Mechanisms of Chronic Muscle Wasting and Dysfunction after an Intensive Care Unit Stay

ORIGINAL ARTICLE

Mechanisms of Chronic Muscle Wasting and Dysfunction after an Intensive Care Unit Stay

A Pilot Study

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Abstract

Rationale: Critical illness survivors often experience permanent functional disability due to intensive care unit (ICU)-acquired weakness. The mechanisms responsible for long-term weakness persistence versus resolution are unknown.

Objectives: To delineate cellular mechanisms underlying long-term weakness persistence in ICU survivors.

Methods: We conducted a nested, prospective study of critically ill patients mechanically ventilated for 7 days or longer. The patients were recruited from the RECOVER program and serially assessed over 6 months after ICU discharge. Twenty-seven of 82 patients consented to participate; 15 and 11 patients were assessed at 7 days and 6 months after ICU discharge, respectively.

Measurements and Main Results: We assessed motor functional capacity, quadriceps size, strength, and voluntary contractile capacity and performed electromyography, nerve conduction studies, and vastus lateralis biopsies for histologic, cellular, and molecular analyses. Strength and quadriceps cross-sectional areas were decreased 7 days after ICU discharge. Weakness persisted to 6 months and correlated with decreased function. Quadriceps atrophy resolved in 27% patients at 6 months. Muscle mass reconstitution did not correlate with resolution of weakness, owing to persistent impaired voluntary contractile capacity. Compared with Day 7, increased ubiquitin–proteasome system–mediated muscle proteolysis, inflammation, and decreased mitochondrial content all normalized at 6 months. Autophagy markers were normal at 6 months. Patients with sustained atrophy had decreased muscle progenitor (satellite) cell content.

Conclusions: Long-term weakness in ICU survivors results from heterogeneous muscle pathophysiology with variable combinations of muscle atrophy and impaired contractile capacity. These findings are not explained by ongoing muscle proteolysis, inflammation, or diminished mitochondrial content. Sustained muscle atrophy is associated with decreased satellite cell content and compromised muscle regrowth, suggesting impaired regenerative capacity.

Keywords: muscle atrophy; ubiquitin–proteasome system; satellite cell; autophagy; mitochondria

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At a Glance Commentary

Scientific Knowledge on the Subject: Critical illness survivors often experience permanent functional disability due to intensive care unit (ICU)-acquired weakness. The mechanisms responsible for long-term weakness persistence versus resolution are unknown.

What This Study Adds to the Field: We show that ICU-acquired weakness that persists long term in survivors of critical illness results from heterogeneous muscle pathophysiology with variable degrees of muscle atrophy and decreased voluntary contractile capacity. Following acute skeletal muscle proteolysis in the ICU, sustained muscle atrophy in the long term derives from impaired regrowth and is associated with decreased satellite cell content. This has critical implications for the development of treatment and rehabilitative strategies.

Individuals who survive critical illness experience functional disability that is evident in the intensive care unit (ICU) (1) and may persist for years after discharge (2, 3). Premorbid conditions, critical illness, prolonged immobilization, and complications of ICU care including mechanical ventilation contribute to muscle and nerve damage, referred to as ICU-acquired weakness (ICUAW) (4). In the short term, ICUAW is associated with failure to wean from the ventilator and increased in-hospital mortality (1, 5, 6). ICUAW that persists for months to years following critical illness imparts functional disability and significantly impairs quality of life.

The mechanisms underlying the acute loss of muscle mass and function during critical illness have been investigated extensively over the past decade in both animal models and patients. Proteolytic degradation of muscle in combination with electrical silencing and excitation–contraction uncoupling all contribute to muscle dysfunction in the ICU during the acute phases of critical illness (7–9). Comprehensive longitudinal data concurrently assessing structural, functional, and molecular features of ICUAW sustained long term in survivors of critical illness are, in contrast, completely lacking. Specifically, the cellular and molecular mechanisms responsible for recovery of strength versus the permanent persistence of weakness remain to be elucidated. This may have important implications for rehabilitation and management, and therapies based on the abnormalities observed early in the ICU may not apply to this long-term recovery phase. We hypothesized that a sustained failure to repair and regrow injured or atrophic muscles following critical illness underlies the pathogenesis of persistent ICUAW. To test this hypothesis, we quantified motor functional capacity, skeletal muscle size, strength and voluntary contractile capacity, and peripheral nerve function, and we performed muscle biopsies to evaluate cellular signaling and processes associated with muscle proteolysis and repair in critical illness survivors serially over the time at which functional recovery plateaus (6 mo) following discharge from the ICU. Some of the results of these studies have been reported previously in the form of an abstract (10).

Table 1. Patient Demographics

Patient	Age (*yr*)	Sex	ICU LOS (*d*)	APACHE II Score	DASI/Est V_{O_2}max (*ml/kg/min*)	Reason for ICU Admission	Comorbidities	Corticosteroid Use
1	29	F	9	22	58.2/34.6	Necrotizing fasciitis	None	No
2	78	M	9	30	Missed	GI perforation/rupture	DM (complicated), substance abuse	No
3	47	M	26	17	58.2/34.6	Sepsis, non–urinary tract origin	DM (uncomplicated)	Yes
4	69	F	32	19	58.2/34.6	Renal failure	Hypertension	Yes
5	71	M	10	31	40.5/27.0	Cardiac arrest	DM (uncomplicated), hypertension	No
6	32	M	13	13	58.2/34.6	Head trauma	None	No
7	36	F	52	13	58.2/34.6	Bacterial/viral pneumonia	None	Yes
8	23	M	19	10	58.2/34.6	Multiple trauma	None	No
9	66	M	14	22	39.5/26.6	Sepsis of urinary tract origin	COPD, hypertension	Yes
10	58	F	14	29	50.2/31.2	Subarachnoid hemorrhage	DM (uncomplicated)	No
11	47	F	88	31	58.2/34.6	Renal pulmonary syndrome	None	Yes
12	58	F	51	34	28.0/21.6	Congestive heart failure	DM (complicated)	Yes
13	53	M	17	15	58.2/34.6	Multiple trauma	Hypertension	No
14	52	M	13	9	58.2/34.6	Subarachnoid hemorrhage	None	No
15	47	F	19	30	58.2/34.6	Pulmonary embolectomy	Lymphoma	No

Definition of abbreviations: APACHE II = Acute Physiology and Chronic Health Evaluation II; COPD = chronic obstructive pulmonary disease; DASI/Est V_{O_2}max = Duke Activity Status Index and corresponding estimated V_{O_2}max; DM = diabetes mellitus; GI = gastrointestinal; ICU = intensive care unit; LOS = length of stay. Demographics include preadmission comorbidities, estimates of preadmission physical functional capacity (DASI/est V_{O_2}max), severity of illness (APACHE II score), ICU LOS, corticosteroid use in the ICU and reason for admission. Sixty percent of patients (9 of 15) had preadmission comorbidities. Ninety-three percent of patients (14 of 15) self-reported the ability to undertake, at minimum, moderate exercise, and 67% (10 of 15) were able to undertake strenuous activity preadmission, as indicated by their DASI scores. Forty-four percent of patients (6 of 15) received corticosteroids within the ICU. No patients received neuromuscular blockade. Trauma patient 8 sustained left humerus, right femur, sacral ala, and left pubic rami fractures. Trauma patients 6 and 13 did not sustain extremity fractures (patient 6, facial fractures; patient 13, penetrating chest/abdominal trauma).

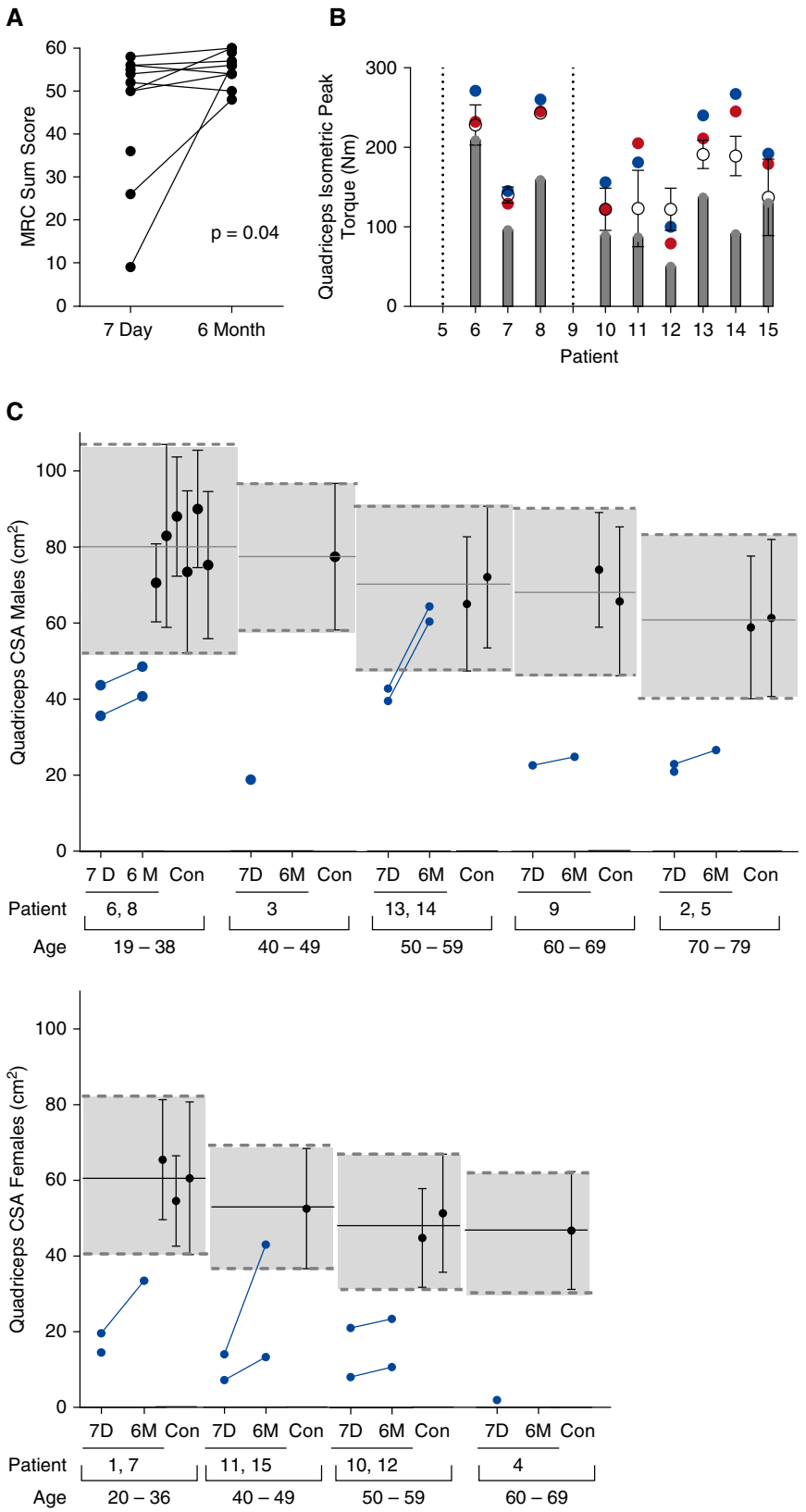

Figure 1. Persistent muscle weakness and atrophy 6 months after intensive care unit (ICU) discharge. (*A*) At 7 days after ICU discharge, patients demonstrated weakness, as indicated by a decreased Medical Research Council (MRC) Sum Score (13 of 13 patients), which improved

Methods

Study Design

We conducted a nested prospective study of patients recruited from the RECOVER Program, a multicenter, prospective longitudinal study evaluating functional outcomes in critically ill patients following prolonged mechanical ventilation over a 1-year period after ICU discharge (*see* online supplement for details). Individuals mechanically ventilated for a minimum of 1 week due to critical illness were recruited from three ICUs (two medical-surgical ICUs and one trauma ICU) in Toronto, Ontario, Canada, between September 2010 and April 2013. Informed consent was obtained from the participants, and the study protocol was approved by the research ethics boards of all participating institutions. For molecular and cellular analyses, banked muscle biopsy specimens previously collected from consenting healthy individuals were used for comparison purposes (*see* Table E1 in the online supplement). Patient assessments were conducted at 7 days and 6 months after ICU discharge. The greatest incremental improvement in physical function is known to occur within the first 6 months after ICU discharge and subsequently plateaus (11, 12). We reasoned that the selected post-ICU time points would provide muscle undergoing maximal repair (7 d) and muscle nearing completion of its maximal recovery (6 mo). Details on inclusion and exclusion criteria and all clinical evaluation tools and experimental protocols are included in the online supplement.

Patient Recruitment, Demographics, and Severity of Illness

We obtained consent from 27 of 82 eligible patients, 15 and subsequently 11 of whom completed 7-day and 6-month follow-up assessments, respectively (Figure E1). The participants' baseline demographics are presented in Table 1. Study patients were representative of the ICU population undergoing prolonged mechanical ventilation (>1 wk) for critical illness (Table E2).

Outcome Measures of Physical Functioning, Strength, and Muscle Mass

Physical functional capacity was determined using the 6-minute walk distance and the

Functional Independence Measure motor subscore. Global muscle strength was ascertained by using the Medical Research Council Sum Score. The quadriceps isometric peak torque was measured from maximal voluntary contractions using a Biodex System 4 dynamometer (Biodex Medical Systems, Shirley, NY). The cross-sectional area (CSA) of the quadriceps at its midsection was determined by computed tomography. Muscle specific force (voluntary contractile capacity) was calculated by normalizing quadriceps isometric peak torque to CSA. Electromyography (EMG) and nerve conduction studies were performed to assess neuropathy and myopathy. All testing was conducted at 7 days and 6 months after ICU discharge, with the exception of the Biodex dynamometer measurements (conducted solely at 6 mo).

Muscle Biopsy

Percutaneous biopsies of the vastus lateralis muscle were obtained from participants under local anesthetic at 7 days and 6 months after ICU discharge.

Molecular Analysis

Muscle fiber CSA, ubiquitin–proteasome system (UPS) activity, autophagy, satellite cell content, vascularity, mitochondrial content, and presence or absence of an inflammatory infiltrate were assessed by a combination of Western blot analyses, 20S proteasome activity measurements, immunohistochemistry, and electron microscopic imaging and analyses.

Statistical Analyses

Continuous data are presented as the mean (±SD). Nonnormally distributed discrete data are presented as the median (25th and 75th percentile) values. Comparisons between cohorts were conducted with one-way analysis of variance or the Kruskal-Wallis test and *post hoc* analyses. Correlations between outcome measures were determined by calculating Pearson or Spearman correlation coefficients. Statistical significance was assumed if P value was less than 0.05. All analyses were conducted with GraphPad Prism 6 software (GraphPad Software, La Jolla, CA).

Results

Persistent Weakness 6 Months after ICU Discharge Correlated with Sustained Physical Functional Impairment

Strength was decreased 7 days after ICU discharge and, while significantly improved by 6 months, did not normalize in the majority of patients (Figures 1A and 1B). Persistent weakness at 6 months correlated with physical functional impairment as assessed using the Functional Independence Measure motor subscore (Figures E2A, E2C, and E2D) and 6-minute walk distance (Figures E2B, E2E, and E2F).

Muscle Atrophy Was Sustained in the Majority of Patients 6 Months after ICU Discharge

All patients demonstrated significant quadriceps wasting 7 days following ICU discharge when compared with published age- and sex-matched, population-based norms (13–21) (Figure 1C). Quadriceps CSA was increased in all patients at 6 months, but there was significant variability in the extent of muscle regrowth, ranging from minimal (ΔCSA <3 cm^2; patients 9, 10, and 12) to substantial regrowth (ΔCSA >21 cm^2; patients 13–15). Assuming that a quadriceps CSA that falls off the 95% confidence intervals for an age- and sex-matched healthy population can be deemed abnormal, 73% of patients (8 of 11) demonstrated persistent quadriceps atrophy 6 months following ICU discharge. Myofiber type–specific CSA was similarly decreased at 7 days after ICU discharge, persisting in 70% of patients (7 of 10) at 6 months (Figure E3).

Muscle Mass Did Not Correlate with Strength

Six months following ICU discharge, there was no significant correlation between quadriceps size and strength (Figure 2A). The three patients (27%) whose quadriceps size normalized all showed persistent weakness due to decreased quadriceps voluntary contractile capacity, as determined by the muscle specific force (isometric peak torque normalized to the muscle CSA) (Figure 2B). Overall, patients with significant weakness demonstrated marked variation in the underlying muscle pathophysiologic processes, atrophy versus impaired voluntary contractile capacity, at 6 months following critical illness (Figure 2C).

Electrophysiologic Testing Detected Persistent Myopathy at 6 Months

At 7 days after discharge, EMG and nerve conduction studies showed electrophysiologic evidence of myopathy and neuropathy in 79% (11 of 14) and 36% (5 of 14) of patients, respectively (Table E3). Myopathy detected by EMG persisted in 38% (3 of 8) of patients tested at 6 months post–ICU discharge, but neuropathy remained in only one of eight patients; that individual had complicated diabetes and also demonstrated myopathy. At 6 months following ICU discharge, patients with electrophysiologic evidence of myopathy were weaker than those without

Figure 1. (Continued). significantly but did not normalize (i.e., achievement of a score of 60) at 6 months in 82% of patients (9 of 11). Patient 8 did not undergo 7-day testing, owing to extremity fractures, and patient 5 was missed. (*B*) Similarly, 6 months after ICU discharge, patients demonstrated decreased quadriceps isometric peak torque (*gray bars*) compared with their predicted peak torque (as determined by using predictive formulae derived by Gosselink and colleagues (52) [*red circles*] or Harbo and colleagues (53) [*blue circles*]) or compared with age-identical and sex-matched quadriceps peak torque norms for healthy persons (*open circles*; mean peak torque ± SD, derived at the University of Toronto Muscle Function and Performance Laboratory). Testing of patient 8 was conducted on the left quadriceps owing to his previous right femur fracture. Patients 5 and 9 declined testing (*dotted lines*). (*C*) One hundred percent of patients (15 of 15; *blue circles*) demonstrated muscle wasting, as determined by computed tomographic measurement of the quadriceps muscle cross-sectional area (CSA), at 7 days after ICU discharge (7D) compared with population-based, age- and sex-matched norms for healthy persons (Con; *black circles* are published quadriceps CSA means ± 95% confidence intervals [13–21]). Quadriceps CSA increased in all patients 6 months (6M) following ICU discharge, but only 27% of patients (patients 13–15) normalized quadriceps size (i.e., fell within 95% confidence interval). Patients 5–12 demonstrated persistent quadriceps atrophy. Patients 1–4 did not complete the 6-month assessments. The left quadriceps CSA of patient 8 was measured at both time points.

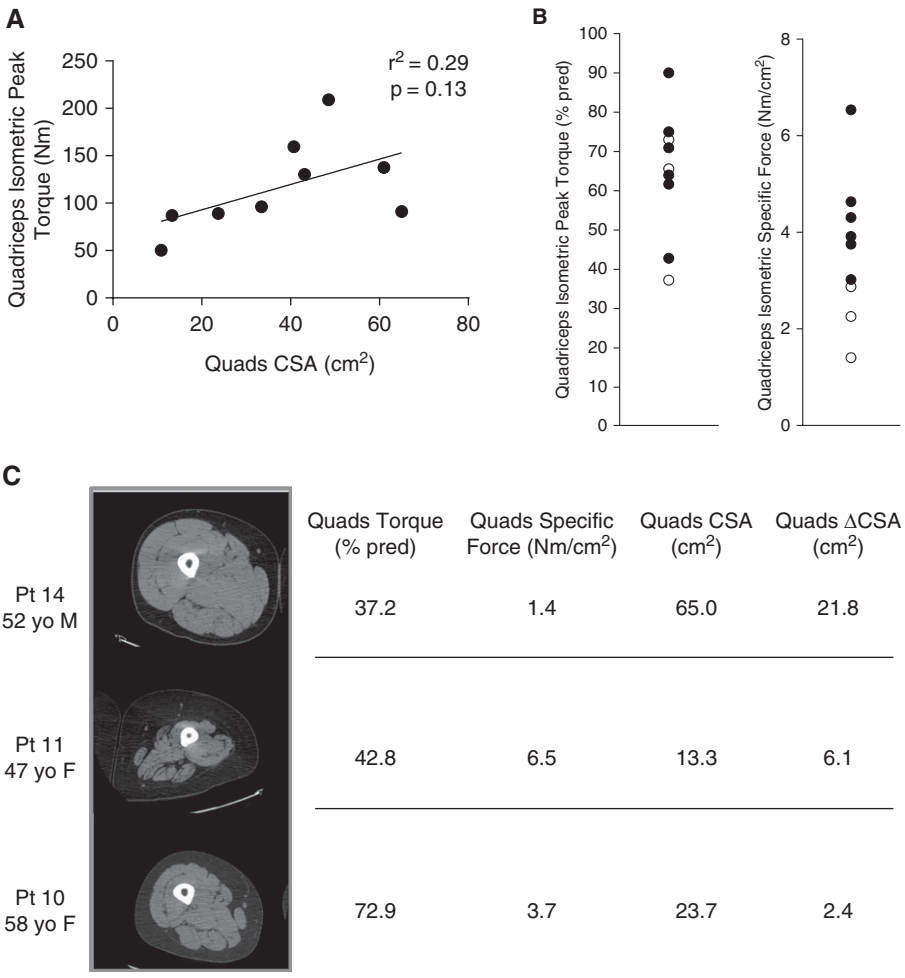

Figure 2. Dissociation between muscle mass reconstitution and weakness resolution in intensive care unit (ICU) survivors. (*A*) There was no significant correlation between quadriceps isometric peak torque and quadriceps cross-sectional area (CSA) 6 months following ICU discharge. (*B*) Percent predicted (% pred) quadriceps isometric peak torque (as determined using the Gosselink equation [52]) was decreased both in patients with persistent muscle atrophy at 6 months (*solid circles*) and in patients who reconstituted quadriceps CSA (*open circles*). Voluntary quadriceps contractile capacity, as determined by the quadriceps specific force of contraction (quadriceps peak torque [Nm]/quadriceps CSA [cm^2]), was actually lowest in patients who reconstituted quadriceps size (*open circles*). (*C*) Representative thigh computed tomographic scans obtained 6 months post–ICU discharge demonstrated sustained weakness resulting from markedly different degrees of muscle atrophy versus impaired contractile capacity. Patient 14 normalized quadriceps size at 6 months with substantial muscle regrowth (ΔCSA) but remained weak owing to low quadriceps specific force of contraction. Patients 10 and 11 remained weak owing to persistent muscle wasting, despite a higher muscle specific force of contraction (ΔCSA = quadriceps CSA at 6 mo − CSA at 7 d). Pt = patient.

it, as determined by the percent predicted quadriceps peak torque (54.8 ± 6.6% vs. 77.5 ± 6.6%, respectively; $P = 0.07$) and Medical Research Council Sum Score (51.5 ± 1.5 vs. 57.6 ± 0.8, respectively; $P < 0.05$).

Quadriceps Inflammatory Infiltrate

Seven days following ICU discharge, an inflammatory infiltrate of leukocytes was always present, but it resolved by 6 months in all patients (Figure E4).

Quadriceps Wasting at 6 Months Was Not Associated with UPS-mediated Muscle Proteolysis

Qualitative assessment of electron microscopic sections of the vastus lateralis revealed sarcomere destruction in 100% of patients (15 of 15) at 7 days after ICU discharge, with resolution noted in all patients at 6 months (Figure E5). Increased UPS-mediated proteolysis was evident in the vastus lateralis 7 days after ICU discharge, as determined by measurement of 20S proteasome activity and total ubiquitinated protein levels (Figures 3 and E6). However, by 6 months, UPS activation decreased in patients (with and without sustained quadriceps atrophy) to levels comparable to those of healthy individuals.

Altered Autophagy Was Not Evident in Atrophic Quadriceps

To assess autophagy engagement, we measured expression of three key autophagy coregulatory proteins (beclin 1 and VPS34, which are involved in autophagosome formation, and Bnip3, which is involved in selective targeting of mitochondria by autophagosomes) in addition to determining the extent of LC3B lipidation, which indicates enhanced LC3B mobilization to autophagosome membranes (Figures E7 and E8). Two measures—LC3 lipidation and Bnip3 expression—remained consistent across serial muscle biopsies from patients 7 days and 6 months after ICU discharge, as well as from healthy individuals. VPS34 was significantly increased at 7 days after discharge but fell to levels in keeping with those of healthy individuals at 6 months, whereas beclin 1 was increased at both 7 days and 6 months after discharge, regardless of the presence or absence of muscle atrophy at either time point. The variability among assays at 7 days after discharge precluded conclusions regarding differences between patients and healthy individuals. By 6 months, however, there were no differences in the majority of autophagy markers, with the exception of increased beclin 1 levels in the patients, but this was not associated with the development of sustained atrophy.

Decreased Satellite Cell Content Associated with Persistent Muscle Atrophy

Vastus lateralis satellite cell content was decreased at 7 days and 6 months after ICU discharge in patients who had the smallest incremental increases in quadriceps CSA and sustained muscle

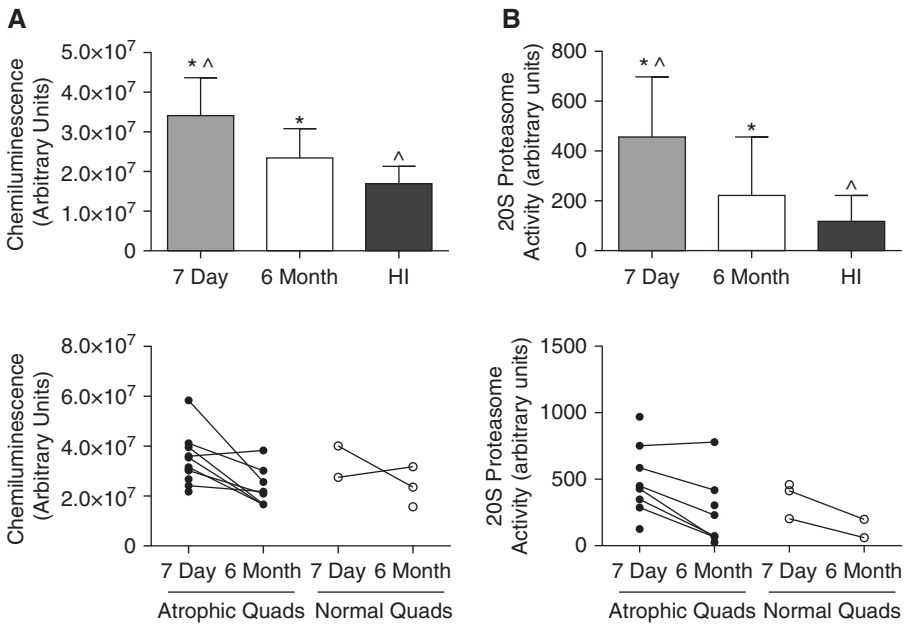

Figure 3. Ubiquitin-mediated proteolysis transiently increased in quadriceps muscle 7 days after intensive care unit (ICU) discharge. (*A*) Western blotting for ubiquitin demonstrates significantly increased ubiquitination of muscle total proteins in patients at 7 days after ICU discharge, but this decreased at 6 months after ICU discharge to levels comparable with those of healthy individuals (HI) (n = 6). There was no difference in the extent of ubiquitination between those patients with persistent atrophy (patients 5–12; atrophic quads) and those patients whose quadriceps cross-sectional area (CSA) normalized (patients 13–15; normal quads). Ubiquitin Western blots are shown in Figure E6. The left vastus lateralis of patient 8 was biopsied because of right femur fracture. (*B*) 20S proteasome activity was significantly increased at 7 days after ICU discharge, but this decreased in all patients at 6 months after ICU discharge to levels comparable with those of healthy individuals (n = 3). There was no difference in proteasome activity between patients whose muscle CSA normalized and those whose did not. Bar graphs show mean ± SD. $^{*,\wedge}P < 0.05$.

wasting at 6 months (Figures 4 and E9). Muscle vascularization (capillary-to-myofiber ratio) correlated with the satellite cell content (Figure E10).

Quadriceps Mitochondrial Content Normalized Despite Sustained Weakness

Vastus lateralis mitochondrial content was significantly decreased at 7 days after ICU discharge but was increased at 6 months after ICU discharge and was similar to that of healthy individuals (Figure E11). No differences were detected between patients and healthy individuals in mitochondrial size at either time point following ICU discharge (Figure E11). Bioenergetic status of the cell as determined by the ratio of phosphorylated AMP-activated protein kinase to AMP-activated protein kinase was not changed between the groups at either 7 days or 6 months after ICU discharge (1.41 ± 0.99 and 2.30 ± 1.98, respectively) and in healthy individuals (1.90 ± 2.00; $P = 0.45$ by analysis of variance).

Discussion

In this cohort of critical illness survivors, we demonstrate that persistent ICUAW 6 months following resolution of critical illness is associated with a heterogeneous muscle pathophysiology. Long-term weakness derives from markedly variable combinations of muscle atrophy and decreased muscle specific force (voluntary contractile capacity), which occurs with intact muscle ultrastructure and nerve function. In contrast to what is known about the development of acute ICUAW during critical illness, we show that persistent weakness at 6 months cannot be explained by ongoing UPS-mediated proteolysis, inflammation, muscle autophagy, or changes in mitochondrial structure or content. Instead, for those with persistent muscle wasting 6 months post–ICU discharge, quadriceps regrowth is compromised and associated with a lower satellite cell content.

Muscle wasting during critical illness can be severe and in excess of what is expected solely with inactivity (22, 23) or systemic illness (24–26). It occurs early and rapidly during the first week of critical illness and is more profound among those with multiorgan failure (7). Increased muscle proteolysis relative to protein synthesis is essential for the development of muscle atrophy (27, 28). Previous studies demonstrated that increased protein breakdown, in excess of synthesis, maintains an overall catabolic state in the muscle of critically ill patients (7, 29). UPS-mediated proteolysis is the predominant system that induces loss of muscle mass (27, 28). Increased expression of proteasome components and increased proteasome activity have previously been reported in the atrophying muscle of the critically ill patient (30–32). Alternative and indirect assessment of UPS engagement by evaluation of expression levels of key regulatory signaling proteins or their transcripts has yielded inconsistent results (7, 29, 33), likely due in part to variable sampling times during acute muscle wasting within the ICU and the complexity of temporal and cellular spatial interplay of these signaling proteins in regulating muscle mass. Whole-system principal component analysis has been used recently in an attempt to deal with these complexities of molecular signaling regulating muscle breakdown, and it revealed patterns of intracellular signaling that supported increased proteolysis and depressed protein synthesis (7). While our study did not address the mechanisms of muscle wasting during critical illness, we did note sarcomeric destruction and increased UPS activation in our patient cohort at 7 days after ICU discharge, suggesting we were seeing residual UPS-mediated proteolysis that had been induced by critical illness during the ICU stay.

In contrast, we were unable to convincingly demonstrate altered autophagy in atrophied muscle following resolution of critical illness. Autophagy clears damaged and unwanted cellular components, and maintenance of

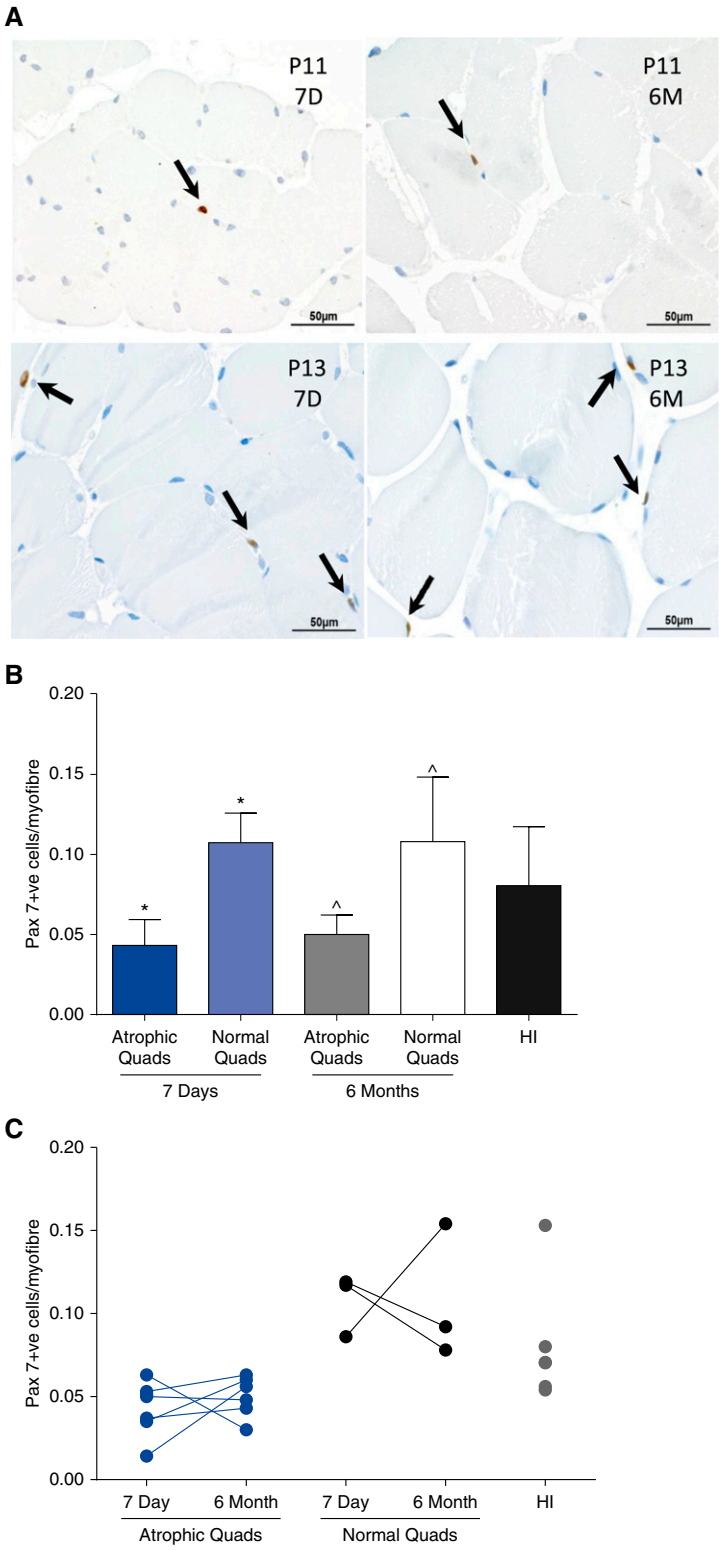

Figure 4. Vastus lateralis satellite cell content was decreased in patients with persistent atrophy compared with those whose cross-sectional area (CSA) normalized at 6 months after intensive care unit (ICU) discharge. (*A*) Histologic cross sections were immunostained for paired box gene 7 (Pax7) (*brown staining*, indicated by *arrows*), a marker of satellite cells. Representative images are shown for patient 11 (P11), who demonstrated persistent quadriceps atrophy at 6 months after ICU discharge, and patient 13 (P13), whose quadriceps CSA normalized. (*B* and *C*) Those patients with

autophagic balance is critical to the preservation of healthy muscle mass. Excessive autophagy results in muscle catabolism, and impaired autophagy results in the accumulation of damaged muscle components and muscle degradation (28). Previous studies have demonstrated that autophagy contributes to muscle catabolism in the ICU (34, 35). If altered autophagy occurred in our patients, it may have happened early in the course of critical illness but had begun to abate by the time of our evaluation 7 days after ICU discharge.

We found that the parameters of muscle proteolysis normalized 6 months following resolution of critical illness, indicating that neither enhanced UPS activity nor altered autophagy can explain the sustained muscle atrophy seen in some critical illness survivors. We speculated that persistent atrophy must result from impaired muscle growth following its catabolism in the ICU. In young, healthy individuals, normalization of muscle mass following prolonged inactivity has been reported to occur within weeks of resuming ambulation. For example, men (aged 32 ± 12 yr) subjected to bed rest for 17 weeks had normalized quadriceps, ankle extensor, and flexor mass within 5 weeks of ambulation (22). Muscles immobilized and/or inactive due to fracture or joint injury can demonstrate slower recovery rates, with rapid recovery early on but tapering as time passes. Researchers in some studies have reported near or complete recovery of mass by 10–24 weeks postinjury (36, 37), while others have demonstrated 20% loss of muscle 1–5 years following lower limb trauma (38). While our patients were exposed to prolonged bed rest none, with one exception, had experienced lower limb fracture; in that individual, quadriceps CSA was measured on the contralateral leg. On the basis of these data, at 6 months we would expect our ICU population to have ultimately reconstituted their muscle mass to its final possible maximum or very close to it.

The corticosteroid administered to some of our patients may have contributed to the induction of acute muscle wasting while in the ICU, but the role of these agents in ICUAW remains unclear, with studies both suggesting and refuting an association (8, 39). In those studies suggesting an association, strength testing was often

conducted in the absence of measurement of muscle mass, so the underlying contribution of wasting versus impaired contractility is unknown. Similarly, diabetes or chronic obstructive pulmonary disease, which in themselves can induce muscle wasting (24, 26), were comorbidities in four of our patients with sustained muscle atrophy; thus, these could also have played a contributory role. This information notwithstanding, two patients with no preadmission comorbidity and who did not receive corticosteroids demonstrated sustained muscle wasting 6 months after discharge, reiterating the capability of an episode of critical illness to cause enduring muscle compromise.

Following injury, muscle grows via hypertrophy of existing myofibers when structural and/or contractile proteins are synthesized and via regeneration where muscle progenitor satellite cells proliferate and differentiate to fuse to become mature muscle (40). While satellite cells are not required for the hypertrophic response of healthy muscle to mechanical load, or for the regrowth of muscle atrophied simply by immobility and/or unloading (41), they are essential for the regeneration of injured muscle (42–44). Decreased satellite cell content also contributes to the sarcopenia of aging (45). In our cohort, patients who had only a small increment in muscle growth and whose quadriceps CSA failed to normalize over time had a lower quadriceps satellite cell content compared with those patients who recovered muscle mass. The diminished satellite cell content may play a causative role in poor muscle regrowth and sustained atrophy following critical illness. Of interest, we found that muscle capillarization correlated positively with satellite cell content, although we were unable to demonstrate a statistically significant difference in the capillary-to-myofiber ratio across groups. Myogenesis and angiogenesis are known to be linked, and a correlation between satellite cell and muscle capillary content has been reported previously (46).

Mitochondria are essential for muscle contractile function and maintenance of mass (47). Loss of muscle mitochondria is associated with poor outcomes in ICU patients, including increased mortality (48), and we demonstrate a decreased quadriceps mitochondrial content 7 days after ICU discharge. However, ICU survivors at 6 months displayed a normal muscle mitochondria population (content and size), suggesting that mitochondrial abnormalities are not responsible for the differential restoration of muscle mass or the variable muscle voluntary contractile capacity noted at 6 months after ICU discharge.

The mechanisms underlying the dissociation between restoration of muscle mass and persistent weakness induced by impaired contractile capacity noted in some of our patients remain to be elucidated. Other pathologic processes have similar effects on muscle biomechanics. In congestive heart failure, animal models suggest that observed dissociation between skeletal muscle mass and contractility results in part from excitation–contraction uncoupling induced by abnormalities in calcium sequestration (49). Weakness with aging is beyond what would be anticipated for loss in muscle mass, due in part to altered muscle quality with increased fat infiltration (50). Post-translational modifications of myofilament proteins such as phosphorylation, acetylation, and oxidation of myosin, troponins, and actin may also be involved in the dissociation between muscle mass restoration and poor muscle contractility (51). Our three patients whose muscle mass normalized but continued to be weak had only hypertension or previous lymphoma as a comorbidity, and they were not elderly, suggesting that critical illness itself and/or ICU-related therapies may induce permanent impairment of muscle contractile capacity independent of muscle mass reconstitution.

The discovery study presented is limited by the small number of patients assessed. While our recruitment was reasonable, with 27 of 82 eligible patients consenting, the attrition rate was high, given their illness severity, and we lost several patients in follow-up due to death, ICU repatriation, and medical complications precluding muscle biopsy. We acknowledge that the limited sample size may underpower some comparisons and will require further investigation with larger populations. The generalizability of our findings needs to be evaluated in other ICU cohorts. In addition, our banked biopsy specimens from healthy individuals were not tightly age and sex matched, which may have influenced our cellular and/or molecular analyses. We did not know the patients' preadmission quadriceps size; thus, our comparisons with control populations to ascertain the presence or absence of muscle wasting with critical illness may overestimate the degree of muscle loss. However, all patients self-reported at minimum a moderate activity level, with the majority capable of strenuous activity (as defined by the Duke Activity Index) before their ICU admission, suggesting that their preadmission muscle mass was not diminished. Similarly, we did not know the patients' preadmission quadriceps peak torque. However, the availability of validated predictive equations with which to determine an individual's expected torque provides a robust measure against which our outcomes can be compared.

We assessed only UPS- and autophagy-mediated proteolysis, but increased activity of calpains and caspases has been shown to induce muscle atrophy (27). While we found mitochondrial size and content to be normalized at 6 months, we did not evaluate mitochondrial function. Similarly, while we found a lower satellite cell content in the ICU patient with sustained muscle atrophy, we did not evaluate satellite cell proliferation or differentiation capacity to determine if the decrease was functionally relevant. We evaluated patients only to 6 months after ICU discharge because improvement in functional disability induced by critical illness has been reported to plateau between 6 months and 1 year (11, 12), suggesting that the observed muscle outcomes at 6 months are representative of the ultimate outcomes and not simply a delay in recovery.

In summary, we demonstrate that persistent muscle weakness following resolution of critical illness is associated with markedly variable combinations of muscle atrophy and impaired voluntary

Figure 4. (Continued). persistent quadriceps atrophy (atrophic quads; patients 5–12) at 6 months after ICU discharge demonstrated a decreased satellite cell population at both 7 days and 6 months after ICU discharge compared with patients whose quadriceps CSA normalized (normal quads; patients 13–15). Bar graphs show mean ± SD; $^{*,\wedge}P < 0.05$. n = 6 healthy individuals (HI). +ve = positive.

contractile capacity. While UPS-mediated muscle proteolysis contributes to atrophy of muscle during illness, it is not sustained over the long term. Persistent muscle wasting results instead from impaired muscle regrowth, potentially related to diminished regenerative capacity resulting from the loss of muscle progenitor satellite cells.

Our findings highlight that the biology of the muscle can be durably and even definitely altered by critical illness. The cellular signaling networks that regulate muscle mass and contractility are intertwined but can potentially be targeted individually, enabling pharmacologic therapies to be specifically directed to address atrophy, impaired contractility, or both. While targeting selective inhibition of UPS-mediated skeletal muscle proteolysis may be a potential therapeutic intervention for patients during the early proteolytic phase of ICUAW, this will not be appropriate in patients with prolonged nonresolving ICUAW, where the problem is compromised muscle growth and not ongoing enhanced proteolysis. Moreover, attempts at reconstitution of muscle mass may be less successful in those patients with satellite cell loss, relying predominantly on hypertrophic growth, nor may they mitigate weakness in those patients with decreased strength due to (mass-independent) contractile dysfunction. ■

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