Burn Serum Stimulates Mitochondrial Fission in C2C12 Myoblasts

by

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Burn Serum Stimulates Mitochondrial Fission in C2C12 Myoblasts

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Background: *Burn patients suffer muscle mass loss associated with a hypercatabolic status. Impairment of mitochondrial function has been observed in the muscle of burn patient's.*

Objective: *We hypothesize that muscle atrophy due to burn injury is associated with an alteration in mitochondrial dynamics. This study was designed to investigate changes in mitochondrial fission and fusion in response to a burn serum challenge in myoblasts in vitro*

Methods: *Cultured murine myoblasts, C2C12 cells, were exposed to 10% rat serum isolated either from 40% total body surface area (TBSA) scald burn rats or control rats. Cells were then labeled with MitoTracker Green dye and live cell images were recorded by confocal microscopy. The expression of mitochondrial fission/fusion factors was examined by Western blots.*

Results: *In cells cultured with 10% serum from control rats, mitochondrial morphology maintained the elongated linear shape during the 48 hours we observed. However, 24 hours of culturing in 10% scaled rat serum resulted in a significant reduction in mitochondrial size. .Further, the number of total mitochondria increased, indicating a stimulation of mitochondrial fragmentation in response to the burn serum challenge. In addition, burn serum increases the total volume of mitochondria about 1.4 fold. Consistently, Western blot analysis showed a significant decrease in the expression of mitochondrial fusion protein Mfn1.*

Conclusion: *Burn serum stimulates an increase in mitochondria fission in myoblasts.*

TABLE OF CONTENTS

CHAPTER 1 – Introduction

Burn patients suffer muscle mass loss associated with a hypercatabolic state. The catabolic state initially increases the rate of muscle proteolysis so that new skin and scar formation can be produced from the stores of amino acids in the muscle.[1] However, burn patients could stay in this hypercatabolic state for at least 9 months post burn trauma.[2] The factors that put a patient at an increased risk for catabolism are increased age, increased muscle mass (weight), and the delay of surgical treatment.[2] The extended hypermetabolic state can lead to detrimental effects such as cachexia, which increases overall mortality.[4] Cachexia interferes with muscle rehabilitation and the nutritional supplementation needed for patients to have improved health outcomes.[5]

Mitochondria are abundant in muscle, and the impairment of mitochondrial function has been observed after thermal injury.[10,11] Mitochondria oxidative function can be decreased as much as 50% post burn.[12,13] One hour post burn, high levels of cytochrome C can be detected in the serum, indicating that mitochondria are dumping cytochrome C in response to the damage.[14] Mitochondrial function has been improved with treatment with anabolic agents, such as insulin.[15] However, it is currently unclear how burns change the mitochondrial morphology and metabolism in skeletal muscle.

Mitochondrial structure is maintained dynamically. Mitochondrial dynamics includes a fission/fusion cycle which serves as the apoptotic method of how mitochondria eliminate defective segments (mitophagy).[16] When mitochondria die, they release cytochrome C and that cytochrome C can activate Caspase 3 to begin the process of mitophagy. The cytochrome C activates apoptosis activating factor 1 (Apaf-1) and Caspase 9. This leads to the processing

of pro-caspase 3 and ultimately mitophagy.[17] In this study, we hypothesized that severe burn increases mitochondrial fission and thus activates subsequent muscle cell death.

Burn serum has been shown to contain dysregulated cytokines and metabolites after burn,[21] and we applied serum stimulation to observe mitochondrial changes in real time and to begin to understand the process of how burn trauma affects mitochondria in muscle cells in vitro. To do this, we started with C2C12 mouse muscle cells and used mitochondrial trackers to observe these changes.[18] The ability to observe these morphological changes in vitro will help us understand the processes occurring at the molecular level and develop effective therapies to combat hypercatabolism.

CHAPTER 2 – Experimental Procedures

Culture C2C12 cells

C2C12 murine myoblasts (ATCC, Manassas, VA) were incubated in a humidified 37°C incubator with consistent 5% CO_2 and 95% O_2. Cells were grown in Dulbecco's Modified Eagle Medium (DMEM) culture medium containing 1% penicillin/streptomycin, 1% glutamate, and 10% fetal bovine serum (FBS). Culture media was changed every 48 hours.

Once C2C12 cells reached 70% confluence, cells were detached with 0.25% trypsin incubation. Cell were stained with 0.4% trypan blue and loaded in a hemocytometer for cell counting under a Nikon light microscope. Then 0.1 x 10^6 cells/well were plated into a 12-well culture plate for 24 hours before the experiment. Additionally, 0.08 x 10^6 cells were plated into a 12 mm petri dish with a glass coverslip (Thermo Scientific, Waltham, MA). The cells were precultured for 24 hours with culture media before the serum stimulation experiment. Each experiment was repeated in triplicate.

Burn rat serum collection

Rat serum was harvested from adult male Sprague Dawley rats (Charles River Laboratories, Wilmington, MA). Burn rats received a 40% TBSA scald burn. The burn procedure was described before and approved by UTSW IACUC.[19] Briefly, anesthetized rats received 40% TBSA of scald burn on the back following the Parkland formula for resuscitation and analgesics. Three rats were euthanized at 6 and 48 hours after the burn for blood collection. Rat blood was collected in a serum separation tube (BD, Franklin Lakes,

NJ), then supernatant serum was separated at 2500 rpm centrifugation for 30 minutes at 4ºC. The serums that would be selected to stimulate cells were chosen randomly and from different burn rats. Serum collection was also collected from 3 non-burn normal rats to serve as a control.

C2C12 cells stimulated with the medium containing rat serum

Culture media was taken out from the12 mm glass base dishes with about 30% confluent C2C12 cells. Afterwards, 1.5 ml of DMEM containing either 10% normal rat serum, 10% post 6-hour burn rat serum, or 10% post 48-hour burn serum was placed into each dish. Cells were then allowed to incubate at 37ºC and 5% CO_2 for 2 days.

Fluorescent image of live cell mitochondria staining

Additionally, 0.4 µl of 1 µM MitoTracker Green (Life Technologies, Waltham, MA) was added to the dishes 15 minutes prior to an image being taken at the first time point of the experiment. MitoTracker Green is a dye that is only fluorescent when accumulated in the mitochondrial lipid environment and bound to the mitochondrial membrane, regardless of membrane potential.[22] Live cell images were taken under a 60x oil objective lens by using a Nikon Eclipse TI fluorescent microscope with NIS element AR 4.200 software (Nikon, Tokyo, Japan). The settings of the microscope were: offset = -2, objective = 60x, filter = PGFP, .448 = 1.08, HV(G) = 50, and pinhole = 1.2. A stack of pictures was taken with 0.2-0.5 micron per slice and 15-25 slices in total per view. Five scope views were randomly taken of each dish. Images were collected at the 0-, 6-, 12-, 24-, and 48-hour time points.

The images were processed with NIS element AR4.200 software to convert them to a JPEG file. The images were then analyzed using Nikon software to evaluate mitochondrial volume, fluorescent intensity, and elongation. To identify mitochondria, a binary histogram was established with a range from 2083 to 4095 and images were scrolled throughout the Z-stack to compare and make sure that the binary was as close as possible to the actual image. To analyze mitochondrial volume, the software processed the entire Z-stack of images in a cell to calculate a final volume. To analyze both intensity and elongation of mitochondria, the middle slice of the Z-stack was selected to be representative of the cell and the data collected from that slice only. This was repeated with 5 different cells to check precision.

Western blot

In addition, 0.1 x 10^6 cells/well were plated into a 12-well culture plate for 24 hours. Cells were stimulated with 1.5 ml of DMEM containing either 10% normal rat serum or 10% post 6-hour burn rat serum. The cell lysate was collected at 48 hours and 30 µg were subsequently analyzed by SDS-PAGE and Western blot following the published procedure.[23] Band intensities were quantified with the GeneSnap/GeneTools software (Syngene, Frederick, MD). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was utilized as a loading control. All antibodies, including Mitofusin 1 and 2 (Mfn1&2), optic atrophy (Opa1) and dynamin-related protein (Drp1), were purchased from Cell Signaling Technology (Danvers, MA). SuperSignal West Pico Chemiluminescent Substrate was purchased from Thermo Scientific (Rockford, IL).

Statistical analysis

Results are presented as a weighted average ± standard error of the mean (SEM). The data were analyzed using paired Student's *t* tests, single factor ANOVA, and Tukey-Kramer post-hoc tests. Differences were considered significant at $p < .01$ for the paired Student's *t* test and $p < .05$ for the single factor ANOVA.

CHAPTER 3 - Results

Data analysis began by visually examining the morphological characteristics of the mitochondria. Using the stack of images taken with the confocal, a 3D volume image was created comparing the 10% normal serum-treated cell and the 10% post burn 6-hour serum-treated cell [Figure 1]. Visual observation showed that the normal serum mitochondria retained a normal rod-like shape and were less dense in the cell compared to the burn serum mitochondria, which lost their rod-like morphology and also increased their density within the cell. We then converted our confocal image slices into JPEG images and arranged them in a time progression comparing the normal serum (Nmrs) and post 6-hour burn serum (B6hrs) images [Figure 2]. We observed a change in the mitochondrial brightness at both the 24- and 48-hour time point in the B6hrs group.

Compared to normal rat serum stimulation, the volume of mitochondria increased significantly with post 6-hour burn serum stimulation only at the 6-hour time point ($p < .01$) [Figure 3]. There was about a 1 fold increase of mitochondrial volume with burn serum stimulation at the 12- and 48-hour time points compared to the first 0-hour time point ($p < .05$).

The fluorescent intensity emitted from cell mitochondria stained with MitoTracker Green was also measured [Figure 4]. The signal intensity of the post 6-hour burn serum group was significantly increased when compared to the normal serum group at all time points except at the 12-hour time point, where it was decreased ($p < .01$). There was a significant difference between both the post 6-hour burn serum and the normal serum groups at each time point ($p < .01$). Compared to the 0-hour time point, fluorescent intensity was decreased

in all groups except for the normal serum at the 12-hour time point. Both groups also were significantly different from the 0-hour time point as time progressed in each group ($p < .05$).

Mitochondrial elongation, which is a unitless parameter of the Nikon software measuring shape morphology, was also measured. This software uses max-Feret and min-Feret values to analyze shape characteristics, in this case, the characteristic of a rod. The higher the elongation index, the more rod-like the object is, and the lower the elongation index, the less it is rod-like. Mitochondrial elongation was significantly decreased between the post 6-hour burn and normal serum group only at the 48-hour time point ($p < .01$). Both groups also were significantly different from the 0-hour time point as time progressed in each group ($p < .05$) [Figure 5].

Fluorescent images and elongation data were collected at the 48-hour time point comparing normal rat serum, post 6-hour burn serum, and post 48-hour burn serum. The morphology change in the post 6-hour burn serum stayed consistent at the 48-hour time point as seen with the previous experiments. The post 48-hour burn serum was observed visually to have long elongated mitochondria and morphologically resembled the normal serum group. The elongation index of the post 6-hour burn serum was significantly lower than the normal serum group at the 48-hour time point ($p < .01$). The elongation index of the post 48-hour burn serum was significantly higher than the normal serum group at the 48-hour time point ($p < .01$) [Figure 6].

The cell lysate was collected from C2C12 cells 48 hours after stimulation with 10% normal serum and post 6-hour burn serum. The mitochondrial fusion proteins Opa1 and Mfn1&2 were measured along with the mitochondrial fission protein Drp1. There was a

significant decrease of Mfn1 ($p < .05$), which is the initiator of the mitochondrial fusion process, at the 48-hour time point [Figure 7].

CHAPTER 4 – Conclusions and Recommendations

Our study is the first instance that mitochondrial morphology in response to burn serum stimulation was observed in vitro and that morphology was converted into a statistically significant numerical value. We found a continued increase in mitochondrial volume in muscle C2C12 cells with 10% burn rat serum stimulation, with the significant difference being at the 6-hour time point. The fact that we also established a significant increase in mitochondrial volume between the 0-hour and 12-hour time point and the 0-hour and 48-time point in the post 6-hour burn serum also shows a significant trend of increasing volume. This increasing volume is reflective of an increase in the fission/fusion ratio of the mitochondrial cycle and also a possible increase in mitochondrial swelling. In contrast, we did not see the similar alteration trend with normal rat serum, suggesting that the normal serum does not change its fission/fusion cycle and maintains normal mitochondrial equilibrium.

The mitochondrial intensity was also significantly different in cells treated with burn and normal rat serum, with burn serum stimulation showing an increase in mitochondrial fluorescent intensity. This increase in intensity also is reflective of an increase in the fission/fusion ratio. This is due to the fact that the intensity signal is generated by MitoTracker Green, which only fluoresces if it is within the mitochondrial lipid environment, regardless of the membrane potential. MitoTracker Green binds to available mitochondrial membrane, so the increase in surface area from increased fission would provide more area for the MitoTracker to bind to and increase the signal intensity.[22] Within the burn serum, the significant difference between time points also indicated an increase in intensity. The 12-hour

time point breaks this trend and we believe this could be due to the methods of collecting our images. Each time point takes about 3 hours to collect images and we believe that this might have disturbed the cells in between collection from the 6-hour and 12-hour time point and caused an additional stressor besides the burn serum.

Finally, mitochondrial elongation is a morphological measure in the TI Nikon software that establishes parameters to measure what is rod-like. It uses max-Feret and min-Feret values to measure the shape characteristics of an object and determine if it is rod-like. The higher the elongation index, the more rod-like the object in question, and the lower the elongation index, the less rod-like the object. Normal mitochondria have a rod-like structure and a decrease in elongation would suggest a loss of this rod-like structure.[26] There was a significant decrease in this elongation, suggesting a loss of normal morphology, between the post 6-hour burn serum and the normal serum stimulation at the 48-hour time point. This matches nicely with the 3D volume image we took at the 48-hour time point that shows a drastic difference in volume and morphology. However, there was a significant increase in this elongation between the post 48-hour burn serum and the normal serum at the same time point. Previous studies have shown that inflammatory cytokines such as IL-6 and IL-1β are at higher concentrations in post 6-hour burn serum than post 48-hour burn serum, which may explain the difference in mitochondrial elongation.[21] There was also a significant difference in all groups when comparing each group to its time 0 starting point. This suggests that no matter whether burn or normal serum is used, the stimulation of cells induces an oscillation of mitochondrial shape characteristics that needs to be examined further.[24,25] These data parameters of volume, intensity, and elongation provide numerical significance and

correlation with our observations from 3D imaging and our snapshot progression of each time point.

Normal mitochondria have a fission and fusion cycle, which maintains mitochondrial functionality. Fusion is upregulated during times of anabolic states, and fission is upregulated during times of catabolic states.[7] The main fusion proteins are Mfn1&2 and Opa1. As GTPase proteins, Mfn1&2 fuse the outer mitochondrial membrane while Opa1 fuses the inner mitochondrial membrane. The main fission protein is Drp1 and its associated proteins Bak and Bax.[8] Drp1 also has a GTPase domain, that once activated, spirals and cleaves the mitochondria. Bax and Bak create channels that release cytochrome C within the mitochondria.[9]

Mitochondrial fission is a part of the fusion/fission cycle that relates to the impairment of mitochondrial function. When mitochondria are damaged due to a burn or intrinsic defects, they shift towards a fission state. To begin fission, Drp1 is upregulated, which is the main fission protein. This protein binds on the mitochondrial membrane and recruits additional Drp1 proteins. A consequent chain complex wraps around the defective segment of mitochondria till Drp1 activates its GTPase and further recruits transmembrane proteins Bax and Bak to form the channel for cytochrome C releasing out of the mitochondria. It is important to realize that the increase in mitochondrial volume observed in our experiment was due to this increased fission pathway that increases the volume of defective, not healthy, mitochondria. We are currently investigating the mechanisms of mitochondrial fission in muscle cell death after burn.

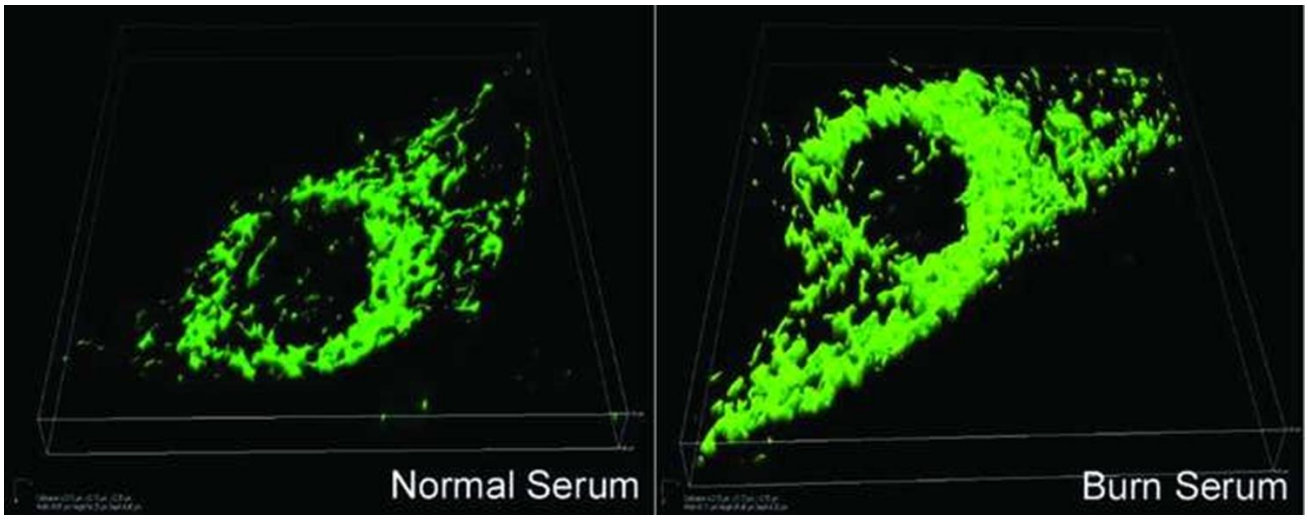
Burn trauma patients show an increase in hypercatabolism that leads to cachexia and a

poor prognosis. Mitochondria is at the center of this shift of metabolism from an anabolic to hypercatabolic state, and seeing how the mitochondria respond to burn serum and observing their morphological changes is the first step in understanding what is occurring at the cellular level. The treatment of post-burn trauma patients with anabolic agents such as insulin, recombinant human growth hormone, oxandralone, and propranolol have been shown as effective solutions to combat the hypercatabolic state and reverse it.[3,6,20] The fact that these anabolic agents improve the cachexia seen in post-burn trauma patients and allows them to continue with their muscle rehabilitation treatments suggests that metabolic control post burn is an effective route to open up new therapies to perhaps slow down the rate of catabolism and allow for patients to have a better prognosis.

The clinical relevance of this study involves understanding the mechanisms of mitochondrial dynamics after burn and potential treatments that can reverse the hypercatabolic state and slow it down. Further studies are necessary to analyze the content of the burn serum and measure which inflammatory cytokines or molecules are present that could be leading to these morphological changes. Once understood, further experiments with inhibitors of those cytokines can be performed to see if there is a change in morphology or function of the mitochondria

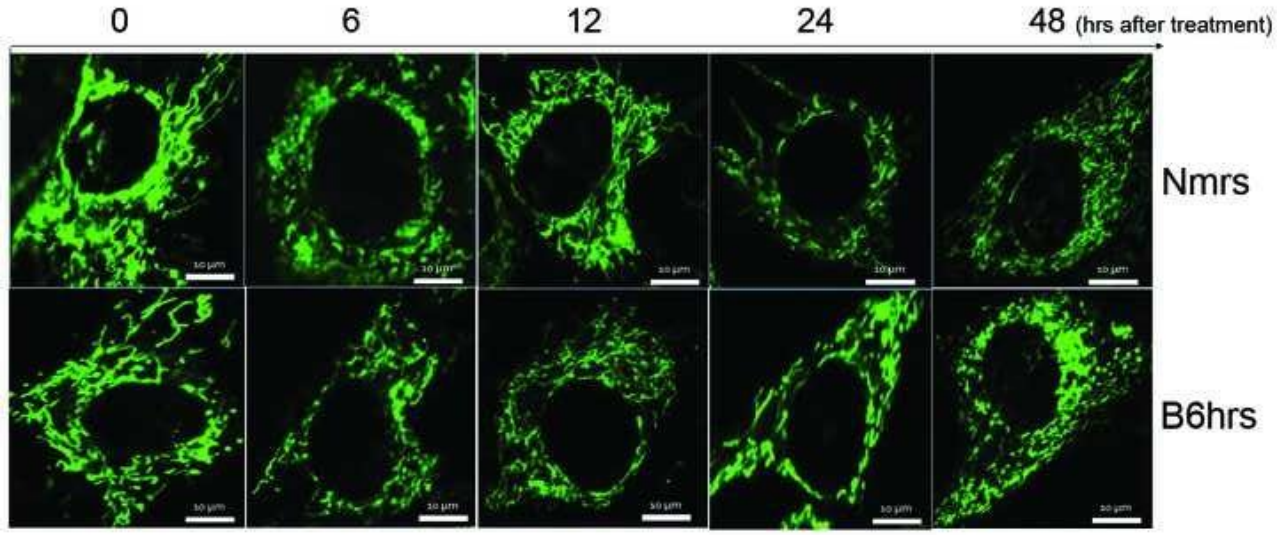
LIST OF FIGURES

Figures 1:



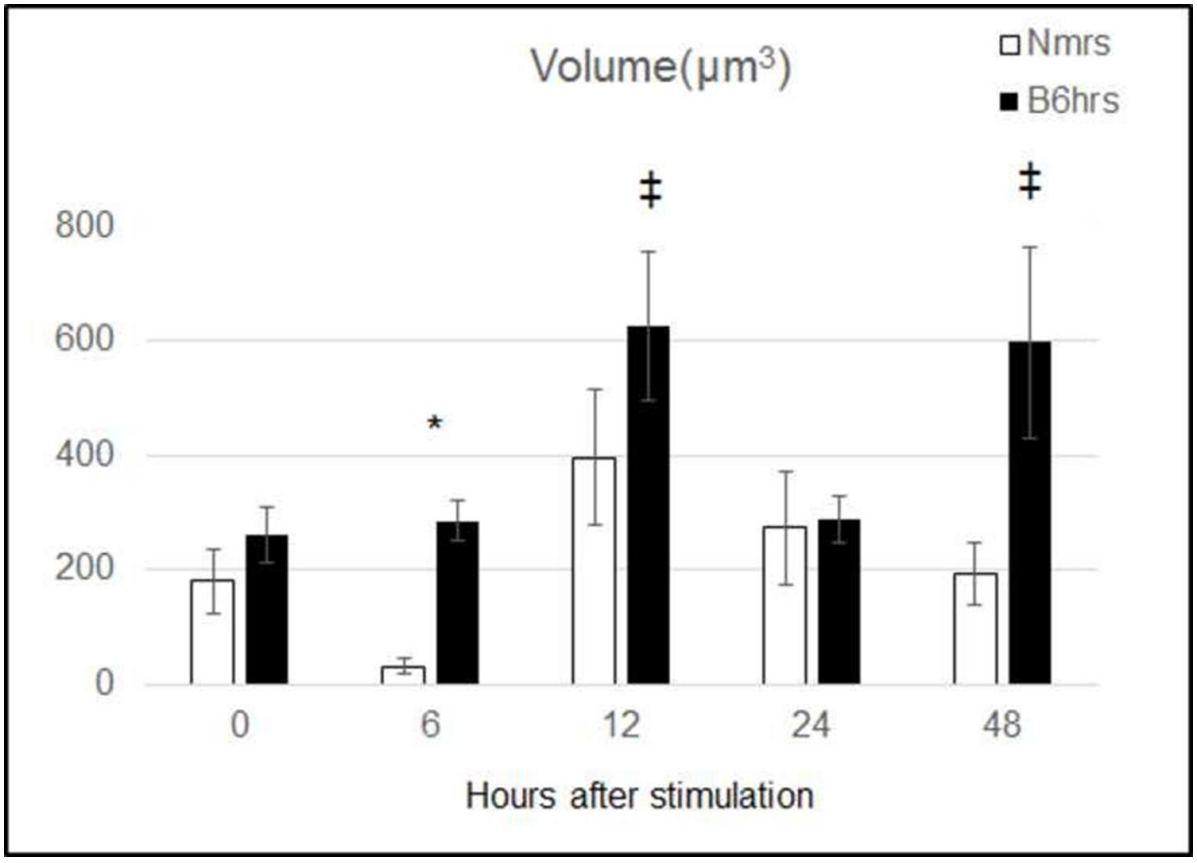
A 3D cell volume image showing the intensity and volume size of MitoTracker Green-stained cell mitochondria with normal and post 6-hour burn serum treatment at the 48-hour time point. The left image shows normal rod-like elongation of mitochondria while the right image shows a loss of normal elongation morphology and an increased density of mitochondria.

Figure 2:



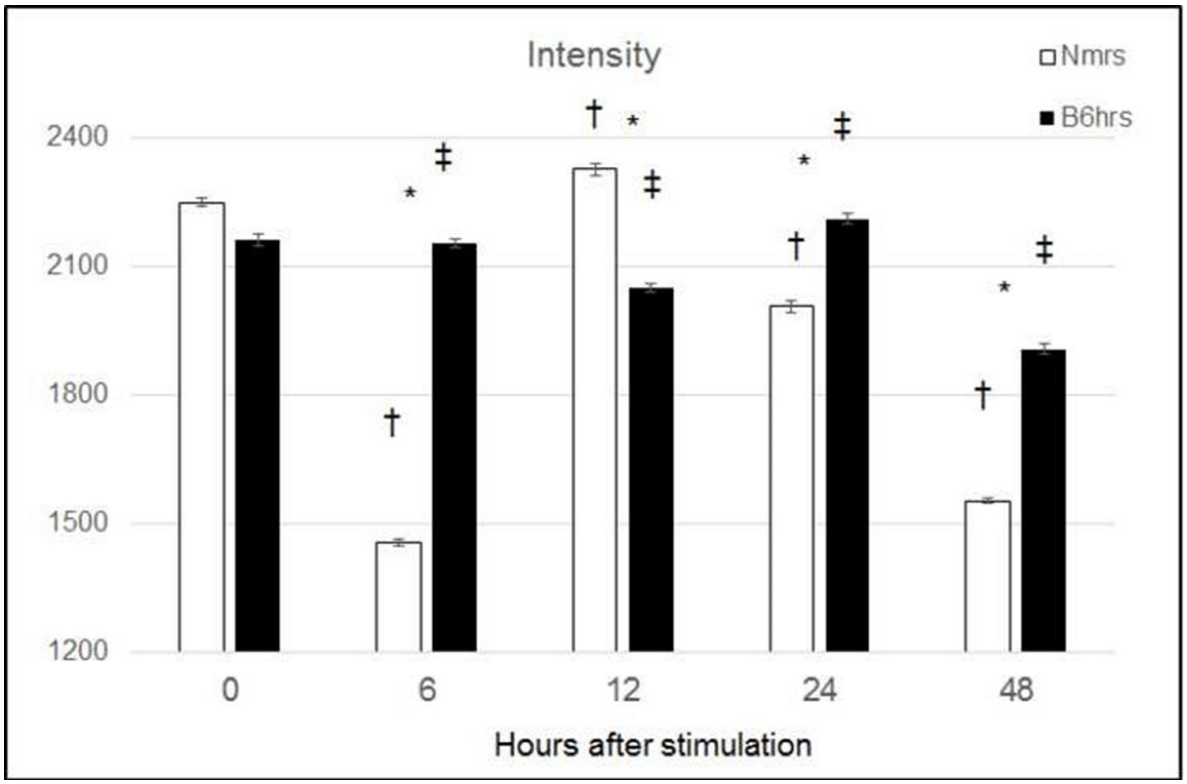
Fluorescent images stained with MitoTracker Green, showing a time course of cell mitochondrial distribution in C2C12 cells treated with normal serum (Nmrs) and post 6-hour burn serum (B6hrs).

Figure 3:



The total volumes of the mitochondria in cells exposed to normal and post 6-hour burn serum were compared at the same time points. A significant difference in volume was found between the cell groups at the 6-hour time point ($p < .01$) and also within the post 6-hour burn group over time ($p < .05$). * Significant difference at the same time point between cells exposed to either the normal serum or burn serum † Significant difference at 0, 6, 12, 24, and 48 hours between cells exposed to either the normal serum or burn serum

Figure 4:

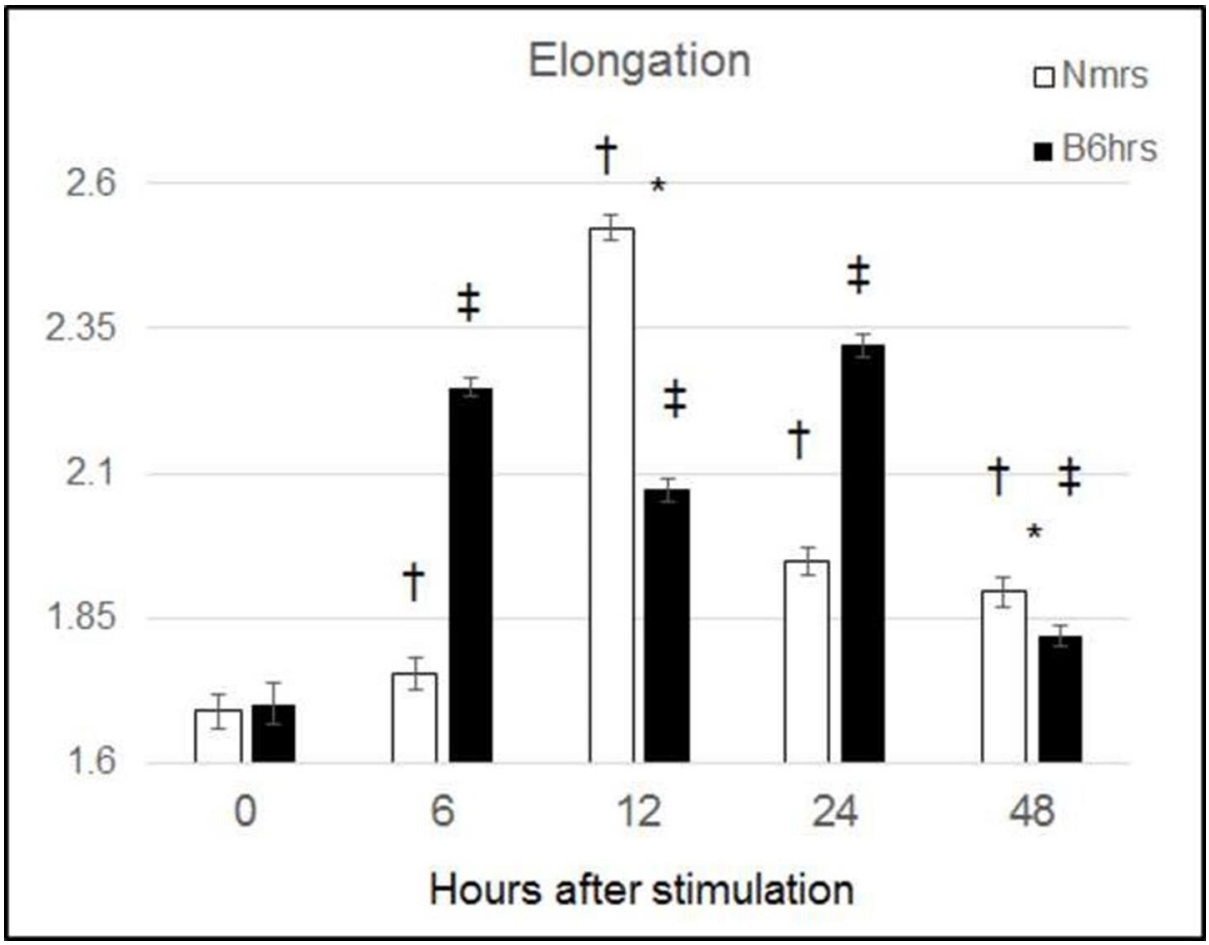


The intensity of the signal from the MitoTracker Green-labeled mitochondria was measured. This signal is only captured if MitoTracker Green has accumulated in the mitochondrial lipid environment, regardless of membrane potential.

* Significant difference at the same time point between cells exposed to either the normal serum or burn serum

† Significant difference at 0, 6, 12, 24, and 48 hours between cells exposed to either the normal serum or burn serum

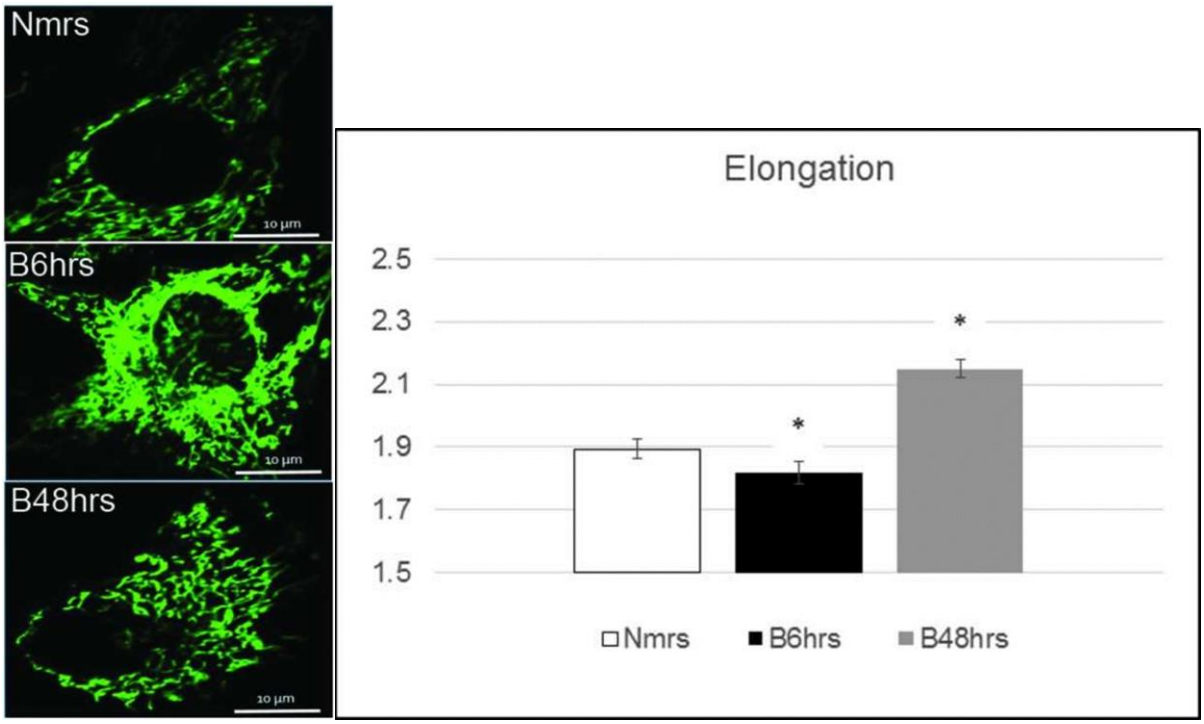
Figure 5:



The elongation of the mitochondria, which is a parameter measured by the Nikon Ti Eclipse software. The software measures how rod-like an object is and expresses it as an elongation index. The higher the index, the more rod-like it is, and the lower, the less rod-like. * Significant difference at the same time point between cells exposed to either the normal serum or burn serum

† Significant difference at 0, 6, 12, 24, and 48 hours between cells exposed to either the normal serum or burn serum

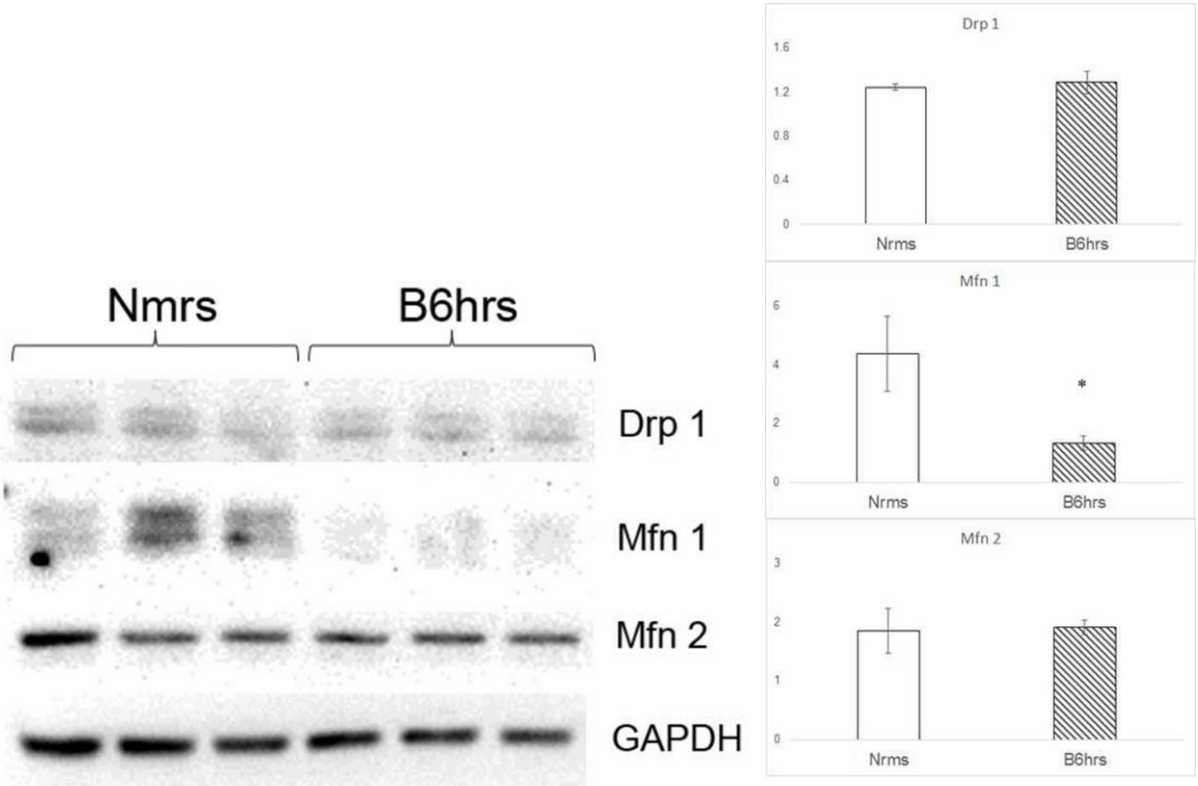
Figure 6:



On the left are fluorescent images stained with MitoTracker Green, showing the 48-hour time point of cell mitochondrial distribution in C2C12 cells treated with normal serum (Nmrs), post 6-hour burn serum (B6hrs), and post 48-hour burn serum (B48hrs). On the right is the elongation of the mitochondria at the 48-hour time point of cell mitochondria in C2C12 cells treated with Nmrs, B6hrs, and B48hrs.

* Significant difference at the same time point between cells exposed to either the normal serum or burn serum

Figure 7:



Western blot on cell lysate of C2C12 cells collected 48 hours after stimulation with 10% normal serum and 10% post 6-hour burn serum to measure levels of mitochondrial fusion and fission proteins. There was a significant decrease ($p < .05$) in the mitochondrial fusion protein Mfn1.

REFERENCES

1. Biolo G, Fleming RY, Maggi SP, et al. Inverse regulation of protein turnover and amino acid transport in skeletal muscle of hypercatabolic patients. *J Clin Endocrinol Metab*. 2002;87:3378–3384.
2. Hart DW, Wolf SE, Mlcak R, et al. Persistence of muscle catabolism after severe burn. *Surgery*. 2000;128:312–319.
3. Hart DW, Wolf SE, Ramzy PI, et al. Anabolic effects of oxandrolone after severe burn. *Ann Surg*. 2001;233:556–564.
4. Jeschke MG, Gauglitz GG, Kulp GA, et al. Long-term persistance of the pathophysiologic response to severe burn injury. *PLoS One*. 2011;6:e21245.
5. Yasuhara S, Perez ME, Kanakubo E, et al. Skeletal muscle apoptosis after burns is associated with activation of proapoptotic signals. *Am J Physiol Endocrinol Metab*. 2000;279:E1114–E1121.
6. Gore DC, Wolf SE, Sanford AP, et al. Extremity hyperinsulinemia stimulates muscle protein synthesis in severely injured patients. *Am J Physiol Endocrinol Metab*. 2004;286:E529–34.
7. Chen H, Chan DC. Mitochondrial dynamics—fusion, fission, movement, and mitophagy—in neurodegenerative diseases. *Hum Mol Genet*. 2009;18(R2):R169–R176.
8. Gao AW, Canto C, Houtkooper RH. Mitochondrial response to nutrient availability and its role in metabolic disease. *EMBO Mol Med*. 2014;6(5):580–9.

9. Babbar M, Sheikh MS. Metabolic stress and disorders related to alterations in mitochondrial fission or fusion. *Mol Cell Pharmacol*. 2013;5:109–133.
10. Porter C, Herndon D, Sidossis L, et al. The impact of severe burns on skeletal muscle mitochondrial function. *Burns*. 2013;39:1039–1047.
11. Padfield KE, Astrakas LG, Zhang Q, et al. Burn injury causes mitochondrial dysfunction in skeletal muscle. *PNAS*. 2005;102(15):5368-5373.
12. Porter C, Herndon D, Borscheim E, et al. Uncoupled skeletal muscle mitochondria contribute to hypermetabolism in severely burned adults. *Am J Physiol Endocrinol Metab*. 2014;307:462–467.
13. Cree MG, Fram RY, Herndon DN, et al. Human mitochondrial oxidative capacity is acutely impaired after burn trauma. *Am J Surg*. 2007;196:234–239.
14. Suen DF, Norris KL, Youle RJ. Mitochondrial dynamics and apoptosis. *Genes Dev*. 2008;22:1577–90.
15. Fram RY, Cree MG, Wolfe RR, et al. Intensive insulin therapy improves insulin sensitivity and mitochondrial function in severely burned children. *Crit Care Med*. 2010;38:1475–1483.
16. Jheng HF, Tsai PJ, Guo SM, et al. Mitochondrial fission contributes to mitochondrial dysfunction and insulin resistance in skeletal muscle. *Mol Cell Biol*. 2012;32:309–319.
17. Porter AG, Janicke RU. Emerging roles of caspase-3 in apoptosis. *Cell Death Differ*. 1999;6(2):99–104.

18. Burattini S, Ferri R, Battistelli M, et al. C2C12 murine myoblasts as a model of skeletal muscle development: morpho-functional characterization. *Eur J Histochem*. 2004;48(3):223–233.

19. Carlson DL, Maass DL, White J, et al. Caspase inhibition reduces cardiac myocyte dyshomeostasis and improves cardiac contractile function after major burn injury. *J Appl Physiol.* (1985). 2007 Jul;103(1):323-30.

20. Herndon DN, Tompkins RG. Support of the metabolic response to burn injury. *Lancet*. 2004;363(9424):1895–1902.

21. Gauglitz GG, Song J, Herndon DN, et al. Characterization of the inflammatory response during acute and post-acute phases after severe burn. *Shock*. 2008; 30:503–507.

22. Pendergrass W, Wolf N, Poot M. Efficacy of MitoTracker Green and CMXrosamine to measure changes in mitochondrial membrane potentials in living cells and tissues. *Cytometry A*. 2004;61:162–169.

23. Song J, Zhang XJ, Boehning D, et al. Measurement of hepatic protein fractional synthetic rate with stable isotope labeling technique in thapsigargin stressed HepG2 cells. *Int J Biol Sci*. 2012;8:265–27.

24. Ihekwaba AE, Broomhead DS, Grimley R, et al. Sensitivity analysis of parameters controlling oscillatory signalling in the NF-κB pathway: the roles of IKK and IκBα. Syst Biol. 2004;1:93-103.

25. Beraud N, Pelloux S, Usson Y, et al. Mitochondrial dynamics in heart cells: very low amplitude high frequency fluctuations in adult cardiomyocytes and flow motion in non beating Hl-1 cells. *J Bioenerg Biomembr*. 2009;41:195–214.

26. Mai S, Klinkenberg M, Auburger G, et al. Decreased expression of Drp1 and Fis1 mediates mitochondrial elongation in senescent cells and enhances resistance to oxidative stress through PINK1. *J Cell Sci*. 2010;123:917–926.

VITAE

Alvand Sehat (November 19th, 1990 - present) is a fourth-year medical student at UT Southwestern Medical School pursuing a career in General Surgery. During his time at UTSW he completed over 20 weeks of dedicated research time in the Burn/Trauma/Critical Care lab under the mentorship of the former Vice-President of Research in the Department of Surgery, Steven Wolf. He has matched to a 5-year categorical position at Rutgers New Jersey Medical School. His interests lie in surgical oncology, hepatobiliary surgery, and MIS/robotic surgery.

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