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RESEARCH REPORT

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Dynamics of Mitochondrial Structure, Biogenesis and Function in Skeletal Muscle of Critically Ill Patients

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OBJECTIVES: To define the timing and phenotype of mitochondrial ultrastructural remodeling and electron transport chain (ETC) function in skeletal muscle during early critical illness.

DESIGN: Secondary analysis of a prospective observational study with serial muscle biopsies.

SETTING: Adult ICU.

PATIENTS: Thirty-three mechanically ventilated ICU patients (median age, 49 yr; 27% women) with Sequential Organ Failure Assessment greater than or equal to 9 on three consecutive days; skeletal muscle biopsies of the *vastus lateralis* muscle at ICU day 5 ($n = 29$) and day 15 ($n = 22$). Healthy subjects served as controls.

INTERVENTIONS: None.

MEASUREMENTS AND MAIN RESULTS: *Vastus lateralis* biopsies were analyzed by electron microscopy with stereology, quantitative real-time PCR (qRT-PCR), Western blotting, and spectrophotometric ETC enzyme assays (reported per tissue weight and normalized to citrate synthase [CS]). Intact mitochondrial profiles were reduced vs. controls (fold change vs. controls: day 5, 0.24 [0.18–0.34]; $p = 0.039$ and day 15, 0.20 [0.16–0.33]; $p = 0.011$) and disturbed profiles increased (day 5, 5.62 [3.20–7.17]; $p < 0.001$ and day 15, 5.20 [3.67–6.36]; $p = 0.001$), with reduced volume density of intact mitochondria (day 5, 0.33 [0.20–0.49]; $p = 0.009$ and day 15, 0.32 [0.21–0.50]; $p = 0.005$). Inner mitochondrial membrane surface-to-volume ratio was reduced at day 5 (0.69 [0.49–0.89]; $p = 0.022$) and remained unchanged at day 15. Complex III tissue activity was reduced at day 5 (85.6 [22.1–133.3] vs. 151.4 [100.5–205.0]; $p = 0.015$) and day 15 (50.5 [36.0–69.8]; $p = 0.001$), remaining reduced after CS normalization (day 5, 0.83 [0.34–1.49]; $p = 0.040$ and day 15, 0.87 [0.65–0.93]; $p = 0.007$). Complex IV activity remained reduced before and after CS normalization at both time points (all $p < 0.001$). CS activity declined by day 15 (56.3 [52.7–65.1] vs. 95.6 [80.1–133.0]; $p = 0.001$ and day 15 vs. day 5; $p = 0.041$). qRT-PCR and Western blot supported reduced ETC components and altered biogenesis signaling.

CONCLUSIONS: Skeletal muscle mitochondrial ultrastructure is altered by ICU day 5 and remains abnormal at day 15, with persistent complex III/IV dysfunction after CS normalization. These findings define an early sustained mitochondrial remodeling phenotype in skeletal muscle during critical illness.

KEYWORDS: critical illness; electron transport chain complex proteins; mitochondria; muscle; oxidative phosphorylation; skeletal

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KEY POINTS

Question: When do mitochondrial structural and biochemical abnormalities develop in skeletal muscle during critical illness, and do they recover during prolonged ICU stay?

Findings: In this prospective observational cohort study of mechanically ventilated ICU patients, serial muscle biopsies showed significant mitochondrial ultrastructural abnormalities by ICU day 5 that persisted to day 15. This was accompanied by significantly reduced electron transport chain complex III and IV activity despite increased expression of mitochondrial biogenesis-associated regulators.

Meaning: Critical illness is associated with early and sustained mitochondrial remodeling in skeletal muscle, suggesting that impaired mitochondrial recovery may contribute to persistent physical dysfunction in ICU survivors.

Survival rates among ICU patients have improved significantly over the past decades (1). However, this progress has been paralleled by an increase in long-term impairments, with more patients requiring postacute care (1, 2). Five years after discharge, ICU survivors exhibit impaired physical function and reduced physical health-related quality of life (3, 4). This highlights a challenge in intensive care medicine, as patients consistently rate functional recovery as more important than survival itself (5, 6). Nevertheless, effective mechanism-based therapies to prevent or reverse long-term physical impairment remain unavailable (7, 8).

ICU patients lose ~2% of muscle mass per day during the first ICU week, and most mechanistic work has focused on muscle atrophy and strength loss (9–11). However, muscle mass and strength may recover while physical function, particularly endurance (6-min walk distance, VO_2max), remains impaired long after discharge (3, 9, 10, 12, 13).

Mitochondrial homeostasis in skeletal muscle depends on the coordinated regulation of mitochondrial biogenesis, protein import and assembly, mitochondrial dynamics, and removal of damaged organelles. Mitochondrial biogenesis is regulated through signaling networks centered on peroxisome proliferator-activated receptor (PPAR) gamma coactivator 1-alpha (PGC-1α), which integrates energetic and stress-related signals including AMP-activated protein kinase, calcium-dependent signaling, and redox-sensitive pathways. Activated PGC-1α coactivates transcription factors including nuclear respiratory factor 1 (NRF1) and PPARs, thereby promoting expression of mitochondrial transcription factor A (TFAM), a key regulator of mitochondrial DNA replication and transcription (14). Coordinated expression, import, and assembly of nuclear- and mitochondrial-encoded oxidative phosphorylation proteins are required to maintain electron transport chain (ETC) organization and adenosine triphosphate (ATP) production. In parallel, mitochondrial quality and functional integrity are maintained through continuous fusion and fission events that regulate mitochondrial network remodeling and segregation of dysfunctional mitochondrial components (15).

Mitochondrial oxidative phosphorylation constrains endurance capacity and is linked to outcomes in sepsis (16, 17). Mitochondrial impairment in the *vastus lateralis*, measured through high-resolution respirometry, has been observed in patients with ICU-acquired weakness (ICUAW) compared with healthy controls (18). However, the timing, nature, and clinical relevance of these mitochondrial alterations remain incompletely defined. A key unresolved question is whether mitochondrial abnormalities are already present early during critical illness and whether they show evidence of recovery during a prolonged ICU stay.

The objective was to characterize mitochondrial ultrastructure, ETC function in *vastus lateralis* muscle during early critical illness (ICU day ~5) and to determine whether these abnormalities persist into the second ICU week (day ~15) using serial biopsy specimens. We prespecified that inference about timing would emphasize within-patient comparison (day 5 vs. day 15) and that healthy controls would serve as reference baselines rather than "ideal" critical illness controls.

MATERIALS AND METHODS

Patients and Ethics Approval

This secondary analysis is from a prospective observational study (19, 20) approved by the Charité Ethics Committee (Title: "Skeletal Muscle Metabolism and Critical Illness Myopathy in the Early Phase of Systemic Inflammation"; EA2/061/06; ISRCTN77569430; approval date June 12, 2006) and conducted in accordance

with the Declaration of Helsinki (1964) and its subsequent amendments. Eligible patients were mechanically ventilated and had a Sequential Organ Failure Assessment (SOFA) score of greater than or equal to 9 on 3 consecutive days within the first 5 ICU days. Written informed consent was obtained from patients or their legal representatives. Exclusion criteria were age younger than 18 years, ICU treatment greater than 7 days before enrollment, body mass index greater than 35 kg/m^2, preexisting neuromuscular disease, insulin-dependent diabetes mellitus, or moribund status. Control muscle specimens were obtained from participants of a previous clinical study without muscle pathology (Title: "Normobaric hypoxia training compared to ambient training on the course of the metabolic syndrome: influence on muscle function and structure," EA1/140/12; NCT 01468220, approval date June 22, 2012 (20, 21)). All patients were treated according to Standard Operating Procedures.

All animal experiments were approved by the local authority (LAGeSo, G207/13) and conducted in accordance with National Institutes of Health (NIH) guidelines (NIH Publication No. 85-23, revised 1985) and German animal welfare legislation.

Skeletal Muscle Biopsy, ETC Enzyme Activity Measurements, and Electron Microscopy

Open surgical muscle biopsies from the *vastus lateralis* were performed on ICU day 5 (median, 5 [interquartile range [IQR], 4–7]) and ICU day 15 (median, 15 [IQR, 14–19]) on critically ill patients, respectively, or as close as clinically feasible. Biopsy specimens were analyzed using quantitative real-time PCR (qRT-PCR), Western blotting, ETC enzyme activity assays, and transmission electron microscopy (TEM) with stereology (22). Investigators were blinded to patient identity and sampling time point.

ETC enzyme activities (complexes I–IV, complex I + III and II + III activities, and citrate synthase [CS] activity) were quantified spectrophotometrically using substrate- and inhibitor-specific assays as previously described (23). Enzyme activities were expressed both per tissue amount ("tissue enzyme activity") and normalized to CS activity ("mitochondrial enzyme activity") to account for mitochondrial content.

For TEM, muscle specimens were fixed in 2.5% glutaraldehyde in cacodylate buffer, post-fixed with osmium tetroxide, embedded in Epon resin, and analyzed using a Zeiss Leo 906 electron microscope (Zeiss, Oberkochen, Germany; at 80kV acceleration voltage), equipped with a slow-scan 2048 × 2048 pixel charge-coupled device camera (TRÖNDLE Restlichtverstärkersysteme, Moorenweis, Germany) and the software ImageSP version 1.2.16.19 (TRÖNDLE Restlichtverstärkersysteme). Quantitative stereologic analysis was performed using systematic uniform random sampling at ×27,800 magnification and Stepanizer software (Stepanizer2b28, STEPanizer BETA 2, Bern, Switzerland) (24). Mitochondria were classified as intact (preserved membrane integrity and cristae structure without swelling) or disturbed (swelling, membrane disruption, or loss of cristae organization) using established ultrastructural criteria (22, 25). Volume density and inner mitochondrial membrane surface-to-volume ratio were quantified.

Additional methodological details are provided in the **Supplementary Information** (https://links.lww.com/CCM/I102).

RNA-seq Analysis of Mouse Tibialis Anterior Muscle

We reused our published *tibialis anterior* (TA) RNA sequencing dataset from male C57BL/6J mice subjected to sham surgery or cecal ligation and puncture (CLP), harvested at 24 and 96 hours (n = 6/group/timepoint) (26–28). These data were included to examine early mitochondrial transcriptional remodeling during a biologic window generally inaccessible through serial ICU muscle biopsies, rather than as a direct human comparator. We report published differential-expression statistics and mitochondrial and ETC-focused visualizations. Full sequencing and alignment methods are provided in the Supplementary Information (https://links.lww.com/CCM/I102).

Statistical Tests

Descriptive data are shown as median and interquartile range. Stereological data were normalized to healthy controls and displayed as fold change. qRT-PCR and Western Blot data were normalized to glyceraldehyde 3-phosphate dehydrogenase, with the mean control value set to 1. Spectrophotometric enzyme activities are shown as tissue enzyme activity and normalized to CS activity (mitochondrial enzyme activity). Differences between related samples (ICU patients on days 5 and 15) were evaluated using the Wilcoxon Signed Rank test, and differences

between unrelated samples (healthy controls and ICU patients) were evaluated using the Mann-Whitney *U* test. Statistical significance was set at $p \leq 0.05$. Analyses were performed in SPSS (IBM SPSS Statistics [Version 30.0], IBM Corp., Armonk, NY; https://www.ibm.com/products/spss-statistic); pathway enrichment was performed using the Database for Annotation, Visualization, and Integrated Discovery tool.

RESULTS

Patients

During the 29-month study period, 5.960 ICU patients were screened for eligibility, of whom 33 patients (27% women; median age, 49 yr) fulfilling inclusion criteria were enrolled in the observational study (**Fig. 1**). Baseline characteristics are shown in **Table 1**; control characteristics are provided in **Table e1** (https://links.lww.com/CCM/I102). Twenty-nine patients underwent a muscle biopsy on ICU day 5. Twenty-two of these patients also underwent a repeat biopsy on ICU day 15. However, seven patients could not undergo a follow-up biopsy due to being discharged from the ICU or having died before the second sampling time point. No adverse events related to the biopsy were observed.

Mitochondrial Structure, Volume Density, and Inner Mitochondrial Membrane Length

TEM revealed swollen and destroyed mitochondria in *vastus lateralis* muscle of critically ill patients. Representative images of intact and disturbed mitochondria are shown in **Figure e1*A*** (https://links.lww.com/CCM/I102). There was a concomitant increase in profiles of disturbed mitochondria at both time points. The profiles of intact mitochondria did not change between day 5 and 15 in ICU (**Fig. e1**, ***B*** and ***C***, https://links.lww.com/CCM/I102). The volume density of intact mitochondria was significantly lower on day 5 and day 15, whereas the volume density of disturbed mitochondria was significantly higher (**Fig. e1**, ***D*** and ***E***, https://links.lww.com/CCM/I102). No difference in the density of intact mitochondria was observed between day 5 and 15 in ICU. The surface-to-volume ratio of the inner mitochondrial membrane, where the ETC is located, was significantly decreased within intact mitochondria of critically ill patients on day 5, with no significant difference on day 15 compared with controls. No change between day 5 and 15 was observed (**Fig. e1*F***, https://links.lww.com/CCM/I102). Sensitivity analyses by SOFA strata, sepsis status, and paired-biopsy subset showed no differences across TEM endpoints (**Tables e2–e4**, https://links.lww.com/CCM/I102).

Mitochondrial Transcription Factors and Genes are Dysregulated in Muscle of ICU Patients

We observed an increased expression of *PPARGC1B* in muscle of ICU patients, while the expression of *PPARG* remained unchanged (**Fig. e2**, ***A–C***, https://links.lww.com/CCM/I102). The expression of *NRF1* and *TFAM* was increased in muscle of ICU patients (**Fig. e2**, ***D*** and ***E***, https://links.lww.com/CCM/I102). In contrast, the expression of the ETC genes, succinate dehydrogenase subunit B (*SDHB*) (CII) and *MTCO1* (CIV), was decreased in muscle of ICU patients on day 5 compared with healthy controls (**Fig. e2**, ***G–J***, https://links.lww.com/CCM/I102). The expression levels of *NDUFB8*

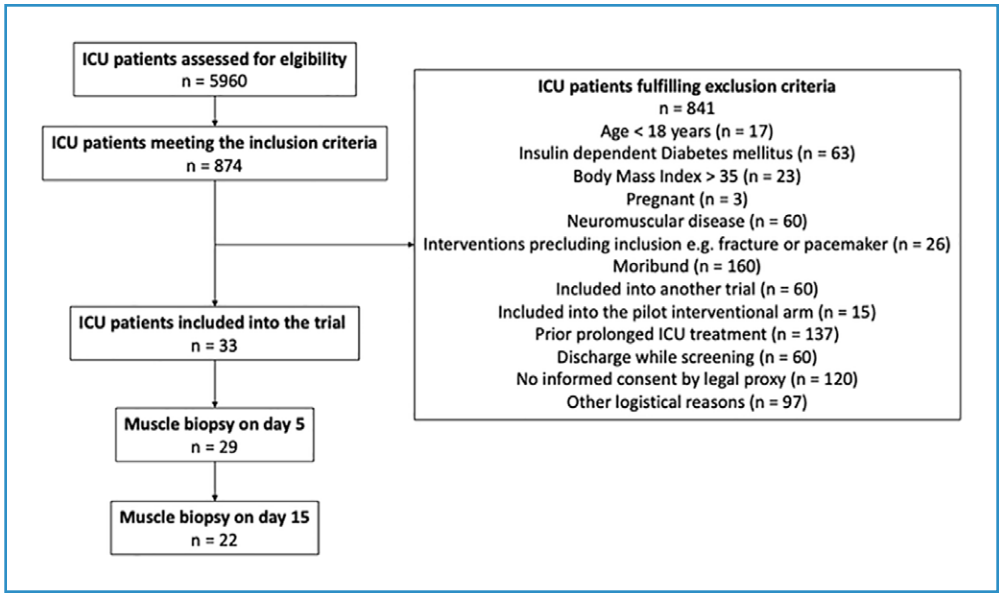


Figure 1. Trial inclusion flow chart.

TABLE 1.
Baseline Characteristics

Characteristics	ICU Patients ($n = 33$)
Age, yr	49 (41–67)
Female	9 (27.3)
Body mass index, kg/m^2	26.9 (23.2–30.3)
ICU length of stay, d	26.0 (20.0–41.0)
Disease severity at ICU admission	
Sequential Organ Failure Assessment score	12 (10–14)
Acute Physiology and Chronic Health Evaluation II score	18 (15–23)
Simplified Acute Physiology Score II	43 (36–53)
Time of first adequate awakening, d	11.0 (8.0–16.5)
Nonsurvivors	8 (24.2)
ICU admission diagnosis	
Acute Respiratory Distress Syndrome	13 (39.4)
Sepsis	8 (24.2)
Trauma	6 (18.2)
CNS	6 (18.2)
Preexisting comorbidities	
Arterial hypertension	10 (30.3)
Heart valve disease	6 (18.2)
Atrial fibrillation	6 (18.2)
Coronary artery disease	1 (3.0)
Chronic heart failure	3 (9.1)
Chronic obstructive lung disease	3 (9.1)
ICU-acquired complications	
Pressure ulcer	14 (42.2)
Acute renal failure	17 (51.5)
Anemia	30 (90.9)
Survived resuscitation	4 (12.1)
Muscle biopsy	
ICU day 5	29 (87.9)
ICU day 15	22 (66.7)

Metric variables are shown as median (interquartile range), and categorical variables as count, *n* (%).

(CI), ubiquinol-cytochrome c reductase core protein 2 (*UQCRC2*) (CIII), *CYTB* (CIII), *COX4* (CIV), and *ATP5A1* (CV) remained unchanged (**Fig. e2*F***, e2*H*, e2*I*, **e2*K***, **e2*L***, https://links.lww.com/CCM/I102).

ETC Protein Contents are Reduced in Skeletal Muscle of ICU Patients

Representative Western blots are shown in **Figure e3*A*** (https://links.lww.com/CCM/I102). The protein contents of SDHB (CII) and ATP5A1 (CV) remained unchanged in ICU patients compared with controls. By contrast, UQCRC2 (CIII) and cytochrome c oxidase subunit II (CIV) were significantly lower in muscle of critically ill patients on day 5 in the ICU. No further changes in ETC protein amounts were observed between day 5 and 15 in the ICU (**Fig. e3**, ***B–E***, https://links.lww.com/CCM/I102).

ETC Activity is Decreased in Skeletal Muscle of ICU Patients

We quantified CS activity as an estimate of mitochondrial content. CS activity was not changed on day 5 compared with controls. However, we found a decrease in CS activity on day 15 compared with controls and also a decrease between day 5 and 15 in ICU patients (**Fig. e4*A***, https://links.lww.com/CCM/I102).

Tissue activities of CI were unchanged on day 5 but significantly reduced on day 15 in ICU compared with controls. We also found a decrease between day 5 and 15 in ICU patients (**Fig. e4*B***, https://links.lww.com/CCM/I102). CII activity remained unchanged on day 5 in ICU with a significant reduction on day 15 compared with controls. No difference in CII activity was observed between day 5 and day 15 (**Fig. e4*C***, https://links.lww.com/CCM/I102).

In contrast, tissue CIII and CIV activities were reduced in muscle of ICU patients on day 5 and 15 compared with controls without differences between both timepoints (**Fig. e4**, ***D*** and ***E***, https://links.lww.com/CCM/I102). Coenzyme Q shuttles electrons from CI and CII to CIII. To detect a possible coenzyme Q deficiency, we performed the combined CI + CIII and CII + CIII assay. CI + CIII did not reveal any differences between patients at both time points and controls (**Fig. e4*F***, https://links.lww.com/CCM/I102). CII + CIII showed a difference only between patients and controls on day 15, without any difference within patients at the two timepoints (**Fig. e4*G***, https://links.lww.com/CCM/I102).

Mitochondrial CI activity was unchanged on day 5, but was significantly reduced on day 15 compared with controls, without a difference between

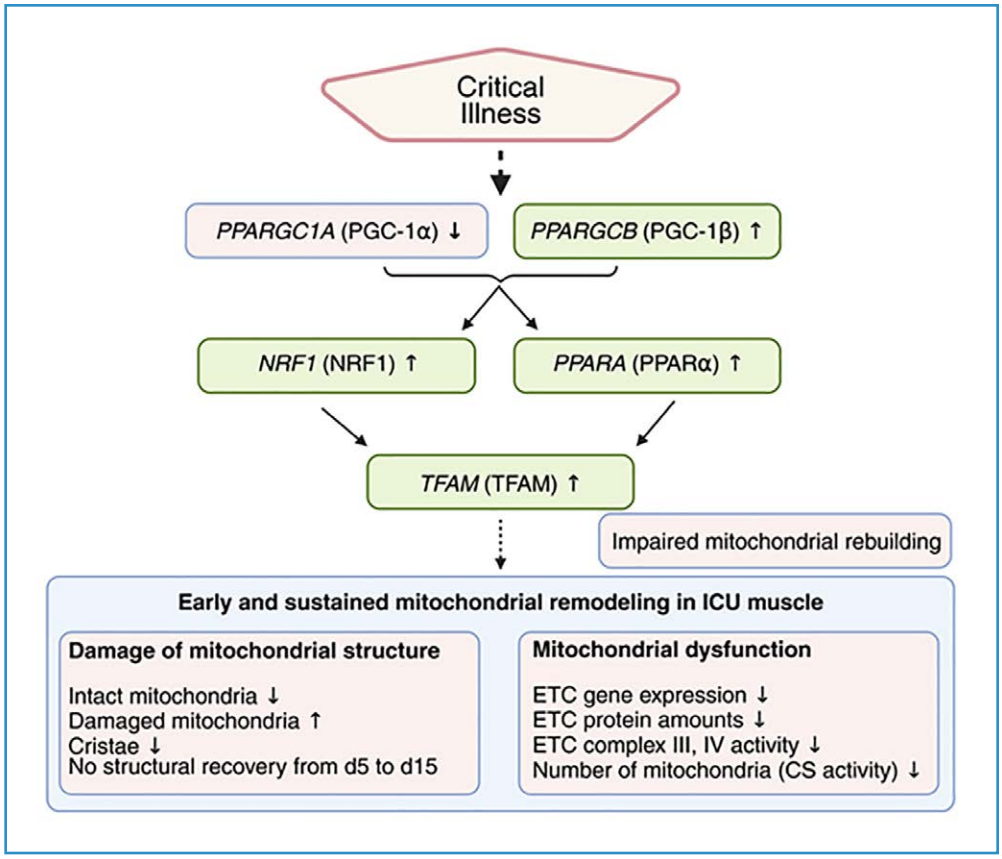


Figure 2. Mitochondrial remodeling in skeletal muscle during critical illness. The canonical pathways of mitochondrial biogenesis are shown, including the upstream metabolic and stress-related signaling pathways, the activation of proliferator-activated receptor (PPAR) gamma coactivator 1-alpha (PGC-1α) and the downstream transcriptional regulators (nuclear respiratory factor 1 [NRF1] and PPARA), which control mitochondrial transcription via mitochondrial transcription factor A (TFAM). In skeletal muscle biopsy specimens from ICU patients, mitochondrial biogenesis signaling is increased, whereas mitochondrial structure and function are impaired. Ultrastructural analysis reveals reduced numbers of intact mitochondria and disrupted cristae architecture, accompanied by decreased mitochondrial content. Electron transport chain (ETC) function is selectively impaired at complexes III and IV, even after normalization to citrate synthase (CS), indicating a persistent functional impairment that extends beyond reduced mitochondrial abundance. Together, these findings suggest early and sustained mitochondrial remodeling characterized by structural disruption, reduced mitochondrial content, and selective ETC dysfunction, despite the activation of mitochondrial biogenesis signaling pathways.

the two timepoints (**Fig. e4*H***, https://links.lww.com/CCM/I102). No changes in mitochondrial CII activity were observed between controls and patients (**Fig. e4*I***, https://links.lww.com/CCM/I102). Mitochondrial CIII activity and CIV activity were significantly reduced on day 5 and day 15 activity compared with controls without a difference between both timepoints (**Fig. e4**, ***J*** and ***K***, https://links.lww.com/CCM/I102). CI + CIII activity remained unchanged patients at both time points and also activity vs. controls. CII + CIII activity was higher in patients at day 15 compared with day 5 without a difference to the controls (**Fig. e4**, ***L*** and ***M***, https://links.lww.com/CCM/I102).

Mitochondrial Gene Expression in Skeletal Muscle During Sepsis Depends on the Disease Stage

To interrogate whether mitochondrial transcriptional remodeling precedes the earliest feasible human biopsy timepoint, we analyzed our previously published mouse TA RNA-seq dataset after CLP at 24 and 96 hours. This analysis was intended to determine whether mitochondrial transcriptional perturbation is already detectable before the earliest feasible human biopsy time point and to provide pathway-level context for the human structural and biochemical phenotype. ETC gene regulation was modest at 24 hours but showed coordinated down-regulation across complexes I–V by 96 hours. These dynamics are summarized in **Figures e5–e7** (https://links.lww.com/CCM/I102).

DISCUSSION

To our knowledge, this study provides the first serial ultrastructural and biochemical characterization of mitochondrial remodeling in human skeletal muscle during the early course of critical illness. Our principal finding is that mitochondrial abnormalities are detectable by ICU day 5 and remain evident through

the second ICU week. Vastus lateralis muscle exhibited fewer intact mitochondria, more morphologically disturbed mitochondria, and reduced inner mitochondrial membrane surface-to-volume ratio (a proxy for cristae architecture). In parallel, ETC enzymatic activity was reduced at the tissue level, with persistent reductions in complexes III and IV at both timepoints. Importantly, complex III and IV activities remained reduced after normalization to CS, indicating selective biochemical impairment beyond reduced mitochondrial content alone, while CS activity itself declined by day 15, consistent with a progressive mitochondrial loss. Together, these findings indicate coordinated structural and biochemical remodeling of skeletal muscle mitochondria during critical illness. Although selected markers of mitochondrial biogenesis were increased, ETC proteins, enzymatic activities, and ultrastructural abnormalities failed to recover. **Figure 2** summarizes this integrated mitochondrial remodeling phenotype, consistent with an early and sustained yet incompletely reparative mitochondrial response during critical illness.

A second key observation is the apparent mismatch between biogenesis-associated signaling and structural and functional recovery. Expression of selected biogenesis-related transcripts (e.g., *PPARGC1B*, *PPARA*, *TFAM*, *NRF1*) was increased at ICU day 5, while ETC gene expression, protein content, and enzymatic activity failed to normalize over time. This pattern is compatible with a compensatory response that does not restore structurally intact, high-capacity mitochondria during the acute ICU course. Several ETC-encoding genes were downregulated and corresponded with reduced ETC protein content and enzymatic activity. Similar transcriptional patterns were observed in our murine sepsis dataset, which demonstrated reduced ETC gene expression up to 96 hours after CLP. Together, these findings highlight a disconnect between the activation of mitochondrial biogenesis signaling and effective regeneration of functional mitochondria.

One potential explanation for the dissociation between increased biogenesis-associated signaling and persistent mitochondrial dysfunction is impaired mitochondrial rebuilding downstream of transcriptional activation. Restoration of functional mitochondria requires coordinated mitochondrial DNA transcription, translation of mitochondrial proteins, import of nuclear-encoded mitochondrial proteins, respiratory complex assembly, and cristae remodeling. Defects at one or more of these downstream steps could limit recovery despite activation of upstream biogenesis pathways. In this context, altered TFAM-dependent mitochondrial transcription may be particularly relevant. Rahmel et al (29) demonstrated impaired intramitochondrial localization of TFAM during sepsis despite increased cellular TFAM expression, suggesting defective activation of mitochondrial transcriptional programs. Furthermore, mitochondrial transcription depends on the coordinated activity of the TFAM/transcription factor B2, mitochondrial (TFB2M)/RNA polymerase, mitochondrial (POLRMT) transcription initiation complex. Experimental evidence indicates that posttranslational modification of TFB2M, including phosphorylation at T184 and T313, may impair interaction with POLRMT and reduce mitochondrial transcriptional activity (30). Although these mechanisms have not yet been investigated in skeletal muscle during critical illness, they provide biologically plausible explanations for the observed mismatch between biogenesis-associated signaling and persistent structural and functional mitochondrial abnormalities. The involvement of mitochondrial and metabolic pathways early in critical illness is consistent with recent multi-omics analyses of skeletal muscle from septic patients (31). Notably, mitochondria may remain central to the prolonged muscle dysfunction observed after ICU discharge, as suggested by recent clinical and translational studies (31, 32).

Long-term ICU survivorship is frequently characterized by persistent impairment in physical function and endurance capacity (3). Because mitochondrial volume and cristae architecture correlate with VO_2max in human *vastus lateralis* (25), the reduced inner mitochondrial membrane surface-to-volume ratio in structurally intact mitochondria may represent a bioenergetic constraint with potential clinical relevance. However, postdischarge functional trajectories were not measured in the present cohort.

Although mitochondrial biogenesis signaling was transcriptionally increased, ETC gene expression remained suppressed; ETC protein content and enzymatic activity failed to recover. Interestingly, a similar pattern was observed by Carre et al (33), who demonstrated increased PGC-1α expression in survivors of critical illness that did not translate into increased expression of respiratory chain genes. They hypothesized that the benefit of this response may instead relate to enhanced antioxidant defense pathways, including manganese superoxide dismutase

induction. In contrast, we previously demonstrated markedly reduced PGC-1α expression in skeletal muscle from patients with critical illness myopathy, suggesting that impaired upstream mitochondrial biogenesis signaling may coexist with incomplete activation of downstream mitochondrial recovery pathways during prolonged critical illness (19). Together, these observations support the concept that activation of mitochondrial biogenesis signaling alone is insufficient for effective mitochondrial rebuilding in the absence of coordinated mitochondrial transcription, translation, protein import, and respiratory complex assembly (34).

We observed increased *PPARA* (PPARα) expression, a regulator of mitochondrial fatty acid β-oxidation (35), in skeletal muscle of ICU patients. Although substrate utilization was not assessed directly, increased *PPARA* expression suggests altered metabolic signaling during critical illness. Together with reduced ETC gene expression, protein abundance, and enzymatic activity, these findings indicate sustained mitochondrial remodeling and suggest that therapies targeting biogenesis signaling alone may be insufficient.

ETC activity remained impaired at both time points investigated. After normalization to CS activity, complex II activity was relatively preserved, suggesting differential vulnerability of ETC complexes during critical illness. These findings support the presence of both reduced mitochondrial content and selective ETC dysfunction, consistent with previous reports of impaired skeletal muscle bioenergetics in critical illness (17, 36–38). Our observations differ from those of Jiroutkova et al (18), who attributed impaired oxidative phosphorylation primarily to reduced mitochondrial content. This may reflect differences in biopsy timing, methodology, or patient populations.

We deliberately avoid equating reduced ETC enzyme activity with definitive "mitochondrial failure." Spectrophotometric assays quantify forward complex activities under substrate excess and cannot resolve mitochondrial membrane potential, redox state, or electron flow direction. Consequently, reduced activities may reflect injury-related impairment, adaptive metabolic remodeling, or a combination of both. Although coenzyme Q10 levels were not measured directly, combined CI + CII and CI + CIII activity assays did not indicate a major coenzyme Q10-limited phenotype in this cohort.

Mitophagy was not assessed directly in this study. However, emerging evidence suggests that autophagy may be impaired after sepsis and that restoration of autophagic flux improves mitochondrial respiration and strength in translational models (31). Conversely, muscle of septic mice can display increased general autophagy without a corresponding increase in mitophagy, potentially allowing damaged mitochondria to accumulate (39). These observations provide a plausible explanation for the increased proportion of structurally "disturbed" mitochondria and persistent ETC deficits observed in our cohort.

The murine RNA-seq dataset was included to provide temporal context for the earliest phase of sepsis, which is generally inaccessible through serial human muscle biopsies. In that framework, the murine data are informative because they show that mitochondrial ETC gene-expression perturbations are already present within 24–96 hours of sepsis, suggesting that the structural and biochemical abnormalities observed in human muscle by ICU day 5 are likely preceded by substantially earlier mitochondrial transcriptional remodeling.

LIMITATIONS

Our study has several limitations. First, tissue availability restricted molecular analyses to selected transcripts at ICU day 5 and precluded direct assessment of mitochondrial respiration and energetic flux. Second, sampling was confined to the acute ICU phase, limiting inference regarding long-term mitochondrial recovery after discharge. Third, the first biopsy was only feasible around ICU day 5; therefore, the murine RNA-seq dataset was included to provide temporal context for the earlier sepsis-associated mitochondrial remodeling. Finally, the cohort reflects the clinical heterogeneity of critical illness, including varying admission diagnoses and imperfectly matched controls. Citrate synthase normalization was therefore interpreted alongside ultrastructural and enzymatic measurements.

CONCLUSIONS

Critically ill patients exhibit early and persistent mitochondrial remodeling in *vastus lateralis* muscle, characterized by disturbed ultrastructure and reduced ETC enzyme function detectable by ICU day 5 and persisting into the second ICU week. Persistent reductions in complex III and IV activities after CS normalization support selective biochemical remodeling in addition

to loss of mitochondrial content. Because sampling was limited to the acute ICU phase, the functional consequences and postdischarge pattern of these abnormalities remain unknown. These findings support further longitudinal studies linking mitochondrial remodeling to functional outcomes and evaluating interventions beyond biogenesis signaling alone.

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Drs. Wickert and Grunow designed and performed all experiments, performed statistical analyses, discussed data, and prepared the figures, tables, and article. Dr. Engelhardt performed experiments and edited the article. Dr. Groß performed statistical analyses, discussed data, and edited the article. Dr. Boschmann designed and performed experiments, discussed data, and prepared and edited the article. Drs. Ochs and Timm designed experiments, discussed data, and edited the article. Dr. Meinhardt discussed data and edited the article. Dr. Stenzel designed experiments, discussed data, and edited the article. Drs. Wollersheim and Weber-Carstens performed the clinical study, discussed data, and edited the article. Dr. Fielitz designed and analyzed experiments, discussed data, prepared and edited figures and tables, and prepared and edited the article.

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The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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