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# The Impact of Muscle Disuse on Muscle Atrophy in Severely Burned Rats

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***Background.*** **Severe burn induces a sustained hypermetabolic response, which causes long-term loss of muscle mass and decrease in muscle strength. In this study, we sought to determine whether muscle disuse has additional impact on muscle atrophy after severe burn using a rat model combining severe cutaneous burn and hindlimb unloading.**

***Methods.*** **Forty Sprague-Dawley rats (≈300 g) were randomly assigned to sham ambulatory (S/A), sham hindlimb unloading (S/HLU), burn ambulatory (B/A), or burn hindlimb unloading (B/HLU) groups. Rats received a 40% total body surface (TBSA) full thickness scald burn, and rats with hindlimb unloading were placed in a tail traction system. At d 14, lean body mass (LBM) was determined using DEXA scan, followed by measurement of the isometric mechanical properties in the predominantly fast-twitch plantaris muscle (PL) and the predominantly slow-twitch soleus muscle (SL). Muscle weight (wt), protein wt, and wet/dry wt were determined.**

***Results.*** **At d 14, body weight had decreased significantly in all treatment groups; B/HLU resulted in significantly greater loss compared with the B/A, S/HLU, and S/A. The losses could be attributed to loss of LBM. PL muscle wt and Po were lowest in the B/HLU group ($<0.05$ *versus* S/A, S/HLU, or B/A). SL muscle wt and Po were significantly less in both S/HLU and B/HLU compared with that of S/A and B/A; no significant difference was found between S/HLU and B/HLU.**

***Conclusions.*** **Cutaneous burn and hindlimb unloading have an additive effect on muscle atrophy, characterized by loss of muscle mass and decrease in muscle strength in both fast (PL) and slow (SL) twitch muscles. Of the two, disuse appeared to be the dominant factor for continuous muscle wasting after acute burn in this model.** 

***Key Words:*** **hindlimb unloading; thermal injury; skeletal muscle; muscle function.**

## INTRODUCTION

Severe trauma and burn induce a sustained hypermetabolic response characterized by increased protein catabolism causing profound loss of lean body mass [1, 2], which is associated with immunologic compromise [3], slowed wound healing, and, in children, growth delay [4]. Catabolism with sustained loss of muscle mass, as well as loss of muscle strength, delays the return to customary pre-injury activities after severe burn. Although administration of nutrient support during hospitalization has been shown to reduce weight loss in severely burned [5] and other critically ill patients [6], these reductions are only partial, and do not fully compensate for the massive wasting of peripheral musculature [7].

It is well established that the hypermetabolic state is not resolved rapidly after burn and complete wound healing, but lasts for at least 9 to 12 mo after burns over 40% TBSA [2, 8]. This results in continuous erosion of lean body mass, from a reduction in muscle protein synthesis, and increase in protein catabolism [2] thus delayed recovery in muscle strength [9] during convalescence. Mechanisms leading to the long-term effect of burn on muscle catabolism have not been fully elucidated. However, one of the known factors associated with long-term muscle catabolism is muscle disuse caused by inactivity, which is common during and after hospitalization in severely burned patients. Wolfe *et al.* showed that muscle inactivity caused by bed rest

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resulted in a significant decrease in skeletal muscle and whole body protein synthesis in human subjects [10]. They also found that bed rest amplified the normal catabolic responses of skeletal muscle to cortisol, one of the catabolic hormones released after surgery or injury. Prolonged bed rest and hypercortisolaemia exacerbated strength and lean muscle loss *via* a chronic reduction in muscle protein synthesis [11, 12]. These results suggest that either burn and muscle disuse or, more likely, as a combination contribute to chronic and long-term hypermetabolism after severe injury. However, this notion has not been clearly identified in either animal models or in patients.

A rat model of 40% total body surface area (TBSA) full-thickness burn induces the hypermetabolic response and muscle catabolism independent of effects of inactivity, as activity in these animals was not restricted [13, 14]. However, inactivity as an additional component to the catabolic factors associated with severe burn has not been tested in this familiar model, and thus the clinical scenario of severe burn combined with inactivity during the healing period seen in patients has not been fully elucidated in the laboratory. We hypothesize that muscle disuse plays an additive role in burn-induced muscle catabolism. Therefore, in this study, we sought to test whether combining the standard Walker-Mason rat burn model with hindlimb unloading (HLU) to simulate the clinical scenario accompanying treatment of the severely burned elucidates additive effects compared wit burn or inactivity alone. In this study, we tested the effects of burn and HLU independently and combined to identify the role of burn, HLU, or the combination on muscle mass and strength after injury.

## METHODS

All procedures were reviewed and approved by Institutional Animal Care and Use Committees (IACUC) at the US Army Institute of Surgical Research. Forty male Sprague-Dawley rats weighing approximately 300 g were randomly and evenly assigned to four groups; one animal died due to anesthesia at the time of injury, thus 39 animals were studied: sham/ambulatory (S/A; $n = 10$); burn/ambulatory (B/A; $n = 9$); sham/hindlimb unloading (S/HLU; $n = 10$), and burn/hindlimb unloading (B/HLU; $n = 10$). The study was conducted in the animal facility at the US Army Institute of Surgical Research. Rats were housed individually in specialized HLU metabolic cages [15] in a temperature-controlled environment with a 12-h light/dark cycle. The animals were acclimatized in HLU metabolic cages and fed powdered food (Teklad Global Diets #2018; Harlan, Houston, TX, USA) for 5 d before the study.

### Burn

Animals were anesthetized with continuous 1.5%–3% isoflurane (Forane; Baxter Healthcare Corp., Chicago, IL) in 100% oxygen using a nose cone. Rats in both sham and burn groups were shaved on the dorsal and ventral surface of the trunk. Animals in the burn groups received a 40% total burn surface area (TBSA) by immersing the dorsum in 100°C heated water for 10 s and ventral surface for 2 s according to the modified Walker-Mason burn model [13, 16]. Burned rats were resuscitated with 20 mL intraperitoneal Ringer's lactate solution immediately following the burn.

### Hindlimb Unloading

HLU was performed according to the model described by Morey-Holton and Globus [17]. Briefly, the rat's tail was cleaned with alcohol; tincture of benzoin was applied and allowed to become tacky to the touch. A half inch strip of Skin Tac (Zimmer, San Jose CA) was secured on the tail, wrapped in a stockinette, and 1 in. strips of filament fiber tape applied at the base, middle, and top. The tip of the tail remained exposed in order to monitor circulation. Animals were allowed to fully recover from anesthesia, and then attached to a fish-line swivel hanging from the unloading device, which rides along two parallel sides of the cage. The angle and height of the rats were adjusted to a 30° angle from the cage floor, and thus the hind feet of the rats were not able to touch the grid floor of the cage. The rats were allowed to move on an $x$-$y$ axis and rotate 360°, and the range of movement was adjusted so that they could freely access food and water but their hindlimbs were kept from contacting the walls of the cage. The ambulatory animals were kept in identical cages without tails attached to the harness. Analgesia was administered for the first 24 h following injury (0.05 mg/kg buprenorphine, q12 h). Body weight, food and water consumption, and urine and fecal output were monitored and recorded daily.

### Muscle Protein Content

At d 14 after injury, the SL and PL from the right hindlimb were isolated under isoflurane anesthesia, dissected, and weighed. A small piece of muscle sample from each muscle was dissected, weighed and placed in a drying oven at 50°C for 5 d and weighed again for its dry weight. The ratio of wet weight/dry weight was determined. The remaining muscle samples were snap-frozen and stored at –70°C for later determination of muscle protein content.

PL and SL muscle samples (about 30 mg) were processed for total protein extraction. Briefly, the muscle samples were snap-frozen in liquid nitrogen and pulverized in BioPulverizer (Biospec Product, Bartlesville, OK, USA). The samples were then immediately homogenized in the lysis buffer (Cell Signaling Technology, Danvers, MA) containing 20 mM Tris-HCL, 150 mM Nacl, 1 mM $Na_2EDTA$, 1 mM EGTA, 1% Triton, 2.5 mM sodium pyrophosphate, 1 mM $\beta$-glycerophosphate, 1 mM $Na_3VO_4$, 1 ug/mL leupeptin. The protein suspension was extracted after centrifugation, and protein concentration was measured by BCA Protein Assay (Thermo Scientific, Rockford, IL). The total amount of protein weight per muscle (PL or SL) and percentage of protein content (protein weight per muscle weight of PL or SL; based on whole muscle weight) were then determined.

### Isometric Mechanical Properties

Following muscle collection from the right hindlimb, muscle isometric force of PL and SL was measured simultaneously in the left hindlimb under anesthesia. First, the posterior thigh was opened to isolate and expose the sciatic nerve. The distal portion of the sciatic nerve was implanted into an electrode cuff with wires connected to a pulse stimulator (A-M Systems, Inc., model 2100, Sequim, WA, USA). After securing the electrode cuff, the proximal portion of the sciatic nerve was cut from its connection to the spinal cord. The distal tendons from both PL and SL were dissected and cut carefully without interrupting blood and nerve supply. They were then connected to two lever arms and secured with 4-0 silk suture separately. The lower leg was secured horizontally on the working platform by a combination of a pin drilled through the knee, and tape and bar to stabilize the ankle. The skin and superficial fascia around the opened wound were retracted to make a reservoir to hold warm mineral oil to maintain the temperature between 36.5 to 37.5°C monitored using a digital