Impaired autophagy, chaperone expression, and protein synthesis in response to critical illness interventions in porcine skeletal muscle

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Banduseela VC, Chen YW, Kultima HG, Norman HS, Aare S, Radell P, Eriksson LI, Hoffman EP, Larsson L. Impaired autophagy, chaperone expression, and protein synthesis in response to critical illness interventions in porcine skeletal muscle. Physiol Genomics 45: 477–486, 2013. First published April 9, 2013; doi:10.1152/physiolgenomics.00141.2012.—Critical illness myopathy (CIM) is characterized by a preferential loss of the motor protein myosin, muscle wasting, and impaired muscle function in critically ill intensive care unit (ICU) patients. CIM is associated with severe morbidity and mortality and has a significant negative socioeconomic effect. Neuromuscular blocking agents, corticosteroids, sepsis, mechanical ventilation, and immobilization have been implicated as important risk factors, but the causal relationship between CIM and the risk factors has not been established. A porcine ICU model has been used to determine the immediate molecular and cellular cascades that may contribute to the pathogenesis prior to myosin loss and extensive muscle wasting. Expression profiles have been compared between pigs exposed to the ICU interventions, i.e., mechanically ventilated, sedated, and immobilized for 5 days, with pigs exposed to critical illness interventions, i.e., neuromuscular blocking agents, corticosteroids, and induced sepsis in addition to the ICU interventions for 5 days. Impaired autophagy as well as impaired chaperone expression and protein synthesis were observed in the skeletal muscle in response to critical illness interventions. A novel finding in this study is impaired core autophagy machinery in response to critical illness interventions, which when in concert with downregulated chaperone expression and protein synthesis may collectively affect the proteostasis in skeletal muscle and may exacerbate the disease progression in CIM.

Skeletal muscle is continuously remodeled as an adaptive mechanism to stress. Muscle proteins including the sarcomeric components are typically in a dynamic state of synthesis, degradation, assembly, and maintenance (39). Therefore, the protein homeostasis (proteostasis) or protein quality control is a critical and essential requirement for proper function of skeletal muscle (12, 39). Chaperones play a key role in proteostasis by either refolding nonnative proteins or targeting them for degradation (4, 18). The ubiquitin proteasome system (UPS), as well as various autophagic pathways, is directly involved in the degradation arm of proteostasis (37, 89). Skeletal muscle specific disruption of autophagy-lysosome system results in profound atrophy, myofibrillar disorganization, and age-dependent drop in force (54).

Using a porcine ICU model we have previously shown that 5 days of immobilization and mechanical ventilation result in upregulation of the UPS and oxidative stress-responsive genes, as well as upregulation of heat shock proteins that may play a protective role in response to immobilization and mechanical ventilation in the ICU (6). In the present study the expression profile of porcine skeletal muscles exposed to neuromuscular blocking agents, corticosteroids, and induced sepsis for 5 days [hereafter critical illness interventions (CII)] was compared with that of pigs that were immobilized and mechanically ventilated [hereafter ICU interventions (ICU-I)] for the same period. The aim of this study was to decipher the immediate cellular and molecular cascades by isolating the collective effect of neuromuscular blocking agents, corticosteroids, and induced sepsis on gene expression and protein expression, which may otherwise be masked by the background effect of

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immobilization and mechanical ventilation. The results indicate that the core autophagy machinery, as well as the chaperone expression, is severely impaired in the porcine skeletal muscle in response to CII.

MATERIALS AND METHODS

Animals and tissue collection. Eight female domestic pigs (Sus scrofa, average body wt 26.5 kg) were included in this study after approval from the ethical committee on animal research at the Karolinska Institute, Stockholm, Sweden (Dnr N71/98, NS4/02, and N75/04). All pigs originated from the same farm (Vallrums Lantbruk, Ransta, Sweden) and were kept in 12-square meter pens with hay, straw, and wood shavings as bedding material. They were housed at 18–19°C and relative humidity of 45–55% under natural day-night rhythm with liberal access to feed (Smågrissfoder Solo 331; Lantmännen, Stockholm, Sweden), water, and environmental enrichment. Food, but not water, was withheld for 12 h before induction of anesthesia. The pigs were sedated with medetomidine (1 mg/ml Domitor vet; Orion Pharma, Stockholm, Sweden) and Zolazepam (Zoletil 250; Reading, Carros, France) before an intravenous access was prepared, and 100 mg of ketamine (vet 50 mg/ml Ketaminol; Intervet, Boxmeer, Netherlands) was administered iv to induce anesthesia. Following tracheostomy, all animals were mechanically normoventilated by volume controlled ventilation (Servo 900A ventilator; Siemens-Elema, Solna, Sweden) by adjusting the FiO2 to 0.21–0.30 and an inspired tidal volume of 10 ml/kg, respiratory rate of ~20 breaths/min at an I:E ratio of 1:2, and inspiratory rise time of 5–10%. During the 5-day study period, the settings were adjusted to avoid high airway pressures and risk of barotraumas while maintaining arterial normoxia and normocarbia as guided by repetitive arterial blood gas analysis (ABL; Radiometer, Copenhagen, Denmark). Sedation was titrated to promote immobilization and allow ventilator synchrony by inhibiting spontaneous breathing activity. Anesthesia was maintained and adjusted through a novel anesthetic conserving device (AnaConda; Sedena Medical, Sundbyberg, Sweden), which permits administration of an inhaled anesthetic via a syringe pump to the inspired gas mixture, as previously described (70). During the 5-day study period isoflurane (Abbott Laboratories, Chicago, IL) was delivered at 0.8–1.3% end-tidal concentration and supplemented by intravenous bolus doses of morphine and ketamine as needed to provide adequate depth of anesthesia.

Core body temperature (blood) was maintained in the range of 38.5–40°C by a servo-controlled heating pad. Animals received 2,000–4,000 ml/day as a continuous intravenous infusion of a crystalloid solution (Ringer acetate; Fresenius Kabi, Stockholm, Sweden) at a rate of 2–4,000 ml/24 h, the infusion rate being adjusted to maintain stable blood pressure and urinary output throughout the experimental period. Food, but not water, was withheld for 12 h before induction of anesthesia. The pigs were sedated with medetomidine (1 mg/ml Domitor vet; Orion Pharma, Stockholm, Sweden) and Zolazepam (Zoletil 250; Reading, Carros, France) before an intravenous access was prepared, and 100 mg of ketamine (vet 50 mg/ml Ketaminol; Intervet, Boxmeer, Netherlands) was administered iv to induce anesthesia. Following tracheostomy, all animals were mechanically normoventilated by volume controlled ventilation (Servo 900A ventilator; Siemens-Elema, Solna, Sweden) by adjusting the FiO2 to 0.21–0.30 and an inspired tidal volume of 10 ml/kg, respiratory rate of ~20 breaths/min at an I:E ratio of 1:2, and inspiratory rise time of 5–10%. During the 5-day study period, the settings were adjusted to avoid high airway pressures and risk of barotraumas while maintaining arterial normoxia and normocarbia as guided by repetitive arterial blood gas analysis (ABL; Radiometer, Copenhagen, Denmark). Sedation was titrated to promote immobilization and allow ventilator synchrony by inhibiting spontaneous breathing activity. Anesthesia was maintained and adjusted through a novel anesthetic conserving device (AnaConda; Sedena Medical, Sundbyberg, Sweden), which permits administration of an inhaled anesthetic via a syringe pump to the inspired gas mixture, as previously described (70). During the 5-day study period isoflurane (Abbott Laboratories, Chicago, IL) was delivered at 0.8–1.3% end-tidal concentration and supplemented by intravenous bolus doses of morphine and ketamine as needed to provide adequate depth of anesthesia.

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and using ECL Advance Western blotting detection kit (RPN 2135, GE Healthcare) according to the manufacturer’s instructions. Autoradiograms (28-9068-36, GE Healthcare) were exposed to the protein blots until a measurable signal intensity was achieved. The autoradiograms were scanned (16 bit, grayscale) using a color image scanner (1,600 dpi, EPSON Expression 1600 Pro), and the intensity volumes were measured (ImageQuant TL, version 7, 2007, GE Healthcare). We used rolling ball (radius = 200 pixels) background subtraction method before measuring the intensity volume on each autoradiogram. All signal intensity volumes were normalized to respective actin signal intensity volumes.

Enzyme-histochemistry and fiber cross-sectional area measurements. Cryosections (serial, transverse, 10 μm thick) were stained for myofibrillar ATPase after acid (pH 4.35, 4.60) and alkaline (pH 10.30) preincubations. Fibers types (type I, IIA, and IIB) were classified according to pH sensitivity of myofibrillar ATPase. Fiber cross-sectional area (CSA) was measured on myofibrillar ATPase stained sections (pH 4.6) using a microscope (Zeiss Axiovert 35; Carl Zeiss, Oberkochen, Germany) combined with a computer assisted image analysis system (Videoplan; Kontron Bildanayze, Munich, Germany).

Statistics. Student t-test (unpaired) was used to determine statistical significance using Sigma Plot 9 (Systat Software). Values are means ± SE. Statistical outliers were removed by the extreme studentized deviate method. Statistical significance level was set at P < 0.05. The Mann-Whitney rank sum test was carried out when equal variances failed.

RESULTS

Downregulated transcripts and related proteins. A total of 54 probe sets were downregulated in the CII group compared with the ICU-I group. A summary of selected downregulated transcripts, functional groups, and fold change are presented in Fig. 1A. Downregulated transcripts and related proteins associated with each functional group are discussed below.

**Autophagy and cytoskeleton.** Dyneins and kinesin superfamilies (KIF) motor proteins are associated with microtubule-dependent plus-end and minus-end directed intracellular transport, respectively (30, 86). Cytoplasmic dynein light chain 1 (DCL1), a subunit of retrograde motor protein, is required for autophagy and clearance of aggregate prone proteins (7, 67). Microtubule-associated protein 1A (MAP-1A) is a microtubule-stabilizing protein that interacts with microtubule-associated protein light chain 3 (LC3) to modulate the shape of microtubules (56). LC3 plays a critical role in autophagosome membrane biogenesis where cytosolic LC3-I is converted to membrane-bound LC3-II lipidated form. The LC3-I to LC3-II conversion by phosphatidylethanolamine (PE) conjugation is a universal cellular marker for autophagosome formation (36) (See Fig. 2).

**ER chaperones, HSPs & Protein Metabolism**

<table>
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<tr>
<td>CANX</td>
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<tr>
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<tr>
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<td>USP34</td>
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**Oxidative stress responsive**

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**Sarcomeric myosins**

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**Protein metabolism**

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**Early growth response**

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**Skeletal muscle related**

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Fig. 1. Graphical representation of gene expression in each functional category. Downregulated (A) and upregulated (B) major functional categories are represented by expression values and fold change. Expression values reflect the variation from median gene expression. Reference key indicates positive expression in red and negative expression in blue. Maximum expression is set to ± 3.0.
gated the protein levels of LC3B-I and LC3B-II. The conversion of LC3B-I to LC3B-II by PE conjugation was impaired in the CII group (Fig. 1A), and the LC3B-II/LC3B-I conversion ratio was decreased in the CII group, indicating an impaired autophagosome formation in the skeletal muscle in response to CII (Fig. 3A). Importantly, only LC3B-II was decreased at the protein level, clearly demonstrating that LC3B-I protein expression was not affected in the CI group (Figs. 3A and 4A).

ATG7 (autophagy related 7) conjugates ATG5 to ATG12 and also is responsible for the key step of conjugating PE to LC3-I in converting to LC3-II. ATG5–ATG12 is a key protein complex involved in catalyzing LC3-I to LC3-II conversion. Therefore, ATG7 and ATG5 (ATG5–ATG12 complex) protein expression was investigated. ATG7 and ATG5 protein expression was reduced in the CII group, suggesting an impaired autophagy activation cascade (Figs. 3A and 4A). Adaptor protein p62 directly binds to ubiquitinated (Ub) proteins and acts as a receptor for Ub proteins. By binding to LC3-II, p62 facilitates autophagy by localizing in autophagic compartments, transporting Ub proteins and organelles for degradation. (Illustration was designed with pathway builder from http://www.proteinlounge.com and modified with Adobe Illustrator.)

Adaptor protein p62/SQSTM1 facilitates autophagic turnover by binding directly to LC3 and polyubiquitinated proteins. This interaction is necessary for the autophagic degradation of p62/SQSTM1 positive inclusion bodies (63). Thus, we tested for the p62/SQSTM1 protein expression, and p62/SQSTM1 was decreased at the protein level in the skeletal muscle of the CII group (Fig. 3A and 4A). Impaired core autophagy machinery (decreased LC3-I to LC3-II conversion, ATG7, ATG5, and p62/SQSTM1; see Fig. 2) in response to critical illnesses interventions is a novel finding that has not been documented before in either CIM patients or in ICU experimental animal models.

Endoplasmic reticulum chaperones, heat shock proteins, and protein metabolism. Several chaperones including endoplasmic reticulum (ER) chaperones and ER membrane proteins were downregulated at the RNA level as indicated by the expression profile (Fig. 1A). Among them, calnexin is a key ER chaperone that regulates the glycoprotein folding and quality control through calnexin cycle (52). Heat shock protein (HSP)47 plays a similar role as an ER chaperone that is indispensable for correct folding of collagen and in preventing aggregate formation of misfolded collagen in ER (34). CRYAB (α-B crystallin) is a small heat shock protein that not only plays a protective mechanism in preventing denaturation and aggregation of unfolded proteins but is also specifically localized in the sarcomere and interacts with titin and actin (71). CRYAB is a particularly important chaperone in skeletal muscle as it has been associated with several myopathies including desmin-related myopathy (71). HSPA4 is a member of HSP family 110, which is related to the HSP70 family and is thought to function in concert with other chaperones.

Chaperones are generally involved in preventing protein misfolding and aggregation of secretory proteins. Since the expression profile data suggest a severely affected and stressed ER, we first examined if BiP/GRP78 protein expression were affected and would follow the same trend of chaperones in the CII group. BiP/GRP78 is a member of HSP70 chaperone family, which regulates ER stress sensors and unfolded protein response (UPR) (10). Surprisingly BiP/GRP78 was not affected in the CII group at the protein level (Figs. 3B and 4B). We then examined the total EIF2A (T-EIF2A) and its phosphorylation status to determine if ER stress and UPR were present in the CII group. Phosphorylated EIF2A (P-EIF2A) is an adaptive response to ER stress and is one of the three major pathways of UPR (77). Increased phosphorylation of EIF2A was observed in the intervention group, determined by P-EIF2A:T-EIF2A (Figs. 3B and 4B). CRYAB (small HSP) and HSPA4 (HSP110 family) were also examined at the protein level and were decreased in the CII group, following the trend indicated by the expression profile (Figs. 1A, 3C, and 4C).
SOD2 was decreased in response to CII (Figs. 3A). We examined the protein expression of SOD2 by immunoblotting, and the expression profile in the intervention group (Fig. 1A). A summary of selected upregulated transcripts, functional groups, and fold change is presented in Fig. 1B. Upregulated transcripts and related proteins associated with each functional group are discussed below.

**Protein metabolism.** Ankyrin repeat SOCS box 11 (ASB11) belongs to the family of suppressor of cytokine signaling (SOCS) and mediates protein degradation through elongin C-cullin-SOCS box (ESC) type complexes, which act as E3 ligases (65). Eukaryotic initiation factor 4E binding protein 1 (EIF4EBP1) acts as a translational repressor by binding to EIF4E and inhibits protein synthesis. PDZRN3 (PDZ ring finger 3) function as an E3 ligase, regulating surface levels of muscle-specific receptor tyrosine kinase (51). TTP is an mRNA binding protein that binds to AU-rich elements in proinflammatory mediators and limits their expression via mRNA decay (74).

Of the genes related to protein metabolism, we examined protein level of the translational repressor EIF4EBP1, as it was upregulated in the expression profile (Fig. 1B). Hypophosphorylated EIF4EBP1 represses the translation of protein synthesis in skeletal muscle that is reversed by the hyperphosphorylation of EIF4EBP1 by activation of the IGF1/AKT/mTOR pathway (14). Total EIF4EBP1 (T-4EBP1) protein expression was increased in response to CII (Fig. 3E). The phosphorylation status of EIF4EBP1 was examined, since phosphorylated EIF4EBP1 (P-4EBP1) indicates an activated protein synthesis cascade. Phosphorylated-to-total protein ratio of the EIF4EBP1 (P-4EBP1/T-4EBP1) was decreased in the CII group, indicating a decreased kinase activity and thereby indicating an impaired protein synthesis in response to CII in skeletal muscle (Fig. 4E). Atrogin-1 (FBXO32) is a muscle-specific E3-ubiquitin ligase that is involved in muscle atrophy (13), and the protein expression of Atrogin-1 did not change in response to CII (Figs. 3E and 4E).

**Early growth response.** Early growth response 1 (EGR1) initiates a defense mechanism against oxidative stress in skeletal muscle by activating its downstream targets SIRT1 and SOD2 (64). FOS family of transcription factors heterodimerize with JUN proteins and constitute AP-1 (activating protein 1) transcription factor complex. The AP-1 complex is induced in response to oxidative stress (94). JUNB inhibits autophagy via a mTOR-independent pathway that requires AP-1 transcription and leads to cell death in response to starvation (90).

**Protein kinase activity.** Activin receptor 2B (ACVR2B) acts as a signaling receptor for myostatin, a potent negative regulator of muscle mass (46). The myosin light chain kinase family of proteins is emerging as a diverse group of regulatory proteins involved in protein turnover and autophagy (26). Tribbles 1 is involved in regulating MAPK signaling cascade (40). Increased expression of ACVR2B as demonstrated by the expression profile was confirmed at the protein level by immunoblotting (Figs. 3E and 4E).

**Skeletal muscle related.** p27kip1 (CDKN1B) is a cyclin-dependent kinase inhibitor that controls cell cycle progression. FOXO1 and FOXO3a induce p27kip1 transcription and decrease proliferation of myoblast precursor cells (47). Mitochondrial creatine kinase (CKMT2) is closely associated with energy metabolism, and previous studies in ICU patients as well as in ICU experimental animal models (44, 59, 60, 62). The myosin-actin ratios at the protein level remained unchanged despite the downregulation at RNA level during the study period (61).

**Oxidative stress responsive.** An array of oxidative stress-responsive genes was downregulated at RNA level as indicated by expression profile in the intervention group (Fig. 1A). We examined the protein expression of SOD2 by immunoblotting, and the SOD2 was decreased in response to CII (Figs. 3D and 4D).

**Sarcomeric myosins.** MYH7 and MYL2 code for the B/slow (type 1) myosin heavy chain isoform and the regulatory myosin light chain, respectively (75). Loss of myosin in CIM is well documented, and the present results of a downregulation of myosin at the transcriptional level are in agreement with

![Fig. 3. Immunoblotting of autophagy-related genes (LC3B-I, LC3B-II, ATG7, ATG5, and p62/SQSTM1) (A), ER stress and UPR proteins (BiP/GRP78, P-EIF2A, and T-EIF2A) (B), chaperones and CMA receptor (CRYAB, HSPA4, HSP70, and LAMP-2A) (C), oxidative stress responsive (SOD-2) (D), and protein metabolism (P-4EBP-1, T-4EBP-1, ACV2RB, and atrogin-1) (E).](https://physiolgenomics.physiology.org/content/101/Supplement_1/481/F3.large.jpg)
CKMT upregulation has been reported in response to oxidative stress, as a compensatory mechanism against low energy state (76). Four and a half LIM domains 3 is a protein with LIM motif, which forms a complex with MYOD in vitro, suppressing MYOD-mediated transcription and myotube formation (21). Myeloid leukemia factor 1 (MLF1) is highly expressed in skeletal muscle. Overexpression of MLF1 in skeletal muscle results in nonpathogenic aggregates (49). Myomesin 2 is a key protein involved in maintenance of sarcomeric integrity and stability.

Fiber CSA. Fiber CSA of fiber types, type I, IIA, and IIB did not change in porcine skeletal muscle in response to CII during the study period (Fig. 5). The mean CSAs for the ICU-I group were 810 ± 60 (type I), 920 ± 20 (IIA), and 1,320 ± 140 (IIB) μm², and the mean CSAs for the CII group were 780 ± 60 (type I), 810 ± 60 (IIA), and 1,370 ± 110 (IIB) μm². Despite the significant changes at RNA and protein levels, fiber CSA was maintained in the early phase in response to CII.

**DISCUSSION**

Dysregulated autophagy and chaperone expression have been reported in many pathobiological conditions including different myopathies and neurodegenerative diseases (18, 72, 73). However, the relevance and impact of these processes on immediate molecular and cellular events in critical illness-associated muscle weakness have not been elucidated. In our previous study we used a porcine model to analyze the temporal effect of 5 days of immobilization and mechanical ventilation on limb...
maintains cellular and protein homeostasis by removing non-
increased membranous structures were reported in postmortem
in this study. Recently, decreased autophagic vacuoles and
does not fit with the decreased p62 protein expression observed
uitin and p62-positive inclusions (54, 66), an observation that
suitable for autophagic degradation (63). p62/SQSTM1 knock-
down results in impaired conversion of LC3-I to LC3-II in a
mice show impaired autophagy, muscle loss, protein aggre-
accumulation of misfolded protein (35). ROS and oxidative stress
ifies protein structures or fragment proteins, which leads to an
expression profile indicates that CMA is impaired in the
mice (54). The skeletal muscle-specific ATG5-deficient mice show impaired autophagy, muscle loss, protein aggregates,
and accumulation of abnormal membranous structures (66).
p62/SQSTM1 is an LC3 interacting protein that is essen-
tial for autophagic degradation (63). p62/SQSTM1 knock-
down results in impaired conversion of LC3-I to LC3-II in a
model of cardiac proteinopathy (93). Recently it was shown
that LC3-II preferentially binds to p62 and efficiently localize
to autophagic compartments (50). However, skeletal muscle-
specific ATG7 and ATG5 knockout mouse models show ubiqui-
utin and p62-positive inclusions (54, 66), an observation that
does not fit with the decreased p62 protein expression observed
in this study. Recently, decreased autophagic vacuoles and
increased membranous structures were reported in postmortem
skeletal muscle biopsies of critically ill patients, suggesting
insufficient activation of autophagy (87). Autophagy actively
maintains cellular and protein homeostasis by removing non-
native proteins as well as damaged cellular components includ-
ing mitochondria (mitophagy) and ER (reticulophagy) (38, 42).
Therefore autophagy inhibition in skeletal muscle could lead to
dysfunctional mitochondria and ER, which in turn could lead to
a new cycle of exacerbated ER stress and oxidative stress,
eventually triggering apoptosis.

Expression profile analyzed by microarray indicates that sever-
al chaperones including ER chaperones were downregu-
lated at RNA level in the CII group. We hypothesized that the
ER stress sensor BiP/GRP78 would be increased in the CII
group, indicating ER stress in these muscles, but there was no
change in BiP/GRP78 protein expression. However, the phos-
phorylated EIF2A/total EIF2A ratio was increased, suggesting
that UPR is initiated in the CII group. In stressed conditions,
BiP/GRP78 dissociates from transmembrane protein PKR-like
endoplasmic reticulum kinase (PERK), which results in auto-
phagy and subsequent EIF2A hyperphosphorylation. Phosphorylated EIF2A results in attenuation of protein synthe-
sis. During this translational attenuation, only select mRNA
such as ATF4 are translated. Prolonged ER stress causes ATF4
to induce apoptosis via C/EBP homologous protein. Moreover,
skeletal muscle-specific attenuation of ATG7 expression re-
ults in a profound increase in BiP/GRP78, as well as phos-
phorylated EIF2A at the protein level (54).

We demonstrate that CRYAB (small HSP family), as well as
HSP70 and HSPA4 (HSP110 family), is indeed decreased at
the protein level. CRYAB is associated with preventing aggre-
gate formation by binding to unfolded proteins. A missense
mutation in CRYAB (CRYABR120G) causes desmin-related my-
opathy with intracellular aggregates and muscle weakness (88).
HSP70 is a key chaperone family involved in recognition of
nonnative proteins in collaboration with HSP40 cochaperones and
subsequent refolding or even disaggregation by cooperating with
HSP100 (16). Previously, we and others have shown that upregu-
lation of chaperones is associated with a protective role in skeletal
muscle in response to immobilization (6, 78).

Moreover, the decrease in LAMP-2A protein expression in
the CII group strongly suggests that CMA is impaired in the
CII group. LAMP-2-deficient mice develop a progressive my-
opathy of skeletal and cardiac muscles (57). Force generation
and Z disk integrity is compromised due to Z disk disintegra-
tion and the rupture of sarcomeric structures in these mice (3).

Oxidative damage by reactive oxygen species (ROS) modi-
ifies protein structures or fragment proteins, which leads to an
accumulation of misfolded protein (35). ROS and oxidative
damage have been implicated in a range of disorders. Oxida-
tive stress-responsive genes act in defense against oxidative
stress and maintain redox balance. Conversely, deficiency in
oxidative stress response mechanisms exacerbates the oxida-
tive damage (15). In our previous study, using immobilized and
mechanically ventilated pigs, we have shown that oxidative
stress-responsive genes are upregulated in the expression pro-
file (6). In this study, in response to CII, oxidative stress-
responsive genes were downregulated, suggesting that protec-
tion from ROS is compromised in these pigs. In fact, skeletal
muscle-specific ATG7-null mice show increased oxidative
stress and upregulation of oxidative stress-responsive genes
(54). Moreover, p62 is shown to stabilize nuclear factor ery-
throid 2-related factor 2 (NRF2) and activate NRF2 target
genes including oxidative stress-responsive genes (41). We
have observed decreased protein expression of p62 with par-
impairment of oxidative stress-responsive genes that associate impaired oxidative response in response to CII with p62/NRF2 pathway in skeletal muscle.

The increased expression of the translational repressor EIF4EBP1 at the RNA and protein levels and the decrease in phosphorylated EIF4EBP1 indicate that protein synthesis is attenuated, inhibiting the IGF/AKT/mTOR signaling axis (14, 68). Together with EIF2A-mediated attenuation of protein synthesis via BiP/PERK axis, the CII exerts a drastic negative impact on skeletal muscle protein synthesis. IGF/AKT/mTOR pathway is inhibited by glucocorticoid (dexamethasone), leading to attenuation of protein synthesis and activation of FOXO transcription factors and muscle-specific ubiquitin ligases (82). Increased protein expression of ACVR2B suggests increased myostatin signaling, which could further downregulate protein synthesis in the CII group. However, the protein expression of muscle-specific E3 ubiquitin ligase atrogin-1 did not change in response to CII. Unchanged atrogin-1 expression, together with impaired autophagy, indicates that protein degradation pathways do not positively contribute to proteolysis in the early phase in ICU. On the other hand, proteasome inhibition results in muscle atrophy, loss of sarcomeric architecture, vacuolization, and aggregate formation, suggesting that basal level of UPS activity is required for maintaining skeletal muscle cellular architecture (27).

Despite the profound molecular and cellular changes in response to CII, the fiber CSA was maintained in the CII group, indicating that muscle fiber atrophy does not occur in the early phase of ICU stay, which is in accordance with our previous observations in the porcine ICU model (1, 61). This observation is not surprising and could be partly explained by the impaired autophagy and unchanged E3 ligase, atrogin-1 protein expression. Similar observations have been reported in patients with CIM, indicating that onset of the disease and atrophy of the muscle occur at a later phase of ICU stay, ranging from at least 4 days to 1 wk after the admission to ICU (2, 84). Myosin-actin ratios were maintained in these muscles during the 5-day study period despite the downregulation of myosin type I at RNA level, suggesting that major contractile proteins are not affected at this early phase of exposure to CII, and atrophy is an unlikely event at this early time point.

ER plays a key role in autophagosome formation by providing a platform for expansion of autophagosomal membranes and emergence of fully formed autophagosomes (5). ER stress and UPR trigger autophagy, which suggest a close interplay between the two systems and may play a regulatory role in protein quality control and proteostasis (9, 91).

The proteostasis network is a complex system that integrates the diverse chaperone systems with UPS and autophagy lysosome system (28). Since the expression profile and the protein expression results suggest a general and ER-specific breakdown of chaperone RNA expression and protein expression, taken together with profound impairment in autophagy lysosome system, we propose that proteostasis may be the key process affected in skeletal muscles during critically ill.

Conclusion

Skeletal muscle undergoes continuous remodeling as an adaptive response to physiological, mechanical and metabolic stresses. Proteostasis, or protein quality control, plays a crucial physiological role in the maintenance of skeletal muscle and sarcomere, in response to various stresses. Chaperones are not only involved in folding and refolding of misfolded or damaged proteins, but also in targeting the terminally misfolded or irreversibly damaged proteins for degradation. On the other hand, autophagy plays a major role in cellular homeostasis as well proteostasis in skeletal muscle. Thus, taking that knowledge together with the novel findings in this study, we conclude that the CII impairs autophagy, chaperone expression, and protein synthesis and may adversely affect overall proteostasis in skeletal muscle, leading to long-term catastrophic cellular and molecular events.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


