Muscle atrophy and preferential loss of myosin in prolonged critically ill patients*

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Objective: **Muscle weakness contributes to prolonged rehabilitation and adverse outcome of critically ill patients. Distinction between a neurogenic and/or myogenic underlying problem is difficult using routine diagnostic tools. Preferential loss of myosin has been suggested to point to a myogenic component. We evaluated markers of muscle atrophy and denervation, and the myosin/actin ratio in limb and abdominal wall skeletal muscle of prolonged critically ill patients and matched controls in relation to insulin therapy and known risk factors for intensive care unit-acquired weakness.**

Design: **Secondary analysis of two large, prospective, single-center randomized clinical studies.**

Setting: **University hospital surgical and medical intensive care unit.**

Patients: **Critically ill patients and matched controls.**

Interventions: **Intensive care unit patients had been randomized to blood glucose control to 80–110 mg/dL with insulin infusion or conventional glucose management, where insulin was only administered when glucose levels rose above 215 mg/dL.**

Measurements and Main Results: **As compared with controls, rectus abdominis and vastus lateralis muscle of critically ill patients showed smaller myofiber size, decreased mRNA levels for myofibrillar proteins, increased proteolytic enzyme activities, and a lower myosin/actin ratio, virtually irrespective of insulin therapy. Increased forkhead box O1 action may have played a role. Most alterations were more severe in patients treated with corticosteroids. Duration of corticosteroid treatment, independent of duration of intensive care unit stay or other risk factors, was a dominant risk factor for a low myosin/actin ratio. The immature acetylcholine receptor subunit γ messenger RNA expression was elevated in vastus lateralis, independent of the myosin/actin ratio.**

Conclusions: **Both limb and abdominal wall skeletal muscles of prolonged critically ill patients showed downregulation of protein synthesis at the gene expression level as well as increased proteolysis. This affected myosin to a greater extent than actin, resulting in a decreased myosin/actin ratio. Muscle atrophy was not ameliorated by intensive insulin therapy, but possibly aggravated by corticosteroids. (Crit Care Med 2012; 40:79–89)**

KEY WORDS: critical illness polyneuropathy/myopathy; hyperglycemia; intensive care; insulin; muscle catabolism

Muscle weakness is a severe complication of prolonged critical illness that hampers weaning from ventilatory support, delays rehabilitation, and is associated with increased risk of death (1–5). Even long after hospital discharge, patients can experience muscle weakness and fatigue, contributing to decreased exercise tolerance and quality of life (5).

The severe weakness may originate from a neurogenic and/or myogenic disturbance. The neurologic component, called "critical illness polyneuropathy" in the absence of other neuropathies, comprises axonal degeneration of sensory and motor neurons. The myogenic component may also involve impaired muscle membrane excitability and muscle atrophy, and may coincide with neurologic problems. Both entities share clinical symptoms and electrophysiological signs (6–8). Hence, electrophysiological differential diagnosis of critical illness neuropathy and myopathy is only possible with sophisticated techniques, and ideally requires a conscious and cooperative patient. A biochemical marker of muscle mass or loss during critical illness could theoretically be a tool for characterizing myopathy. Previous studies on muscle biopsies from selected patients with established myopathy revealed a low myofibrillar protein content, loss of myosin and myosin-associated proteins associated with a low ratio of thick-to-thin filaments, and with a very low force-

***See also p. 314.**

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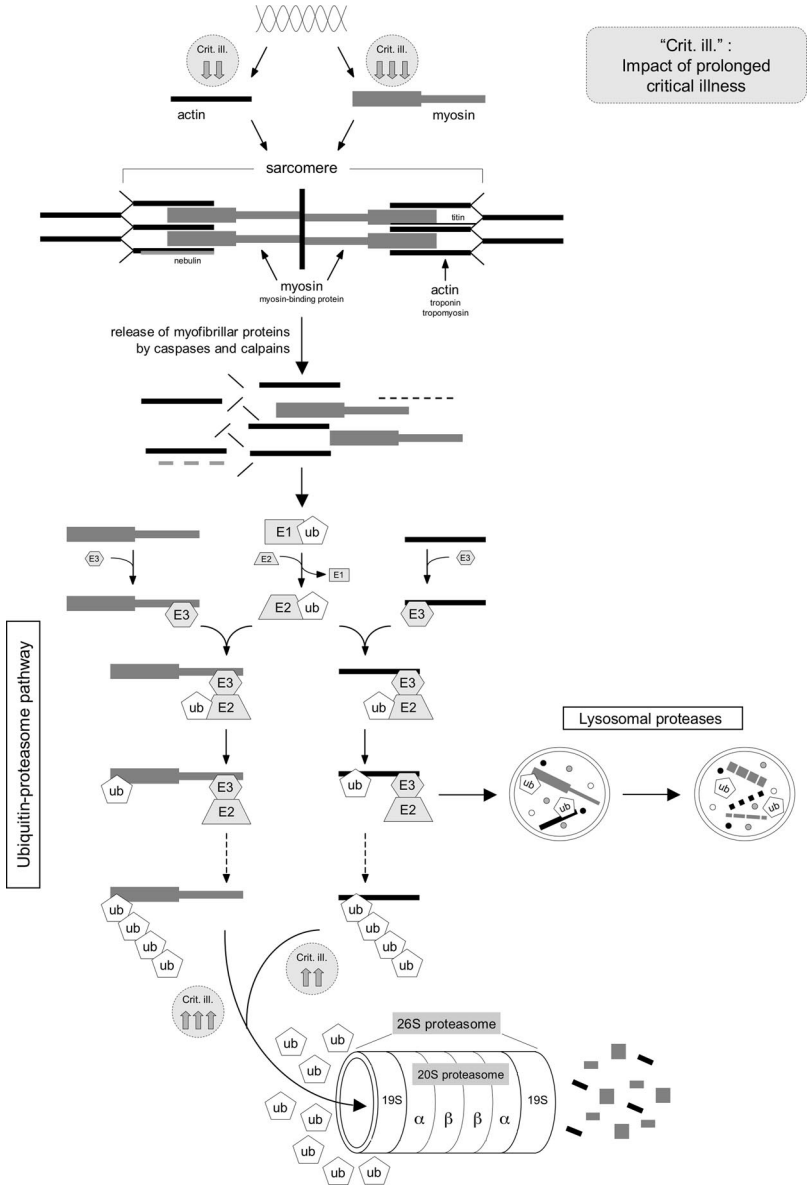

Figure 1. Simplified scheme illustrating the mechanisms of muscle wasting in prolonged critical illness. Imbalance between decreased muscle protein synthesis and increased muscle protein degradation affects myosin more severely than actin, resulting in a reduction of the myosin/actin ratio. Several proteolytic systems contribute to muscle breakdown. Myofibrils first have to be dismantled by caspases and/or calpains with release of myosin and actin from the sarcomere before these can be further degraded. The ubiquitin-proteasome pathway targets proteins destined for degradation by conjugation of ubiquitin (*ub*) molecules in a three-step process involving E1, E2, and E3 enzymes, the latter determining the selectivity of the system. Muscle ring finger-1 and atrogin-1 are two muscle-specific E3 ligases that are highly upregulated in and widely used as markers of muscle wasting. The three-step process is repeated until a chain of at least four ubiquitin molecules has been attached to the protein, which allows recognition by the 26S proteasome and cleavage into short peptides by its 20S proteasome catalytic core. In contrast to polyubiquitinated proteins, mono- or diubiquitinated proteins are delivered to the lysosome for degradation by lysosomal proteases, of which cathepsin-L has recently been recognized as a general marker of muscle atrophy. A more detailed explanation of this schematic presentation with corresponding references is presented in the supplemental data (Supplemental Digital Content 2, http://links.lww.com/CCM/A295).

generating capacity of muscle fibers (8, 9). The decrease in myofibrillar proteins in severe muscle wasting appears to affect myosin to a greater extent than actin, and the resulting pronounced drop in the myosin/actin ratio has been suggested as diagnostic tool for myopathy (9–11). Such loss of optimal stoichiometry may result in disruption of force-generating interactions between myosin and actin (12–15).

We quantified markers of protein synthesis and proteolytic activity, the myosin/actin ratio (Fig. 1), and markers of denervation/neuronal inactivity in skeletal muscle from a large heterogeneous group of prolonged critically ill patients included in two randomized studies on tight glycemic control (16, 17) and healthy controls. We hypothesized that muscle atrophy in critically ill patients could be attenuated by intensive insulin therapy, since several studies have suggested a protein-sparing effect of controlling glycemia using insulin (18–20). Therefore, we assessed effects of randomization to conventional or intensive insulin therapy, as well as of known risk factors for weakness, such as sepsis, use of corticosteroids, and neuromuscular blocking agents (NMBAs), on these parameters.

MATERIALS AND METHODS

Patients and Controls

We investigated 208 skeletal muscle biopsies obtained from intensive care unit (ICU) patients and 35 biopsies obtained from controls. The ICU patients had been included in two large, prospective, randomized controlled studies on strict blood glucose control with intensive insulin therapy in a surgical (16) and medical (17) ICU (Supplemental Fig. 1 [Supplemental Digital Content 1, http://links.lww.com/CCM/A294] and legend [Supplemental Digital Content 2, http://links.lww.com/CCM/A295]). They received conventional glucose management, in which insulin was administered only if glucose levels exceeded 215 mg/dL (11.9 mmol/L) and tapered below 180 mg/dL, or intensive insulin therapy to normalize blood glucose levels (80–110 mg/dL). The KULeuven Institutional Review Board approved the protocols (ML1094, ML1820, ML2707) (16, 17). Written informed consent was obtained from the patients or next of kin and from the healthy volunteers.

Biopsies of right musculus rectus abdominis were taken immediately after death from 144 nonsurvivors (median day 10, Table 1, Supplemental Fig. 1*A* [see Supplemental Digital Content 1, http://links.lww.com/CCM/A294). Samples were snap-frozen in liquid nitrogen within 30 ± 20 min or 19 ± 10 min of death (mean ± SD) for surgical and medical ICU patients. Characteristics of the patients according to randomization are described in Supplemental Table S1 (Supplemental Digital Content 2, http://links.lww.com/CCM/A295). As a

Table 1. Characteristics of the patients

Patient Characteristics	Rectus Abdominis (n = 144)	Vastus Lateralis (n = 64)
Demography and anthropometry		
Gender (number, % male)	94 (65)	44 (69)
Age (yr) (median, IQR)	71 (58–77)	58 (51–72)
Body mass index (kg/m^2) (median, IQR)	24.2 (22.0–27.3)	23.4 (20.8–26.7)
History		
Diabetes (number, %)	16 (11)	8 (13)
Malignancy (number, %)	48 (33)	14 (22)
Underlying Illness		
Medical ICU/surgical ICU (number, %)	69/75 (48/52)	100/0 (100/0)
Reason for admission or type of surgery (number)[a]		
Cardiovascular	37	1
Abdominal	19	10
Respiratory	45	39
Other	43	14
Sepsis day 1 (number, %)[b]	74 (51)	46 (72)
Acute Physiology and Chronic Health Evaluation II during first 24 h (median, IQR)	19 (12–28)	23 (15–29)
Admission glycemia and glucose control in ICU		
Blood glucose upon admission (mg/dL) (median, IQR)	151 (117–189)	147 (123–189)
Hyperglycemia ≥200 mg/dL on admission (number, %)	29 (20)	12 (19)
Randomization to intensive insulin therapy (number, %)	60 (42)	30 (47)
Morbidity		
Total days in ICU (median, IQR)	10 (5–20)	27 (20–40)
Days in ICU until biopsy (median, IQR)	10 (5–20)	15 (14–18)
Mechanical ventilation (number, %)[c]	140 (97)	58 (92)
Days on mechanical ventilation (median, IQR)[c]	9 (5–18)	14 (12–16)
Renal replacement therapy (number, %)[c]	66 (46)	13 (21)
Treatment with noradrenaline (number, %)[c]	129 (90)	42 (67)
Days on noradrenaline (median, IQR)[c]	5 (2–10)	3 (0–7)
Treatment with corticosteroids (number, %)[c]	93 (66)	46 (73)
Days on corticosteroids (median, IQR)[c]	2 (0–8)	5 (0–13)
Treatment with neuromuscular blocking agents (number, %)[c]	67 (48)	40 (63)
Days on neuromuscular blocking agents (median, IQR)[c]	0 (0–2)	1 (0–4)
Cause of death (number)		
Cardiac/hypovolemic shock	23	1
Multiple organ failure	78	6
Respiratory failure	27	9
Septic shock/therapy resistance	7	1
Severe brain damage	9	3

IQR, interquartile range; ICU, intensive care unit. The Acute Physiology and Chronic Health Evaluation score denotes the severity of illness, with higher scores for more severely ill patients. 1 mg/dL glucose = (1/18) mmol/L.

[a]Cardiovascular: cardiac disease, cardiac or complicated aortic surgery; Abdominal: gastrointestinal disease or hepatic disease, complicated abdominal surgery or peritonitis; Respiratory: respiratory disease, complications of pulmonary or esophageal surgery; [b]Sepsis on day 1 criteria per definition could not be applied for trauma patients and patients who underwent cardiac surgery (31 patients from whom a rectus abdominis had been taken were hence excluded; percentages are expressed relative to all patients); [c]Evaluated until the day of the biopsy.

"healthy" reference, rectus abdominis biopsies were taken intraoperatively from 25 patients, further referred to as "controls," who did not suffer from generalized disease and who underwent elective surgery for restorative rectal resection, with comparable gender distribution (64% male, $p = .9$), age (median 69, interquartile range 58–77; $p = .7$), and body mass index (25.0 [23.6–26.9] kg/m^2, $p = .1$) as the ICU patients.

Biopsies from musculus vastus lateralis of the quadriceps femoris were taken *in vivo* on median day 15 from 64 medical ICU patients (Table 1, Fig. S1*B*) and immediately snap-frozen in liquid nitrogen. Patient characteristics according to randomization are described in Supplemental Table S1 (Supplemental Digital Content 2, http://links.lww.com/CCM/A295). Vastus lateralis biopsies were taken from ten healthy volunteers, referred to as "controls," with comparable gender distribution (70% male, $p = .9$), age (62 [57–66] years, $p = .2$), and body mass index (24.7 [23.4–27.0] kg/m^2, $p = .2$) as the ICU patients. All samples were stored at −80°C until analysis.

Tissue Analyses

Morphology was evaluated on 5-μm paraffin sections stained with hematoxylin-eosin. Myofiber cross-sectional area distribution was analyzed using Matlab7.4.0, and corrected for myofiber density (total myofiber area/evaluated muscle area). RNA extraction and real-time polymerase chain reaction conditions are described in Supplemental Table S2 (Supplemental Digital Content 2, http://links.lww.com/CCM/A295). Messenger RNA (mRNA) levels are expressed relative to hypoxanthine-guanine phosphoribosyltransferase mRNA (rectus abdominis) or 28S rRNA (vastus lateralis). Commercial kits were used to measure the chymotrypsin-like (b5) activity of the 20S proteasome (Chemicon, Temecula, CA) and cathepsin-L activity (Calbiochem, Darmstadt, Germany). Relative contents of myosin heavy chain (MyHC) and actin were determined after gel electrophoresis and Coomassie staining (21, 22). Based on the severe myosin/actin ratio reduction of approximately one-third in muscle from patients with critical illness myopathy and its association with muscle function impairment (13), we *a priori* defined a reduction with at least one-third compared to the median of the reference samples (myosin/actin of 1.5) as cutoff for a physiologically relevant disturbance in the stoichiometry of both proteins. All analyses were performed blinded for patients' treatment assignment.

Electrophysiological Screening

Electrophysiological abnormalities were prospectively assessed with electromyography of all limbs on day 7, and repeated once a week during ICU stay (23, 24). One electrophysiologist evaluated all patients, blinded for randomization. Patients with preexisting neuromuscular disorders were excluded. Critical illness polyneuropathy/myopathy was diagnosed by abundant spontaneous electrical activity in proximal and distal muscles in both upper and lower extremities in the form of positive sharp waves or fibrillation potentials (23, 24). Differential diagnosis of a potential myogenic component from a neurogenic problem could not be performed by the routine electrophysiology, since patients were often not cooperative or unconscious and no direct muscle stimulation was performed.

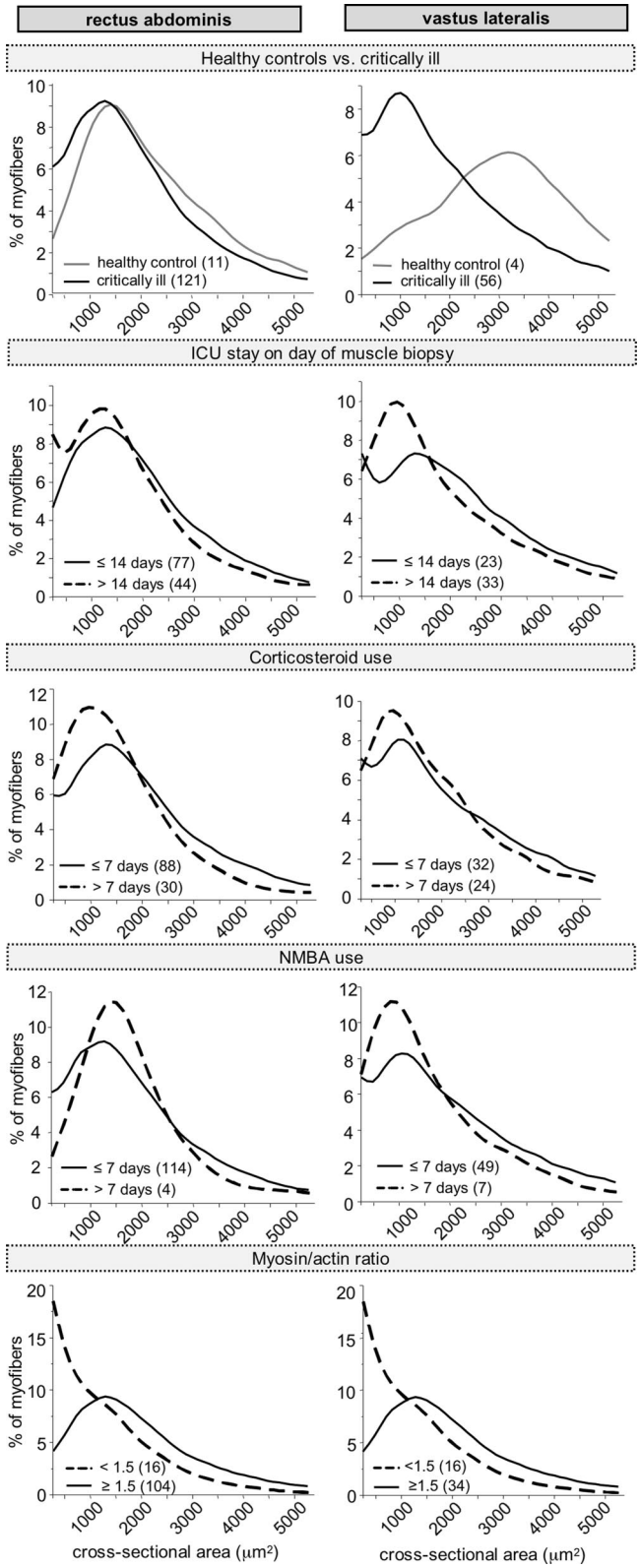

Figure 2. Distribution of myofiber cross-sectional area. Myofiber cross-sectional area was categorized in blocks of 250 μm^2. The graphs show *smoothed curves* for the percentage of myofibers in each category and are shown for rectus abdominis and vastus lateralis. The smoothed curves were created with graph builder in JMP9 (SAS Institute, Cary, NC). *ICU*, intensive care unit; *NMBA*, neuromuscular blocking agent.

Statistical Analyses

Data are presented as median (interquartile range) or number and percentage. Group differences were analyzed by the chi-square or Fisher's exact test for comparison of proportions, and Mann-Whitney *U* test for continuous variables. Pearson or Spearman correlation coefficients were calculated to study correlations between variables. To identify the independent risk factors for a myosin/actin ratio <1.5, multivariable logistic regression analysis was performed. The well-documented risk factors for muscle weakness (sepsis at admission; duration of treatment with the vasopressor noradrenaline, corticosteroids, and NMBAs; duration of ICU stay until biopsy [25]) and the randomized insulin treatment scheme (26) were primarily considered. The relatively limited number of patients with a myosin/actin ratio <1.5 invalidates simultaneous evaluation of a large number of factors. Therefore, we built supplemental models, also including other baseline characteristics to test for robustness of our findings. Statistical analyses were performed using StatView 5.0.1 (SAS Institute), with differences considered significant when two-sided p values were $<.05$.

RESULTS

Morphologic Analysis of Skeletal Muscle

Compared with controls, prolonged critically ill patients revealed smaller myofibers in rectus abdominis and even smaller myofibers in vastus lateralis, illustrated by a leftward shift in the cross-sectional area distribution (Fig. 2). There was no difference between the two types of insulin therapy (data not shown). The shift to smaller myofibers was more pronounced in patients in the ICU for at least 14 days. Prolonged corticosteroid and NMBA treatment for at least 7 days negatively affected myofiber size (Fig. 2).

Whereas signs of inflammation or necrosis were absent in both muscle types of controls, they were clearly present in a substantial proportion of critically ill patients (Table 2, Fig. 3). A remarkably high number of patients showed pronounced infiltration of muscle with (or myofiber conversion to) adipose and connective tissue compared with controls.

Table 2. Histologic analysis of skeletal muscle biopsies

	Rectus Abdominis			Vastus Lateralis		
Type of Abnormality	Absent	Present	*p*	Absent	Present	*p*
Inflammation and/or Necrosis (n, %)			.09			.1
Control	11 (100)	0 (0)		4 (100)	0 (0)	
CIT	52 (72)	20 (28)		26 (93)	2 (7)	
IIT	34 (68)	16 (32)		21 (75)	7 (25)	
			.053			.5
CS ≤7 days	67 (75)	22 (25)		28 (88)	4 (12)	
CS >7 days	17 (57)	13 (43)		19 (79)	6 (21)	
Presence of Adipocytes (n, %)[a]			.01			.2
Control	7 (64)	4 (36)		4 (100)	0 (0)	
CIT	17 (24)	55 (76)		16 (57)	12 (43)	
IIT	20 (40)	30 (60)		18 (64)	10 (36)	
			.08			.048
CS ≤7 days	30 (34)	59 (66)		23 (72)	9 (28)	
CS >7 days	5 (17)	25 (83)		11 (56)	13 (54)	
Fibrosis (n, %)			.06			.03
Control	5 (45)	6 (55)		4 (100)	0 (0)	
CIT	17 (24)	55 (76)		9 (32)	19 (68)	
IIT	7 (14)	43 (86)		11 (39)	17 (61)	
			.1			.8
CS ≤7 days	21 (24)	68 (76)		11 (34)	21 (66)	
CS >7 days	3 (10)	27 (90)		9 (38)	15 (62)	
Any Abnormality (n, %)[b]						
			.02			.003
Control	4 (36)	7 (64)		4 (100)	0 (0)	
CIT	8 (11)	64 (89)		5 (18)	23 (82)	
IIT	3 (6)	47 (94)		9 (32)	19 (68)	
			.3			>.9
CS ≤7 days	10 (11)	79 (89)		8 (25)	24 (75)	
CS >7 days	1 (3)	29 (97)		6 (25)	18 (75)	

CIT, conventional insulin therapy; IIT, intensive insulin therapy.

[a]denotes infiltration of muscle with adipocytes or myofiber conversion to adipocytes; [b]"Inflammation and/or necrosis," "Presence of adipocytes," or "Fibrosis."

Synthesis Capacity of Myofibrillar Proteins and Markers of Muscle Proteolysis

Levels of MyHC-I, MyHC-IIa, and actin mRNA were severely reduced in rectus abdominis and vastus lateralis of critically ill patients compared with controls (Fig. 4). The mRNA levels of muscle ring finger-1 and atrogin-1, two E3 ligases of the ubiquitin-proteasome pathway, were unaltered in both muscles, whereas cathepsin-L and 20S proteasome (catalytic core of the 26S proteasome of the ubiquitin-proteasome pathway) activities in rectus abdominis of critically ill patients were significantly upregulated (Fig. 5). Myostatin mRNA was lower in rectus abdominis of patients than in controls, but comparable in vastus lateralis (Fig. 4). These changes coincided with upregulated forkhead box (Fox), but not FoxO3 mRNA (Fig. 4).

Intensive insulin therapy did not significantly affect these markers (Figs. 4 and 5). Patients who received corticosteroids had lower MyHC-I, MyHC-IIa, and actin mRNA, higher muscle ring finger-1, atrogin-1, and FoxO1 mRNA, but comparable myostatin mRNA, FoxO3 mRNA, and cathepsin-L and 20S proteasome activities compared with those who did not (Figs. 4 and 5). NMBA treatment or sepsis diagnosis at admission in general did not affect these markers (Supplemental Fig. 2 [Supplemental Digital Content 3, http://links.lww.com/CCM/A296], its legend [Supplemental Digital Content 2, http://links.lww.com/CCM/A295]; and Supplemental Fig. 3 [Supplemental Digital Content 4, http://links.lww.com/CCM/A297] and its legend [Supplemental Digital Content 2, http://links.lww.com/CCM/A295]).

Relative Protein Levels of Myosin and Actin

In rectus abdominis of controls, the median myosin/actin ratio was 2.27 (interquartile range 2.13–2.39, range 1.71–2.56, n = 25). The myosin/actin ratio in rectus abdominis from conventionally treated critically ill patients was lower than in controls ($p < .0001$, Fig. 6). Intensive insulin therapy did not affect the myosin/actin ratio, but tended to lower the proportion of patients with a myosin/actin ratio <1.5 (10.2% vs. 22.6%). Characteristics of the patients with a myosin/actin ratio above vs. below 1.5 are described in Supplemental Table S3 (Supplemental Digital Content 2, http://links.lww.com/CCM/A295). In the multivariable logistic regression analysis correcting for known risk factors for muscle weakness, duration of corticosteroid treatment tended to be an independent predictor of a myosin/actin ratio <1.5 ($p = .06$), whereas randomization to intensive insulin therapy protected against a low myosin/actin ratio (Table 3). Patients with a low myosin/actin ratio also had smaller myofibers (Fig. 2).

In vastus lateralis of controls, the median myosin/actin ratio was 2.18 (interquartile range 2.12–2.23, range 1.99–2.25, n = 5). The myosin/actin ratio was lower in vastus lateralis from conventionally treated critically ill patients (on median ICU day 15) than in healthy controls ($p = .03$, Fig. 6). Thirty-one percent of critically ill patients had a low myosin/actin ratio (<1.5) in vastus lateralis, which was more than that observed for rectus abdominis. Intensive insulin therapy did not affect the myosin/actin ratio nor the proportion of patients with a myosin/actin ratio below 1.5, whether patients survived or not (Fig. 6). Corticosteroid treatment negatively affected the myosin/actin ratio (Fig. 6). Its duration correlated inversely with the myosin/actin ratio (Fig. 7). The myosin/actin ratio did not correlate with total ICU stay ($\rho = 0.065$, $p = .6$), ICU stay, or duration of mechanical ventilation after the biopsy (both $\rho = 0.045$, $p = .7$ for ICU survivors). Multivariable logistic regression analysis confirmed the independent association between duration of corticosteroid treatment and a severely reduced myosin/actin ratio (Table 3). A low myosin/actin ratio coincided with smaller myofibers (Fig. 2).

Electromyography and Denervation/Neuronal Inactivity

Electrophysiological screening was performed in 87 of the 143 patients from whom the myosin/actin ratio was determined in rectus abdominis (Fig. S1). Characteristics of the patients with or without spontaneous electrical activity

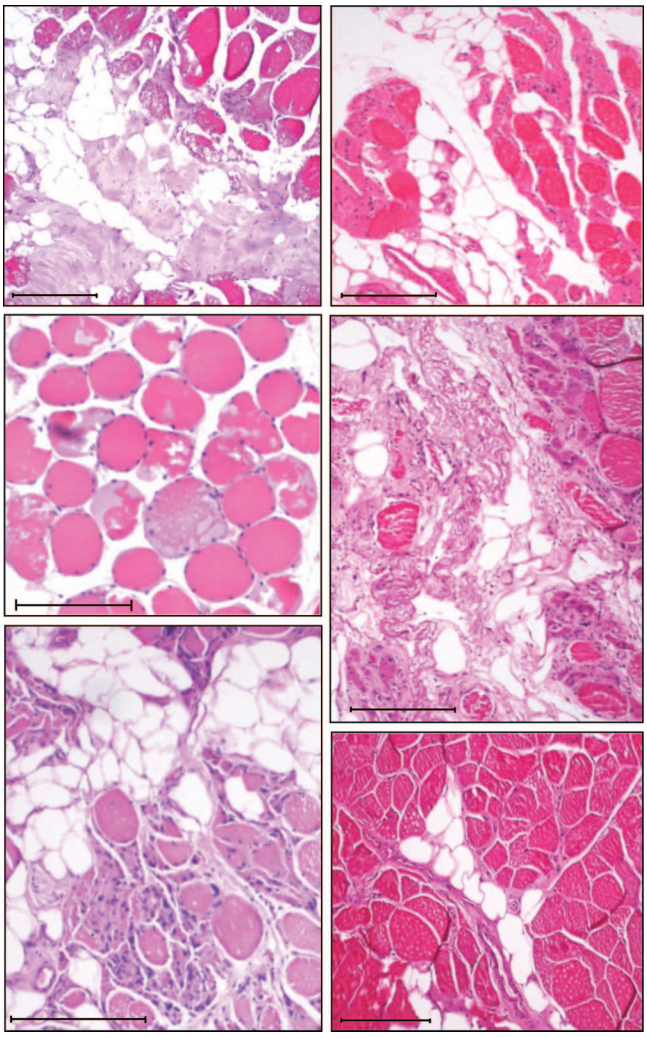

Figure 3. Illustration of histologic abnormalities in skeletal muscle of critically ill patients. Scale bar represents 200 μm.

are described in Supplemental Table S4 (Supplemental Digital Content 2, http://links.lww.com/CCM/A295). Whereas 48.3% of these patients showed abnormal spontaneous electrical activity at least once during ICU stay, and 18.4% had a myosin/actin ratio below 1.5, only 10.3% presented with both. Of the 57 patients from whom a vastus lateralis biopsy was analyzed for the myosin/actin ratio, 45.6% developed abundant spontaneous electrical activity before or at the biopsy time, and 31.6% had a myosin/actin ratio below 1.5. Only 8.8% presented with both.

mRNA for the denervation/neuronal inactivity markers histone deacetylase-4 and acetylcholine receptor (AChR) subunit γ (representing the immature acetylcholine receptor isoform) (27) was elevated in vastus lateralis of critically ill patients compared with controls (Fig. 8). AChRγ expression was higher in patients with spontaneous electrical activity, but not in patients with muscle loss reflected by myosin/actin ratio <1.5. Alterations in rectus abdominis were less pronounced. Insulin therapy did not significantly affect these markers (data not shown).

Of the screened patients with rectus abdominis biopsy, 58% (31 of 53) in the conventional insulin therapy group developed spontaneous electrical activity vs. 32% (11 of 34) in the intensive insulin therapy group ($p = .02$). Development of electrophysiological abnormalities was observed in 20 of 29 (69%) conventionally treated patients with vastus lateralis biopsy vs. 12 of 28 patients (43%) in the intensive insulin group ($p = .049$).

DISCUSSION

A large cohort of prolonged critically ill patients showed severely reduced mRNA levels of the myofibrillar proteins myosin and actin in rectus abdominis and vastus lateralis muscle. This was accompanied by activation of proteolysis and a decreased myofiber size. Myosin was more severely affected than actin, as illustrated by the overall lower myosin/actin ratio compared with controls. The presence of selective myosin loss only rarely co-occurred with an electrophysiological diagnosis of critical illness polyneuropathy/myopathy suggested by the presence of spontaneous electrical activity. Although intensive insulin therapy reduced the presence of spontaneous electrical activity, it did not affect myofibrillar protein loss. Treatment with glucocorticoids, however, was associated with more myofibrillar protein loss.

We observed decreased myofiber size in skeletal muscle of critically ill patients. As previously described (9), this coincided with severely reduced mRNA levels for MyHC isoforms and actin in these patients vs. controls, suggestive of compromised synthesis capacity of the corresponding proteins. Regarding protein degradation, we found no increase by critical illness in mRNA for muscle ring finger-1 and atrogin-1, which are widely used as markers of activated catabolism in muscle wasting (28–30). Similar observations *in vivo* and postmortem excluded confounding by postmortem artifacts. However, increases in muscle ring finger-1 mRNA have been shown to be transient, with a return to control levels between 3 to 5 days after burn injury in rats (31), and between day 10 and 21 in a study on disuse in human muscle (32). Our biopsies were taken on median day 10 and 15, in contrast to day 8 in the study of Mansoor et al (28). Nevertheless, the highly increased activities of the 20S proteasome and lysosomal cathepsin-L in our patients suggest increased proteolysis, in agreement with previous data (30, 33). Myostatin inhibits muscle mass development and causes muscle atrophy by compromising proliferation and differentiation of satellite cells and protein synthesis (34). Although its expression is upregulated in several conditions with muscle atrophy, myostatin mRNA was lower in our ICU patients than in controls. The myostatin response may differ according to the insult, with an increase described after burn injury and a decrease with sepsis (35, 36). FoxO transcription factors are central players in muscle wasting (37, 38). Sepsis has been de-

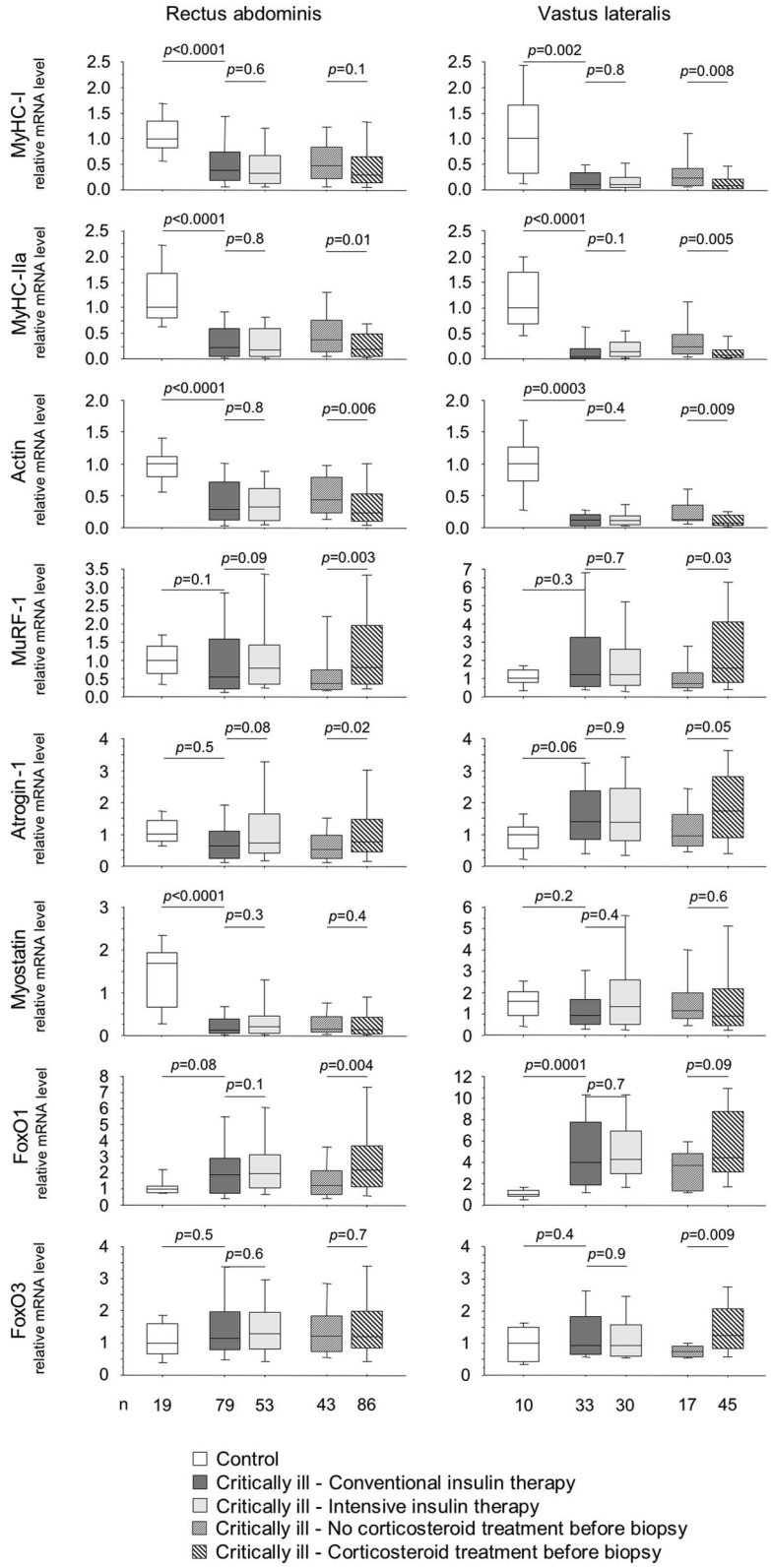

Figure 4. Impact of critical illness, intensive insulin therapy, and corticosteroid treatment on gene expression levels of several skeletal muscle atrophy markers. Relative mRNA levels are expressed as box plots, where the *central lines* indicate the medians, the *boxes* the interquartile ranges, and the *whiskers* the tenth and 90th percentiles. *p* values indicate comparisons between controls and critically ill patients from the conventional insulin therapy group, between critically ill patients receiving conventional or intensive insulin therapy, and between critically ill patients who did or did not receive corticosteroids. *MyHC*, myosin heavy chain; *MuRF*, muscle ring finger; *FoxO*, forkhead box O.

scribed to increase expression and activity of FoxO1 in a glucocorticoid-dependent manner, whereas increased FoxO3 mRNA expression was not followed by increased activity (39). We observed increased transcription of FoxO1, but not FoxO3, in muscle of a heterogeneous group of prolonged critically ill patients. Only for FoxO1, we observed higher mRNA levels with corticosteroid treatment.

Published data on the myosin/actin ratio in ICU patients are scarce and derived from selected patients with established myopathy, almost exclusively after treatment with corticosteroids and NMBAs, and only reported for muscles of the extremities (9–11). In this large cohort of prolonged critically ill patients, we demonstrated that critical illness affected the myosin/actin ratio in peripheral and abdominal wall skeletal muscle. A severely reduced myosin/actin ratio (<1.5) was found more frequently in limb (31.0%) than in abdominal wall (22.6%) muscle. Possible explanations include differences in patient populations, timing of tissue sampling, myofiber type composition, and treatment of the patients. Rectus abdominis was biopsied from surgical and medical ICU patients, whereas vastus lateralis was taken only from medical ICU patients. Median time to tissue sampling was longer for vastus lateralis, but no correlation was found between myosin/actin ratio and ICU stay. Since the incidence of a severely reduced myosin/actin ratio was higher for *in vivo* vastus lateralis than for postmortem rectus abdominis, confounding by postmortem artifacts is unlikely. Type II fibers decline earlier with aging than type 1 fibers (12). However, fiber type composition is comparable for both muscles, with 58%–59% type 1 and 41%–42% type 2 fibers for the patient population's age group (40).

We did not formally evaluate muscle force. However, a pronounced decrease in the myosin/actin ratio has shown physiologic relevance. It reflects loss of optimal stoichiometry and interaction between myosin heads and actin, which is essential for muscle force generation. Reduced myosin/actin ratio and force-generating capacity co-occur with aging (12, 15) and cancer cachexia (13, 14). Hence, preferential myosin loss may also contribute to muscle weakness in critical illness. Although physical inactivity activates proteolysis in muscle (41), preferential myosin loss is not explained merely

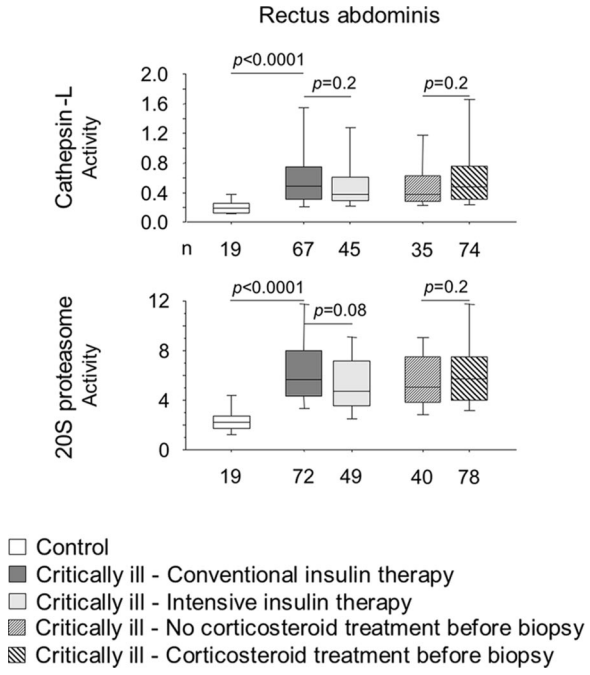

Figure 5. Impact of critical illness, intensive insulin therapy, and corticosteroid treatment on the activity of proteolytic enzymes in skeletal muscle. Enzyme activities in rectus abdominis are expressed as box plots, where the *central lines* indicate the medians, the *boxes* the interquartile ranges, and the *whiskers* the tenth and 90th percentiles. *P* values indicate comparisons between controls and critically ill patients from the conventional insulin therapy group, between critically ill patients receiving conventional or intensive insulin therapy, and between critically ill patients who did or did not receive corticosteroids. Limited tissue availability hampered enzyme activity measurements in vastus lateralis.

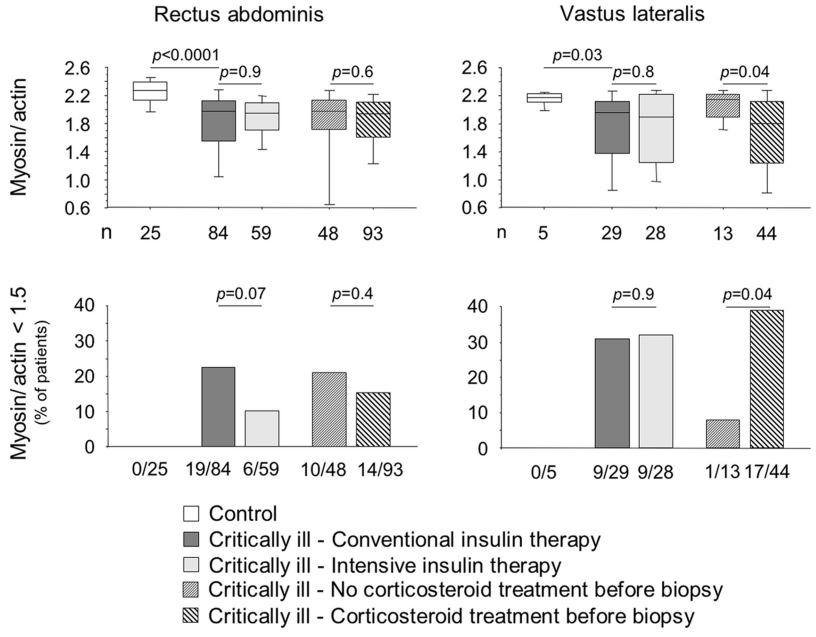

Figure 6. Impact of critical illness, intensive insulin therapy, and corticosteroid treatment on the myosin/actin ratio in skeletal muscle. Myosin/actin ratios are presented as box plots. The *central line* indicates the median, the *box* the interquartile range, and the *whiskers* the tenth and 90th percentiles. *Bars* indicate the percentage of patients with a severely reduced myosin/actin ratio to below 1.5. *p* values indicate comparisons between controls and critically ill patients from the conventional insulin therapy group, between critically ill patients receiving conventional or intensive insulin therapy, and between critically ill patients who did or did not receive corticosteroids. In vastus lateralis, median myosin/actin ratio and interquartile range are 1.91 (1.35–2.13, 3 <1.5) for conventionally treated nonsurvivors, 2.23 (1.54–2.28, 1 <1.5) for intensive insulin treated nonsurvivors ($p = .3$), 1.96 (1.39–2.10, 6 < 1.5) for conventionally treated survivors, and 1.86 (1.21–2.15, 8 <1.5) for intensive insulin treated survivors ($p = .8$).

by immobilization (42). Involvement of abdominal muscle in weakness of critical illness is unclear, but decreased contractile force of the abdominal muscle of critically ill patients *in vitro* has been demonstrated (43). The similarly reduced myosin/actin ratios in the abdominal wall may be clinically relevant since these are the major muscles involved in the expiratory phase of coughing and are required for active respiratory movements and thus weaning from mechanical ventilation.

In general, intensive insulin therapy did not significantly affect the myosin/actin ratio or markers of atrophy and proteolysis. The nutrition these patients received was, on average, below normal caloric requirements. In critically ill rabbits, increased intravenous feeding within the physiologic range, while maintaining normoglycemia with insulin, dose-dependently reduced the catabolic responses as compared to fasting (44). Thus, confounding of our observations by insufficient feeding is possible. However, (continuous) feeding suppresses autophagy, a crucial cellular quality control mechanism that removes toxic protein aggregates and damaged organelles. Feeding may explain why we previously observed an autophagy deficiency phenotype in liver and muscle of prolonged critically ill patients, with accumulation of autophagy-specific substrates and signs of myofiber degeneration as seen in muscle-specific autophagy-deficient mice (45). Importantly, inadequate removal of damaged proteins and mitochondria could explain lack of recovery from organ failure in prolonged critically ill patients, emphasizing that counteracting catabolism may not be exclusively beneficial.

We previously found that intensive insulin therapy significantly reduced the incidence of abundant spontaneous electrical activity in ICU patients (16, 17, 24), contributing to reduced need for prolonged mechanical ventilation (23, 24). The absence of an effect on myofibrillar proteins despite clinical and electrophysiological benefits of insulin therapy may have various explanations. First, the relatively rare co-occurrence of a low myosin/actin ratio and abnormal spontaneous electrical activity may suggest a distinct underlying pathophysiology. Abnormal spontaneous electrical activity in the absence of a severe reduction in the myosin/actin ratio could indicate predominance of a neurologic component of muscle weakness, since the myosin/actin ratio is

Table 3. Multivariable logistic regression analysis for a myosin/actin ratio below 1.5

Variable	Odds Ratio and 95% Confidence Interval	*p*
Rectus Abdominis		
Sepsis on day 1 versus no sepsis[a]	1.918 (0.583–6.311)	.3
Noradrenaline (per day added)	1.060 (0.971–1.156)	.2
Corticosteroids (per day added)	1.083 (0.996–1.177)	.06
Neuromuscular blocking agents (per day added)	0.994 (0.889–1.112)	.9
Intensive care unit stay until biopsy (per day added)	0.955 (0.880–1.037)	.3
Randomization to intensive insulin therapy	0.314 (0.108–0.917)	.03
Vastus Lateralis		
Sepsis on day 1 versus no sepsis	0.902 (0.114–7.143)	.9
Noradrenaline (per day added)	0.858 (0.677–1.086)	.2
Corticosteroids (per day added)	1.374 (1.131–1.669)	.001
Neuromuscular blocking agents (per day added)	1.028 (0.803–1.316)	.8
Intensive care unit stay until biopsy (per day added)	0.755 (0.517–1.101)	.1
Randomization to intensive insulin therapy	0.314 (0.050–1.986)	.2

[a]Sepsis on day 1 criteria per definition do not apply for trauma patients and patients who underwent cardiac surgery (31 patients were hence categorized as "not applicable").

The odds ratio (95% confidence interval) in rectus abdominis in the univariable setting was 0.387 (0.144–1.039) ($p = .06$) for randomization to intensive insulin therapy and 1.043 (1.004–1.083) ($p = .03$) for duration of glucocorticoid treatment. In vastus lateralis, the odds ratio (95% confidence interval) in the univariable setting for duration of corticosteroid treatment was 1.337 (1.151–1.553) ($p = .0001$). The relatively limited number of patients in our study with a low myosin/actin ratio did not allow simultaneous evaluation of a large number of factors. Therefore, we selected only the classic risk factors, as indicated in the Methods section. To test the robustness of our findings for duration of corticosteroid treatment and randomization to intensive insulin therapy, we built the model with these two variables and systematically added separately each of the other factors described in Supplemental Table S3 (Supplemental Digital Content 2, http://links.lww.com/CCM/A295) as a third variable. This yielded similar conclusions.

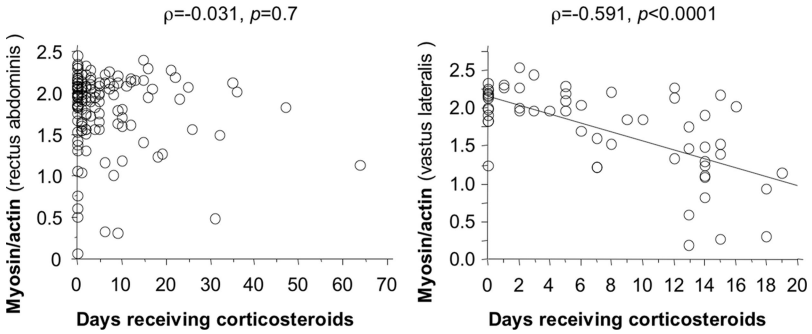

Figure 7. Correlation between the myosin/actin ratio in skeletal muscle and duration of corticosteroid treatment.

not affected in patients with axonal neuropathy (11). This is supported by our observation of increased histone deacetylase-4 and AChRγ expression in patients with abundant spontaneous electrical activity but not in patients with a low myosin/actin ratio. Reduced neural input dissociates histone deacetylase-4 from the neuromuscular junction and induces its expression and nuclear accumulation, thereby connecting neural activity to muscle transcription and affecting AChR expression (27). Increased expression of the AChRγ subunit and thus of the immature AChR, with a longer mean channel open time than that of the mature isoform containing AChRε, has been associated with muscle weakness (46). The effect of insulin therapy on spontaneous electrical activity, without impact on the myosin/actin ratio, could theoretically suggest that it can prevent or treat critical illness polyneuropathy, but not myopathy. However, this constellation may be too simplistic, since the intervention did not affect denervation markers and spontaneous electrical activity is known to occur in both critical illness polyneuropathy and myopathy. Second, increased spontaneous electrical activity in critical illness myopathy, secondary to a muscle membrane defect, may have preceded altered myosin and actin protein expression. The disturbed stoichiometry of myosin and actin has been explained by a more severe suppression of transcription of myosin than that of actin, combined with a slower turnover of actin than of myosin (47). Altered transcriptional regulation occurs early in the progression of myopathy, but it manifests itself only as a late event at the protein level as a decreased myosin/actin ratio (9, 48, 49). Third, some patients whose electrophysiological examinations remained negative had a severely reduced myosin/actin ratio. This may have resulted from technical limitations of the electrophysiology, since only abundant spontaneous electrical activity was recorded. Some myopathies presenting as muscle membrane inexcitability therefore may have been missed (50). Finally, for rectus abdominis, another reason for apparent discrepancy between electrophysiology and biochemistry is that these investigations were performed on different muscles and rectus abdominis may not be representative of limb muscles. Nevertheless, our data may further support that spontaneous electrical activity is not valid on its own as a sole measure for diagnosing myopathy. Recording of motor unit potentials during voluntary muscle contraction or performance of direct muscle stimulation would be the only means to distinguish between neuropathy and myopathy using electrophysiology.

Our multivariable logistic regression analysis showed that duration of corticosteroid administration was a risk factor for a low myosin/actin ratio, independent of time in ICU. This corresponded with worsened atrophy-related gene expression in corticosteroid-treated patients, and a further reduction in myofiber size when corticosteroid treatment was prolonged for >7 days. The role of corticosteroids in ICU-acquired weakness is debated. Several observational studies have shown corticosteroids to be a risk factor of ICU-acquired paresis (2, 51, 52), whereas others found no effect of corticosteroids (1, 3, 53). We previously reported that steroid-induced hyperglycemia

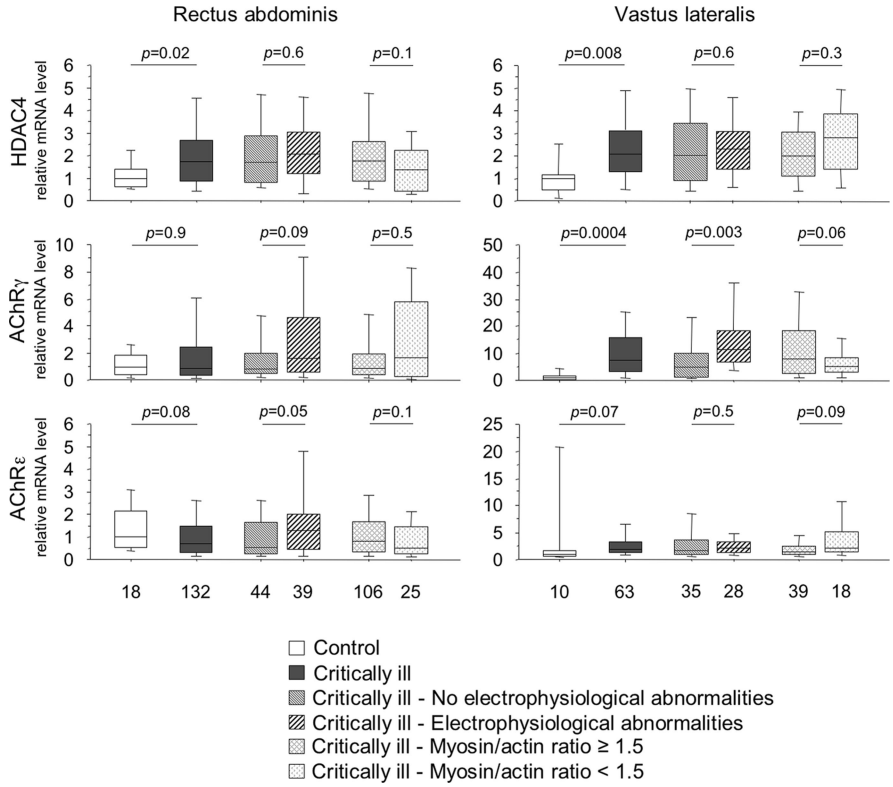

Figure 8. Impact of critical illness, electrophysiological abnormalities, and a low myosin/actin ratio on gene expression levels of several markers of denervation/neuronal inactivity. Relative mRNA levels are expressed as box plots, where the *central lines* indicate the medians, the *boxes* the interquartile ranges, and the *whiskers* the tenth and 90th percentiles. *HDAC4*, histone deacetylase-4; *AChR*, acetylcholine receptor.

rather than corticosteroid treatment itself was deleterious (24). Indeed, when glycemia was strictly controlled, corticosteroids protected against critical illness neuromyopathy diagnosed upon electrophysiological criteria. This apparent contradiction to the present results may be due to the fact that the electrophysiological criteria that we have used may favor diagnosis of axonopathy rather than myopathy. Thus, although we did not randomize for corticosteroid treatment, one may infer that steroids and intensive insulin therapy may be protective for the peripheral nerves, and that steroids may be deleterious and insulin therapy globally neutral to the muscle. Corticosteroids are known to cause atrophy due to decreased protein synthesis and increased protein degradation (54). Also, myostatin has a crucial role in steroid-induced atrophy (55). The fact that we did not find increased proteolytic activity with glucocorticoid treatment nor an effect on myostatin may suggest that this occurs earlier in the process. Finally, neither NMBAs nor sepsis at admission were independent risk factors for a low myosin/actin ratio.

CONCLUSIONS

Both limb and abdominal wall skeletal muscles of critically ill patients showed downregulation of muscle protein synthesis at the level of gene expression as well as increased proteolysis, which affected myosin to a greater extent than actin, resulting in a low myosin/actin ratio and smaller myofibers. Muscle atrophy was not ameliorated by intensive insulin therapy, but possibly aggravated by corticosteroids.

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