

Skeletal Muscle Mitochondrial Impairment in Cirrhosis-Induced Sarcopenia

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Abstract

Cirrhosis-associated muscle mass loss or sarcopenia is a common complication (17-30% prevalence) in cirrhotic patients. However, the pathogenesis of this complication is poorly understood. Therefore, finding the mechanisms of sarcopenia could lead to the development of therapeutic strategies against this complication. In the current study, rats underwent bile duct ligation (BDL) surgery, and their skeletal muscle (gastrocnemius; GS) was isolated and assessed 28 and 56 days after BDL operation. Significant increase in biomarkers of oxidative stress, including reactive oxygen species (ROS) formation, lipid peroxidation, and increased oxidized glutathione (GSSG) levels were detected in the muscle of cirrhotic animals. Skeletal muscle tissue antioxidant capacity and reduced glutathione (GSH) were also significantly decreased in BDL rats. Moreover, deterioration of several mitochondrial indices, including mitochondrial depolarization, increased mitochondrial permeabilization, depleted ATP reservoirs, and decreased mitochondrial dehydrogenases activity, were evident in the GS isolated from cirrhotic rats. Based on these data, oxidative stress and mitochondrial impairment seem to play as primary mechanisms of cirrhosis-induced sarcopenia.

Keywords: Cell death, Cirrhosis, Energy crisis, Mitochondria, Muscle waste, Weakness

1. Introduction

Sarcopenia is a common complication in cirrhotic patients (1). Sarcopenia could severely affect patients' quality of life (1, 2). Moreover, this complication directly influences the outcome of other therapeutic interventions such as liver transplantation in cirrhosis (1, 2). It has been found that the survival rate in cirrhotic patients with sarcopenia is lower than their non-sarcopenic counterparts (1, 2). Unfortunately, no specific therapeutic modality has been developed for cirrhosis-associated sarcopenia. The main reason for the lack of ther-

apeutic intervention in cirrhosis-induced sarcopenia is connected with the poor understanding of the mechanism(s) of this complication.

Malnutrition, hormonal and biochemical alterations, as well as circulating endotoxins, could contribute to the pathogenesis of sarcopenia in cirrhosis (3, 4). Perturbation in skeletal muscle protein synthesis and turnover is a hallmark of sarcopenic muscle injury (3, 4). Enhanced proteolysis could lead to the loss of muscle mass in cirrhotic patients (4). Several mediators of the liver-muscle axis, including ammonia, testosterone, growth hormone, and lipopolysaccharides, have been proposed to be involved in the pathogenesis of cirrhosis-associated sarcopenia (4). However, there is no

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investigation on the role of mitochondrial impairment in the pathogenesis of sarcopenia in cirrhotic models.

Previous studies mentioned that bile duct ligation (BDL) could serve as a reliable animal model for investigating cirrhosis-induced sarcopenia (5). Muscle atrophy is adequately induced in this model (5). In the current study, BDL rats were used to evaluate the role of mitochondrial impairment and oxidative stress in cirrhosis-induced sarcopenia.

Finding the mechanism(s) of skeletal muscle injury in cirrhosis could lead to the development of novel therapeutic strategies against this complication. Therefore, the current study was designed to evaluate the role of oxidative stress and mitochondrial impairment in the pathogenesis of cirrhosis-associated sarcopenia. Rats underwent BDL surgery to induce cirrhosis. Then, 28 and 56 days after the BDL operation, the gastrocnemius muscle was isolated and assessed.

2. Material and Methods

2.1. Chemicals

Trichloroacetic acid, 2',7' dichlorofluorescein diacetate (DCFH-DA), reduced glutathione (GSH), malondialdehyde, oxidized glutathione (GSSG), 3 (N-morpholino) propane sulfonic acid (MOPS), 3-[4,5dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), sucrose, D-Mannitol, trypsin, fatty acid-free bovine serum albumin (BSA) fraction V, rhodamine123, Coomassie brilliant blue, 2, 4, 6-tripyridyl-s-triazine (TPTZ), glacial acetic acid, ferric chloride hexahydrate (FeCl₃.6H₂O), dithiothreitol (DTT), thiobarbituric acid, and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma (Sigma-Aldrich, St. Louis, MO). Iodoacetic acid, ethylenediaminetetraacetic acid (EDTA), di-nitro fluoro benzene, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), meta-phosphoric acid, 2 Amino 2-hydroxymethyl-propane-1, 3-diol-hydrochloride (Tris-HCl), and n-Butanol, were obtained from Merck (Darmstadt, Germany).

2.2. Animals

Male Sprague-Dawley rats (n=18) weighing 200-250 g were obtained from Shiraz Univer-

sity of Medical Sciences, Shiraz, Iran. Animals were housed at the temperature of 23±1 °C with 40% relative humidity. Rats had free access to tap water and a commercial rodent pellet diet (Royan Feed®, Isfahan, Iran). All procedures were in accordance with the protocol for care and use of laboratory animals and approved by the ethics committee at Shiraz University of Medical Sciences, Shiraz, Iran (94-01-36-10650).

2.3. Bile duct ligation surgery for cirrhosis induction and experimental setup

Animals were anesthetized with a mixture of ketamine (60 mg/kg, i.p) and xylazine (10 mg/kg, i.p). A midline incision (2 cm) through the *linea alba* was made (6-8). The common bile duct was ligated and cut between ligatures. In sham-operated animals, the bile duct was identified and manipulated without ligation (9, 10). The assessment of serum biochemical measurements confirmed the occurrence of cholestasis in BDL rats (11, 12). Rats were allotted into three groups (n = 6 in each group). At days 28 and 56, after BDL surgery, animals were anesthetized (80 mg/kg, thio-pental, i.p), and muscle (Gastrocnemius) samples were collected.

2.4. Plasma and muscle ammonia levels

Plasma levels were measured with standard kits (13, 14). GS tissue extract was prepared for assessing the NH₄⁺ levels (13, 14). For this purpose, samples (100 mg) of the GS muscle samples were dissected, homogenized, and deproteinized in 3 mL of ice-cooled lysis solution (Trichloroacetic acid, 6%, w/v, 4 °C). After centrifugation (17,000g, 5 min, 4 °C), the supernatant was collected and neutralized with potassium carbonate (KHCO₃; 2 mol/L, pH=7). Afterward, brain ammonia content was measured using standard kits (13, 14).

2.5. Rotarod test

Rats underwent four sessions of rotarod performance on a rotarod apparatus to assess muscle function and locomotor activity (15, 16). The speed of the rotarod was 10 rpm. Each rotarod session had three trials for each rat with 10 min interval. The time that rats stayed on the rotating

rod was recorded (15-17).

2.6. Reactive oxygen species (ROS) formation in skeletal muscle

Reactive oxygen species (ROS) formation was estimated using 2', 7' dichlorofluorescein diacetate (DCF-DA) (10, 18-22). Briefly, tissue samples (200 mg) were homogenized in 5 mL of ice-cooled Tris-HCl buffer (40 mM, pH=7.4). Then, samples of the tissue homogenate (100 μ L) were treated with Tris-HCl buffer (1 mL) and DCF-DA (Final concentration 10 μ M) (23-26). The mixture was incubated at 37 °C (10 min, in the dark). Finally, the fluorescence intensity was assessed using a fluorimeter (FLUOstar Omega[®], $\lambda_{\text{excit}}=485$ nm, and $\lambda_{\text{emiss}}=525$ nm) (9, 10, 27, 28).

2.7. Lipid peroxidation in skeletal muscle of cirrhotic animals

The thiobarbituric acid reactive substances (TBARS) were measured in the GS tissue as an index of lipid peroxidation (10, 29-32). Briefly, 500 μ L of tissue homogenate (10% w/v in KCl, 1.15% w: v) was treated with 1 mL of thiobarbituric acid (0.375%, w: v), and 3 mL of phosphoric acid (1% w:v, pH=2) (33-36). Samples were mixed well and heated (100 °C) for 45 minutes. Then, 2 mL of n-butanol was added, mixed well, and centrifuged (10000 g, 20 min) (37-42). Finally, the absorbance of developed color in the n-butanol phase (upper phase) was measured ($\lambda=532$ nm, EPOCH[®] plate reader, BioTek[®], USA) (10, 13, 43-46).

2.8. Total antioxidant capacity of Gastrocnemius muscle

Ferric reducing antioxidant power (FRAP) assay for the assessment of the GS total antioxidant capacity in the sham-operated and cirrhotic animals (10, 47-49). Briefly, the working FRAP reagent was freshly-prepared by mixing 10 volumes of 300 mM acetate buffer (pH=3.6), with 1 volume of 10 mM TPTZ (in 40 mM HCl) and 1 volume of 20 mM ferric chloride. Tissue was homogenized in an ice-cooled 250 mM Tris-HCl buffer containing 200 mM sucrose and 5 mM DTT (pH=7.4) (33, 50-54). Afterward, 100 μ L of tissue homogenate was added to 900 μ L of the FRAP reagent. Samples were incubated in the dark (5 min

at 37 °C). Finally, the absorbance was measured using EPOCH[®] plate reader ($\lambda=595$ nm, BioTek[®], USA) (10, 41, 55).

2.9. Protein carbonylation

Protein carbonylation in the skeletal muscle of cirrhotic rats was assessed using dinitrophenylhydrazine (DNPH) (56-58). Briefly, 200 mg of the GS tissue was homogenized in 5 mL of the triton X-100 (0.1% v:v)-containing phosphate buffer solution (pH=7.5). Tissue homogenate was centrifuged (700 g, 10 min, 4 °C), and 500 μ L of the resulting supernatant was treated with 300 μ L of 10 mM DNPH (dissolved in HCl) (59). Samples were then incubated (1 h, 25 °C, in the dark, with vortexing every 10 minutes) (56, 57, 60-62). Then, 100 μ L trichloroacetic acid (20% w:v) was added, tubes were centrifuged (17,000 g, 5 min), and the supernatant was discarded. Afterward, the pellet was washed three times, with ethanol: ethyl acetate (1 mL of 1:1 v:v), and the precipitate was re-dissolved in 6 M guanidine chloride solution (pH=2.3) (35). Finally, samples were centrifuged (17,000 g, 5 min), and absorbance of the supernatant was measured at $\lambda=370$ nm (EPOCH[®] plate reader, BioTek[®] Instruments, USA) (56-58).

2.10. Skeletal muscle mitochondria isolation

Gastrocnemius was isolated, washed, and minced in an ice-cold buffer medium (70 mM mannitol, 220 mM sucrose, 2 mM HEPES, 0.5 mM EGTA and 0.1% BSA, pH=7.4). Minced tissue was transported into a fresh buffer (5 mL buffer/1g tissue) containing trypsin (0.1% w:v). Samples were incubated on ice (15 min). Then, samples were centrifuged (1000 g, 10 min, 4 °C), and the supernatant was discarded. The pellet was homogenized in fresh mitochondria isolation buffer (5 mL buffer: 1g tissue). Mitochondria were isolated by differential centrifugation method as previously described (63-65). First, unbroken cells and nuclei were pelleted at 1000 g for 10 min at 4 °C; second, the supernatant was centrifuged at 10000 g for 10 min at 4 °C to pellet the mitochondria. The second step was repeated at least four times using the fresh isolation buffer medium. Final mitochondrial pellets were suspended in a buffer containing 70 mM mannitol, 2 mM HEPES, 220 mM sucrose, and 0.5

mM EGTA, pH=7.4, except for the mitochondria used to assess mitochondrial depolarization and mitochondrial swelling, which were suspended in depolarization assay buffer (220 mM Sucrose, 68 mM Mannitol, 10 mM KCl, 2 mM MgCl₂, 5 mM KH₂PO₄, 50 μM EGTA, and 10 mM HEPES, pH=7.2) and swelling assay buffer (125 mM Sucrose, 10 mM HEPES, and 65 mM KCl, pH=7.2) (63, 66). Samples protein content was determined by the Bradford method (67).

2.11. Mitochondrial dehydrogenase activity (MTT assay)

3-(4, 5-dimethylthiazol-2-yl)-2, the 5-dimethyltetrazolium bromide (MTT) was used to estimate mitochondrial dehydrogenase activity in the GS (68-72). Briefly, a mitochondrial suspension (0.5 mg protein/ml) was treated with 40 μL of MTT (0.4% w:v) and incubated at 37 °C (30 min, in the dark). Then, samples were centrifuged (10000 g, 20 min), and the product of purple formazan crystals (pellet) was dissolved in 1 mL dimethyl sulfoxide. Finally, the absorbance at λ=570 nm was assessed using an EPOCH plate reader (Bio-Tek® Instruments, USA) (28, 48, 73).

2.12. Mitochondrial depolarization

The rhodamine 123 uptake by isolated GS mitochondria was used to estimate mitochondrial depolarization (10, 43, 63, 74). Briefly, the mitochondrial fractions (0.5 mg protein/ml) were incubated with 5 μM of rhodamine 123 in the depolarization assay buffer (Final concentration 10 μM) for 10 minutes (21, 75-81). Then, samples were centrifuged (17,000 g, 1 min, 4 °C), and the fluorescence intensity of the supernatant was measured using a fluorimeter (FLUOstar Omega®, λ_{excitation}=485 nm and λ_{emission}=525 nm) (63, 82, 83).

2.13. Mitochondrial swelling

The changes in light scattering at λ=540 nm (25 °C) was used as a method to evaluate mitochondrial swelling (63, 82, 84). Briefly, isolated mitochondria (0.5 mg/mL) were suspended in swelling buffer, and the absorbance was monitored at λ=540 nm (30 min, using an EPOCH plate reader; Bio-Tek® Instruments, USA) (70, 85-87). The

decrease in sample absorbance is associated with an increase in mitochondrial swelling (63, 82, 88).

2.14. Mitochondrial and muscle tissue reduced (GSH) and oxidized (GSSG) glutathione

Mitochondrial and tissue glutathione content (oxidized and reduced) was measured using an HPLC method based on a previously reported protocol (89). Briefly, isolated mitochondria (1 mL, 1mg protein/mL) or homogenized GS tissue (1 mL of 10% w:v homogenate) were treated with 100 μL of trichloroacetic acid (50 % w:v). Samples were incubated on ice (15 min) and centrifuged (17,000 g, 30 min, 4 °C). The supernatant was collected in 5 mL tubes and treated with 300 μL of NaOH: Na₂CO₃ (2 M: 2 M solution). Afterward, 100 μL of iodoacetic acid (15% w:v) was added and incubated at 4 °C for one hour. Then, 100 μL of dinitrofluorobenzene was added. Samples were mixed well and incubated in the dark (25 °C, 24 h). Finally, samples were centrifuged (17000 g, 20 min), filtered, and injected (25 μL) to an HPLC apparatus. The HPLC system composed of an NH₂ column (Bischoff chromatography, Leonberg, Germany, 25 cm length, 10 μm particle size, 10 Å pore size), and a UV detector (λ=252 nm). A gradient method using the mobile phases A (Water: Methanol; 1:4 v: v) and B (Acetate buffer: Buffer A; 1: 1:4 v: v) was used (flow rate of 1 mL/min) (90, 91).

2.15. Lipid peroxidation in isolated mitochondria

Thiobarbituric acid-reactive substances (TBARS) test was used for lipid peroxidation assay in isolated kidney mitochondria (63). As previous studies mentioned, sucrose interferes with the TBARS assay (63). Hence, isolated mitochondria were washed once in an ice-cooled MOPS-KCl buffer (50 mM MOPS and 100 mM KCl, pH=7.4). Afterward, GS isolated mitochondria preparations were re-suspended in MOPS-KCl buffer and used for the lipid peroxidation assay (63). For this purpose, the mitochondrial suspension (1 mL) was added to 2 mL of a mixture containing trichloroacetic acid (15% w/v), thiobarbituric acid (0.375%), HCl (0.24 N), and Trolox (0.5 mM). Samples were heated for 15 min at 100 °C (63). After centrifugation (17000 g, 10 min), the absor-

bance was measured at $\lambda=532$ nm (EPOCH plate reader, BioTek® Instruments, USA) (63).

2.16. Mitochondrial ATP content

Based on a previously reported procedure, GS mitochondrial ATP level was assessed using an HPLC (91, 92). Briefly, isolated GS mitochondria (1 mg protein/mL) were treated with 100 μ L of ice-cooled perchloric acid (200 mM), incubated on ice (5 min), and centrifuged (30 min, 17,000 g, 4 °C). Afterward, the supernatant (100 μ L) was treated with its equivalent volume of ice-cooled KOH (1 M). Samples were filtered and injected (25 μ L) into an HPLC system consisted of an LC-18 column (μ -Bondapak, 15 cm) (93). The mobile phase was composed of tertiary butyl ammonium sulfate (2.3 mM), KH_2PO_4 (215 mM), KOH (1 M, 0.4% w:v), and acetonitrile (4 % v:v). The flow rate was 1 mL/min, and the UV detector was set at $\lambda=254$ nm (94).

2.17. Statistical analysis

Data are represented as mean \pm SD. Data comparison was performed by the one-way analysis of variance (ANOVA) with Tukey's multiple comparison test as the post hoc. $P<0.05$ was considered a statistically significant difference.

3. Results

A significant decrease in muscle mass index was evident in the GS muscle of cirrhotic animals (56 days after BDL surgery) (Figure 1). Animals locomotor activity and muscle function were also significantly declined in cholestatic rats (Figure 1). Moreover, significant changes in muscle tissue, and plasma ammonia levels were evident in the cirrhotic rats (Figure 1).

Significant changes in biomarkers of oxidative stress were evident in the GS muscle isolated from cirrhotic animals (Figure 2). ROS for-

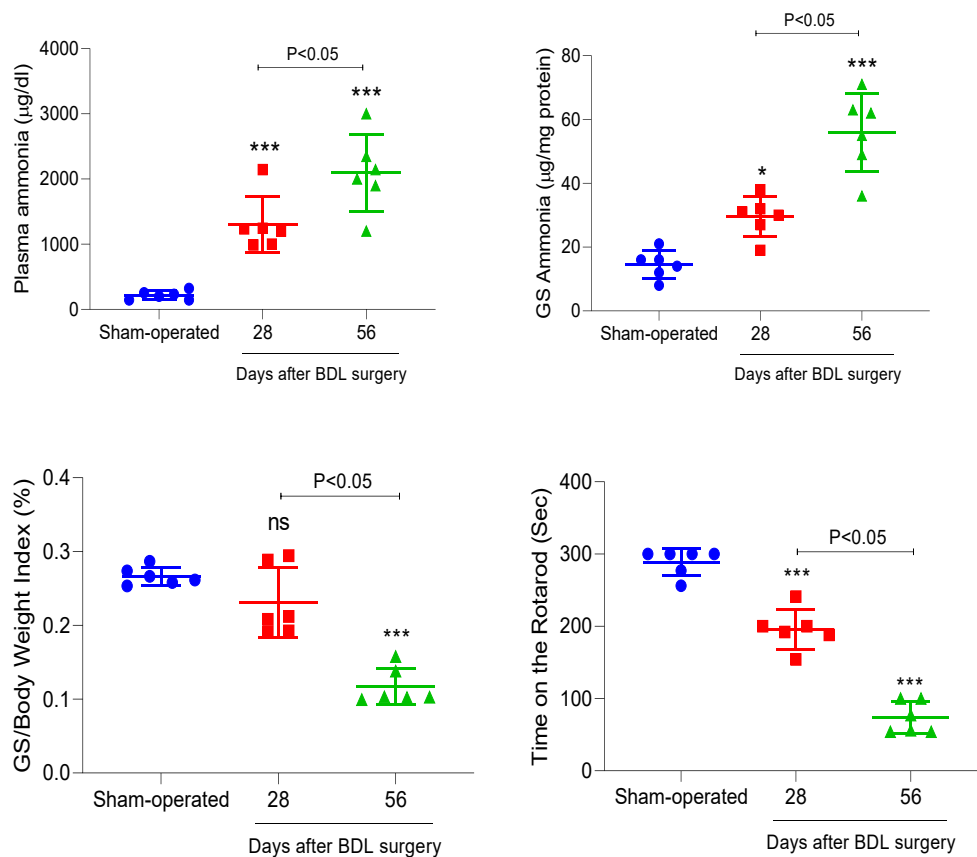


Figure 1. Gastrocnemius muscle (GS) weight index, ammonia level, and rotarod test in bile duct ligated (BDL) rats. Data are given as mean \pm SD (n=6).

***Indicates significantly different as compared with the sham-operated group ($P<0.05$).

ns: not significant.

mation, lipid peroxidation, increased GSSG levels, and protein carbonylation was detected in GS of cirrhotic rats (Figure 2). On the other hand, GS antioxidant capacity and GSH levels were significantly decreased at 28 and 56 days after the BDL surgery (Figure 2). The development of oxidative stress in the GS tissue of cirrhotic rats was time-dependent in the current study (Figure 2).

Disturbances in mitochondrial function were evident in mitochondria isolated from GS of cirrhotic rats (Figure 3). Significant mitochondrial depolarization, mitochondrial permeabilization, depleted glutathione reservoirs, as well as ATP deprivation were detected in GS mitochondria of cirrhotic animals as assessed 28 and 56 days after

BDL operation (Figure 3). It was found that GS mitochondrial function was time-dependently deteriorated in the current model (Figure 3).

Histopathological evaluation of the GS revealed significant muscle atrophy in comparison with the sham-operated group (Figure 4). Muscle atrophy in BDL rats was deteriorated in a time-dependent manner (Figure 4).

4. Discussion

Sarcopenia is a frequent but mostly hidden complication of cirrhosis (1, 2). Muscle weakness and atrophy could significantly influence cirrhotic patients' quality of life (1, 2). Lower skeletal muscle mass in cirrhosis also affects the response to

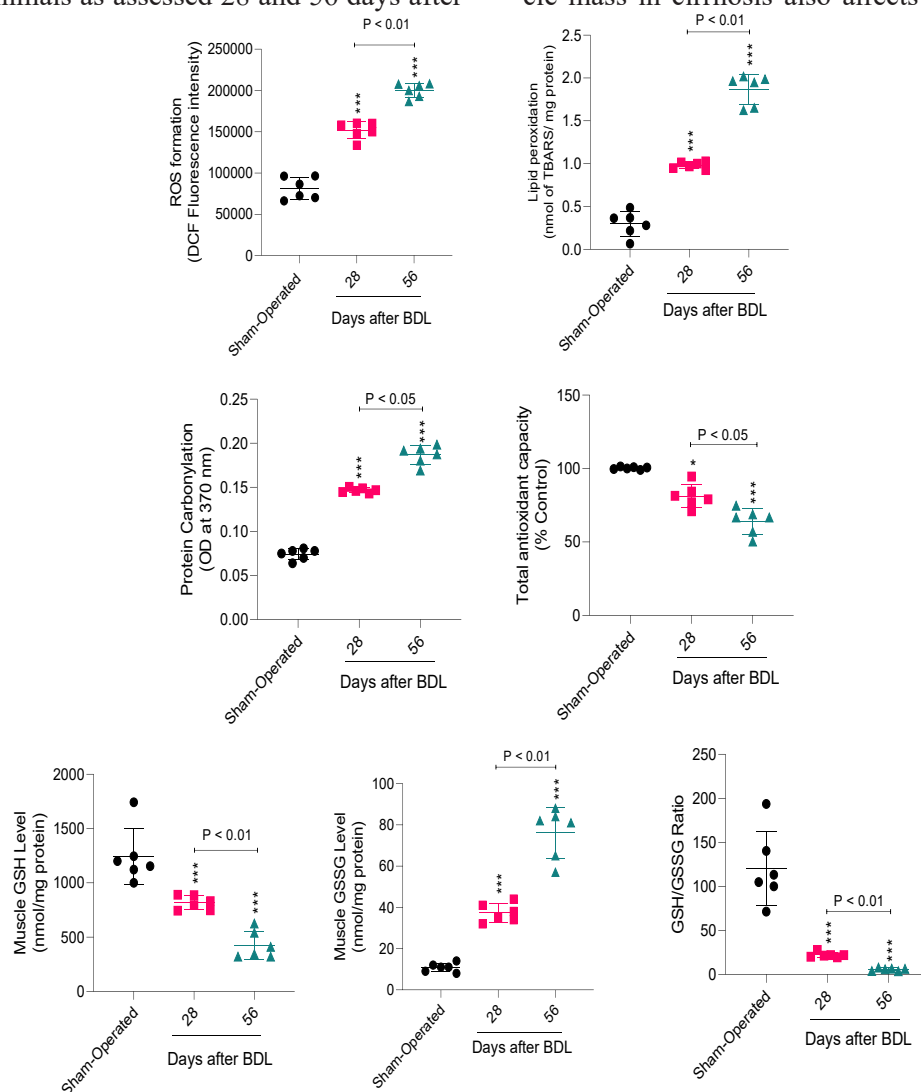


Figure 2. Biomarkers of oxidative stress in the skeletal muscle of cirrhotic rats. BDL: Bile duct ligation. Data are given as mean±SD (n=6).

***Indicates significantly different as compared with the sham-operated group ($P<0.05$).

stressors, such as body infection and surgery (1, 2). Hence, finding the mechanisms of muscle weakness and injury could lead to the development of novel therapeutic strategies in cirrhotic patients. In the current study, it was found that markers of oxidative stress were significantly increased in the GS muscle of cirrhotic rats. Moreover, several mitochondrial indices were impaired in the skeletal muscle during cirrhosis. Based on these data, oxidative stress and mitochondrial dysfunction play a fundamental role in cirrhosis-induced sarcopenia.

The role of several cytotoxic molecules has been highlighted in the mechanism of sarcopenia (1, 2). It has been mentioned that all these agents could act in an interconnected mechanism

to induce muscle waste and sarcopenia (1, 2). Ammonia is the primary suspected agent for inducing muscle mass loss and sarcopenia (4). The role of ammonium ion in sarcopenia has been extensively investigated (4). Neurotoxicity is a well-characterized side effect of ammonia (95). Recently, it has been reported that this molecule is also able to induce adverse effects in tissues such as skeletal muscle (4). Following the entry of ammonia in the muscle, it activates a series of signaling mechanisms, which could lead to cytotoxicity and organ injury (4). It has been found that ammonia could induce impaired protein synthesis in the skeletal muscle during cirrhosis (4, 96). Ammonia causes the release of myostatin, which is involved

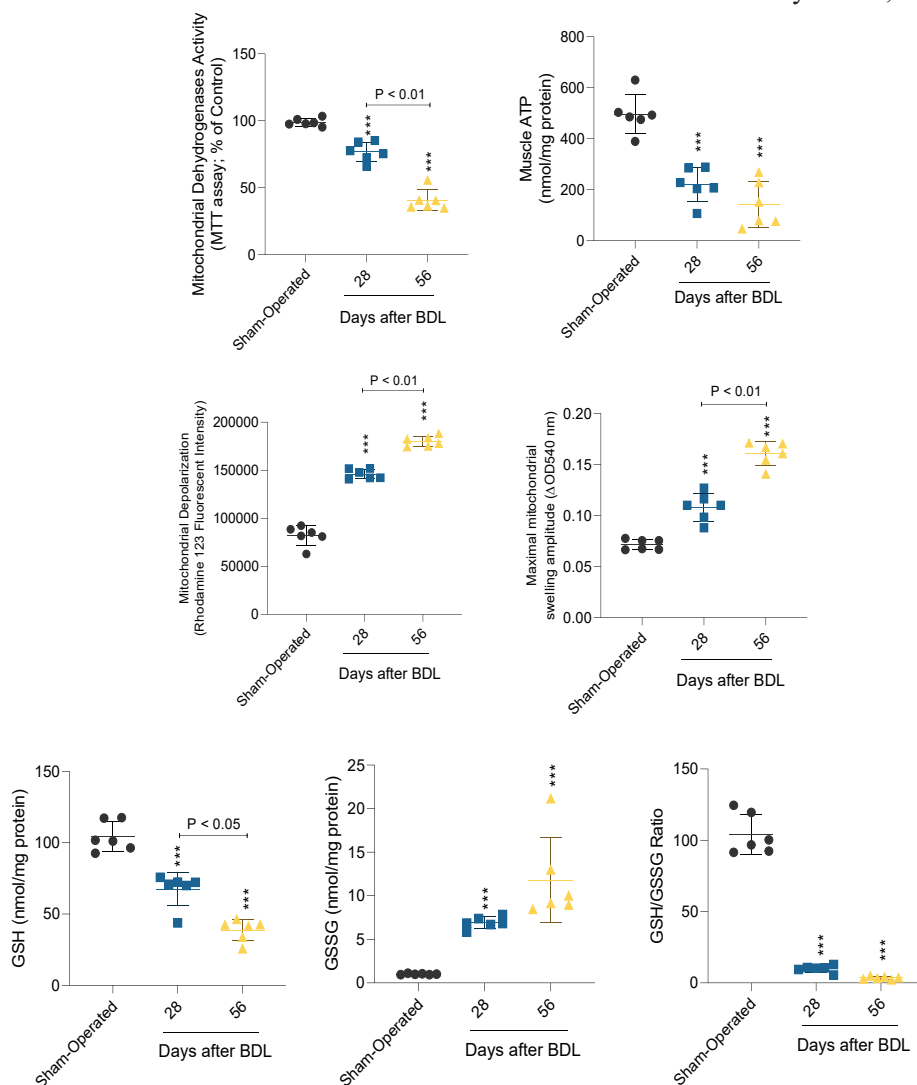


Figure 3. Mitochondrial indices in the skeletal muscle of cirrhotic rats.

Data are represented as mean \pm SD (n = 6).

***Indicates significantly different as compared with the sham-operated group ($P < 0.05$).

in the inhibition of protein synthesis and enhancing proteolysis in skeletal muscle (4). It has been found that the expression and levels of myostatin are significantly increased in the skeletal muscle of cirrhotic patients (4). In the current study, we found that critical mitochondrial indices such as mitochondrial depolarization, mitochondrial permeabilization and swelling, tissue ATP levels, and mitochondrial dehydrogenases activity were significantly decreased in the GS of cirrhotic rats.

Ammonia is able to disrupt mitochondrial function in different tissues such as the brain (95). It has been repeatedly mentioned that ammonia cause mitochondrial impairment in neural tissue (14, 97-101). Ammonia could also inhibit critical enzymes involved in energy (ATP) metabolism in the skeletal muscle (4). Our data from the current study revealed that similar perturbation could occur in the skeletal muscle of cirrhotic animals.

Ammonia also could activate the myostatin protein (102, 103). It has been found that myostatin could decrease cellular ATP levels

through different pathways (102, 103). It has been well-known that high skeletal muscle ammonia levels could significantly decrease α -ketoglutarate (104). This phenomenon is known as "cataplerosis" (104). Increase muscle ammonia could lead to the activation of a protein named "hypoxia-inducible factor- α ; HIF- α " (105). Activation of HIF- α could lead to catastrophic events such as increased myostatin levels, decreasing acetyl-CoA, and finally, skeletal muscle energy crisis (106). In the current study, we found that skeletal muscle energy (ATP) levels were significantly depleted in the GS of cirrhotic animals (Figure 3). Although it has not been evaluated in the current study, a part of ATP depletion in the muscle of cirrhotic rats could be connected with the HIF- α pathway.

Cellular mitochondria are the primary sources of intracellular reactive oxygen species (ROS) (107). Therefore, oxidative stress and mitochondrial impairment are two firmly-interconnected events (107, 108). In the current study, we found that markers of oxidative stress were significantly increased in the GS of cirrhotic rats (Figure

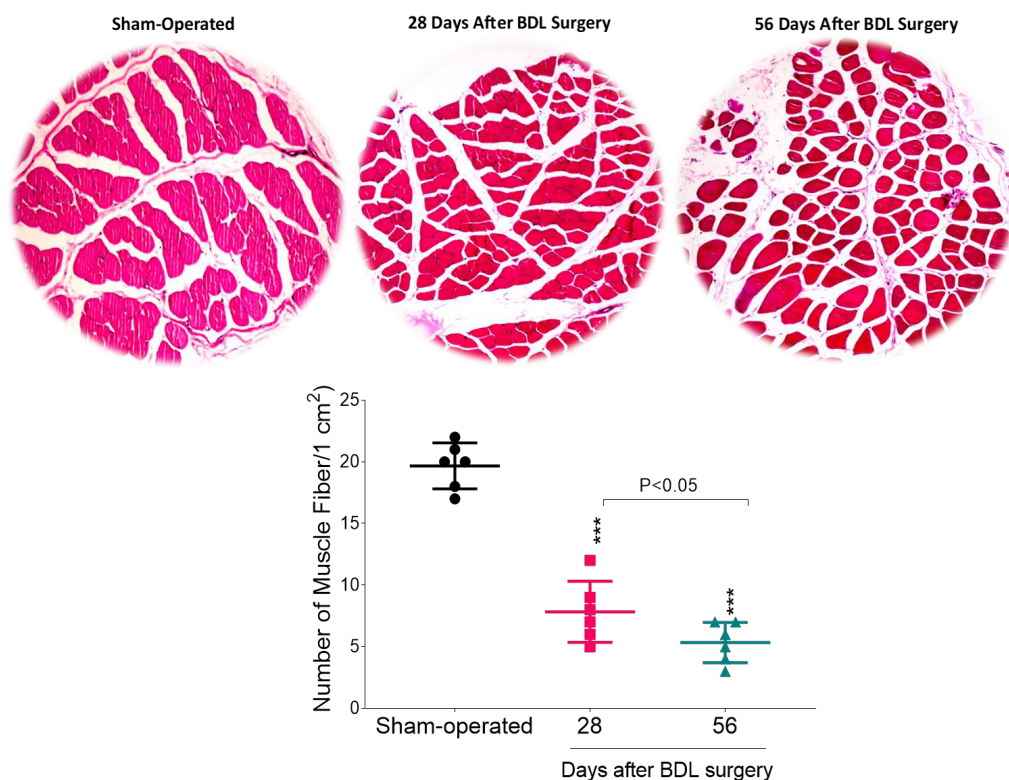


Figure 4. Skeletal muscle histopathological alterations in cirrhotic rats. Significant muscle atrophy was detected in cirrhotic animals at different time intervals post-bile duct ligation (BDL) surgery. Data are represented as mean \pm SD (n=6).

***Indicates significantly different as compared with the sham-operated group ($P < 0.05$).

2). Although more investigations are warranted to be precisely revealed, cellular mitochondria could act as a significant source of ROS in the GS tissue during cholestasis.

Several therapeutic options have been suggested for managing cirrhosis-associated sarcopenia (105, 109). Nutritional supplementation is the first approach for preventing skeletal muscle loss in cirrhotic patients (109, 110). Therefore, adequate calorie and protein intake have been recommended in cirrhosis (109, 110). Although nutritional supplementation could enhance muscle strength, there are some discrepancies about the effects of this strategy on cirrhotic patients' survival (110). It has been reported that nutritional supplementation might increase muscle mass and its capacity in ammonia removal, glucose disposal, and insulin response (110). However, the contractile function of the skeletal muscle might not back to its average level (110). Protein supplementation and using ammonia lowering agents are the other recommended strategies for blunting cirrhosis-induced sarcopenia (4). However, the benefits of these strategies in improving cirrhotic patients' quality of life and survival have been questioned (4). Mild exercise also has been recommended for the cirrhotic patient to blunt muscle mass loss (111-113). The mechanism of exercise in improving cirrhotic patients' muscle strength might be associated with enhanced mitochondrial biogenesis in these patients (114). Mitochondria biogenesis could improve muscle energy (ATP) and power in cirrhotic patients (114).

Based on the data obtained from the cur-

rent study, mitochondrial impairment and energy crisis could play a pivotal role in the pathogenesis of cirrhosis-associated sarcopenia. Therefore, mitochondria-targeted therapies could serve as a viable option in enhancing muscle strength and the quality of life in cirrhotic patients. Several safe and clinically-applicable agents have been developed, which could robustly improve mitochondrial function. Our previous studies mentioned the positive effects of several amino acids, antioxidant molecules, and peptides on mitochondrial function and energy metabolism in various experimental models (8, 20, 47, 52, 70, 115-118). These safe agents might find therapeutic value in enhancing muscle strength during cirrhosis. Obviously, much more investigations are needed for identifying the precise mechanism(s) of muscle weakness in cirrhotic patients and evaluating antioxidants and mitochondria protecting agents as therapeutic options.

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Conflict of Interest

None declared.

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