Award Number: **W81XWH-13-1-0462**

TITLE: **Combination Therapies for the Mitigation of Musculoskeletal Pathologic Damage in a Novel Model of Severe Injury and Disuse**

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REPORT DATE: **OCTOBER 2018**

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED *(From - To)*
OCTOBER 2018	Annual	*30SEP2017 - 29SEP2018*

4. TITLE AND SUBTITLE

Combination Therapies for the Mitigation of Musculoskeletal Pathologic Damage in a Novel Model of 8evere Injury and Disuse

5a. CONTRACT NUMBER

5b. GRANT NUMBER

W81XWH-13-1-0462

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

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Dallas, TX. 75390-9105

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and
Materiel Command
Fort Detrick, MD 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Severe injury in patients results in adverse physiologic and musculoskeletal changes that are immediate and long lasting. In response to injury, metabolic and physiologic responses determine length of hospitalization and subsequent activity limitations. Reduced mobility from bed rest and injury severity affect muscle and bone health and are detrimental to rehabilitative success. Therefore, means to counteract adverse effects on muscle and bone after injury and disuse are needed. Pharmacologic (i.e insulin or oxandrolone), non-pharmacologic (i.e. exercise) and nutritional interventions have been used independently with limited success [17]. The combination of pharmacological interventions and exercise has not been systematically investigated. **We propose to determine if the administration of pharmacologic agents combined with exercise attenuates muscle atrophy and bone degradation following severe injury, disuse and re-ambulation. The current proposal is for mitigation of musculoskeletal pathologic change in polytrauma patients.**

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
U	U	U	UU	19	

Table of Contents

1. INTRODUCTION

Background: Severe injury results in musculoskeletal pathophysiologic changes to the patient that are immediate and long lasting. Reduced mobility from bed rest and injury severity affect muscle and bone health and are detrimental to rehabilitative success. We propose to determine if the combination of exercise and the use of insulin or oxandrolone will further improve muscle and bone strength and subsequent function for improved quality of life. The specific aims of this study are to: 1) Characterize the effect of resistance exercise on muscle and bone health in a validated model of burn and disuse. 2) Evaluate the effect of resistance exercise in combination with currently used pharmacological therapies (insulin or oxandrolone) on muscle and bone health in a validated model of burn and disuse. 3) Determine the interrelationship between muscle and bone after re-ambulation following pharmacological interventions and exercise. To accomplish these aims we will use our established rat model of burn and disuse for a 14 day period. Rats will be assigned to vehicle or drug treatment and will be further randomized into either exercise or no exercise groups. After 14 days, additional studies will examine the effects of re-ambulation. Analysis will be completed on blood, tissues, and bones of the study animals. This proposed project will delineate the synergistic effects of current therapy that will be directly translational to the clinical care of military polytrauma victims.

2. KEYWORDS

- Rats
- Burn
- Hindlimb Unloading
- Exercise
- Disuse
- Re-ambulation
- Insulin
- Oxandrolone

3. OVERAL PROJECT SUMMARY

Specific Aim 1 was to characterize the effect of resistance exercise on muscle and bone health in a validated model of burn and disuse. The milestones for Year 1 of the project, which were completed, included beginning and completing experiments for Aim 1, including animal, assay work, data analysis, and presentation. There were eight major tasks associated with this milestone.

Specific Aim 2 was to evaluate the effect of resistance exercise in combination with currently used pharmacological therapies (insulin or oxandrolone) on muscle and bone health in a validated model of burn and disuse. The milestone for Year 2 of the project was to complete experiments for Aim 2, including animal assay work and data analysis. There were six major tasks associated with this milestone.

Specific Aim 3 was to investigate the role of reloading and resistance exercise in the animal model, The experiment schedule for Specific Aim 3 was approved by DOD during two one-year-extension period due to key personal shift . The milestone for Year 5 was to complete experiments for Aim 3, including animal assay work and data analysis. There were eight major tasks associated with this milestone.

RESULTS SUMMARY (JSONG):

FOR SPECIFIC AIM 1:

- The resistant exercise alleviates muscle mass loss and function impairment in burn and hindlimb unloading rats

We examined muscle isometric force as the primary outcome. Isometric force significantly decreased in both plantaris and soleus under the sustained hindlimb unloading in burn rats. The positive effect of exercise was observed in slow-twitch myofiber dominated soleus. Muscle tissue mass and protein content significantly reduced in hindlimbunloading rats. Soleus mass and myofiber size was greater in burn and hindlimb unloading (B/H) rats with exercise. [Saeman MR. et al. J Surg Res]

- The reversal effect of exercise training on muscle genomic profile in rats with burn and hind limb

Micro RNA (miRNA) is a class of non-coding RNA that regulates gene expression by silencing messenger RNA. Pooling 3 animal muscle samples from each treatment group, we measured miRNA expression by using Affymetrix miRNA 4.0 Arrays and gene expression by using Affymetrix rat gene 2.0 chips.

MiRNAs and gene profiles are distinguished in response to burn, hindlimb unloading and exercise respectively. We identified 703 (57.7%) up-regulated miRNAs and 515 (42.3%) down-regulated miRNAs in the burn group compared to sham. 35 up-regulated and 12 down-regulated genes after burn in rat plantaris. 623 miRNAs were upregulated and 587 were down regulated with exercise. 41 gene transcript probes were identified including 40 down-regulated and 1 up-regulated in B/H rats with exercise. Genes related pathways are activated after burn, such as inflammation response, oxidative stress, cell cycle, cell apoptosis, calcium regulation, striated muscle contraction.

The effect of exercise alleviated miRNA and gene expression in B/H rats. miR-182 decreased -7.04 fold in B/H rats with exercise training; gene expressions of *Fgl2* in blood clotting cascade and *Col1a1* in inflammatory response pathway decreased in response to exercise training as well. In summary, miRNAs and transcript gene profiles were affected in burn and hindlimb unloading, those changes are associated with muscle pathophysiological changes, including muscle mass loss and function impairment. The muscle improvement with exercise training were also observed in gene levels with miRNA alterations. [Song J et al. J Burn Care Res. 2017]

FOR SPECIFIC AIM 2:

- The improvement of exercise and insulin pharmacological combination in rat muscle

Twenty Four animals received burn and hindlimb unloading procedure (as previous experiment) and were randomly assigned (n=6) to vehicle without exercise (VEH/NEX), insulin (pro zinc 40U daily) without exercise (INS/NEX), vehicle with exercise (VEH/EX), or insulin with exercise (INS/EX). On day 14 muscle functions were tested and tissue collected.

In summary, we observed that muscle functions including tetanic (Po) and twitch (Pt) were significantly elevated in both plantaris and soleus with insulin and excise combined treatment. No function improvement with solely insulin treatment was found at the moment. (Data values presented as mean ±SEM listed the following table)

Muscle Dimensions and Isometric Muscle Function

Parameter		Plantaris				Soleus			
Group		No Exercise		Exercise		No Exercise		Exercise	
		Vehicle	Insulin	Vehicle	Insulin	Vehicle	Insulin	Vehicle	Insulin
Muscle	Wet weight (mg)	332 ± 18	329 ± 9	348 ± 6.3	354 ± 14	119 ± 2	143 ± 23	151 ± 32	131 ± 7
	Lo (mm)	35 ± 2	31 ± 0.3	32 ± 1	32 ± 1	33 ± 1.4	29 ± 0.4	31 ± 0.9	30 ± 0.4
	PCSA (mm^2)	27 ± 3	30 ± 2	30 ± 2	#31 ± 2	5.0 ± 0.2	6.9 ± 2.3	6.7 ± 1.3	6.0 ± 0.3
Twitch Force	Pt (g)	89 ± 9	85 ± 3	92 ± 2	‡102 ± 8	10 ± 2	10 ± 2	14 ± 2	*18 ± 1
Tetanic Force	Po (g)	430 ± 31	459 ± 12	508 ± 14	†522 ± 17	38 ± 8	38 ± 9	59 ± 5	*69 ± 5
	Po/CSA (N/cm^2)	16 ± 2	15 ± 2	16 ± 1	17 ± 1	7.4 ± 2	7.0 ± 1	10 ± 2	‡12 ± 1
Pt/Po (%)		21 ± 1	19 ± 1	18 ± 0.4	20 ± 1	26 ± 1	24 ± 2	24 ± 2	26 ± 2
Fatigue	Maximum (g)	——	——	——	——	33 ± 6	34 ± 9	53 ± 4	*64 ± 4
	Minimum (g)	——	——	——	——	27 ± 6	27 ± 6	†46 ± 4	*54 ± 4
	Index (%)	——	——	——	——	81 ± 7	84 ± 5	87 ± 5	84 ± 5

Lo = optimal muscle length
PCSA = Physiological Cross Sectional Area
Po/CSA = Tetanic force normalized to PCSA
Pt/Po (%) = Ratio of twitch to tetanic force
Fatigue Index = Ratio of fatigue minimum to maximum

* vs. No Exercise (ANOVA, $p<0.05$)
† vs. Vehicle No Exercise (ANOVA, $p<0.05$)
# vs. Vehicle No Exercise (one-tailed t-test $p<0.05$)
‡ vs. Other groups combined (two-tailed t-test, $p=0.05$)

Signal regulation pathway study revealed that both insulin treatment and exercise training increased PDK/Akt protein synthesis pathway and decreased protein degradation pathway by reduction of muscle ring-finger protein-1(MuRF-1), an E3 ubiquitin ligase expression.

- The effect of exercise and oxandrolone pharmacological combination in rat muscle function

Twenty Four animals received burn and hindlimb unloading procedure (as previous experiment) and were randomly assigned (n=6) to vehicle without exercise (VEH/NEX), oxandrolone (daily) without exercise (OXD/NEX), vehicle with exercise (VEH/EX), or oxandrolone with exercise (OXD/EX). On day 14 muscle functions were tested and tissue collected.

Two way ANOVA statistical analysis showed that there is significant decreased fatigue index (FI) with exercise training, like we observed in previous study. There is no significant changes with oxandrolone treatment under the current analysis. (Data values presented as mean ±SD listed the following table)

Plantaris	NEX		EX		Soleus	NEX		EX	
	VEH	OXD	VEH	OXD		VEH	OXD	VEH	OXD
Tissue weight(g)	0.330 ± 0.018	0.312 ± 0.028	0.331 ± 0.042	0.300 ± 0.033	Tissue weight(g)	0.109 ± 0.015	0.100 ± 0.031	0.102 ± 0.014	0.110 ± 0.016
Lo(mm)	22.2 ± 1.8	22.2 ± 2.5	22.3 ± 1.1	22.3 ± 1.2	Lo(mm)	21.8 ± 2.4	22.0 ± 3.0	21.4 ± 3.4	20.8 ± 0.8
1/2 RT(s)	0.0166 ± 0.001	0.0169 ± 0.001	0.0187 ± 0.001	0.0165 ± 0.001	Pt(g)	47.80 ± 29.44	52.30 ± 14.13	64.95 ± 39.77	48.82 ± 11.19
Pt(g)	98.00 ± 13.39	115.85 ± 27.13	112.40 ± 22.47	105.86 ± 11.51	Po(g)	113.49 ± 69.89	126.02 ± 49.97	106.30 ± 18.62	124.09 ± 17.81
Po(g)	426.67 ± 137.64	469.08 ± 47.16	489.74 ± 58.13	555.44 ± 45.41	FI	26.43 ± 11.79%	27.24 ± 13.02%	15.56 ± 7.75%	16.08 ± 6.36%
Po/Pt	4.29 ± 1.01	4.17 ± 0.70	4.48 ± 0.94	5.31 ± 0.55	Fatigue (max)(g)	79.89 ± 45.63	95.075 ± 36.44	100.25 ± 13.55	104.71 ± 11.34
sPt(N/cm2)	6.787 ± 0.804	8.969 ± 2.974	7.853 ± 1.449	8.187 ± 1.020	sPt(N/cm2)	9.693 ± 5.549	10.814 ± 4.504	14.055 ± 8.328	9.521 ± 1.741
sPo(N/cm2)	29.686 ± 10.058	35.212 ± 7.434	34.733 ± 6.979	38.251 ± 11.784	sPo(N/cm2)	22.671 ± 14.044	29.082 ± 13.787	23.413 ± 6.091	24.459 ± 4.136

FOR SPECIFIC AIM 3:

After comparing the effect of oxandrolone and insulin in previuse experiment, we chose insulin to study the muscle function recovery in reloading period. Twenty Four rats received 40% TBSA burn and hindlimb unloading (HLU) described previously. All animals received exercise training for 14 days; meanwhile half of them were treated with pro zinc insulin 5U/kg subcutaneous daily and half with vehicle saline injection. Reloading period starts on day 14. All rats were removed from HLU and all injections stopped. Rats within each treatment group were separated into exercise (EX)/no exercise (NEX) (n=6) for another 14 days.

On day 28 *in situ* isometric forces of the left soleus and plantaris muscles were examined. The isometric twitch tension Force (Pt) and muscle optimal length (Lo) decreased in left plantaris from rats with exercise treatment significantly. Unlike fast twitch dominated plantaris, rat slow-twitch myofiber dominated soleus tetanic force (Po) with insulin treatment increased

	Plantaris				Soleus			
	VEH-NE	VEH-EX	INS-NE	INS-EX	VEH-NE	VEH-EX	INS-NE	INS-EX
Tissue weight (g)	0.44540.057	0.4782±0.007	0.484±0.030	0.461±0.040	0.166±0.021	0.177±0.008	0.170±0.023	0.169±0.019
Lo (mm)	39.17±11.82	37.42±16.88	39.67±16.31	36.75±15.88 *	41.75±13.48	40.17±5.98	41.00±26.58	39.00±14.01
Pt (g)	149.45±17.42	127.64±13.25	149.35±18.13	136.33±21.89 *	58.20±44.47	52.19±21.65	69.02±48.91	52.87±33.38
Po (g)	672.11±33.87	613.71±50.31	647.59±67.86	527.93±190.32	180.94±35.71	185.08±34.04	217.33±25.50	224.54±29.02 +
sPo (N/cm^2)	618.40±118.43	509.68±120.16	549.23±59.18	444.93±183.95	475.12±113.67	437.45±99.75	546.15±65.54	530.88±104.25
Pt/Po	0.229±0.029	0.210±0.025	0.230±0.014	0.269±0.096	0.326±0.073	0.270±0.023	0.312±0.087	0.249±0.023 *
			Fatigue	max (g)	180.4 ± 23.4	187.4 ± 45.6	182.6 ± 25.4	202.1 ± 24.9
				min(g)	13.4 ± 6.3	19.1 ± 14.0	14.2 ± 11.2	22.7 ± 8.2

significantly. [Data values presented as mean ±SD listed the following table, * $p<0.05$, exercise (EX) vs. non-exercise (NEX), + $p<0.05$, insulin (INS) vs. vehicle (VEH), two way ANOVA]

We investigated altered genes related signal pathways in rat muscle with continuous exercise and insulin treatment differed from those seen at earlier treatment stage.
Under the condition of continuous exercise to 28 days, there were 249 genes altered (75 downregulated and174 upregulated) with default set (absolute linear fold change greater than 2), and 37 altered miRNA with 1 down-regulated. Genes related pathways including MAPK cascade and fatty acid synthesis are upregulated.
Under the condition of insulin treatment, there were 210 altered genes (133 upregulated and 77 downregulated), and 33 altered mRNA with 4 down-regulated. Gene related pathways of MAPK cascade, fatty acid synthesis, and smooth muscle contraction are also upregulated.

4 KEY RESEARCH ACCOMPLISHMENTS

- The milestone for Year 5 was the completion of Aim 3. See **Appendix 1** for project timeline. There were 8 major tasks associated with this aim. The tasks and completion status are included below:

- Major Task 1 was to obtain Animal Use Approval from the University of Tesas Health Science Center at Houston. This task was completed before any associated animal wrok could be completed (Wade).

- Major Tasks 2 and 3 included training personnel, acquiring any equipment needed for the experiments associated with Aim 3 and setting up for the animal testing. These tasks were *completed* prior to any work being done. Coordination with investigators from the other institutions was initiated and timelines were able to be generated experiment completion (Wade/Song).

- Major task 4 includes starting and completing Aim 3 experiments. All pre-training and experimental procedures were *completed* according to the timeline. Samples were collected and stored appropriately for further analysis (Wade/Song).

- Major task 5 was *completed* at the conclusion of animal experiment. Muscle function was completed on all animals in all groups for Aim 3. Plasma and tissue were collected and stored for processing at a later time (Wade). Muscle function testing was completed and muscle sample was stored (Song).

- Major task 6: Muscle sample analysis is completed.
    - Subtask 1: Order ELISAs - *See Partnering PI Technical Report*
    - Subtask 2: Complete ELISAs - *See Partnering PI Technical Report*
    - Subtask 3: Complete muscle tissue weight measurement—*completed (Song)*
    - Subtask 4: Complete muscle RNA/protein extraction - - *completed (Song)*
    - Subtask 5: Complete histology & immunohistochemistry staining – *completed (Song)*
    - Subtask 6: Complete muscle tissue protein electrophoresis- *completed (Song)*
    - Subtask 7: Genomic profile analysis – *completed (Song)*

- Major Task 7: Bone Analysis is currently *on-going. (See Partnering PI Technical Report)*

- Major Task 8: Final Aim III Data Analysis is on-going. 3 abstracts were presented and 2 abstracts were submitted to professional scientific meetings detailing this work to-date. 1 manuscripts is published, 1 is submitted in a peer-reviewed journal detailing this work to-date.

5 CONCLUSION

Both Aim 1 and Aim 2 used our validated rodent model of burn and disuse, with a daily resistance exercise regimen started before injury and continued for the duration of the experimental period. Aim 2 focused on the addition of daily doses of either vehicle or a pharmacological agents (insulin or oxandrolone) in unison with the daily resistance exercise regimen. For both aims, all rats were able to complete the exercise program after injury and no rats were excluded from the experiment at any time. Data presented, irrespective of resistance

exercise and daily dosing, are comparable to previous studies. Daily resistance exercise resulted in a significant change in body mass, which can be attributed to the reduction in fat mass. The changes in muscle and bone support our previous research. Bone was affected primarily by the disuse component. Exercise alone did not seem to contribute to the overall changes. Muscle changes, however, were a result of the addition of resistance exercise. Transcriptomic gene profiles are comparable reflected to skeletal muscular pathophysiological changes in response to treatments. All eight major tasks for Year 1 have been completed. All six major tasks for Year 2 have been completed or are completed.

Animal experiment and muscle isometric force test for Aim 3 are completed. The primary focus is to determine the inter-relationship between muscle and bone following re-ambulation following pharmacological interventions and exercise. Muscle function data implied that myofiber type affect the response of exercise. All eight major tasks for Year 5 have been completed or are currently on-going.

6 PUBLICATIONS, ABSTRACTS AND PRSENTATIONS

Abstract/Manuscripts (Appendix 1):

ABSTRACT PRESENTED AND SUBMITTED IN 2018: (2017 Sep to 2018 Aug)

2018- ABA Annual Meeting PRENSENTED *(Abstract content attached)*

1. Calvin Geng, Nishika Karbhari, Juquan Song, Lisa Baer, Charles Wade, Steven E.Wolf, **Insulin and exercise combination therapy recovers muscle function in a burn and disuse rat model by activating protein synthesis and inhibiting.**

2. Juquan Song, Kevin DeSpain, Lisa Baer, Charles E Wade, Steven E Wolf. **A long-term of resistant exercise decreased rat muscle function in fast twitch myofiber dominated plantaris.**

3. Paula Hernandez, Andrea Fa, Thomas Mitchell, Dustin Buller, Ryan Huebinger, Michael Van Hal, Steven E. Wolf and Juquan Song. **Molecular and structural changes in Intervertebral Discs following Severe Burn in Rats.**

2019- ABA Annual Meeting SUBMITTED *(Abstract content attached)*

1. Audra Clark, Ryan Huebinger, Deborah L. Carlson, Steven E. Wolf, Juquan Song. **Serum Level of Musclin Is Elevated Following Severe Burn**.

2. Audra T. Clark, Juquan Song, Xiao Yao, Deborah Carlson, Alvand Sehat, Ryan M. Huebinger, Ming Mei Liu, Tarik Madni, Jonathan B. Imran, Luis Taveras, Holly Cunningham, Brett D. Arnoldo, Herb A. Phelan, Steven E. Wolf. **Muscle Homeostasis is Disrupted in Burned Adults**.

MANUSCRIPT PUBLISHED:

Paula Hernandez, Dustin Buller, Thomas Mitchell, Jamie Wright, Haixiang Liang, Kshitij Manchanda, Tre Welch, Ryan M. Huebinger, Deborah L. Carlson, Steven E. Wolf, Juquan Song. **SEVERE BURN-INDUCED INFLAMMATION AND REMODELING OF ACHILLES TENDON IN A RAT MODEL**. SHOCK. Sep;50(3):346-350. doi: 10.1097/SHK.0000000000001037

MANUSCRIPT PROCESSING:

MR. Saeman, SE. Wolf, **J Song. Muscle function improved in injured mice with a biological de-cellularized matrix application.** J Surg. Res. October 2018. In review.

(Manuscript attached separately)

7. INVENTIONS, PATENTS and LICENSES

Nothing to report.

8. REPORTABLE OUTCOMES

Nothing to report.

9. OTHER ACHIEVEMENTS

Nothing to report.

10. REFERENCES

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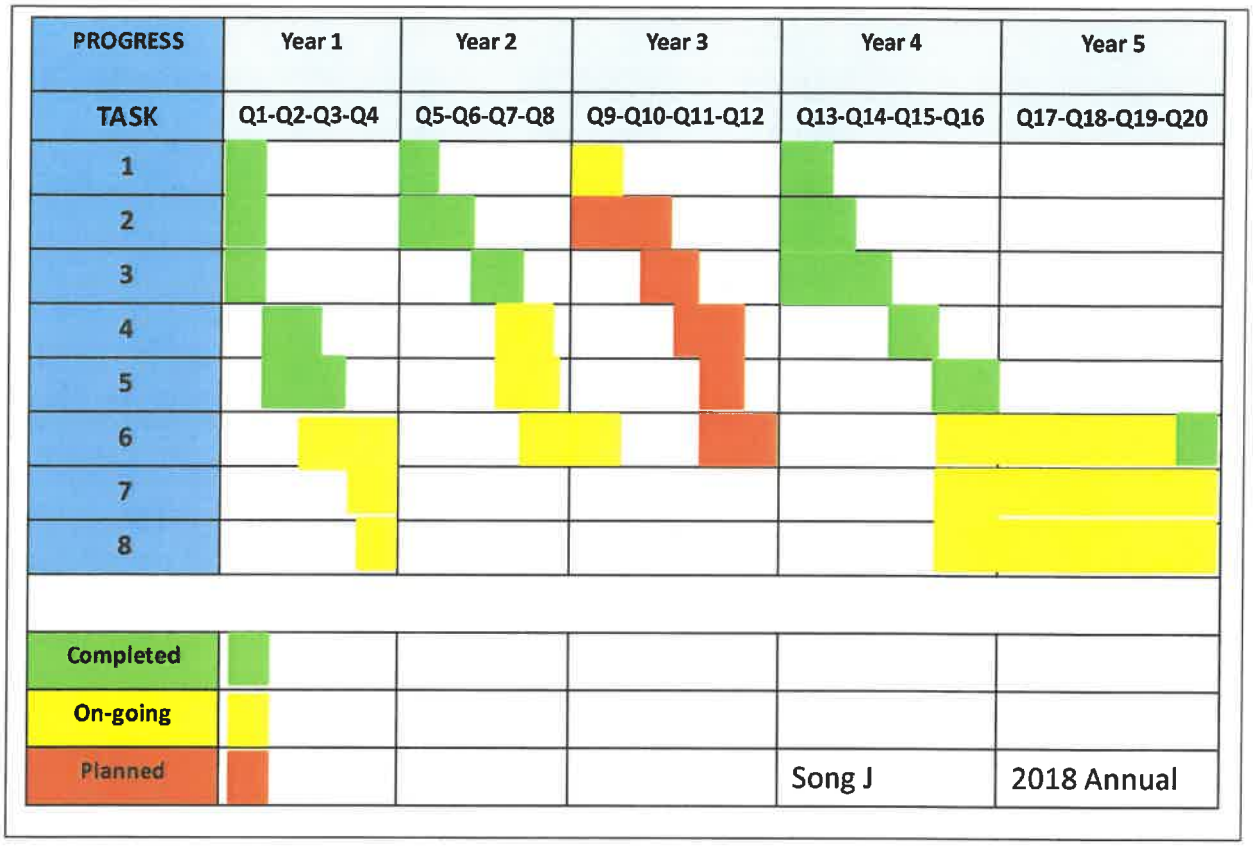
11. APPENDICES

1. Project Timeline
2. Submitted Abstracts
3. Published/Accepted Manuscripts (separate attached files)
4. Quad Chart (separate attached file)

APPENDIX 1- Project Timeline -TASK PROGRESS SUMMARY CHART



APPENDIX 2- PUBLICATIONS, ABSTRACTS AND PRSENTATIONS

2018- ABA Annual Meeting

Insulin and exercise combination therapy recovers muscle function in a burn and disuse rat model by activating protein synthesis and inhibiting proteolysis

Calvin Geng, Nishika Karbhari, Juquan Song, Lisa Baer, Charles Wade, Steven E. Wolf
Department of Orthopaedic Surgery, University of Texas Southwestern, Dallas, TX

Introduction

Burn injuries bring about a hypermetabolic state that results in a loss of muscle mass and function. Like burns, disuse of muscle also results in muscle loss[2]. Resistance exercise and insulin have individually been shown to attenuate burn and disuse induced muscle atrophy, though neither is fully compensatory. To date, there is no data on the efficacy of insulin and exercise as a combination therapy to recover muscle mass and function. This project investigates the molecular mechanisms behind musculoskeletal pathophysiological improvements in a burn and disuse rat model given these treatments. Muscle function, protein synthesis/proteolysis pathway protein levels, and genomic profiles are examined.

Methods

24 Sprague-Dawley rats received full thickness 40% total body surface area burns and hindlimb unloading and were randomly grouped into vehicle without exercise (V/N), pro zinc 5U/kg of insulin without exercise (I/N), vehicle with exercise (V/E), and insulin with exercise (I/E) groups. 14 days after injury, hindlimb muscle function was measured and muscle tissues were harvested for genomic profile and western blot analysis.

Results

The isometric force including tetanic (Po) and twitch (Pt) were significantly elevated in the plantaris of I/E rats. The soleus also had significant elevation of Po, Pt, fatigue maximum, and fatigue minimum in I/E rats. Affymetrix transcriptome analysis determined that 70, 62, and 116 genes were upregulated more than 2 fold in insulin, exercise, and combination treatment, respectively. Western blots showed that p-PDK 1, which activates AKT activity, was significantly increased in all treatment groups compared to control ($p<0.01$). p-AKT S473 was significantly increased in the combination group ($p<0.05$). eEF2 controls the elongation step in translation and was increased in the exercise and combination. ($p<0.05$). Muscle RING-finger protein-1(MuRF-1), an E3 ubiquitin ligase, was reduced in the combination group ($p<0.05$).

Conclusions

Insulin and resistance exercise have a positive combined effect on muscle function recovery. Signal pathway examination showed that the combination treatment decreased protein degradation and increased protein synthesis. The observed changes at the transcriptional and protein levels are supported by muscle function improvements.

Applicability of Research to Practice

Muscle loss is a sequela of burn and disuse that increases cost and risk of complications. By identifying the molecular basis of these changes, treatments that target critical proteins can be developed to mitigate muscle loss and improve patient outcomes.

External Funding DOD W81XWH-13-1-0462; Baxter Surgery Department Fund; Medical School Summer Research Program

A long-term of resistant exercise decreased rat muscle function in fast twitch myofiber dominated plantaris

J. Song, MD, K. DeSpain, BS, L. Baer, BS, J. Burchfield, BS, K. Nutall, BS, S. Vincent, BS, C. Wade, PhD, S. E. Wolf, MD
UT Southwestern Medical Center, Dallas, TX; UT Health Science Center at Houston, Houston, TX

Abstract:
Introduction: We previously showed muscle function was impaired with hindlimb unloading in burned rats which was alleviated by insulin treatment and resistance exercise. In the current study, we investigated the role of continued resistance exercise to further improve function in a model designed to mimic the late recovery period akin to the rehabilitation phase in patients.

Methods: Twenty-four Sprague-Dawley rats received a full thickness 40% total body surface area (TBSA) burn and hindlimb unloading (HLU) to mimic severe burn with muscle disuse. All animals underwent exercise training twice a day with 5 climbs per training session. Resistance exercise was achieved by adding weight to the base of the tail and increased every 3 days. All rats were given a subcutaneous injection of either saline or pro zinc insulin 5U/kg daily. On day 14, all rats were removed from HLU and all injections stopped. Then, rats within each treatment group were separated into no exercise (NEX) and exercise (EX) groups (n=6 per group) for an additional 14 days. On day 28, *in situ* isometric forces of the left soleus and plantaris muscles were measured. Values are presented as mean ± SD. Statistical analysis was by two-way ANOVA.

Results: Plantaris isometric twitch tension force (Pt) and muscle optimal length (Lo) significantly decreased with exercise treatment for 28 days (149.40±16.95g NEX vs Pt: 131.99±17.84g EX) (p=0.028); (39.42±1.77mm NEX vs Lo: 37.08±1.99mm EX) (p=0.008). However, soleus tetanic force (Po) increased significantly in those treated with insulin previously with or without continued exercise (183.01±33.33g vehicle vs 220.61±26.01g insulin) (p=0.01). Further, the ratio of single twitch force to maximal tetanic force (Pt/Po) significantly decreased in the soleus with exercise treatment (0.32±0.08 NEX vs. 0.26±0.02 EX) (p=0.037).

Conclusions: In our previous study, we found the combination of insulin treatment and exercise after burn and hindlimb unloading improved muscle function in both plantaris and soleus. In the current study, the effect of previous insulin treatment further augments improvements at 28 days after injury in slow twitch muscle. However, continued resistance exercise actually decreased muscle isometric force in the fast twitch myofiber dominated plantaris with no change in the slow-twitch soleus. The decrease in Pt/Po ratio in slow-twitch myofiber dominated soleus suggests a myofiber type change in response to continued resistance exercise.

Applicability of Research to Practice: The current study provides evidence of appropriate type of exercise in burn patient rehabilitation.

External Funding: DOD W81XWH-13-1-0462;

Molecular and structural changes in Intervertebral Discs following Severe Burn in Rats

Paula Hernandez[1], Andrea Fa[1], Thomas Mitchell [1], Dustin Buller[1], Ryan Huebinger[2], Michael Van Hal, Steven E. Wolf and Juquan Song[2]

[1]Department of Orthopaedic Surgery, University of Texas Southwestern, Dallas, TX; [2]Department of Surgery, University of Texas Southwestern, Dallas, TX

INTRODUCTION:

Intervertebral discs (IVD) connect to the spinal vertebrae. IVD impairment and dysgenesis are clinically relevant to pain management and movement restriction. Severe burn disrupts skeletomuscular metabolic status. IVD response following severe burn is currently unknown. Transient receptor potential cation channel subfamily V member 4 (TRVP4) protein is a Ca^{2+}-permeable, nonselective cation channel which has been recently reported to be elevated in human disc degeneration. The aim of this study is to investigate the role of TRVP4 in rat IVDs following thermal injury.

METHODS:

Under a UTSW IACUC approved protocol, 40 adult male Sprague-Dawley rats were examined in this study. Animals received 40% of total body surface area (TBSA) scald burn with the standard procedure under anesthesia and randomly grouped: Control (n = 11), 1 day (n = 6), 3 days (n = 6), 7 days (n = 6), and 14 days (n = 11) post burn. Total RNA was extracted from whole IVDs and analyzed for expression of IL-6, TNF, IL-1β, MMP9, MMP13 and TRPV4 by qPCR. Lumbar IVD was also fixed for histological analysis. Data are presented as mean ± standard deviation. Data were analyzed in GraphPad Prism 7 with one-way ANOVA and Fisher's LSD posthoc test, or by unpaired Student's t test when comparing two variables, ($p < 0.05$ being significant).

RESULTS:

Gene expressions of IL-6, TNF and IL-1β were not altered in rat IVD after burn. ILMMP9 and MMP13 gene expression showed a significant upregulation in thoracic IVD at day 1 after burn. Histological analysis of lumbar IVD showed an increase in nucleus pulposus (NP) height in discs at days 1 and 3 after burn compared to control discs, indicating tissue swelling following thermal injury. The gene expression of the calcium-permeant channel TRPV4, activated by osmotic changes, showed a significant upregulation in both thoracic and lumbar IVD at day 3 after burn.

SMMARY:

Local response of IVD was observed with the increased height of NP and the increased gene expression of MMP9 and MMP13. The elevation of TRPV4 gene expression after burn indicates local mechanical/osmotic changes in IVDs. Future investigations will focus on the acute structural changes and if these alterations lead to late degeneration of the IVDs in the current animal model.

Clinical relevant: Patients start experiencing back pain and disc degeneration long after trauma occurs, it is crucial to understand the early events occurring at the cellular level in IVD triggered by trauma.

ACKNOWLEDGEMENTS: Funded by Hofmann funds for Resident research from the Department of Orthopaedic Surgery. TM and DB were funded by UT Southwestern Summer Research program. Baxter Surgery Department Funding. DOD- W81XWH13-1-0462

2019- ABA Annual Meeting

Serum Level of Musclin Is Elevated Following Severe Burn

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[1], Department of Surgery, University of Texas Southwestern Medical Center, Dallas
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Introduction
Muscle wasting induced by severe burn worsens clinical outcomes and is associated with hyperglycemia. A novel muscle-specific secretory factor, musclin, was reported to regulate glucose metabolism with a homologous sequence of natriuretic peptides. The purpose of this study was to investigate musclin expression in response to burn injury in both human and animal models.

Methods
Serum was collected from 13 adult burn patients and circulating levels of musclin protein were measured via ELISA. The cytokine profile was measured by Bio-Plex multiple immunoassay. Following the clinical study, we used a burn rat model with 40% TBSA to study the time course of musclin expression till day 14 after injury. Rat serum and muscle tissue sample were harvested. Finally an *in vitro* study was applied to investigate whether the muscle cell C2C12 myoblast expressed musclin under 10% burn serum stimulation.

Results
Pearson analysis showed that there was a significant positive correlation of musclin expression to total body surface area of burn in patients ($p = 0.038$). Musclin expression was significantly positively correlated to IL-4, IL-7, IL-12, and IL-13 in burn patients' serum ($p < 0.05$). In the animal study, we found that level of musclin was elevated at 6 hour and day 1 in burn rat serum ($p<0.05$). *In vitro*, musclin mRNA expression in C2C12 muscle cells was significantly increased with burn serum stimulation at 24 hours ($p<0.05$).

Conclusions
Serum level of musclin was elevated both in human patients and in a rodent burn model; musclin levels were correlated to the severity of burn injury as well as to an elevated cytokine profile in patients; in vitro stimulation with burn serum increased musclin expression.

Applicability of Research to Practice
The current study implies a novel biomarker in response to the progress of burn severity.

External Funding
Department of Defense #W81XWH-13-1-0462

Muscle Homeostasis is Disrupted in Burned Adults

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[a], Department of Surgery, University of Texas Southwestern Medical Center, Dallas

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Introduction

Severe burn leads to substantial skeletal muscle wasting associated with adverse outcomes and protracted recovery. Signal pathways that regulate apoptosis, myogenic proliferation and differentiation are incompletely understood and have not been studied in adult burn patients. The purpose of the study is to investigate muscle tissue homeostasis in response to severe burn.

Methods

Muscle biopsies from the right vastus lateralis were obtained from 10 adult burn patients at the time of their first operation. Patients were grouped by burn size (TBSA <30% vs. TBSA ≥30%). Muscle fiber size was measured on histology and factors of cell death and muscle regeneration were examined by cytokine analysis and Western blot.

Results

There were five adult burn patients in both the small burn and large burn goup. There were no differences between age, gender, BMI, or time to biopsy. Muscle cell cross sectional area (CSA) was significantly smaller in the large burn group (2174.3 ± 183.8 μm^2 vs. 3687.0 ± 527.2 μm^2, $p=0.04$, Figure 1). Expression of ubiquitin E3 ligase Murf1 and cell death downstream effector Caspace3 were increased in the large burn group ($p<0.05$). No significant difference was seen between groups in expression of the myogenic factors Pax7, MyoD, or myogenin. Interestingly, Pax7 and PCNA expression in muscle tissue were significantly correlated to injury severity only in smaller burn group ($p<0.05$).

Conclusions

Adult patients with large burn (>30%) have decreased muscle cell cross sectional area, and increased circulating caspase3 and Murf1 when compared to patients with small burn. Muscle atrophy after burn is driven by apoptotic activation without an equal response of satellite cell activation, differentiation, and fusion.

Applicability of Research to Practice

A better understanding of the mechanisms behind the imbalance of muscle cell loss and regrowth may contribute to therapeutic advances to improve the morbidity associated with muscle atrophy after burn.

External Funding

Department of Defense #W81XWH-13-1-0462

Combination Therapies for the Mitigation of Muscloskeletal Pathologic Damage in a Novel Model of Severe Injury and Disuse

OR120033/OR120033P1 Award Number W81XWH-13-1-0489/W81XWH-13-1-0462

PI: Charles Wade, PhD and Juquan Song, MD **Org:** UTHealth, Houston/UTSouthwestern, Dallas **Award Amount:** $1,081,066

Study/Product Aim(s)

- Aim 1: Characterize the effect of resistance exercise on muscle and bone health in a validated model of burn and disuse.
- Aim 2: Evaluate the effect of resistance exercise in combination with currently used pharmacological therapies on muscle and bone health in a validated model of burn and disuse.
- Aim 3: Determine the interrelationship between muscle and bone after re-ambulation following pharmacological interventions and exercise.

Approach

A 40% TBSA severe burn will be induced followed by disuse for 14 days. Rats will be assigned to vehicle or drug treatment and further into exercise or no exercise groups. We will examine effects of re-ambulation with or without further resistance exercise after the 14 days. Blood and select organs, muscles and bones will be removed and weighed for testing of mechanical properties, typing, bone morphology, mineral content, and microarchitecture measurements.

Accomplishment: The experiments for Aims 1, 2, and 3 have been completed. Data analysis and manuscript writing for Aims 1, 2 and 3 are on-going. One manuscripts published and one under review, 5 abstracts have been presented and submitted:

1. Hernandez P, Buller D, Mitchell T, Wright J, Liang H, Manchanda K, Welch T, Huebinger RM, Carlson DL, Wolf SE, **Song J**. Severe Burn-Induced Inflammation and Remodeling of Achilles Tendon in a Rat Model. Shock. 2018. doi: 10.1097/SHK.0000000000001037
2. M. R.S. Threlkeld, S.E. wolf, J. Song Muscle function improved in injured mice with a biological de-cellularized matrix application. J. Surgical Res. Oct, 2018. In review.
3. C. Geng, N. Karbhari, J. Song, L. Baer, C. Wade, S.E. Wolf. Insulin and exercise combination therapy recovers muscle function in a burn and disuse rat model by activating protein synthesis and inhibiting proteolysis. Oral presentation at 50th Annual Meeting in Chicago, IL on April 10-13, 2018.
4. J. Song, K. DeSpain, L. Baer, J. Burchfield, K. Nutall, S. Vincent, C. E Wade, S. E. Wolf. A long-term of resistant exercise decreased rat muscle function in fast twitch myofiber dominated plantaris. 50th Annual Meeting in Chicago, IL on April 10-13, 2018.
5. P. Hernandez, A. Fa, T. Mitchell, D. Buller, R. Huebinger, M. Van Hal, S.E. Wolf and J. Song. Molecular and structural changes in Intervertebral Discs following Severe Burn in Rats. 50th Annual Meeting in Chicago, IL on April 10-13, 2018.
6. A. Clark, RM. Huebinger, DL. Carlson, SE. Wolf, J. Song. Serum Level of Musclin Is Elevated Following Severe Burn. 51st Annual Meeting in Las Vegas, NV on April 1-5, 2019.
7. AT. Clark, J. Song, X. Yao, D. Carlson, A. Sehat, R M. Huebinger, MM. Liu, T. Madni, JB. Imran, L. Taveras, H. Cunningham, BD. Arnoldo, HA. Phelan, SE. Wolf. Muscle Homeostasis is Disrupted in Burned Adults. 51st Annual Meeting in Las Vegas, NV on April 1-5, 2019.

Timeline and Cost

Activities CY	14	15	16/17	18
Aim 1 – Burn/Disuse + Exercise				
Aim 2 – Burn/Disuse + Exercise + Pharmacological Therapy				
Aim 3 – Burn/Disuse + Exercise + Pharmacological Therapy + Exercise				
Estimated Budget ($K)	$399	$348	$278	$56

Updated: 29 MAR 2019

Goals/Milestones for Current Award Year

CY14 Goals

- ✓ Obtain all animal approvals and order supplies
- ✓ Aim 1 animal experiments and sample collections
- ✓ Complete muscle function testing, uCTs and bone testing
- ✓ Complete ELISAs

CY15/16 Goal

- ✓ Aim 2 animal experiments and sample collections
- ✓ Complete muscle function testing, ELISAs, uCTs and bone testing

CY17/18 Goal

- ✓ Aim 3 animal experiments and sample collections
- ✓ Complete muscle function testing, ELISAs, uCTs and bone testing
- ✓ Complete data analysis
- o Submission of final results manuscript (*In Process*)

Comments/Challenges/Issues/Concerns

- o Requested no-cost extension for FY19/20 for project finalization.

Budget Expenditure to Date

- o **$ 1,080,000** Projected Expenditures to Date
- o **$ 1,079,263.00** Actual Expenditures to Date