

Original Investigation | CARING FOR THE CRITICALLY ILL PATIENT

Acute Skeletal Muscle Wasting in Critical Illness

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IMPORTANCE Survivors of critical illness demonstrate skeletal muscle wasting with associated functional impairment.

OBJECTIVE To perform a comprehensive prospective characterization of skeletal muscle wasting, defining the pathogenic roles of altered protein synthesis and breakdown.

DESIGN, SETTING, AND PARTICIPANTS Sixty-three critically ill patients (59% male; mean age: 54.7 years [95% CI, 50.0-59.6 years]) with an Acute Physiology and Chronic Health Evaluation II score of 23.5 (95% CI, 21.9-25.2) were prospectively recruited within 24 hours following intensive care unit (ICU) admission from August 2009 to April 2011 at a university teaching and a community hospital in England. Patients were recruited if older than 18 years and were anticipated to be intubated for longer than 48 hours, to spend more than 7 days in critical care, and to survive ICU stay.

MAIN OUTCOMES AND MEASURES Muscle loss was determined through serial ultrasound measurement of the rectus femoris cross-sectional area (CSA) on days 1, 3, 7, and 10. In a subset of patients, the fiber CSA area was quantified along with the ratio of protein to DNA on days 1 and 7. Histopathological analysis was performed. In addition, muscle protein synthesis, breakdown rates, and respective signaling pathways were characterized.

RESULTS There were significant reductions in the rectus femoris CSA observed at day 10 (-17.7% [95% CI, -25.9% to 8.1%]; $P < .001$). In the 28 patients assessed by all 3 measurement methods on days 1 and 7, the rectus femoris CSA decreased by 10.3% (95% CI, 6.1% to 14.5%), the fiber CSA by 17.5% (95% CI, 5.8% to 29.3%), and the ratio of protein to DNA by 29.5% (95% CI, 13.4% to 45.6%). Decrease in the rectus femoris CSA was greater in patients who experienced multiorgan failure by day 7 (-15.7%; 95% CI, -27.7% to 11.4%) compared with single organ failure (-3.0%; 95% CI, -5.3% to 2.1%) ($P < .001$), even by day 3 (-8.7% [95% CI, -59.3% to 50.6%] vs -1.8% [95% CI, -12.3% to 10.5%], respectively; $P = .03$). Myofiber necrosis occurred in 20 of 37 patients (54.1%). Protein synthesis measured by the muscle protein fractional synthetic rate was depressed in patients on day 1 (0.035%/hour; 95% CI, 0.023% to 0.047%/hour) compared with rates observed in fasted healthy controls (0.039%/hour; 95% CI, 0.029% to 0.048%/hour) ($P = .57$) and increased by day 7 (0.076% [95% CI, 0.032%-0.120%/hour]; $P = .03$) to rates associated with fed controls (0.065%/hour [95% CI, 0.049% to 0.080%/hour]; $P = .30$), independent of nutritional load. Leg protein breakdown remained elevated throughout the study (8.5 [95% CI, 4.7 to 12.3] to 10.6 [95% CI, 6.8 to 14.4] μmol of phenylalanine/min/ideal body weight $\times 100$; $P = .40$). The pattern of intracellular signaling supported increased breakdown ($n = 9$, $r = -0.83$, $P = .005$) and decreased synthesis ($n = 9$, $r = -0.69$, $P = .04$).

CONCLUSIONS AND RELEVANCE Among these critically ill patients, muscle wasting occurred early and rapidly during the first week of critical illness and was more severe among those with multiorgan failure compared with single organ failure. These findings may provide insights into skeletal muscle wasting in critical illness.

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Survivors of critical illness experience significant skeletal muscle weakness and physical disability, which can persist for at least 5 years.^{1,2} Muscle wasting contributes substantially to weakness acquired in the intensive care unit (ICU),^{1,3} but its time course and underlying pathophysiological mechanisms remain poorly characterized and understood. In health, muscle mass is maintained through balanced protein breakdown and synthesis.⁴ For wasting to occur, breakdown must be increased relative to synthesis.

Increased breakdown has been described in animal models of critical illness⁵ and in patients with severe burns.⁶ Decreased protein synthesis due to immobility and endotoxin exposure has been reported in human studies.^{7,8} However, the few relevant human studies of muscle loss in critically ill patients are cross-sectional in design and lack standardized time points for measurement comparison.⁹⁻¹¹ Furthermore, data relating to qualitative changes, such as skeletal muscle necrosis, are also limited.¹² We thus sought to prospectively characterize and evaluate the time course and pathophysiology of acute muscle loss in critical illness, and determine the role that alterations in protein synthesis and breakdown have in driving such changes.

Methods

Study Design

Ethical approval was obtained from University College London ethics committee A for recruitment from King's College Hospital NHS Trust and the Whittington Hospital NHS Trust from August 2009 to April 2011. All patients were older than 18 years and were anticipated to be intubated for longer than 48 hours, spend more than 7 days in critical care, and to survive ICU stay. Patients were subsequently excluded if these criteria were not met. Patients were also excluded if pregnant, had a lower limb amputated, or had a primary neuromuscular pathology or disseminated cancer. At enrollment, written assent was obtained from the next of kin with retrospective patient consent obtained when full mental capacity was regained.

Measurement of Muscle Mass

Markers of muscle mass loss were assessed ultrasonographically, histologically, and biochemically on days 1, 3, 7, and 10. Rectus femoris cross-sectional area was measured using B-mode ultrasound.^{13,14} Biopsy samples of the vastus lateralis muscle were obtained using the Conchotome¹⁵ technique and snap frozen for analysis of the fiber cross-sectional area (Scion Image, Scion Corporation), and quantification of the ratio of protein to DNA¹⁶ (Qubit, Life Technologies) by staff blinded to all patient data.

Muscle Protein Synthesis and Leg Protein Turnover

Rates of muscle protein synthesis were determined by leucine incorporation into the vastus lateralis, using primed constant infusions of [1,2-¹³C₂] leucine on ICU days 1 and 7.¹⁶ Muscle biopsies were obtained before and after 150-minute

infusions. Critical illness patient data were compared with data from 8 healthy volunteers (mean age, 70.7 years [95% CI, 67.7-73.7 years]; mean body mass index (calculated as weight in kilograms divided by height in meters squared) of 26.2 (95% CI, 24.3-28.2) in both a fasted state and a fed state (intravenous Glamin, Fresenius-Karbi, 289 mL/kg over 150 minutes).¹⁷

Leg protein breakdown and balance were simultaneously determined by femoral vein dilution of D₅ phenylalanine during a primed constant infusion.¹⁶ Values were normalized against calculated ideal body weight.¹⁸ Leg protein synthesis was calculated as the difference between breakdown and balance. Serum creatine kinase and myoglobin concentrations were assayed (Siemens Healthcare Diagnostics) on days 1 and 7.

Intracellular Regulators of Protein Homeostasis

Protein concentrations of key components in the synthesis and breakdown signaling pathways were determined (Figure 1). Muscle protein synthesis is mediated by pathways convergent on protein kinase B.¹⁹ Phosphorylation (or dephosphorylation) of the pathway of insulin-like growth factor 1 and protein kinase B controls muscle protein synthesis and muscle protein breakdown; however, this can also be modulated through other regulators such as nuclear factor κB.¹⁹

The ubiquitin proteasome pathway represents the final common proteolysis pathway in multiple disease models.¹⁹ We determined the total and phosphorylated protein concentrations of key signaling molecules (Figure 1) using Luminex technology (Flexmap3d, Merck Millipore) and Western blotting. Messenger RNA expression of myostatin, a member of the transforming growth factor β family and a known negative regulator of muscle mass, also was determined.¹⁹

Histological Assessment

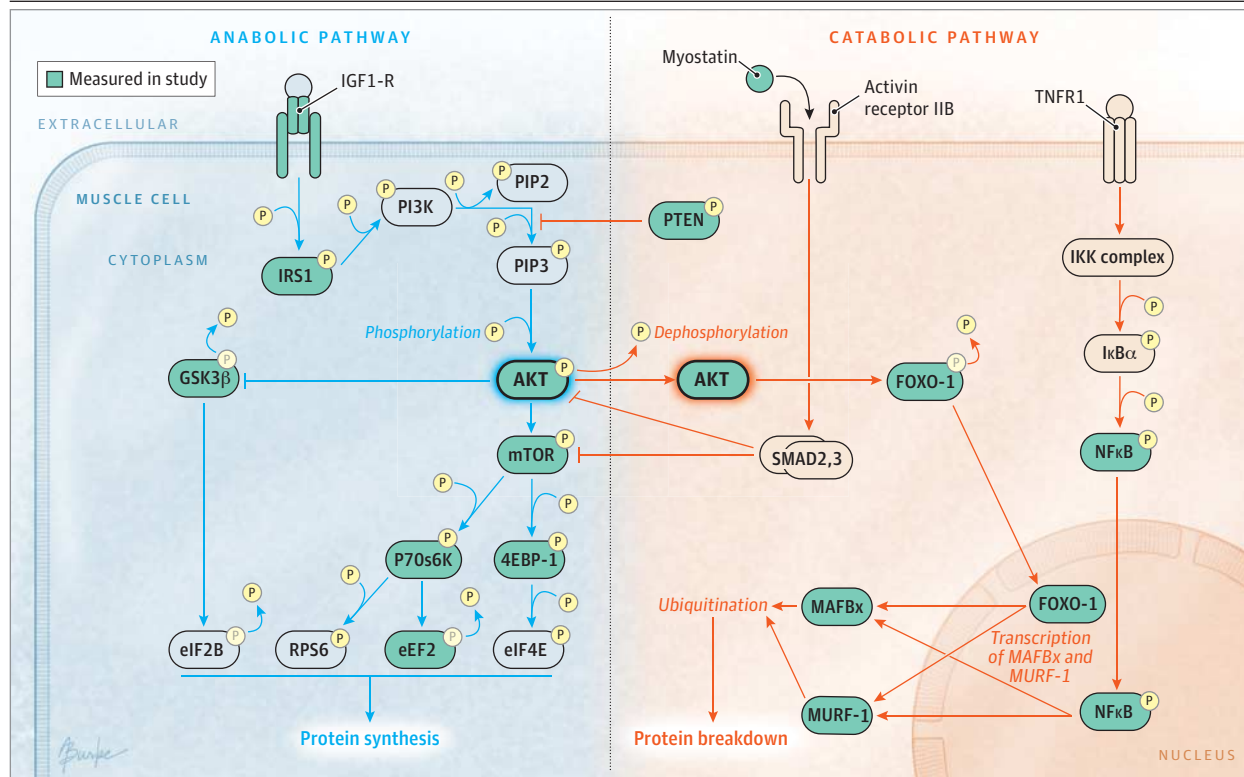
Muscle specimens were stained with hematoxylin and eosin and examined by a senior histopathologist (R.P.), who was blinded to all clinical data. Cellular infiltrates were stained with a macrophage-specific antibody (Dako monoclonal mouse IgG1 isotype κ [anti-CD68]).

Clinical Correlates

Organ failure was measured using components of the Sequential Organ Failure Assessment score²⁰; a score of greater than 2 represented organ failure.²¹ Daily assessment of organ failure was made and an area under the curve was derived from the number of organs that failed per day over the 10-day study period.

Severity of illness was further defined through collation of bedside physiological data and measurement of daily serum C-reactive protein concentration. Nutritional intake (normalized to ideal body weight) was measured daily. Such possible associations were defined a priori. In addition, routinely collected clinical data were presented for bivariable or multivariable analysis and subsequent backward multivariable analysis to identify other relevant associations. This process is both agnostic and parsimonious (see statistical analysis section below).

Figure 1. Schematic Diagram of Anabolic and Catabolic Pathways Involved in Muscle Protein Homeostasis



AKT indicates protein kinase B; 4EBP-1, eukaryotic translation initiation factor 4E-binding protein; eEF2, eukaryotic elongation factor 2; eIF2B, eukaryotic initiation factor 2; eIF4E, eukaryotic initiation factor 4E; FOXO-1, forkhead box class O-1; GSK3 β , glycogen synthase kinase 3 β ; IGF1-R, insulin-like growth factor 1 receptor; I κ B α , inhibitor of nuclear factor κ B α ; IKK, inhibitor of nuclear factor κ B kinase; IRS1, insulin receptor substrate 1; MAFBx, muscle atrophy f-box-1; mTOR, mammalian target of rapamycin; MURF-1, muscle ring finger protein 1;

NF κ B, nuclear factor κ B; P70s6K, 70-kDa s6 protein kinase; PI3K, phosphoinositide 3-kinase; PIP2, phosphatidylinositol 4,5-bisphosphate, phosphatidylinositol 3,4,5-trisphosphate; PIP3, phosphatidylinositol 3,4,5-trisphosphate; PTEN, phosphatase and tensin homolog deleted on chromosome 10; RPS6, ribosomal protein S6; SMAD2,3, vertebrate homolog of *Drosophila* protein MAD (mothers against decapentaplegic) and *Caenorhabditis elegans* protein SMA 2,3; TNFR1, tumor necrosis factor receptor 1.

Statistical Analysis

Data from a pilot study¹⁴ indicated that 32 patients would be required to detect a 10% reduction in rectus femoris cross-sectional area over 10 days (with an α level of .05 and a β level of .10). A 10% cutoff was used to define clinically relevant muscle wasting in accordance with studies in other fields.^{13,14} All data were assessed for normality using D'Agostino-Pearson omnibus normality tests. Data were then analyzed using the *t* test, Pearson coefficient, Mann-Whitney test, and the Wilcoxon signed rank test as appropriate.

Change in rectus femoris cross-sectional area was assessed by repeated measure analysis of variance. Principal component analysis was used to identify the patterns of change within the signaling data and related to limb protein homeostasis. Multiple linear and logistic regression analyses (both bivariable and multivariable) were applied using the Statistical Package for the Social Sciences version 17 (SPSS Inc) and MedCalc version 12.3.0 (MedCalc Software).

In bivariable analysis, categorical variables were analyzed using the χ^2 and Fisher exact tests as appropriate. Measures of central tendency for continuous variables were compared using 2-sided *t* tests (parametric variables) following normality testing and use of the Mann-Whitney test (nonpara-

metric variables). Parametric variables were reported as mean (95% confidence interval) and nonparametric variables as median (interquartile range). Statistical significance was indicated by a *P* value of less than .05; significance for multivariable analysis was set at a *P* value of less than .10.

Linear regression was performed with change in rectus femoris cross-sectional area at days 7 and 10 as a continuous dependent variable. The independent variables that were statistically significant in bivariable analysis for correlation with rectus femoris cross-sectional area were entered into a backward multivariable analysis if the *P* value achieved on bivariable analysis was .10 or less. Logistic regression was used to determine the development of necrosis and predictors of rectus femoris cross-sectional area change at the 10% level based on chronic obstructive pulmonary disease rehabilitation data.¹³

Results

Ninety-one patients were recruited, of whom 63 met inclusion criteria for analysis. One patient could not undergo rectus femoris cross-sectional area assessment due to morbid obesity (body mass index of 67) and 1 patient declined venesection.

Table. Baseline Characteristics of Patients

	All Patients (N = 63)	Serial Muscle Biopsies and Ultrasound (n = 42)	Muscle Ultrasound Alone (n = 21)	Stable Isotope Incorporation (n = 11)
Age, mean (95% CI), y	54.5 (50.0-59.1)	55.3 (49.4-61.1)	53.1 (45.4-60.1)	62.7 (50.1-75.4)
Male sex, No. (%)	37 (58.7)	30 (71.4) ^a	7 (31.3)	9 (81.8) ^a
Hospital length of stay prior to ICU admission, median (range), d	1 (1-45)	1 (1-6)	1 (1-45)	1 (1-6)
Period ventilated, median (range), d	10 (2-62)	8.5 (2-62)	10 (4-24)	12 (2-62)
ICU length of stay, median (range), d	16 (7-80)	15.5 (7-80)	17 (7-73)	18 (8-80)
Hospital length of stay, median (range), d	30 (11-334)	29.5 (11-212)	33 (13-334)	50 (17-212)
APACHE II score, mean (95% CI)	23.5 (21.9-25.2)	23.3 (21.3-25.3)	24 (20.1-27.2)	27 (22.9-31.3)
SAPS II score, mean (95% CI)	45.5 (41.8-49.3)	43.4 (39.2-47.6)	49.7 (42.0-57.4)	47 (39.6-54.4)
Survival, No. (%)				
ICU	61 (97)	40 (95)	21 (100)	10 (91)
Hospital	56 (89)	37 (88)	19 (90)	9 (82)
Renal replacement therapy, No. (%)	19 (30.2)	13 (31.0)	6 (29.0)	4 (36.4)
Use of neuromuscular blocking agents, median (range), d	0 (0-6)	0 (0-6)	0 (0-5)	0 (0-6)
Hydrocortisone dose, median (range), mg ^b				
Day 1	0 (0-800)	0 (0-800)	0 (0-400)	200 (0-800)
Total by day 10	0 (0-4533)	0 (0-4533)	0 (0-3360)	450 (0-4533)
Statin use, No. (%)	11 (17.4)	7 (16.7)	4 (19)	1 (9.1)
Blood glucose level, median (range), mmol/L	7.4 (5.1-11.4)	7.3 (5.1-10.3)	7.6 (5.6-11.4)	7.9 (6.1-9.5)
Cumulative insulin, median (range), IU	93 (0-1704)	90 (0-1704)	125 (0-817)	90 (0-1704)
Admission diagnosis, No. (%)				
Sepsis	31 (49.2)	19 (45.3)	12 (57.1)	6 (54.5)
Trauma	16 (25.4)	13 (31.0)	3 (14.3)	4 (36.4)
Intracranial bleeding	5 (7.9)	4 (9.5)	1 (4.8)	0
Acute liver failure	5 (8.0)	3 (7.0)	2 (9.5)	1 (9.1)
Cardiogenic shock	6 (9.5)	3 (7.1)	3 (14.3)	0
Comorbidities, No. (%)				
Chronic obstructive pulmonary disease	9 (14.3)	7 (16.7)	2 (9.5)	2 (18)
Ischemic heart disease	10 (15.9)	7 (16.7)	3 (14.3)	1 (9.1)
Hypertension	13 (19.0)	8 (19.0)	5 (23.8)	1 (9.1)
Diabetes mellitus	8 (12.7)	6 (14.3)	2 (9.5)	1 (9.1)
Liver cirrhosis	6 (9.5)	4 (9.5)	2 (9.5)	1 (9.1)
Chronic pancreatitis	2 (3.2)	1 (2.4)	1 (4.7)	0
Hematological disease	4 (6.3)	2 (4.8)	2 (9.5)	0
Obesity	3 (4.8)	2 (4.8)	1 (4.7)	0
Previous cerebrovascular accident	1 (1.6)	1 (2.4)	0	0
Renal impairment	2 (1.6)	1 (2.4)	1 (4.7)	0
Crohn disease	1 (1.6)	0	1 (4.7)	0
Thyroid disease	3 (4.8)	1 (2.4)	2 (9.5)	0

Abbreviations: APACHE II, Acute Physiology and Chronic Health Evaluation II; ICU, intensive care unit; SAPS II, Simplified Acute Physiology Score II.

^a Indicates $P < .05$ vs muscle ultrasound alone group; χ^2 test was used.

^b Indicates corticosteroid dosing as hydrocortisone equivalents.

Forty-two patients underwent at least 1 muscle biopsy, and 35 had biopsies on both ICU days 1 and 7. Eleven received isotope infusions on days 1 and 7. The characteristics of those patients who had biopsies (with or without isotope infusions) and those who underwent ultrasound examination only (all $P > .05$) did not differ, except that the proportion of males was higher among those who had biopsies (71.4% vs 31.3%, $P = .003$; Table).

Changes in Markers of Muscle Mass

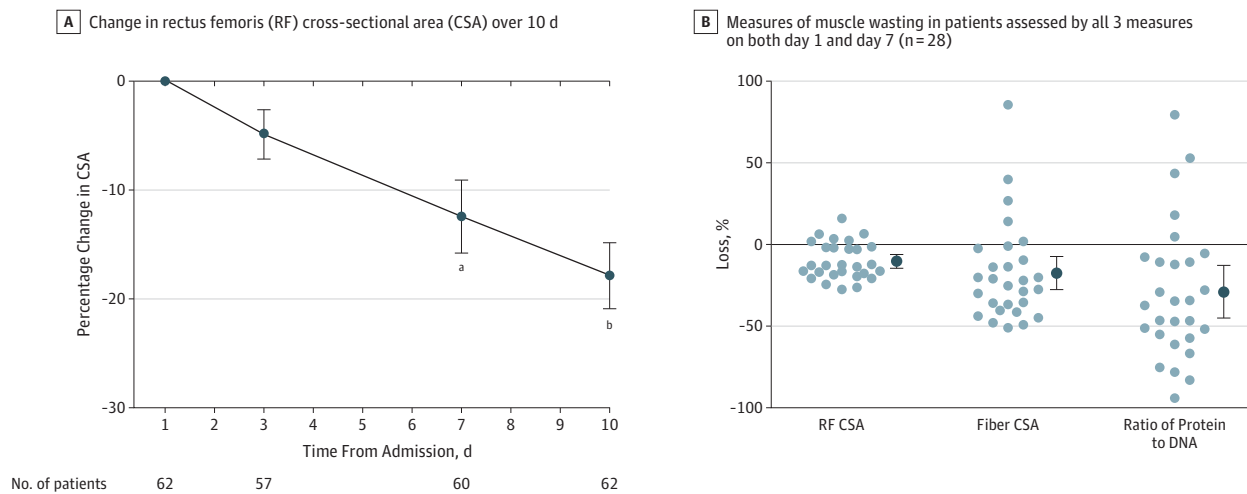
In the group overall, rectus femoris cross-sectional area decreased significantly from days 1 to 7 (−12.5% [95% CI, −35.4%

to 24.1%]; $P = .002$), and continued to decrease to day 10 (−17.7% [95% CI, −25.9% to 8.1%]; $P < .001$). In the 28 patients assessed by all 3 methods on days 1 and 7, the rectus femoris cross-sectional area decreased by 10.3% (95% CI, 6.1% to 14.5%), the fiber cross-sectional area by 17.5% (95% CI, 5.8% to 29.3%), and the ratio of protein to DNA by 29.5% (95% CI, 13.4% to 45.6%) (Figure 2).

Muscle Protein Homeostasis

Nasogastric feeding was successfully initiated in 9 of the 11 patients on day 1 and in all patients by day 7 (<200 mL of nasogas-

Figure 2. Measurements of Muscle Wasting During Critical Illness

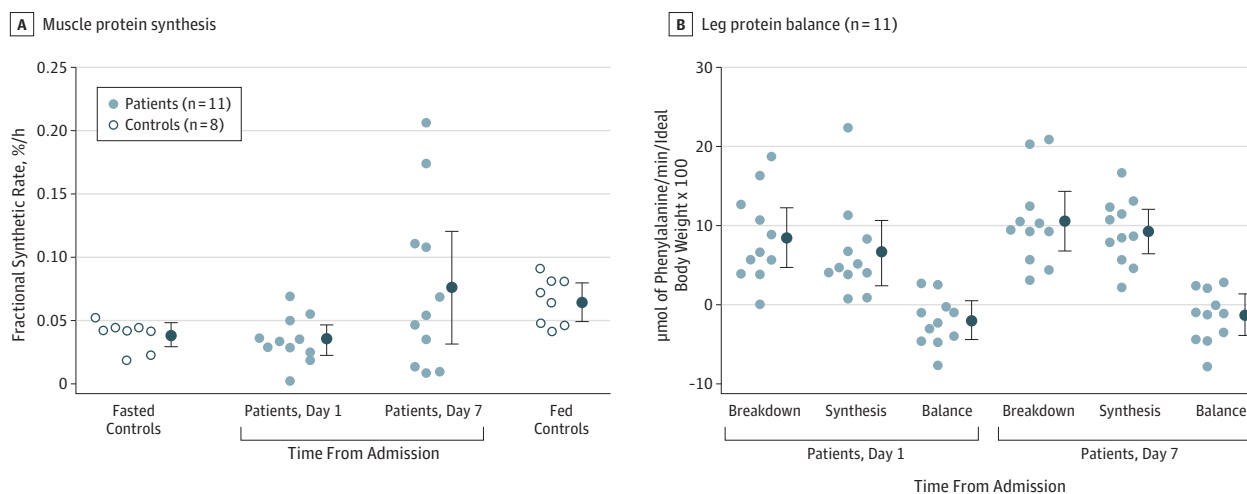


Summary data (dark circles) are expressed as medians and 95% confidence intervals.

^a $P = .002$ for change from day 1 to day 7 by repeated measures 2-way analysis of variance.

^b $P < .001$ for change from day 1 to day 10.

Figure 3. Muscle Protein Synthesis and Leg Protein Balance



Summary data (dark circles) are expressed as medians and 95% confidence intervals. P values calculated using the Wilcoxon signed rank test. In part A, the comparison between fasted patients and controls yielded a P value of .57; the comparison between patients on days 1 and 7 yielded a P value of .03; and the

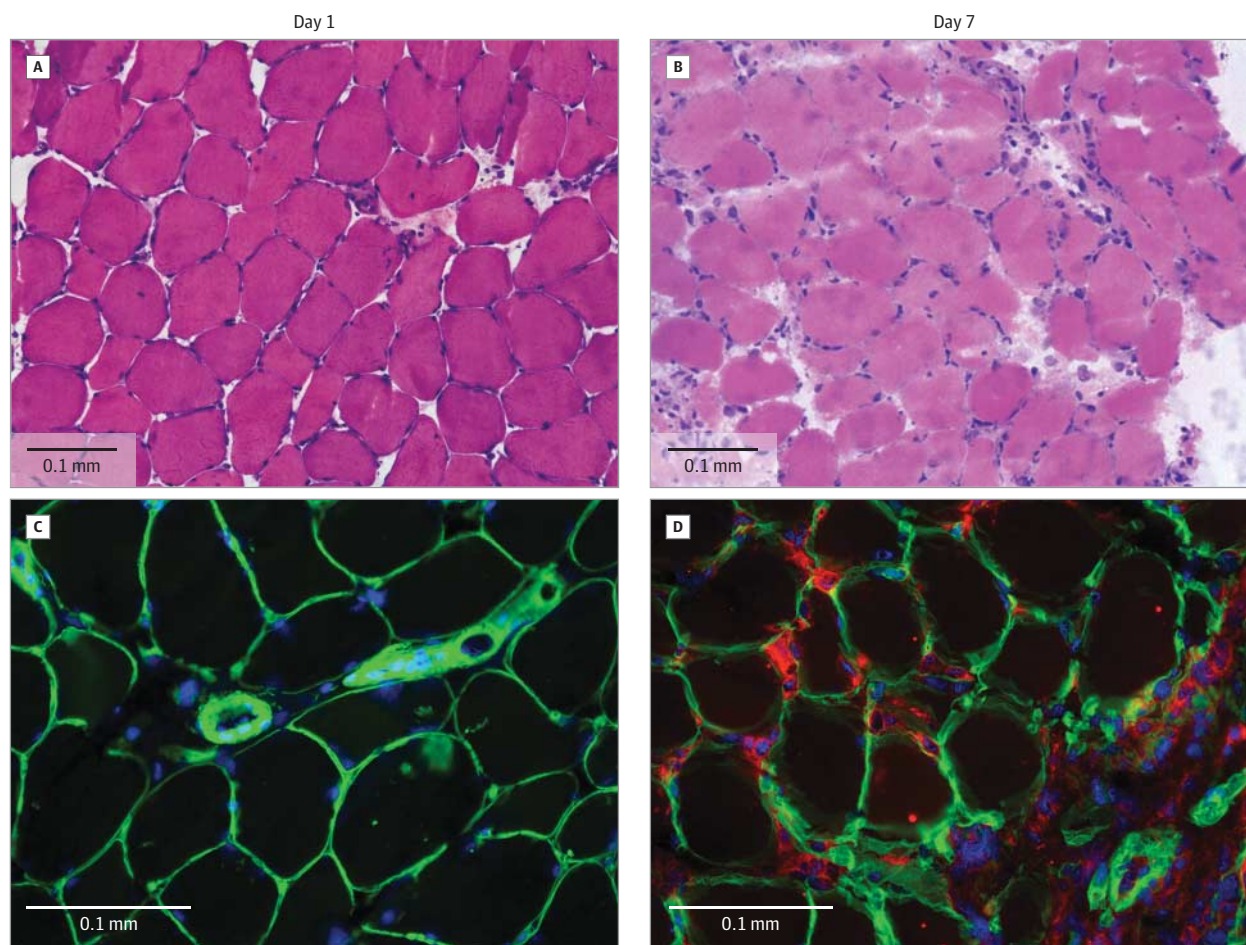
comparison between fed patients and controls yielded a P value of .30. In part B, the comparison between breakdown, synthesis, and balance at 1 day yielded a P value of .05; at 7 days, the P value was .30.

tric aspirate every 4 hours). Muscle protein fractional synthetic rate was depressed in patients on day 1 (0.035%/hour; 95% CI, 0.023%-0.047%/hour) to rates observed in fasted healthy controls (0.039%/hour; 95% CI, 0.029%-0.048%/hour) ($P = .57$) and increased by day 7 (0.076%/hour [95% CI, 0.032%-0.120%/hour]; $P = .03$) to rates observed in healthy fed controls (0.065%/hour [95% CI, 0.049%-0.080%/hour]; $P = .30$) (Figure 3A).

Leg protein breakdown was elevated compared with leg protein synthesis on day 1 (8.5 [95% CI, 4.7 to 12.3] $\mu\text{mol of phenylalanine}/\text{min}/\text{ideal body weight} \times 100$ vs 6.6 [95% CI, 2.5-

10.6] $\mu\text{mol of phenylalanine}/\text{min}/\text{ideal body weight} \times 100$, respectively; $P = .05$) and equivalent on day 7 (10.6 [95% CI, 6.8-14.4] $\mu\text{mol of phenylalanine}/\text{min}/\text{ideal body weight} \times 100$ vs 9.31 [95% CI, 6.6-12.1] $\mu\text{mol of phenylalanine}/\text{min}/\text{ideal body weight} \times 100$; $P = .30$), resulting in a net catabolic balance (Figure 3B). Concentrations were mildly elevated on day 1 for creatine kinase (546.7 U/L; 95% CI, 325.1-768.2 U/L) and myoglobin (0.301 $\mu\text{g}/\text{L}$; 95% CI, 0.214-0.389 $\mu\text{g}/\text{L}$). Both decreased by day 7 (creatin kinase: 197.2 U/L [95% CI, 125.5-268.9 U/L], $P < .001$; myoglobin: 0.163 $\mu\text{g}/\text{L}$ [95% CI, 0.108-

Figure 4. Muscle Biopsy Specimens From a Representative Patient on Day 1 and Day 7



Healthy muscle is seen on day 1 (A, C) with necrosis and a cellular infiltrate on day 7 (B, D). This infiltrate was CD68 positive on immunostaining, indicating macrophage origin (red). A, B are hematoxylin and eosin stain, and C, D was

immunostaining, with CD68 for red, laminin (myofiber outline) for green, and 4',6'-diamidion-2-phenylidole (a nuclear marker) for blue.

0.218 $\mu\text{g/L}$; $P = .01$). Neither absolute values on days 1 and 7 nor change over time for serum creatine kinase or myoglobin correlated with change in rectus femoris cross-sectional area (all $r^2 < 0.1$, $P > .05$).

Intracellular Drivers of Protein Homeostasis

Thirty-five pairs of serial muscle biopsies were analyzed. No clear pattern of change in expression of individual signaling components was observed from days 1 to 7, with the exception of a decrease in ubiquitin ligase muscle ring finger protein 1 (-54.3 arbitrary units [AU] [95% CI, -92.7 to -15.7 AU]; $P = .01$) and muscle atrophy F box (-58.6 AU [95% CI, -128.2 to 10.9 AU]; $P < .001$). These findings were independently confirmed by measurements of messenger RNA concentrations by investigators blinded to the clinical data (qStandard Group).

Myostatin messenger RNA levels remained unchanged from day 1 (36.3; 95% CI, 14.8-55.9) to day 7 (34.6; 95% CI, 8.1-58.1) (copy numbers per reaction: 22; $P = .88$). However, a principal components analysis of putative signaling molecules identified a pattern that correlated with both muscle protein

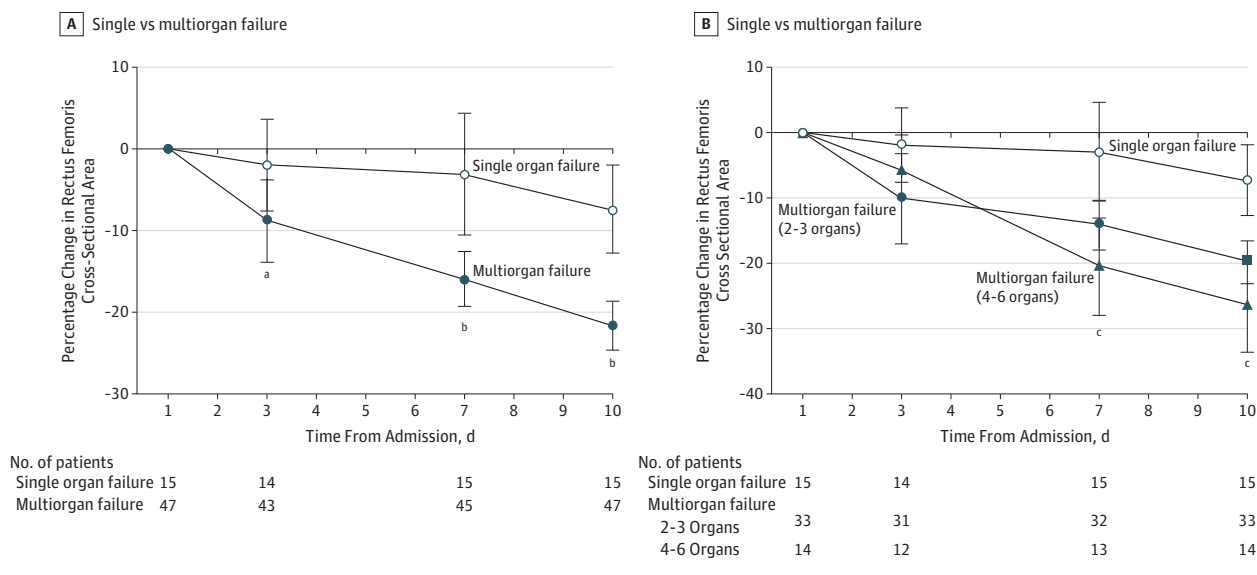
synthesis and breakdown. A 2-component principal component analysis model explained 41% of the variance in the time-related log-fold changes in molecular signaling data.

The second component of the principal component model captured biologically relevant relationships between signals, and 12% of the total variance in the signaling data. The model was tested for correlations with log-fold change in the components of protein homeostasis (defined by D_5 phenylalanine dilution data), synthesis, and breakdown. Significant correlations were found between the model and both muscle protein breakdown ($n = 9$, $r = -0.83$, $P = .005$) and synthesis ($n = 9$, $r = -0.69$, $P = .04$).

Qualitative Analysis

Serial muscle biopsies in 37 patients were analyzed. Fourteen patients had evidence of muscle necrosis by day 7, and 20 had evidence by day 10. In 6 patients, iatrogenic necrosis secondary to previous biopsy could not be completely excluded. All necrotic samples demonstrated macrophage infiltrates that were confirmed with CD68 staining (Figure 4). Two showed neutrophil infiltrates. Excluding

Figure 5. Measurements of Muscle Wasting During Critical Illness by Organ Failure



Data are expressed as medians and 95% confidence intervals.

^a $P = .03$ for change from day 1 to day 3 in multiorgan failure vs single organ failure.

^b $P < .001$ for change from day 1 to day 7 and day 1 to day 10 in multiorgan failure vs single organ failure.

^c $P < .001$ for difference between failure of 2-3 organs and 4-6 organs from day 1 to day 7 and day 10.

necrosis, all other myopathic changes reported on days 7 through 10 were present on day 1 of ICU admission. No clinical variables correlated with the development of necrosis (all $r^2 < 0.10$, $P > .05$).

Clinical Correlates, Patient Stratification, and Risk Factors for Muscle Wasting

Increasing organ failure score correlated with change in rectus femoris cross-sectional area ($r^2 = 0.23$, $P < .001$). Change in rectus femoris cross-sectional area differed between patients with multiorgan failure vs single organ failure (day 3: -8.7% [95% CI, -59.3% to 50.6%] vs -1.8% [95% CI, -12.3% to 10.5%], respectively, $P = .03$; day 7: -15.7% [95% CI, -27.7% to 11.4%] vs -3.0% [95% CI, -5.3% to 2.1% , $P < .001$). Change in rectus femoris cross-sectional area was greater in those with 4 or more failed organs (-20.3% ; 95% CI, -34.7% to 17.5%) than in those with 2 to 3 failed organs (-13.9% ; 95% CI, -25.7% to -9.8%) ($P < .001$). The differential effect of organ failure became more pronounced by day 10 (Figure 5).

When the cohort was examined by tertiles, no association was seen between change in rectus femoris cross-sectional area and age. A significant association was seen between change in rectus femoris cross-sectional area and ICU length of stay ($P < .001$). In a bivariable analysis, change in rectus femoris cross-sectional area was weakly associated with length of stay ($r^2 = 0.09$, $P = .02$). In a multivariable linear analysis, change in rectus femoris cross-sectional area at day 10 was negatively associated with serum bicarbonate, ratio of PaO_2 to fraction of inspired oxygen (FiO_2), and hemoglobin concentration at ICU admission ($r^2 = 0.51$, $P < .001$) and positively associated with the degree of organ failure,

mean C-reactive protein level, and total protein delivered during the study period.

A logistic multivariable regression analysis demonstrated age (odds ratio [OR], 1.05/y; 95% CI, 1.01-1.07/y), bicarbonate level at admission (OR, 0.72 mmol; 95% CI, 0.65-1.00 mmol), and ratio of PaO_2 to FiO_2 (OR, 0.88; 95% CI, 0.87-0.97) to be associated with greater than 10% loss in rectus femoris cross-sectional area at day 10 ($P < .001$); (Hosmer and Lemeshow, $P = .67$, c statistic = 0.89 [95% CI, 0.79-0.96]). More details about the methods and results appear in the Supplement. Specifically, the regression and model appears in the eTable 5.2 in the Supplement.

Discussion

In this study, skeletal muscle wasting, which occurred early and rapidly in critical illness, was characterized for the first time, to our knowledge, in a longitudinal cohort using 3 independent measures. Specifically, ultrasound-derived rectus femoris cross-sectional area, histologically determined vastus lateralis muscle fiber cross-sectional area, and ratio of protein to DNA decreased over the first week. This was shown to be a consequence of both depressed muscle protein synthesis and an elevation in leg protein breakdown relative to protein synthesis, resulting in a net catabolic state.

Muscle protein synthesis was depressed to levels equivalent to the healthy fasted state on day 1, but increased to rates similar to the healthy fed state by day 7; however, the net balance remained catabolic. Importantly, these overall effects occurred despite the administration of enteral nutrition. Unex-

pectedly, higher protein delivery in the first week was associated with greater muscle wasting. The trajectory of change implies an increase in muscle protein synthesis toward more normal values. Complex interactions between the different components of the anabolic and catabolic signaling pathways were identified. It would be unusual for a single molecule to represent a rate-limiting step in muscle homeostasis and, unsurprisingly, individual components of the anabolic and catabolic signaling pathways did not correlate with muscle loss or protein homeostasis. However, a whole-system principle component analysis extracted a novel pattern in the signaling data that inversely correlated with muscle protein turnover. Such analysis thus appears to have uncovered a complex molecular signal underpinning muscle protein turnover in critically ill patients.

The incidence of acute myofiber necrosis affected 40% of patients in our study, which was higher than previously described incidence.¹² A macrophagic cellular infiltrate was only found in those samples with evidence of necrosis. The occurrence of necrosis was independent of the disease state precipitating ICU admission. The chronic diseases our patients experienced (Table) can affect skeletal muscle function (associations between fiber type shift and muscle fiber atrophy have been reported in such groups), but there have been no descriptions of either necrosis or macrophagic infiltrates.²²⁻²⁴ The interplay between chronic disease and acute illness seems limited because necrosis was observed across all of the diagnostic groups. In addition, prolonged antecedent acute illness may have been responsible for these changes; however, only 5 patients were hospitalized for greater than 72 hours prior to ICU admission. It thus appears that critical illness, per se, is associated with an early and aggressive myopathic process. Although arterial hypoxia was not associated with the development of necrosis, this does not exclude roles for cellular dysoxia or microvascular redistribution.

Clinical and Physiological Correlates

Reduction in rectus femoris cross-sectional area was associated with organ failure burden. In particular, patients with single organ failure demonstrated limited wasting, whereas those with failure of 4 organs showed muscle loss of more than 15% by the end of the first week. Patients with multiorgan failure experienced greater physiological derangements previously implicated in the pathogenesis of muscle wasting.^{7,25-27} Specifically, inflammation reduces protein synthesis and increases breakdown,⁷ and lung-derived inflammatory mediators (eg, tumor necrosis factor) are associated with muscle wasting in chronic lung disease.²⁵

We showed a direct correlation between muscle wasting and both inflammation (C-reactive protein) and acute lung injury (ratio of PaO₂/FIO₂). Even though immobility⁸ is associated with wasting, all our patients were effectively confined to bed and we doubt that mobility differences contributed significantly to organ failure-related differential muscle loss. In addition, metabolic acidemia was associated with wasting, which is in keeping with a possible causal role.²⁶ Low hemoglobin concentrations, often a biomarker of chronic disease,²⁷ were also associated with muscle wasting. Eight patients re-

ceived neuromuscular blockade for longer than 48 hours. These small numbers and the confounders of accompanying disease state and severity preclude the dissection of the effect of paralysis per se or its cumulative effect with corticosteroids on muscle wasting.

Clinical Implications

Rapid muscle wasting occurs early in critical illness and is more pronounced in those patients with multiorgan failure. Early interventions to enhance anabolism may be required in addition to those aimed at reducing catabolism if muscle wasting is to be limited or prevented. These data expand on those of Constantin et al,²⁸ whose case-controlled cross-sectional study of 10 critically ill patients suggested implementation (within 6-8 hours of ICU admission) of an increased expression of markers of catabolism together with expression of indices of a possibly adaptive program of anabolism. Protein synthesis remained refractory in the early stages of critical illness, and increasing protein delivery was associated with increased muscle wasting. This finding is in keeping with an adverse effect of early targeted feeding,²⁹ which is supported by the observation that a short period of continuous amino acid feeding reduces muscle protein synthesis.³⁰ In addition, early supplemental parenteral feeding does not affect length of mechanical ventilation, length of hospital stay, or mortality.³¹ In a recent study investigating initial trophic vs early full enteral feeding, feeding did not affect ventilator-free days or 60-day mortality,³² or strength or functional outcomes at 1 year.³³⁻³⁵ The timing and mode of nutritional support (continuous vs intermittent) needs further investigation.

Limitations

Even though our data relate to the largest cohort of critically ill patients to have undergone longitudinal deep phenotyping, the pragmatic nature of this study raises several methodological issues. The first day of ICU admission does not necessarily reflect the first day of critical illness. However, although we were unable to quantify physiological derangement prior to admission, the median time from hospital to ICU admission was only 24 hours. In addition, there were 22 trauma patients who were not exposed to antecedent decline. As a result of the a priori stipulation that the patients would be deemed likely to survive (and therefore face long-term debility), we may have missed those patients who lose muscle far more rapidly as a result of fulminant illness.

The study's sample size precludes meaningful exploration of the association of wasting with specific disease entities. However, homogeneity of muscle loss with stratification by organ failure suggests that the specific disease state may not be the most significant driver of muscle loss during the first week. Although these data may be relevant to all patients during the acute stages of critical illness, expanded disease-specific studies are needed.

C-reactive protein is a nonspecific marker of inflammation, which responds relatively slowly to inflammatory stimuli and has a half-life approaching 19 hours. Its kinetics can be influenced by liver function, given that it is hepatically synthesized. Although frequently used in clinical practice, measure-

ment of C-reactive protein once per day has limited capacity to define the nature, cause, and scale of global and sustained inflammatory load.

Although data were consistent across measurement techniques, variation in muscle loss between methods may relate to differences in technique or the muscles studied. Specifically, rectus femoris was assessed by ultrasound and vastus lateralis muscle biopsy specimens were used to measure fiber cross-sectional area and ratio of protein to DNA. Ratio of protein to DNA measured by spectrophotometry is not affected by water content unlike ultrasound measurement and histology.³⁶ Muscle edema may have contributed to an underestimation of loss of ultrasound-derived rectus femoris cross-sectional area.

It is difficult to compare our data with those of previous studies because few studies were longitudinal, and none had standardized time points for measurements. Those comparable studies were performed more than 2 decades ago in a vastly different clinical arena.^{12,37}

Conclusion

Among these critically ill patients, muscle wasting occurred early and rapidly during the first week of critical illness and was more severe among people with multiorgan failure compared with single organ failure. These findings may provide insights into skeletal muscle wasting in critical illness.

ARTICLE INFORMATION

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