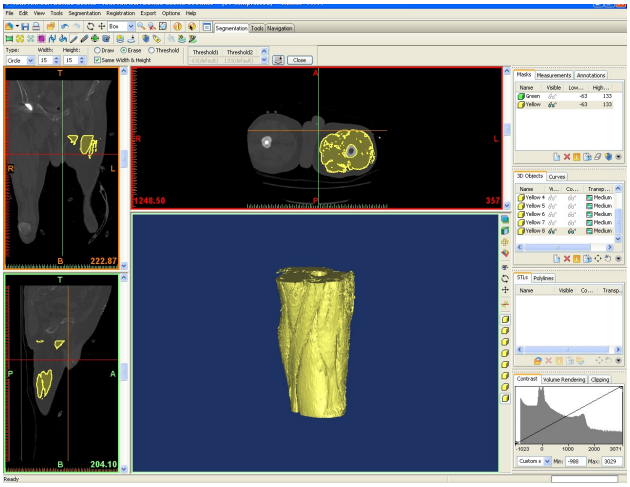

Figure 5: Screen shot from a 3D-reconstruction software (Mimics) used to evaluate muscle volume

ASSESSMENT OF MEASURING TOOLS

The two key concepts when assessing a particular measuring tool is reliability and validity. Reliability can be defined as the consistency of measurements, i.e., the absence of random error. Validity is the ability of the measurement tool to reflect what it is designed to measure, i.e., the absence of systematic error.

Figure 6: The reliability/validity relationship. Target to the left show high validity and reliability, the target in the middle show low validity and high reliability, target to the right show low reliability

As evident from the reliability/validity relationship (simplified in the Figure 6) a new measurement tool will never be valid if it is not adequately consistent. Logically, establishing the degree of reliability should therefore precede any form of validation. When assessing measurement error there are three important areas to consider: retest correlation, within-subject variation and change in the mean.

Retest correlation (relative reliability)

Relative reliability is the degree to which individuals maintain their position in a sample with repeated measurements. This type of reliability is usually assessed with intraclass correlation coefficient (ICC). However, ICC is unit-less and does not provide information on expected difference between repeated measures in individuals, data that are important to clinicians and researchers.

Within-subject variation (absolute reliability)

On the other hand, absolute reliability assesses to which degree repeated measurements vary for individuals and, in contrast to ICC, is unaffected by between-subject variability. This type of reliability is usually expressed in the actual units of the measurements. There are several methods to describe 'absolute reliabil-

ity'. These methods vary considerably in the way they are calculated and the presence or absence of heteroscedasticity dictates the choice of method. Data exhibit heteroscedasticity if the size of the measured error (test–retest differences) correlates with the magnitude of the measured values (test–retest means). If heteroscedasticity is present, the coefficient of variation should be used; conversely, if heteroscedasticity is absent, the standard error of the mean (SEM) should be used. In the present study absolute reliability was calculated using SEM. SEM also allows the calculation of the minimal detectable change (MDC), which is an estimate of the minimum difference between separate measures that can be detected objectively with a 95% degree of certainty.

Change in the mean

This measure of reliability is simply the change in the mean value between 2 trials of a test and consists of 2 components. Differences in the mean are due to either random change (sampling error) or to systematic bias. Systematic bias refers to a non-random trend for measurements to be different in a particular direction between tests typically caused by learning or training effects.

BIOMECHANICAL TESTS IN ICU PATIENTS

Biomechanical properties are sensitive to a variety of factors. Thus, both age and gender influence biomechanical parameters. With advancing age, muscle mass is gradually diminishing due to the loss of muscle fibres and atrophy (sarcopenia) [240], and the number of motor units are reduced [241]. A concomitant reduction of rate of force development has also been observed in elderly [242] primarily caused by a reduced rate of cross-bridge cycling [243, 244] and by alterations in the excitability of the muscle cell [245]. Furthermore, the mechanism of fatigue may vary between age classes and with gender [246]. These age- and gender-related changes limit comparability across populations with large age ranges or different gender distributions. Due to the explanatory nature of our study, we tried to address these comparability issues by investigation a specific age and gender group, thus knowingly limiting the generalizability of our results.

STATISTICAL ANALYSES

GENERAL CONSIDERATIONS

Data were presented as means and standard deviations (SD) or as medians and interquartile ranges (IQR) where appropriate after testing for Gaussian distribution assessing graphical plots and normality test (Kolmogorov–Smirnov and Shapiro-Wilk tests). To obtain a normal distribution, screwed data were log-transformed before parametric analyses were applied. For continuous variables, data were compared using independent t-tests or Mann Whitney U tests, respectively, for parametric and non-parametric analyses. P-values < 0.05 were considered statistically significant. All analyses were conducted using the Statistical Package for the Social Sciences version 15 (SPSS Inc., Chicago, IL) and graphics using MS Excel (Microsoft Corporation, Redmond, WA). The following analyses were used in the specific articles:

ARTICLE I

Mann-Whitney U- and chi-square tests were used to analyze demographic data. Changes in the SF-36 domains between septic shock patients and the age- and gender-matched control group from the general Danish population were analyzed using Wilcoxon Signed-Rank test. Spearman's rho was calculated to examine rank order correlations between variables. Internal consistency of the SF-36 questionnaire was determined with inter-item correlation expressed as Cronbach's alpha coefficient.

ARTICLE II

The single-legged exercise design using the contra lateral as control leg allowed paired statistical analysis to be used. Data were analyzed by Wilcoxon signed-ranks test. Spearman's rho was calculated to assess correlations.

ARTICLE III

Please refer to 'General methodological considerations' under the section 'Assessment of measuring tools', and to 'Discussion of Article III' under 'Study specific considerations'.

ARTICLE VI

Cohort characteristics between tested ICU survivors and controls and difference in measured biomechanical parameters were normally distributed and compared using an independent samples t-test. A general linear regression model was used to assess the effect of co-morbidity on performed maximum force and endurance.

ETHICAL CONSIDERATIONS

In clinical trials, the scientific benefits must be carefully balanced against the risk of side effects and complications for participating patients. In sedated or otherwise incompetent ICU patients, these considerations are particularly important, as the next of kin, on behalf of the patient, gives informed consent. All clinical studies were approved by the Research Ethics Committee of the Capital Region Denmark and the Danish Data Protection Agency and conducted in conformity with the principles expressed in the World Medical Association Declaration of Helsinki. Danish law exempts questionnaire surveys from ethical board approval (Danish Ethics Committee legislation § 8.3), which is why we did not apply for ethical approval in study I. All clinical trials were registered at www.clinicaltrials.gov

STUDY-SPECIFIC ETHICAL CONSIDERATIONS

Study II

Computed tomography (CT) of both thighs was performed in septic shock patients, some during the initial fluid resuscitation and vasopressor treatment. This procedure was carried out in the radiology department and if possible sought combined with other radiological examinations. If patients suffered from severe respiratory or circulatory instability that precluded transportation of the patient to the CT scanner, they were excluded from the study. The radiation dose of CT scan of the thighs was 2 mSv, which corresponds to the normal yearly Danish background radiation dose (2-3 mSv). Given the potential benefits of TEMS, the additional risks due to radiation and transportation of the patients out side the ICU were considered acceptable.

Study IV

The tested ICU survivors had a higher degree of co-morbidity than age- and gender-matched controls. Due to safety concerns, patients suffering from cardiopulmonary conditions that contraindicated the exertion of maximum force or a prolonged strenuous effort were excluded. In accordance with the study population

and type of study, all tests of ICU survivors were performed under the supervision of a medical doctor in case of unexpected medical emergencies. Medical emergency equipment and medicine, a suction device and oxygen supply, and an automated external defibrillator were all available in the laboratory.

DISCUSSION

DISCUSSION OF ARTICLE I

Brief study outline

The objective of the study was to assess the physical function and socio-economical outcomes in survivors of septic shock. A self-composed questionnaire including the SF-36 and Functional Co-morbidity Index was administered. We found that patients who survived septic shock had persistent functional limitations one year after ICU discharge, largely as a result of loss of muscle mass and of weakness. Less than half of formerly employed patients had returned to work, less than 15% of survivors were economically independent (a 30% increase in the number of persons who were dependent on financial support) and the number of patients in need of home-based primary care had doubled.

Study-specific considerations

Lack of baseline values and well-matched controls are inherent to most studies on functional deficits in ICU survivors. Preadmission values are important when assessing the effects of intensive care on physical function. Furthermore, it has been shown that pre-admission HRQOL and co-morbidity may be important determinants for HRQOL of ICU survivors [180]; this is particularly important given that ICU patients are burdened with a higher degree of co-morbidity prior to ICU admission compared to the background population [180].

Because admission to the ICU is often sudden and unexpected, baseline values are, in general, lacking. Retrospective assessments performed by ICU survivors or their proxies and using SF-36 are probably subject to significant bias [184, 247]. However, without an SF-36 measurement at the time of admission or a control group of ICU survivors who did not have septic shock, it is difficult to assess the contribution of sepsis to the poor physical outcome. The specific choice of the study population also limits the generalizability of our result to other ICU patients groups. Thus, whether the reduced functional capacity in our study population is a result of the sepsis, co-morbidity or others factors associated with intensive care is unknown. However, our data are consistent with the reports from studies on ARDS patients [1, 179, 248, 249], suggesting that the functional limitation is not specific to septic shock, but rather is a complication that may occur in any condition characterized by hyper-inflammation and prolonged bed rest. According to the patients, the reduction in physical function is due to loss of muscle mass and increased fatigability. Even though the study does not permit any conclusions regarding causality, our results demonstrate severe residual physical limitations in survivors of septic shock at one year after ICU discharge.

Further implications

This study contributes to the existing knowledge of long-term physical outcome in patients with septic shock by suggesting that intensive care has a deleterious long-term effect on physical function. Following this study, we hypothesized that this persistent poor physical outcome is due to loss of muscle mass caused by inflammation in combination with inactivity, which led us to conduct Study II. Since we found it plausible that the early phase of critical illness, representing the phase with the most pronounced loss of skeletal muscle mass, might also be the phase with the greatest potential to mitigate muscle loss in, we applied TEMS to counteract inactivity in bedridden ICU patients during the initial treatment of septic shock. Furthermore, the underlying biomechanical properties responsible for the post-ICU muscle function impairment are largely unknown, which led us to conduct study IV.

DISCUSSION OF ARTICLE II

Brief study outline

The aim of the present study was to assess the effect of early TEMS on muscle volume in septic patients admitted to the ICU. We hypothesized that TEMS would preserve muscle volume. We found a 16-20% reduction in quadriceps muscle volume in patients with septic shock after one week of stay in the ICU (Figure 7). This loss of muscle mass was unaffected by TEMS.

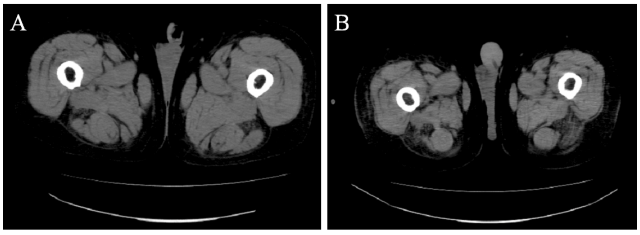

Figure 7. CT scans of the thigh in a patient with septic shock at baseline (A) and seven days later (B), showing the changes in muscle volume.

Study-specific considerations

Several factors may influence our results. First, a major consideration in the present study is its small sample size, which increases the possibility of chance findings and therefore limits the strengths of our conclusions. However, our data did not show a significant difference in muscle volume, neither did we find a trend towards a beneficial effect of TEMS on muscle volume in patients with septic shock. If any, the trend was toward a detrimental effect of TEMS.

Secondly, as mentioned previously, the choice of stimulation protocol is of paramount importance for the effect of TEMS. In the present study we stimulated patients 60 min/day for seven days using biphasic pulses at widths of 300 µs, frequency of 35 Hz and at currents of up to 99 mA. The stimulation period was chosen based on previous reports of effect after one week of stimulation [129, 250, 251] and because the average length of stay in our ICU is 6 days with 75% of our patients having a ICU stay of less than seven days. Therefore, to test the clinical relevance of TEMS, we chose to evaluate a stimulation time with-in a time frame that would benefit the average ICU patient. The intensity applied in our study (31-42 mA) was comparable to a previous study using a maximum tolerable level of intensity (28–59 mA) [200] in conscious patients. To ensure a tetanic contraction and minimize recruitment of sensory fibers pulse duration of 300 µs and stimulation frequency of 35 Hz was chosen, which is in accordance with common recommendations [211].

Thirdly, changes in tissue conductivity may influence the resulting muscle contraction produced by TEMS [30, 133, 252]. Changes in tissue blood flow and subcutaneous edema [235] along with

inflammatory, metabolic and electrolyte changes may influence tissue conductivity [253, 254]. In critically ill patients, all these parameters are subject to large day-to-day variations. In the present study we have tried to address this problem by measurement of the stimulus threshold on a daily basis, which provide means to evaluate this variability and to adjust stimulation settings accordingly. Furthermore, as discussed in Article II, a critical illness-induced muscle membrane dysfunction may have adversely affected the excitability of the quadriceps muscle. This is most likely a graded response associated with the severity of illness, which if severe enough, may render muscle tissue refractory to TEMS. Unfortunately, we did not systematically evaluate the present of muscle excitability dysfunction or CIPNM in our patients and their contribution to our results are therefore unknown.

Fourth, we chose to intervene during the initial phase of septic shock hoping to prevent the muscle wasting. This timing aspect may also be important to our results. It is recognized that skeletal muscle serves as primary protein and metabolic reserve in the body and as such plays a crucial role in endogenous mechanisms to combat illness. The metabolic response to critical illness is, among others, alterations in substrate utilization and increased resting energy expenditure. In the process to meet these increased energy and protein demands, skeletal muscle catabolism serves as a protein and nitrogen donor supplying the liver with increased availability of amino acids for gluconeogenesis, synthesis of immune components and acute phase proteins. Obstructing skeletal muscle catabolism during the initial phase of critical illness may therefore not be beneficial for the patients' outcome in general. On the other hand, the maintenance of skeletal muscle mass may not only facilitate functional recovery, but preservation of muscle mass as a metabolic reserve may also be an important determinant of patient morbidity and mortality [255, 256]. Furthermore, animal studies have shown that older rats exhibit incomplete regeneration of lost muscle mass from immobilization-induced atrophy. None returned to baseline values after immobilization which may suggust that a portion of muscle loss is permanent [257]. Prevention rather than regeneration of lost muscle mass is therefore an appealing approach to improve the physical outcome in ICU patients. How and when to intervene is therefore the subject for an ongoing debate.

Fifth, increased micro vascular water permeability in patients with septic shock may lead to development of peripheral edema [258, 259] and an abnormal extra- and intracellular water distribution [260, 261]. This fluid shift may influence the CT evaluated muscle volume. However, when radiological evaluated (using Hounsfield units) a trend towards an increase in muscle tissue density was recorded bilaterally (no differences between legs) over the seven-day period indication reduced intra muscular water. This lead us to believe that peripheral edema only have a minor impact on our muscle volume measurements and, if present, was equally distributed between the intervention and control leg.

Further implications

Muscle strength is determined both by muscle CSA, synchronization of motor unit recruitment and specific tension [159]. Although previous studies have shown a good correlation between strength and cross sectional area at mid-thigh measured by CT scan [262], this relationship may only be valid under condition where motor unit recruitment strategy and specific tension are stabile. This assumption is questionable in critical ill patients. Measurements of muscle volume, therefore, does not allow for any conclusions regarding the contribution of CIPNM or potential neural adaptation to the muscle function. Thus, muscle volume remains a surrogate for more clinically relevant measurements such as muscle strength and, ultimately, physical function. Although TEMS have the potential to up-regulate genes associated with contractile proteins [70], this may not be sufficient to effectively override the strong endogenous catabolic signal associated with critical illness and provoke the entire biochemical cascade of hypertrophy. However, we suspect that there may be some increase in patients' muscle strength due to neural adaptations [263], which led us to develop a new method to assess muscle force in non-cooperating patients (Study III).

DISCUSSION OF ARTICLE III

Brief study outline

The aim of the study was to develop a non-invasive method to evaluate fatigability of the quadriceps muscle in non-cooperating subjects and to assess the reliability of this method. On two occasions, separated by seven days, a custom-designed strain gauge attached to a hospital bed was used to measure force output during a sequence of tetanic and twitch contractions. Primary outcome measures were various fatigue parameters. For tetanic contractions the fatigue index and the slope of the regression line of peak torque values was calculated, and for twitch contractions; maximum peak torque values and rise time. Relative and absolute reliability and minimum detectable change was then calculated for all parameters. We developed a new non-invasive, quantitative assessment method, which can reliably determine the relative individual changes of muscle fatigue resistance of the thigh muscles in non-cooperating healthy subjects.

Study specific considerations

When construction and conduction a test-retest study assessing measurement error of a particular tool several factors may have important influence on the result.

Retest correlation

There are six different formulas for calculating intra class correlation (ICC), all giving different results, and depends on type of measurement, purpose and design of the study, (Figure 8) [264]. The choice of 'model' depends on selection of raters and the 'form' depends on whether reliability is calculated for a single measure or from an average of several measurements.

ICC type	Description
ICC(1,1)	Each subject is assessed by a *different set* of *randomly selected* raters, and the reliability is calculated from a single measurement. Uncommonly used in clinical reliability studies.
ICC(1,k)	As above, but reliability is calculated by taking an average of the *k* raters' measurements.
ICC(2,1)	Each subject is measured by each rater, and raters are considered representative of a larger population of similar raters. Reliability calculated from a single measurement.
ICC(2,k)	As above, but reliability is calculated by taking an average of the *k* raters' measurements.
ICC(3,1)	Each subject is assessed by each rater, but the raters are the only raters of interest. Reliability calculated from a single measurement.
ICC(3,k)	As above, but reliability is calculated by taking an average of the *k* raters' measurements.

Figure 8: The six kinds of intraclass correlation coefficient. From265

Accordingly, in the present study we chose to express relative reliability as ICC coefficients with the corresponding 95% confidence interval (CI), combined with a non-randomly selected rater and single measures (ICC3.1), which provides the most conserva-

tive estimations of the ICC value [265]. ICC may present some interpretational difficulties. Because ICC examines the stability of the sample ranks it is sensitive to the heterogeneity of the sample values between participants. Consequently, if employed as the sole statistic, it may not be provide sufficient evidence for reliability. This is exemplified by previous studies reporting substantial retest correlations (ICC range from 0.89 to 0.98), but when a measure of absolute reliability was calculated the usefulness of the measurement tools was questionable [266, 267]. Furthermore, there is no general consensus on what is an acceptable degree of relative reliability. In the present study we chose, the classification suggested by Shrout (fair=0.41-0.60, moderate=0.61-0.80 and substantial >0.81) [268]. Although easy applicable, Shrout's classification is a rather insensitive instrument with limited discriminative abilities. This is particularly important in the higher end of the scale and has lead others to subcategorize an ICC close to 1 as 'excellent' and ICC values >0.9 as 'high' reliability. Unfortunately there are no superior alternatives.

Within-subject variation
In the present study, Pearson correlation coefficient and Bland–Altman scatter plots were used to assess heteroscedasticity. As our data did not show signs of heteroscedasticity, absolute reliability was calculated as standard error of measurement (SEM) and minimal detectable change (MDC95%) using the following equations

$$SEM = \sum_{1-n} SD \times \sqrt{(1 - ICC)}$$

$$MDC_{95\%} = SEM \times 1.96 \times \sqrt{2}$$

The advantage of absolute reliability is increased comparability between different study populations and measurement tools. Also, minimum detectable change translate statistical values into more applicable and clinical meaningful terms. When interpreting MDC values it is important to recognize that they are limited by the assumption that measured errors are stabile throughout the scale, although these may vary at different points along the scale [269]. This means that the MDC values in the present study may be limited to the specific range of the scale in which our test were carried out.

Change in the mean
Using paired t-test no systematic bias was found between test and retest measurements.
Random error is usually caused by mechanical inconsistencies in the measurement protocol. We tried to address these issues by ensure uniformity in regard to time of day, location, room temperature, equipment, observer and activity level in the period prior to the test. Strict adherence to standardized procedures such as calibration of equipment, positioning of subjects, placement and re-placement of electrodes and stimulation regime. However, lack of rigidity in the experimental set-up may have caused some baseline drift during the twitch contractions, which may increase the risk of random error. This may explain the less reliable results during twitch contractions.
Systematic bias is usually due to either learning effect or training effect (fatigue). With one week separating the tests fatigue is an unlikely contributor to systematic change. Subjects had no previous experience with electrical muscle stimulation and were instructed to relax as much as possible and to avoid any voluntary contraction during the test. Systematic error can not be ruled out, due to the possibility that subject may have been more accustomed to TEMS at the second test day and therefore easier suppressed voluntary contractions. If present, systematic error were not of a detectable magnitude and the impact on our result is though to be equally minimal.

Further implications
After successfully determining substantial reliability of the measurements, we need to establish the validity of the method in an ICU population. Validity is defined as the degree of agreement between the observed value and the true value of a measure. Inherent to the unavoidable presence of error in the true value, proving validity is often very complex.
Using muscle biopsies a recent study who also assessed an artificially evoked muscle fatigue protocol [161] proved biologically validity by correlating fiber type composition and oxidative capacity of the muscle tissue with the fatigue resistance measured by the strain gauge. Due to the comparability between fatigue protocols we expect that a similar approach could validate our method.
Methods for assessing muscle function in ICU patients are required. Independently of patient' cooperation, our method has the potential to assess the immediate effect of potential therapies during the initial stage of ICU admission in a large muscle group relevant to activities of daily living. In contrast to the present alternatives (radiological imaging), our method enables a functional assessment of the muscle function providing insights to the contribution of CIPNM, and in TEMS treated patients, possible benefits from neural adaptation. A new study, approved by the ethical committee, aiming to validate the method using muscle biopsies and assess the effects of TEMS on muscle tissue working capacity in a broader ICU population is currently being prepared.

DISCUSSION OF ARTICLE IV

Brief study outline
In an effort to broaden our understanding of the mechanisms responsible for the muscle function deficits observed in ICU survivors, we investigated the biomechanical properties of the quadriceps muscle in 16 ICU survivors 12 months after ICU discharge. The results from an extensive battery of biomechanical test were compared with an age and gender matched control group (N=15). In congruence with current literature, we found an expected reduction in muscle strength (20%). More surprisingly was the magnitude of reduction observed in other parameters such as endurance (40%) and absolute and relative rate of force development (50% and 32%, respectively). These parameters are of profound clinical importance as they are key factor in many ADL activities and, for RFD in particular, in time restricted tasks such as regaining balance and avoiding falls. Because rate of force development is responsive to training it may constitute a potential target for rehabilitating interventions and, thus, could potentially ameliorate the current rehabilitation practice.

Study specific considerations
When patients and controls volunteer to participate in clinical trials there is always a risk of selection bias. Our concern was that a study of this nature would attract the healthier and stronger age and gender matched controls. This did not seem to be the case as the controls did not differ significantly for the Danish background population within the physical domains of the SF-36

and were burden with co-morbidity. We did also not detected signs of selection bias between the biomechanical tested patient group and the non-tested patient group in regards to severity of illness and ICU stay. The difference in co-morbidity (expressed as functional co-morbidity index, FCI) may present a potentially comparability issue. ICU patients are known to be burden with a higher degree of co-morbidity and this may influence physical performance. On the other hand did we not find any correlation between FCI values and knee extension force and endurance in Study IV. It may be explained by the concept of the muscle testing procedure in the present protocol. The tests were to a large degree short in duration and specifically target a single muscle. This poses minimal stress on the cardiovascular system, supported by the notion that a patient suffering from COPD completed the protocol without breathing difficulties whereas his walking distance was greatly reduced due to limited cardiopulmonary resources.

Based on our results from co-activation and agonist EMG motor drive we did not find any evidence for changes in muscle activation strategies in ICU survivors. Combined with the electromechanical delay, reaction time and rate of force development data we found it plausible that the deficit responsible for the muscle impairment observer in ICU survivors may be isolated to the muscle tissue. Even though it is likely that the majority of our patients suffered to some degree from CIPNM 10 and previous studies suggest that recovery from pure critical illness polyneuropathy (CIPN) may be slower than from pure critical illness polymyopathy (CIPM), it did not reflect in reduced reaction or EMG onset time in the present study suggesting that there is no neural impairment during muscle activation. However, conclusions on central muscle activation strategies based on surface EMG recordings should be made with caution as it may present several interpretational difficulties [270].

Inherent to this kind of endurance measurement is the risk of contaminating data because of premature termination of the test for reasons other than muscle fatigue (i.e. lack of motivation). However, we used fatigue related indices calculated from EMG data in the present study to assess achievement of muscle fatigue. In order to maintain a constant force output muscle tissue adapt progressively to fatigue by recruiting more motor units and increased firing frequencies. In the EMG signal this is expressed by an increase in amplitude (increase in mRMS EMG) and in a decreasing mean power frequency. These indicators of fatigue were present in both ICU survivors and controls and there were no difference between groups. This indicates equal levels of fatigue and effort at the time of exhaust.

Further implications

Our results have provided new insights to the biomechanical deficits that encumber ICU survivors and indicating rate of force development as a potentially new target in rehabilitation strategies in ICU patients. Evaluating the effect of such a rehabilitation protocol seems to be an obvious area for future research.

FINAL CONCLUSION AND PERSPECTIVES

CONCLUSION

In recent years it has become increasingly evident that neuromuscular dysfunction and weakness greatly reduce the health related quality of life in ICU survivors, especially in the domain of physical function. In the work presented here, we have added to this growing body of evidence. At present there is no accepted standard of care and no treatment available for preserving muscle function in bedridden ICU patients. A focused approach to develop treatment strategies is therefore warranted. We tried to counteract muscle wasting in ICU patients using TEMS treatment and, although failing to show any effect, succeeded in documenting the severe loss of muscle mass encountered by ICU patients during the early stages of critical illness. In that process we developed a new measuring tool capable of producing reliable non-voluntary force measurements characterising muscle performance, thus providing means to evaluate potential new therapeutic regimes initiated during the early phase of ICU admission. In the pursuit of treatment strategies, knowledge of the trajectory of skeletal muscle loss and the underlying mechanisms of skeletal muscle weakness is fundamental for understanding the relationship to functional impairment. In an effort to illuminate some of these mechanisms we conducted a detailed characterisation of the contractile properties of the quadriceps femoris muscle in ICU survivors. Our results have provided new insights to the biomechanical deficits encumbering ICU survivors and provided a potential new target in rehabilitation strategies.

PERSPECTIVES

With the aging of the population and advances in critical care, the numbers of ICU survivors are expected to grow exponentially over the coming years [271]. This prospect may, in the light of the magnitude of the acquired physical deficits presently observed ICU survivors, constitute an immense burden on future patients and health care institutions and thus present a profound clinically challenge.

The challenges of future research must be to further elucidate the pathophysiological mechanisms of muscle wasting in critical ill patients and to develop strategies to counteract muscle function impairment and to improve physical outcome. Since the major skeletal muscle-preserving intervention is muscle contraction, it would be interesting to investigate the excitability of the skeletal muscle membrane in the early phase of illness, and if impaired, establish the cause of this impairment. Next, the signaling pathways involved in the catabolic response should be outlined and their potential regulation should be further elucidated. Although teleological reasoning is risky, the potential benefits of the catabolic response, such as the resulting generation of acute phase and immune mediators, should also be explored.

Minimization of sedation and mobilization both appear to be beneficial to critically ill patients. The impact of these relatively simple measures, as well as nutrition and physiotherapeutic interventions such as passive mobilization, on muscle metabolism and signaling warrant further study.

Finally, although early interventions to counter skeletal muscle atrophy are intuitively attractive, rehabilitation after discharge from the intensive care unit as well as the hospital may have potential to restore physical function as well. This part of the rehabilitation chain should be studied as well.

SUMMARY

Intensive care unit (ICU) admission is associated with muscle weakness and ICU survivors report sustained limitation of physical capacity for years after discharge. Limited information is available on the underlying biomechanical properties responsible for this muscle function impairment. A plausible contributor to the accentuated catabolic drive in ICU patients is a synergistic response to inflammation and inactivity leading to loss of muscle mass. As

these entities are predominantly present in the early phase of ICU stay, interventions employed during this time frame may exhibit the greatest potential to counteract loss of muscle mass. Despite the obvious clinical significance of muscle atrophy for the functional impairment observed in ICU survivors, no preventive therapies have been identified as yet.

The overall aim of the present dissertation is to characterize aspects of physical function and biomechanical properties in ICU patients and to provide new insights into ICU-induced muscle wasting and the underlying biomechanical mechanisms responsible for the residual impairment of physical function in ICU survivors.

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