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TITLE: **COMBINATION THERAPIES FOR THE MITIGATION OF MUSCULOSKELETAL PATHOLOGIC DAMAGE IN A NOVEL MODEL OF SEVERE INJURY AND DISUSE**

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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

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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 three one-year-extension period due to key personal shift . The milestone for Year 6 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. 2016]

- 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. [Song J et al. Physiol Rep. 2019]

- 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] Soleus tissue weight (g/100g body mass) is significantly greater in VEH-EX group than VEH-NE group. (0.07±0.003 vs. 0.062±0.008, $p<0.05$) After examining myfiber type by immunohistolocial staining and western blots, we did not observe myfiber type changing under exercise and insulin treatment.

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 6 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 completed. 2 abstracts were presented to professional scientific meetings detailing this work to-date. 3 manuscripts are 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 and it needs to be further investigated. All eight major tasks for Aim 3 have been completed.

6 PUBLICATIONS, ABSTRACTS AND PRSENTATIONS

Abstract/Manuscripts (Appendix 2):

There are 19 abstracts presented in the national conferences through the funding period. There are 6 publications and 2 manuscripts in review with the funding support. The bibliography of Abstracts and Publications through the whole funding period are listed below: (2014-2019)

ABSTRACTS PRESENTED AT CONFERENCE: (total 19)

2015- (6)

American Burn Association (ABA) Annual Meeting

- L.A. Baer , J. Song , S.E. Wolf and C.E. Wade . **Effects of Resistance Exercise on Caloric Intake and Body Mass in Rats Following Burn and Disuse.**
- B.A. Carlson, J. Song, M. R. Saeman, K. DeSpain, L. A. Baer, C. E. Wade, S. E. Wolf. **Exercise alleviates skeletal muscle protein loss after severe burn and hindlimb disuse**.
- J. Song, L.A. Baer, M.R. Saeman, M.M. Liu, B. Carlson, H.E. Wolf, K. DeSpain, C.E. Wade, S.E. Wolf. **Skeletal Muscle Fiber Type Changes in Severe Burn Rats with Muscle Disuse Atrophy**

10th Annual Academic Surgical Congress

- M. R. Saeman, K. DeSpain, M. Liu, B. Carlson, L. A. Baer, J. Song, C. E. Wade, S. E. Wolf**. The Effects of Exercise on Soleus Function in Severe Burn with Muscle Disuse Atrophy.**

Shock annual meeting

- J Song, MR. Saeman, K DeSpain, M-M Liu, BA Carlson, LA. Baer, C E. Wade, SE. Wolf. **Plantaris miRNA and Target Gene Profile after Exercise Training in an Animal Model of Hindlimb Unloading and Severe Burn.**
- J. Song , M. R. Saeman , L A. Baer, C. E. Wade, S. E. Wolf. **Muscle microRNA profile alteration following severe burn.**

2016- (4)

11th Annual Academic Surgical Congress (2016 ASC)

- MR. Saeman, K DeSpain, J Song, LA. Baer, C E. Wade, SE. Wolf**. Combined Effects of Insulin and Exercise on Muscle Function in Severe Burn.**

American Burn Association (ABA) Annual Meeting

- A Cai, J Song (mentor), P Kumar, A Sehat, MR. Saeman, L Baer, CE. Wade, SE. Wolf. **Exercise Treatment Reversed Micro RNA Profile in Burn Rats with Hindlimb Unloading.**
- L.A. Baer, J. Song, S.E. Wolf and C.E. Wade. **Effects of Resistance Exercise and Daily Insulin on Body Mass, Food Intake, Fat Mass and Total Hindlimb Muscle Mass in Rats Following Burn and Disuse.**

Shock Society Annual Meeting

- J Song, K DeSpain, LA. Baer, C E. Wade, SE. Wolf. **Combined Effects of Oxandrolone and Exercise on Muscle Function Recovery in Rats with Severe Burn and Hindlimb Unloading.**

2017- (3)

ABA Annual Meeting

- L. A. Baer, K. I. Stanford, J. Song, S. E. Wolf. **Resistance Exercise Effects on Body Mass, Free Fatty Acid Concentration and Fatty Acid Metabolism in sqWAT Following Burn and Disuse in Rats**

Shock Society Annual Meeting

- Juquan Song, Lisa Baer, Charles E Wade, Steven E Wolf. **Transcriptomic profile alterations in burn/hindlimb unloaded rats with insulin and exercise combination treatment.**

ORS 47th International Musculoskeletal Biology Workshop

- P. Hernandez, D. Buller, T. Mitchell, J. Wright, H. Liang, K. Manchanda, T. Welch, R.M. Huebinger, D.L. Carlson, S.E. Wolf, J. Song. **Inflammation and Remodeling of Rat Achilles Tendon after Severe Burn.**

2018- (4)

ABA Annual Meeting

- C.Geng, N. Karbhari, J. Song, L. Baer, C. Wade, SE.Wolf.**Insulin and exercise combination therapy recovers muscle function in a burn and disuse rat model by activating protein synthesis and inhibiting proteolysis.**
- 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.**
  K. DeSpain, J. Song, CR. Rosenfeld, SE. Wolf. **Vascular Smooth Muscle Dysfunction After Burn.**
- P. Hernandez, A. Fa, T. Mitchell, D. Buller, R. Huebinger, M. Van Hal, S. E. Wolf, J. Song. **Molecular and structural changes in Intervertebral Discs following Severe Burn in Rats.**

2019- (2)

ABA Annual Meeting

- J. Song, AT. Clark, RM. Huebinger, DL. Carlson, SE. Wolf. **Serum Level of Musclin Is Elevated Following Severe Burn**.
- AT. Clark, J Song, X. Yao, DL. Carlson, A. Sehat, RM. Huebinger, MM. Liu, T. Madni, JB. Imran, L. Taveras, H. Cunningham, BD. Arnoldo, HA. Phelan, SE. Wolf. **Muscle Homeostasis is Disrupted in Burned Adults**.

PUBLICATIONS:

1. Saeman MR, DeSpain K, Liu MM, Carlson BA, Song J, Baer LA, Wade CE, Wolf SE. **Effects of exercise on soleus in severe burn and muscle disuse atrophy**. J Surg Res. 2015 Sep; 198 (1):19-26. doi: 10.1016/j.jss.2015.05.038. Epub 2015 Jun 12. PMID: 26104324

2. Song J, Saeman MR, Baer LA, Cai AR, Wade CE, Wolf SE. **Exercise Altered the Skeletal Muscle MicroRNAs and Gene Expression Profiles in Burn Rats With Hindlimb Unloading**. J Burn Care Res. 2017 Jan/Feb;38(1):11-19. doi: 10.1097/BCR.0000000000000444. PMID:27753701

3. 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. Sep;50(3):346-350. doi: 10.1097/SHK.0000000000001037

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5. Song J, Baer LA, Threlkeld MRS, Geng C, Wade CE, Wolf SE. **Insulin and exercise improved muscle function in rats with severe burns and hindlimb unloading**. Physiol Rep. 2019 Jul;7(14):e14158. doi: 10.14814/phy2.14158. PMID: 31353827

6. Clark AT, Song J, Yao X, Carlson D, Huebinger RM, Liu MM, Madni TD, Imran JB, Taveras LR, Weis HB, Arnoldo BD, Phelan HA, Wolf SE. **Muscle Homeostasis Is Disrupted in Burned Adults**. J Burn Care Res. 2019 Nov 18. pii: irz190. doi: 10.1093/jbcr/irz190. [Epub ahead of print] PMID: 31738430

MANUSCRIPT PROCESSING:

7. 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.

8. Song J, Clark A, Wade CE, Wolf SE. **Skeletal Muscle Wasting After Burn is a Consequence of Cachexia and Sarcopenia. Mini Review**. Frontiers in Physiology. In review, November 01, 2019

7. INVENTIONS, PATENTS and LICENSES

Nothing to report.

8. REPORTABLE OUTCOMES

Nothing to report.

9. OTHER ACHIEVEMENTS

Nothing to report.

10. REFERENCES

1. Herndon DN, Tompkins RG. Support of the metabolic response to burn injury. Lancet 2004;363: 1895-902.
2. Jeschke MG, Kraft R, Song J, Gauglitz GG, Cox RA, Brooks NC, Finnerty CC, Kulp GA, Herndon DN, Boehning D. Insulin protects against hepatic damage postburn. Mol Med 2011;17: 516-22.
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4. Wolf SE, Edelman LS, Kemalyan N, Donison L, Cross J, Underwood M, Spence RJ, Noppenberger D, Palmieri TL, Greenhalgh DG, Lawless M, Voigt D, Edwards P, Warner P, Kagan R, Hatfield S, Jeng J, Crean D, Hunt J, Purdue G, Burris A, Cairns B, Kessler M, Klein RL, Baker R, Yowler C, Tutulo W, Foster K, Caruso D, Hildebrand B, Benjamin W, Villarreal C, Sanford AP, Saffle J. Effects of oxandrolone on outcome measures in the severely burned: a multicenter prospective randomized double-blind trial. J Burn Care Res 2006;27: 131-9; discussion 140-1.
5. Wu X, Baer LA, Wolf SE, Wade CE, Walters TJ. The impact of muscle disuse on muscle atrophy in severely burned rats. J Surg Res 2010;164: e243-51.
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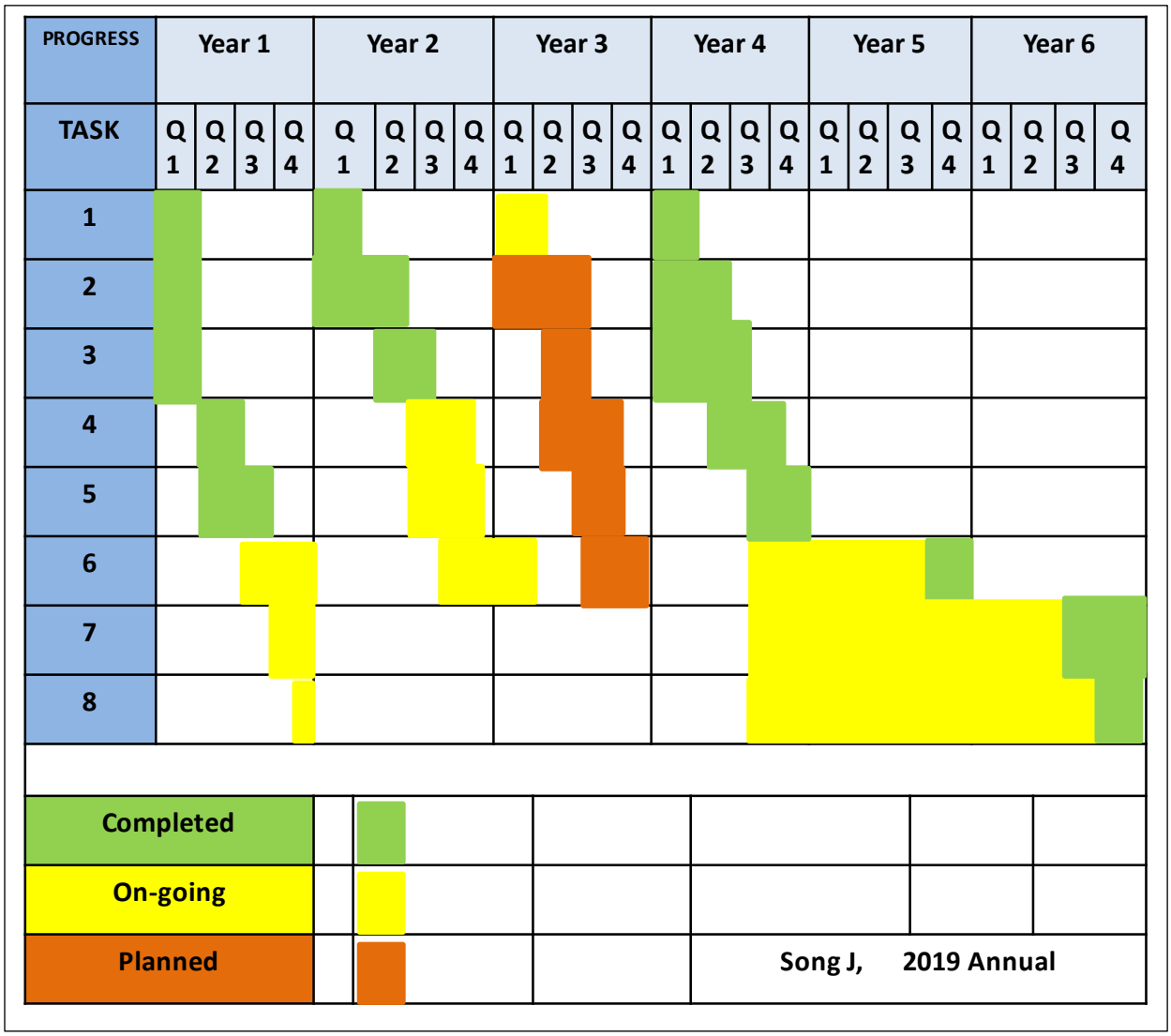
10. Pidcoke HF, Baer LA, Wu X, Wolf SE, Aden JK, Wade CE**.** Insulin effects on glucose tolerance, hypermetabolic response, and circadian-metabolic protein expression. Am J Physiol Regul Integr Comp Physiol. 2014

11. APPENDICES

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

APPENDIX 1- Project Timeline -TASK PROGRESS SUMMARY CHART



APPENDIX 2- PUBLICATIONS, ABSTRACTS AND PRSENTATIONS
BIBLIOGRAPHY OF ALL PUBLICATIONS AND MEETING ABSTRACTS

There are 6 publications and 2 manuscripts in review with the funding support. The bibliography of publication is listed below and manuscripts are attached separately.

There are 19 abstracts presented in the national conferences through the funding period. The bibliography is listed below with abstracts attached.

I.PUBLICATIONS:

1. Saeman MR, DeSpain K, Liu MM, Carlson BA, Song J, Baer LA, Wade CE, Wolf SE. **Effects of exercise on soleus in severe burn and muscle disuse atrophy**. J Surg Res. 2015 Sep; 198 (1):19-26. doi: 10.1016/j.jss.2015.05.038. Epub 2015 Jun 12. PMID: 26104324

2. Song J, Saeman MR, Baer LA, Cai AR, Wade CE, Wolf SE. **Exercise Altered the Skeletal Muscle MicroRNAs and Gene Expression Profiles in Burn Rats With Hindlimb Unloading**. J Burn Care Res. 2017 Jan/Feb;38(1):11-19. doi: 10.1097/BCR.0000000000000444. PMID:27753701

3. 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 Sep;50(3):346-350. doi: 10.1097/SHK.0000000000001037

4. Clark A, Huebinger R, Carlson DL, Wolf SE, Song J. **Serum Level of Musclin Is Elevated Following Severe Burn**. J Burn Care Res. 2019 Aug 14;40(5):535-540. doi: 10.1093/jbcr/irz101. PMID: 31187123

5. Song J, Baer LA, Threlkeld MRS, Geng C, Wade CE, Wolf SE. **Insulin and exercise improved muscle function in rats with severe burns and hindlimb unloading**. Physiol Rep. 2019 Jul;7(14):e14158. doi: 10.14814/phy2.14158. PMID: 31353827

6. Clark AT, Song J, Yao X, Carlson D, Huebinger RM, Liu MM, Madni TD, Imran JB, Taveras LR, Weis HB, Arnoldo BD, Phelan HA, Wolf SE. **Muscle Homeostasis Is Disrupted in Burned Adults**. J Burn Care Res. 2019 Nov 18. pii: irz190. doi: 10.1093/jbcr/irz190. [Epub ahead of print] PMID: 31738430

MANUSCRIPT PROCESSING:

7. 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.

8. Song J, Clark A, Wade CE, Wolf SE. **Skeletal Muscle Wasting After Burn is a Consequence of Cachexia and Sarcopenia**. Mini Review. Frontiers in Physiology. In review, November 01, 2019

II.CONFERENCE PRESENTED ABSTRACTS: (abstract attached below)

1. L.A. Baer, J. Song, S.E. Wolf and C.E. Wade. **Effects of Resistance Exercise on Caloric Intake and Body Mass in Rats Following Burn and Disuse**. 47th ABA annual meeting, April 21-24, 2015. Chicago, IL

2. B.A. Carlson, J. Song, M. R. Saeman, K. DeSpain, L. A. Baer, C. E. Wade, S. E. Wolf. **Exercise alleviates skeletal muscle protein loss after severe burn and hindlimb disuse**. 47th ABA annual meeting, April 21-24, 2015. Chicago, IL

3. J. Song, L.A. Baer, M.R. Saeman, M.M. Liu, B. Carlson, H.E. Wolf, K. DeSpain, C.E. Wade, S.E. Wolf. **Skeletal Muscle Fiber Type Changes in Severe Burn Rats with Muscle Disuse Atrophy**. 47th ABA annual meeting, April 21-24, 2015. Chicago, IL

4. M. R. Saeman, K. DeSpain, M. Liu, B. Carlson, L. A. Baer, J. Song, C. E. Wade, S. E. Wolf. **The Effects of Exercise on Soleus Function in Severe Burn with Muscle Disuse Atrophy**. 10th Annual Academic Surgical Congress, Feb, 2015

5. J Song, MR. Saeman, K DeSpain, M-M Liu, BA Carlson, LA. Baer, C E. Wade, SE. Wolf. **Muscle microRNA profile alteration following severe burn**. 38th Shock annual meeting, June 6-10, 2015. Denver, CO

6. MR. Saeman, K DeSpain, M-M Liu, BA Carlson, J Song, LA. Baer, C E. Wade, SE. Wolf. **Epigenetic changes in rats with burn and hindlimb unloading affected by exercise**. 38th Shock annual meeting, June 6-10, 2015. Denver, CO

7. MR. Saeman, K DeSpain, J Song, LA. Baer, C E. Wade, SE. Wolf. **Combined Effects of Insulin and Exercise on Muscle Function in Severe Burn**. 11th Annual Academic Surgical Congress (2016 ASC), February 2 - 4, 2016.Jacksonville, FL

8. A Cai, J Song (mentor), P Kumar, A Sehat, MR. Saeman, L Baer, CE. Wade, SE. Wolf. **Exercise Treatment Reversed Micro RNA Profile in Burn Rats with Hindlimb Unloading**. Oral presentation. 48th ABA annual meeting, May 3-6, 2016. Las Vegas, NV

9. L.A. Baer, J. Song, S.E. Wolf and C.E. Wade. **Effects of Resistance Exercise and Daily Insulin on Body Mass, Food Intake, Fat Mass and Total Hindlimb Muscle Mass in Rats Following Burn and Disuse**. 48th ABA annual meeting, May 3-6, 2016. Las Vegas, NV

10. J Song, K DeSpain, LA. Baer, C E. Wade, SE. Wolf. **Combined effects of oxandrolone and exercise on muscle function recovery in rats with severe burn and hindlimb unloading**. 39th Shock annual meeting, June 11-14, 2016. Austin, TX

11. L. A. Baer, K. I. Stanford, J. Song, S. E. Wolf. **Resistance Exercise Effects on Body Mass, Free Fatty Acid Concentration and Fatty Acid Metabolism in sqWAT Following Burn and Disuse in Rats**. 49th ABA Annual Meeting. Mar 21-24, 2017. Boston, MA

12. J. Song, L. Baer, C. E. Wade, S. E. Wolf. **Transcriptomic profile alterations in burn/hindlimb unloaded rats with insulin and exercise combination treatment**. 40th Shock annual meeting, June 3-6, 2017. Ft. Lauderdale, FL.

13. P. Hernandez, D. Buller, T. Mitchell, J. Wright, H. Liang, K. Manchanda, T. Welch, R.M. Huebinger, D.L. Carlson, S.E. Wolf, J. Song. **Inflammation and Remodeling of Rat Achilles Tendon after Severe Burn**. ORS 47th International Musculoskeletal Biology Workshop at Sun Valley in beautiful Sun Valley, Idaho, August 6 – 9, 2017

14. 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 ABA Annual Meeting, Chicago, IL. April 10-13, 2018

15. P. Hernandez, A. Fa, T. Mitchell, D. Buller, R. Huebinger, M. Van Hal, S. E. Wolf, J. Song**. Molecular and structural changes in Intervertebral Discs following Severe Burn in Rats**. 50th ABA Annual Meeting in Chicago, IL on April 10-13, 2018

16. K. DeSpain, J. Song, CR. Rosenfeld, SE. Wolf. **Vascular Smooth Muscle Dysfunction After Burn**. 50th ABA Annual Meeting in Chicago, IL on April 10-13, 2018

17. C. Geng, N. Karbhari, J. Song, L. Baer, C. Wade, SE. 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. 50th ABA Annual Meeting in Chicago, IL on April 10-13, 2018

18. AT. Clark, J Song, X. Yao, DL. Carlson, A. Sehat, RM. Huebinger, MM. Liu, T. Madni, JB. Imran, L. Taveras, H. Cunningham, BD. Arnoldo, HA. Phelan, SE. Wolf. **Muscle homeostasis disrupted after burns.** Oral presentation at 51st ABA annual meeting, April 2-5, 2019. Las Vegas, NV

19. J. Song, AT. Clark, RM. Huebinger, DL. Carlson, SE. Wolf. **Musclin expression elevated after severe burn**. Oral presentation at 51st ABA annual meeting, April 2-5, 2019. Las Vegas, NV

ABSTRACT ATTACHED the following pages _____

Abstract #1

Effects of Resistance Exercise on Caloric Intake and Body Mass in Rats Following Burn and Disuse

L.A. Baer[1], J. Song[2], S.E. Wolf[2] and C.E. Wade[1]

[1]Center for Translational Injury Research and Dept. of Surgery, University of Texas Health Science Center-Houston, Houston, TX , [2]University of Texas, Southwestern Medical Center, Dallas, TX.

Introduction: The treatment and recovery of patients with severe traumatic injuries is impacted by a pronounced increase in metabolism. Injury induces a systemic catabolic response with increased energy expenditure and loss of body mass. The ability to resume normal activities is compromised due to inactivity associated with bed rest and the catabolic response due to burn injury. Exercise and nutritional interventions have been used independently with limited success. Using a combined severe burn and hindlimb unloading rodent model with no exercise, hypermetabolism was observed associated with a significantly reduced body mass combined with increased food intake. The purpose of this study was to determine if body mass and caloric intake are affected by daily resistance exercise following burn and disuse.

Methods: Male, Sprague-Dawley rats were randomized into four groups: Sham Ambulatory (SA), Burn Ambulatory (BA), Sham/Hindlimb unloaded (SH) and Burn/Hindlimb unloaded (BH), with daily resistance exercise (EX) or no exercise (NEX) (N=6/group). Rats were introduced to resistance exercise by adding weight to each tail during repetitive ladder climbing ten days prior to injury. Rats were then weight-matched into treatment groups, either daily exercise or no-exercise. Statistical analysis using ANOVA with significance at $p<0.05$.

Results: No differences in body mass were observed between any groups at the time of injury. At day 14, NEX-SA and NEX-BA body mass were significantly greater than EX-SA and EX-BA, but no differences in SH or BH. BH rats were significantly smaller than other treatment groups. Compared to other treatment groups, there was a significant increase in average kcals consumed over the last 5 days in BH, irrespective of exercise. No other differences in food intake between NEX and EX within each treatment group were observed.

Conclusions: Disuse showed no significant changes in body mass due to exercise. Irrespective of exercise, BH demonstrated a hypermetabolic response with increased caloric intake in conjunction with significantly reduced body mass. Burn injury,accompanied by long-term disuse results in complex metabolic changes early after the initial injury. With disuse, exercise did not affect body mass and alterations in caloric intake were independent of exercise. Different underlying factors seem to be influencing the acute metabolic changes offering opportunities for early intervention resulting in positive long-term outcomes.

Grant Information: US Army MRMC CDMRP W81XWH-13-1-0489

	SA	BA	SH	BH
D14 Body Mass (g)				
NEX	336±7	307±7	305±6	282±4¥
EX	319±6*	287±5*	294±7	278±4¥
Caloric Intake (kcal/100g BM/day)				
NEX	20.5±0.8	21.3±0.4	21.7±0.6	22.1±0.9¥
EX	19.7±0.4	21.0±0.6	20.2±0.5	23.0±0.9¥

*p<0.05 from NEX; ¥ p<0.05 from SA

Abstract #2

Exercise decreases skeletal muscle protein loss in severe burn rats with muscle disuse atrophy

Brett A. Carlson, Juquan Song, Melody R. Saeman, Kevin DeSpain, Lisa A. Baer, Charlie E. Wade, Steven E. Wolf

Introduction:

Muscle atrophy is often present in severely burned patients with restricted movement. A prior study in a rat model of hindlimb unloading after burn supports that bedrest contributes significantly to muscle atrophy. The aim of our study was to evaluate whether exercise mitigates the protein content loss in this animal model.

Methods:

Forty-eight Sprague-Dawley male adult rats were randomly assigned to burn ambulatory (BA), burn hindlimb unloading (BH), sham ambulatory (SA), or sham hindlimb unloading (SH). Rats received a full thickness scald burn of 40% total body surface area (TBSA) or sham, and were allowed to ambulate or were placed in a tail traction system for hindlimb unloading. Half of the animals were trained to exercise twice a day; half had no exercise. On day 14 hindlimb skeletal muscle was harvested for weight and tissue analysis. Muscle protein was extracted from the rat medial gastrocnemius (GM), soleus (SL) and plantaris (PL) following T-PER Tissue protein extraction procedure (Thermo Fisher Scientific). Protein concentration was measured with DC Protein Assay (Bio-Rad Laboratories Inc.). Protein expression was analyzed with western blot. Statistical analysis was performed with Sigma Plot using three-way ANOVA. Significance was accepted with $p<0.05$.

Results:

Muscle tissue wet weight in rat GM, SL, PL was significantly reduced with hindlimb unloading. Muscle wet tissue weight in PL and GM decreased in rats 14 days after burn. The ratio of soleus to body mass significantly increased with exercise treatment in rats ($p<0.001$).

There was significant protein content loss of SL in rats with HLU ($p=0.001$), and protein yield in GM significantly increased in rats with exercise training ($p=0.003$).

Western blotting results showed that the absorbance ratio of desmin to GAPDH in tissue lysate from GM significantly decreased in BH group ($p<0.05$), while there was no difference between BH and SA groups after exercise.

Summary:

Skeletal muscle mass loss was significantly affected by hindlimb immobilization. Exercise increase protein yield in rat GM muscle. Western blot data confirmed that muscle specific protein loss was decreased through exercise treatment in severe burn rats with hindlimb unloading.

Applicability of Research to Practice:

The current study demonstrates the benefit of physical therapy in burn victims with muscle disused atrophy

Funding support by DOD –CDMRP, NIH T32 Training Grant #T32GM008593, and Golden Charity Guild Charles Baxter, MD chair Department funding

Abstract #3

Skeletal Muscle Fiber Type Changes in Severe Burn Rats with Muscle Disuse Atrophy

Juquan Song, Lisa A. Baer, Melody R. Saeman, Ming-Mei Liu, Brett Carlson, Hailey E. Wolf, Kevin DeSpain, Charlie E. Wade, Steven E. Wolf

[1]University Of Texas Southwestern Medical Center, Surgery, Dallas, TEXAS, USA; [2]University Of Texas Health Science Center At Houston, Surgery, Houston, TEXAS, USA

Introduction:
Muscle atrophy is common in severe burned patients with limited movement. In a rat model of hindlimb unloading after burn supports the contention that bedrest in the severe burn contributes significantly to muscle atrophy. The aim of our study was to evaluate if exercise in the setting of bedrest affected myofiber type in this burned animal model.

Methods:
Male rate were assigned to four treatments: sham ambulatory (SA), sham hindlimb unloading (SH), burn ambulatory (BA), or burn hindlimb unloading (BH). Rats received 40% total body surface area (TBSA) scald burn or sham. Animals grouped to either ambulate or were placed in a tail traction system for hindlimb unloading. Within each treatment half of rats (n=6 per group) performed twice daily exercises of climbing 1 meter with 5 repetitions (EX) with the others did not exercise (NX). On day 14 skeletal muscles in hind limb were harvested. Myofiber type in plantaris (PL) and soleus (SL) muscles were determined using Immunihistochemistry methods (IHC) staining with anti-myosin heavy chain (MHC) Slow, type I and Fast, Type II antibodies (Sigma Aldrich, MO). The positive stained myofiber number was counted blinded to treatment under light microscopy at 10 x magnification. Statistical analysis was performed with Sigma Plot using 3-way ANOVA method, and significance was accepted by $p<0.05$.

Results:
Type I MHC myofiber was predominate in the soleus with only 11.4% type II myofibers in the sham ambulatory group. Burn had no effect on myofiber type, while the number of type II myofibers increased significantly in the soleus 14 days after hind limb unloading ($p=0.004$). No significant change in soleus myofiber type was found between exercise and non-exercise groups. Type II MHC myofiber was predominant (91±1.2 %) in the plantaris. the interior of the muscle on cross-section had significantly decreased type I fibers after burn ($p=0.047$); no difference was found after HLU or exercise.

Summary:
Following severe burn, muscle immobilization increased the proportion of fast twitch myofiber in the soleus, while the burn injury had more effect on the predominately fast-twitch plantaris. No effect of exercise on myofiber type change in both PL and SL muscle was observed. Changes in myofiber type may occur independently due to injury or disuse, and do not responds to exercise training.

Myofiber type distribution might be related with metabolic changes in burn patients and muscle disused dystrophy.

External Funding support: US Army MRMC CDMRP, NIH T32 Training Grant

Abstract #4

The Effects of Exercise on Soleus Function in Severe Burn with Muscle Disuse Atrophy

M. R. Saeman[1], K. DeSpain[1], M. Liu[1], B. Carlson[1], L. A. Baer[2], J. Song[1], C. E. Wade[2], S. E. Wolf[1]. [1]University Of Texas Southwestern Medical Center, Surgery, Dallas, TEXAS, USA; [2]University Of Texas Health Science Center At Houston, Surgery, Houston, TEXAS, USA

Introduction:
Muscle loss is a known sequela of severe burn and critical illness that increases the risk of complications such as sepsis and prolonged recovery time. A prior study in a rat model of hindlimb unloading after burn supports that bedrest contributes significantly to muscle atrophy. The aim of our study was to evaluate if exercise mitigates the loss of muscle in this animal model.
Methods:
Two groups of 24 Sprague-Dawley rats were randomly assigned to burn ambulatory (B/A), burn hindlimb unloading (B/H), sham ambulatory (S/A), or sham hindlimb unloading (S/H). One group was trained to perform twice daily weighted resistance climbing of 1 meter with 5 repetitions; the other group had no exercise. Rats received a full thickness scald burn of 40% total body surface area or sham and were allowed to ambulate or were placed in a tail traction system for hindlimb unloading. On day 14 in situ isometric forces were measured on the left soleus muscle. Statistical analysis was performed with Sigma Plot using Student's t-test, Mann Whitney, or ANOVA with Holm-Sidak method where appropriate.
Results:
The soleus wet weight was lower in the hindlimb (144 mg) and the exercise (136 mg) versus the ambulatory (190 mg, $p<0.001$), and no exercise (180 mg, $p=0.01$) groups. There was no difference in weights between burn and sham. Twitch was significantly lower in the hindlimb group: 31 vs 12 g ($p<0.001$). Compared to no exercise, the B/H exercise group had a significantly higher twitch force 14 vs. 8 g ($p=0.04$). Across all other factors there was no significant difference in the twitch between exercise and no exercise. There was a significantly lower tetanic force in the hindlimb group: 55 vs 148 g ($p<0.001$). B/A had a lower tetanic force in the exercise group versus no exercise: 118 vs 165g ($p=0.02$). In B/H no difference in tetanic force was seen with or without exercise. All hindlimb groups had significantly lower specific tetanic force than ambulatory: 12 vs. 22 N/cm^2 ($p<0.001$). The specific tetanic force in B/H was significantly higher in exercise versus no exercise: 14 vs. 7 N/cm^2 ($p=0.008$). Fatigue index was significantly lower in the ambulatory (55%) and exercise (52%) groups versus hindlimb (69%) and no exercise (73%) groups ($p=0.03$, $p=0.002$ respectively). Muscle function of all groups included in table.

Soleus Weight and Isometric Contractile Function

	No Exercise				Exercise			
	BA	BH	SA	SH	BA	BH	SA	SH
Muscle Weight (mg)	200 (10)	170 (40)	210 (20)	140 (40)	190 (10)	110 (10)	190 (10)	120 (4)
Twitch Force (g)	36 (4.4)	8 (2.0)	31 (2.8)	14 (2.1)	27 (2.0)	14 (2.1)	30 (2.6)	11 (0.6)
Tetanic Force (g)	165 (9)	42 (5)	159 (18)	56 (4)	118 (14)	54 (5)	150 (16)	66 (8)
Specific Force (N/cm^2)	25 (2)	7 (1)	25 (3)	12 (2)	20 (2)	14 (2)	23 (2)	17 (2)
Fatigue Index (%)	69 (6)	76 (3)	66 (6)	82 (11)	37 (11)	58 (13)	52 (11)	63 (6)

Data are expressed as mean (SEM)
BA: Burn Ambulatory; BH: Burn Hindlimb; SA: Sham Ambulatory; SH: Sham Hindlimb
Specific Force calculated from maximum tetanic force (N)/ physiological cross sectional area (cm2)
Fatigue Index: (minimum force at 4 minutes/maximum force) x 100

ID: **ASC20151202 10th Annual Academic Surgical Congress, Feb, 2015**

Abstract #5

MUSCLE MICRORNA PROFILE ALTERATION FOLLOWING SEVERE BURN

J. Song[1], M. R. Saeman[1], L A. Baer[2], C. E. Wade[2], S. E. Wolf[1]. [1]University Of Texas Southwestern Medical Center, Surgery, Dallas, TEXAS, USA; [2]University Of Texas Health Science Center At Houston, Surgery, Houston, TEXAS, USA

Introduction:

Injury causes systemic epigenetic changes associated with clinical outcomes. MicroRNAs (miRNAs) are a class of small non-coding RNAs that function in RNA silencing and post-transcriptional regulation of gene expression. Muscle is significantly involved in the metabolic response after severe burn; however, the miRNA profile in this state is unknown. The aim of the study is to outline the microRNA profile in response to severe burn in an animal model.

Methods:

Twelve male Sprague-Dawley rats were randomly assigned to sham and burn groups. Rats received 40% total body surface area (TBSA) scald burn or sham. On day 14, hindlimb skeletal muscles were harvested. plantaris tissue samples from three animals were pooled in each treatment group for total RNA extraction. The microRNA profile of each biological sample was measured using Affymetrix miRNA 4.0 Arrays chips at our institutional microarray core facility. Each chip contained a total of 36,353 small non-coding RNA probes, and the chip reproducibility is greater than 0.95. The raw data signal intensity was normalized with Robust Multi-array Average (RMA)**.** Data was analyzed with Transcriptome Analysis Console (TAC 2.0) software.

Results:

A total of 36,222 miRNAs were detected in each grouped sample, of these 1,218 rattus norvegicus miRNAs probes were identified. The highest probe signal intensity (binary log ratio) was 16 for miRNA-206-3p in both groups. There were 74.5% transcripts in the sham group and 73.9% in the burn group, with a signal intensity less than 2. We identified 703 (57.7%) up-regulated miRNAs and 515 (42.3%) down-regulated miRNAs in the burn group compared to sham. Among the up-regulated profiles, 8 miRNAs were increased over 5 fold. All of the down-regulated miRNAs were within a -3 fold change (table1). MiR-182 was the most up-regulated miRNA following burn. It increased 12.81 linear fold (log ratio 6.14 in BA, 2.46 in SA). It inhibits *Col1a2 gene* (for type I collagen) and promotes *NEU2* gene (for Sialidase-2). MiR-409a-3p was the most down-regulated miRNA in response to burn (-2.95 fold change). Interestingly, it functions with miRNA-182 to inhibit muscle Col1a2 gene expression after burn.

Conclusion:

In summary, we describe the miRNA profile in muscle 14 days after burn f Up-regulation predominated in response to burn. The interaction network between epigenetics and genomic profile confirmed with biological response is under investigation.

Acknowledgement: Supported by DOD grants: # W81XWH-13-1-0489; NIH # T32GM008593

Table1 the number of altered miRNAs in rat muscle after burn

Total 1218 miRNAs	Fold change (Linear)	Number of miRNAs
703 Up-regulated	>10	2
	>5	8
	>2	58
	>1.5	121
515 Down-regulated	<-1.5	61
	<-2	15

Abstract #6

Plantaris microRNA and target gene profile with exercise training in an animal model of bed rest in severe burn

M. R. Saeman[1], J. Song[1], K. DeSpain[1], M. Liu[1], L. A. Baer[2], C. E. Wade[2], S. E. Wolf[1]. [1]University Of Texas Southwestern Medical Center, Surgery, Dallas, TEXAS, USA; [2]University Of Texas Health Science Center At Houston, Surgery, Houston, TEXAS, USA

Introduction:
Severe burn causes muscle atrophy which is typically worsened with bed rest. Exercise is known to ameliorate disuse muscle atrophy. MicroRNAs (miRNAs) are small non-coding RNAs that function as post-transcriptional regulators of gene expression. We hypothesized that plantaris microRNA profiles would differ with exercise training in a clinically relevant animal model of severe burn with prolonged bed rest.

Methods:
Male Sprague-Dawley rats received 40% total body surface area (TBSA) scald burn and were placed in hindlimb unloading with a tail traction system. Half (n=6 per group), performed twice daily exercises of climbing 1 meter with 5 repetitions (EX) the others did not exercise (NX). On day 14, the right plantaris was harvested, preserved in RNA later, and stored in -80°C for further analysis. Tissue samples from each group were pooled. Total RNA was extracted using Qiagen miRNeasy Mini kit. MicroRNA profile was measured using Affymetrix miRNA 4.0 Arrays chips at the institutional microarray core facility. One chip, including a total of 36,353 small non-coding RNA probes with varied species and controls, was used per sample. Chip reproducibility (intra and inter-lot) was greater than 0.95. Raw data was normalized with Robust Multi-array Average (RMA). Data was analyzed with Transcriptome Analysis Console (TAC 2.0) software.

Results:
A total 36,222 miRNAs were detected in each group; of these 1,218 miRNAs were rattus norvegicus. 623 genes were upregulated and 587 were down regulated with exercise. 31 probes were increased greater than 2 fold and 21 probes were decreased more than 2 fold with exercise. Of these, 6 upregulated and 7 down regulated miRNAs corresponded with changes in target gene profile (Table).

Conclusion:
We found that exercise in muscle disuse atrophy after burn is associated with down regulation of several genes.

Acknowledgement: supported by DOD grants: # W81XWH-13-1-0489; NIH # T32GM008593

miRNA Expression With Gene Expression

Transcript Cluster ID	Transcript ID (Array Design)	Fold Change vs. NX	Gene Symbol	Gene Description	Fold Change vs. NX
20501493	rno-miR-183-5p	-12.9	Pla2g2a	phospholipase A2, group IIA	-2.4
20506507	rno-miR-182	-7.8	Col1a2	collagen, type I, alpha 2	-3.5
20501461	rno-miR-138-5p	-5.2	Fabp3	fatty acid binding protein 3, muscle and heart	-2.0
20501431	rno-miR-124-3p	-4.4	Prx	periaxin	-2.3
20500978	rno-miR-338-5p	-3.7	Cdh19	cadherin 19, type 2	-2.3
20500958	rno-miR-330-3p	-2.8	Cldn19	claudin 19	-4.2
20506539	rno-miR-495	5.9	S100b	S100 calcium binding protein B	-6.6
20506523	rno-miR-410-3p	4.2	Gpc3	glycan 3	-2.3
20504257	rno-miR-409a-3p	3.8	Col1a2	collagen, type I, alpha 2	-3.5
20504488	rno-miR-505-3p	2.8	Rrm2	ribonucleotide reductase M2	2.3
20501495	rno-miR-184	2.1	Chad	chondroadherin	-2.4

Abstract #7

Combined Effects of Insulin and Exercise on Muscle Function in Severe Burn

Melody R. Saeman[1], Kevin DeSpain[1], Juquan Song[1], Lisa A. Baer[2], Charles E. Wade[2], Steven E. Wolf[1]
[1]University of Texas Southwestern Medical Center, Dallas, Texas; [2]University of Texas Health Science Center at Houston, Houston, Texas

Introduction:
Muscle loss is a known sequela of severe burn and critical illness that increases the risk of complications such as sepsis and prolonged recovery time. A prior study in a rat model of hindlimb unloading after burn supports that bedrest contributes significantly to muscle atrophy. The aim of our study was to evaluate if exercise combined with insulin in the immediate recovery period mitigates the loss of muscle function in this animal model.

Methods:
Twenty Four Sprague-Dawley rats received a full thickness 40% total body surface area (TBSA) burn and were randomly assigned (n=6) to vehicle without exercise (V/No), insulin (pro zinc 40U daily) without exercise (I/No), vehicle with exercise (V/Ex), or insulin with exercise (I/Ex). All animals were placed in a tail traction system for hindlimb unloading to mimic bed rest immediately following burn. The exercise group was trained to perform twice daily weighted resistance climbing of 1 meter with 5 repetitions. On day 14 *in situ* isometric forces of the left soleus and plantaris muscles were measured. Fatigue measurement was performed in only the soleus. Statistical analysis was performed with Sigma Plot using Student's t-test or ANOVA where appropriate.

Results:
There was no significant change in animal body mass between treatments. The physiological cross sectional area (PCSA) of the plantaris increased with combined insulin and exercise. The tetanic (Po) and twitch (Pt) muscle functions were significantly elevated in the plantaris of I/Ex. However, there was no change in the tetanic force when normalized to PCSA (Po/CSA). The soleus had significant elevation of Po, Pt, Po/CSA, fatigue maximum, and fatigue minimum in I/Ex. Please refer to the table for specific values and significance.

Conclusion:
Insulin and resistance exercise have a positive combined effect on the hindlimb muscle function in this model of critical illness. The plantaris muscle demonstrated increased physiological cross sectional area with increased force suggesting a net increase in muscle fibers as the cause of this change. The soleus demonstrated a change in the specific force of the muscle and fatigue functions indicating a change in the composition of muscle fiber types.

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, $p=0.05$)

Abstract #8

Exercise Treatment Reversed Micro RNA Profile in Burn Rats with Hindlimb Unloading

Anthony Cai[1], Juquan Song[1], Puneet Kumar[1], Alvand Sehat[1], Melody R. Saeman[1], Lisa A. Baer[2], Charles E. Wade[2], Steven E. Wolf[1]

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Introduction:

Micro RNA (miRNA) is a class of non-coding RNA that regulates gene expression by silencing messenger RNA. Burn induces muscle breakdown that is made worse by bed rest, while exercise has been found to alleviate this muscle atrophy. We hypothesize that the alteration of miRNA and target gene profiles contributes to skeletal muscle mass loss after burn, and exercise reverses the muscle atrophy. The purpose of our study was to characterize the miRNA profile correlated to gene expression in an animal model for burn and disuse, as well as miRNA changes seen with exercise.

Methods:

Forty-eight Sprague-Dawley rats were randomly assigned to sham ambulatory (SA), burn ambulatory (BA), sham hindlimb unloading (SH), and burn hindlimb unloading (BH) groups. Rats received a 40% total body surface area scald burn or sham treatment, and they were placed in hindlimb unloading by tail harness, a model for bed rest, or ambulatory. Half of each group received twice daily resistance exercise for eight total groups (n=6 per group). After the 14-day treatment period, the plantaris muscles were harvested for miRNA and genomic data analysis.

Results:

Our results show that compared to the SA group, BA and SH independently upregulate 3- to 4-fold more miRNAs and genes than they downregulate. miRNA-182, -187-3p, and -155-5p rank among the most upregulated. Comparing the combination of B and H (BH) to SA reveals that miRNA-182, miRNA-187-3p, and gene Nr4a3 receive additive contributions from B and H. With exercise, miRNA-182 increased 10.06-fold, and miRNA-138-1-3p decreased 2.14-fold. In comparison, exercise in the BH group strongly downregulated miRNA-182 7.04-fold and miR-138-1-3p 6.57-fold. In a similar fashion, exercise upregulated genes Chad and Cpxm2 in SA, then downregulated them in BH. [Table 1]

Conclusions:

Burn and disuse additive contributions to miRNA and gene changes may explain the additional muscle atrophy burn patients experience with bed rest. Furthermore, exercise demonstrates a greater downregulation of miRNA and genes in BH compared to the SA group.

Applicability of Research to Practice: Elucidating specific miRNAs' roles in muscle atrophy secondary to burn and bed rest opens the possibility of new markers and treatments targets.

External Funding: This project was funded by DOD grant #W81XWH-13-1-0489.

Table 1. miRNA and gene expression changes in response to B, H, BH, exercise in SA, and exercise in BH.

miRNA/ Gene	BA vs. SA	SH vs. SA	BH vs SA	SA: Exercise vs. No Exercise	BH: Exercise vs. No Exercise
miR-138-1-3p	n/a	9.05	14.7	-2.14	-6.57
miR-155-5p	8.82	9.47	7.81	5.66	n/a
miR-187-3p	4.19	4.24	7.9	6.15	n/a
miR-182	12.81	23.83	35.35	10.06	-7.04
mir-184	10.5	17.73	n/a	3.34	2.13
Nr4a3	2.56	3.59	6.31	n/a	-2.38
Cpxm2	2.63	n/a	2.7	2.08	-2.49
Fmod	2.64	n/a	2.77	n/a	-3.01
Chad	6.22	n/a	4.52	3.91	-2.44

---- n/a: fold change is lower than criterion threshold of 2

Abstract #9

Effects of Resistance Exercise and Daily Insulin on Body Mass, Food Intake, Fat Mass and Total Hindlimb Muscle Mass in Rats Following Burn and Disuse

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Introduction: Treatment and recovery of patients with severe traumatic injuries is impacted by an increase in metabolism. Injury induces a systemic catabolic response with increased energy expenditure and loss of body mass. After burn injury, the ability to resume normal activities is compromised due to inactivity associated with bed rest as well as the catabolic response. Exercise and nutritional interventions have been used independently with limited success. The purpose of this study was to determine following burn and disuse how body mass, food intake and total hindlimb muscle mass are affected by a combination of daily resistance exercise and daily insulin.
Methods: Male, Sprague-Dawley rats were randomized into four groups: Burn/Hindlimb unloaded (BH) with daily resistance exercise (EX) or no exercise (NEX) and vehicle control (VEH) or insulin-treated (INS). Daily resistance exercise began ten days prior to injury by adding weight to the tail during repetitive ladder climbing. Rats were then weight-matched into treatment groups, either daily exercise or no-exercise, VEH or INS. Body mass, food intake was collected daily throughout the study. Fat mass and total hindlimb muscle (TA+EDL+Plantaris+Soleus+Medial and Lateral Gastroc) mass was measured on D14. Data are mean±SEM and an ANOVA was used with significance at $p<0.05$.
Results: No differences in body mass were observed between any groups at the time of injury or day 14. Exercise caused a significant increase in mean food over the last 5 days independent of daily insulin. Fat mass was not different between any of the groups. Exercise significantly increased total muscle mass irrespective of insulin. Total hindlimb muscle mass was further increased with the combination of daily insulin and exercise.
Conclusions: Exercise independently and with insulin elicited a significant increase in food. However, the increase in food intake did not result in differences between treatment groups in either body mass or fat mass, supporting a hypermetabolic effect. Muscle wasting was reduced with daily exercise. When daily exercise was combined with daily insulin, there was a greater attenuation of muscle wasting, suggesting the combination of exercise and insulin may be a contributing factor in reversing wasting as a result of burn injury. Different underlying factors seem to be influencing the acute metabolic changes offering possible opportunities for combinations of early interventions resulting in positive long-term outcomes.
Grant Information: US Army MRMC CDMRP W81XWH-13-1-0489

	BH-VEH	BH-INS
D14 Body Mass (g) (n=63)		
NEX	270±3	271±4
EX	273±3	262±6
Food Intake (Last 5 days-g/100g BM/day) (n=63)		
NEX	7.2±0.1	7.3±0.2
EX	7.4±0.1*	7.8±0.2*
Fat Mass (per 100g BM) (n=63)		
NEX	0.62±0.03	0.69±0.04
EX	0.66±0.03	0.65±0.05
Total Hindlimb Muscle Mass (per 100g BM) (n=63)		
NEX	0.88±0.01	0.90±0.02¥
EX	0.95±0.01*	0.99±0.02*¥

Mean ± SEM; *p<0.05 from NEX ¥ p<0.05 from VEH

Abstract #10

Combined Effects of Oxandrolone and Exercise on Muscle Function Recovery in Rats with Severe Burn and Hindlimb Unloading

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Introduction: Muscle mass loss and function impairment worsens with restricted mobility in severe burn patients. We previously showed exercise training improved muscle function recovery in severely burned rats with hindlimb unloading. The aim of the study was to evaluate if the combination of daily oxandrolone with resistance exercise mitigates the loss of muscle function in this animal model.

Methods: Twenty Four Sprague-Dawley rats received a full thickness 40% total body surface area (TBSA) burn and were randomly assigned to vehicle (corn oil) without exercise (V/NEX), oxandrolone (0.1mg/kg/day) without exercise (O/NEX), vehicle with exercise (V/Ex), or oxandrolone with exercise (O/Ex) (n=6/group). All animals were placed in a tail traction system for hindlimb unloading to mimic bed rest after burn. The exercise groups completed resistance training twice a day. On day 14 *in situ* isometric forces of the left plantaris and soleus muscles were measured by using the ASI muscle lever system with dynamic muscle control and analysis software (Aurora Scientific, Inc). Fatigue measurement was only performed in the soleus. Statistical analysis was performed with Sigma Plot using Student's t-test or ANOVA where appropriate.

Results: Tetanic (Po) muscle function were significantly elevated in the plantaris with exercise ($p=0.038$), but not with oxandrolone treatment alone. Fatigue index (FI) was lower and integration was significantly elevated in the soleus after exercise ($p<0.05$) with or without oxandrolone treatment [Table1]. A generalized estimating equation linear regression model was applied to further analyze data of fatigue in the soleus. Results showed that average max force was achieved in the soleus either with oxandrolone treatment or resistance exercise independently. The max force for the O/Ex group was significantly higher than that for the V/NEX group ($p=0.01$).

Conclusion: Resistance exercise improves muscle function in burned rats with hindlimb unloading. Oxandrolone treatment increases strength in the soleus, but is not additive to the effects of exercise.

Support: DOD funding W81XWH-13-1-0462

Abstract #11

Resistance Exercise Effects on Body Mass, Free Fatty Acid Concentration and Fatty Acid Metabolism in sqWAT Following Burn and Disuse in Rats

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Abstract
Introduction: Severe burn induces a catabolic response with increased energy expenditure and loss of body and white adipose tissue (WAT) mass. Burn induces WAT lipolysis, increasing intracellular free fatty acid (FFA) turnover, but despite increased lipolysis, circulating FFA can either increase or decrease. The ability to resume normal activities after burn injury is compromised due to the catabolic response and inactivity associated with bed rest. Exercise, improves insulin sensitivity and decreases circulating FFA, likely due to adaptations in adipose tissue. We investigated how resistance exercise in a combined rodent model of burn and disuse affects circulating FFA, WAT mass and expression of genes involved in fatty acid transport and metabolism.

Methods: Methods: Male SD rats randomized into 8 groups: Sham/Amb (SA), Burn/Amb (BA), Sham Unloaded (SH), Burn Unloaded (BH) with (EX) or without (NEX) exercise. Daily resistance exercise by repetitive ladder climbing. Body mass was collected daily throughout the study. Blood was collected and subcutaneous white adipose tissue (sqWAT) was collected on D14. Circulating FFAs were measured in plasma. Enzymatic pathways of fatty acid metabolism were measured in 84 key genes.

Results: Significant decrease in body mass in SAEX and BAEX vs. SANEX and BANEX. Exercise significantly reduced circulating FFA and sqWAT in SA, BA, and BH. SHNEX & BHNEX had a decrease in expression of sqWAT genes involved in fatty acid metabolism compared to SANEX & BANEX. Exercise increased expression of sqWAT genes in fatty acid metabolism (SAEX, BAEX vs. SANEX, BANEX). Exercise restored genes involved fatty acid metabolism to that of SANEX. Overall, hindlimb unloading decreased genes involved in sqWAT fatty acid metabolism, while the expression was increased with burn injury. In all cases, resistance exercise restored expression of genes involved in fatty acid metabolism to that of the SANEX.

Conclusions: Burn injury with disuse is affected by exercise. Exercise reduced body mass in ambulatory groups, but maintained body mass when combined with unloading. Reductions in circulating FFAs is an indication of possible intracellular breakdown. Alterations in fatty acid metabolism gene expression are often associated with metabolic syndrome, resultants of burn injury and disuse. Fatty acid metabolism in sqWAT is decreased by both burn and unloading, independently and in combination, however, with the incorporation of daily resistance exercise, it appears to be restored.

Applicability of Research to Practice: Gene pathway data may help to shed some light on molecular changes that may be occurring and these data suggest the incorporation of a daily exercise program may be an effective treatment resulting in positive long-term outcomes

Abstract #12

Transcriptomic profile alterations in burn/hindlimb unloaded rats with insulin and exercise combination treatment

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We previously demonstrated changes in miRNA related gene profiles in burn and hindlimb unloaded (B/H) rats. We also found that exercise training reversed those gene profiles which was principally related to diminished oxidative stress and inflammatory signaling. Insulin treatment has also shown positive effects in burns; we recently observed that insulin additively improved muscle function when combined with exercise treatment in burn and hindlimb unloaded rats. We wondered whether transcriptome profiles reflect skeletal muscle pathophysiological changes in this event.
Twenty-four Sprague-Dawley rats received a full thickness 40% total body surface area (TBSA) burn and placed in a tail traction system for hindlimb unloading to mimic bed rest immediately following burn. Half were trained to perform twice daily weighted resistance climbing of 1 meter with 5 repetitions, while the other half was not. Six rats the exercise and no exercise groups received insulin injection subcutaneously (5U/kg daily). On day 14 plantaris muscles were harvested and tissues were grouped for RNA extraction and genomic data analysis applied with Affymetrix Analysis Console 3.0 software.
With threshold for absolute fold change greater than 2, we found 59, 48 and 93 miRNAs altered in rat muscle with insulin, exercise, and combination treatment respectively; miR-499-5p was the most increased with 15 fold in the combination group. For gene expression, we found 122, 119 and 170 changes in insulin, exercise and combination treatment respectively. Act1, Sln, Tecrl were the most upregulated, and Mbp, Pmp2, Mpz were the most down-regulated genes with exercise and insulin treatment. Wikipathway analysis showed that striated muscle contraction pathways were improved with both exercise and insulin treatment, and directly correlated with the muscle function improvement. Meanwhile, TGF beta and TLR signaling pathways were inhibited with both treatments.
In conclusion, exercise and insulin contribute additively to miRNA and gene expression changes after burn and immobilization, and reflect physiologic muscular improvement. The finding of the study identifies the specific pathway signals affected by insulin and exercise.

External Funding: This project was funded by DOD grant #W81XWH-13-1-0489

Abstract #13

Inflammation and Remodeling of Rat Achilles Tendon after Severe Burn

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INTRODUCTION: Severe burn, a full-thickness burn of greater than 30% of total body surface area (TBSA), may affect multiple organs, even those distantly located from the burn site. In the musculoskeletal system, studies have focused mainly on muscle and bone, where hypercatabolism due to the activation of systemic inflammation, and disuse from long immobilization periods is induced. Muscle loss and atrophy post burn have been associated with increased levels of TNF-α, while bone mass loss has been associated with circulating IL-1β and IL-6, which are increased from 24 hours post burn. We aim to study the molecular and structural effects of burn injury on tendon in an effort to ultimately understand the behavior of the whole muscle- tendon-bone organ. We report acute local molecular and functional changes in Achilles tendon as result of severe burn-induced systemic inflammation.
METHODS: *Severe burn injury:* 40 adult male Sprague-Dawley rats, 270-300 g, were used with protocol approved by the Institutional Animal Care and Use Committee at the University of Texas Southwestern. Animals were randomly divided into 5 groups: Control (n = 11), 1 day (n = 6), 3 days (n = 6), 7 days (n = 6), and 14 days (n = 11) post burn. Under anesthesia, animal hair was removed from the dorsal and lateral surfaces. Dorsal skin amounting to 40% of TBSA was immersed in 95°C-100°C water for 10 seconds. Control animals received sham treatment. *Gene and protein expression*: Tendon RNA was extracted and analyzed for expression of IL-6, TNF, IL-1β, TGFβ1, col1a1, col3a1, MMP9 and MMP13 by qPCR. 10 μg of total protein was used for Western blot with anti-collagen I (Abcam), anti-collagen III (Abcam). *Histology*: After tendon fixation and decalcification, paraffin sections were processed for H&E. *Biomechanics:* Testing was done in an Instron 5565 universal testing system equipped with a 5 kN load cell. Samples were pulled until failure at a cross-head speed of 6 mm/min collecting force and deformation data throughout the test. *Statistics*: Data are presented as mean ± error propagation for gene expression and mean ± standard deviation elsewhere. 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 expression of IL-6 and IL-1β as well as MMP9 and MMP13 increased in rat tendon 3 days after burn. TNF did not reached significant difference. Moreover, TGFβ1 a marker for tissue repair, was elevated after 14 days. Protein expression of Collagen I decreased at day 7 and returned to control levels by day 14. Together with this, we observed a trend to increase of col1a1 gene expression at day 7 (p=0.055). On the other hand, col3a1 gene expression is significantly higher at day 3 post burn, while an increase in protein Collagen III was observed by day 14 post burn. Protein ratio for Collagens I/III decreased at day 14, $p < 0.05$, indicating signs of tendon remodeling. Histological analysis showed higher cell density in tendon periphery and surroundings at days 1 and 3 post burn. Biomechanical analysis showed a decrease in stiffness and ultimate force of tendons in burn rats after 14 days.
DISCUSSION: The upregulation of both IL-1β and IL-6 indicates the initiation of acute inflammation in the tendon. When tendon experiences remodeling or injury, collagen III content increases, resulting in a decrease in tensile strength. On the other hand, metalloproteinases such as the collagenase MMP13 can cleave the collagen triple helix, creating fragments that are further degraded by gelatinases such as MMP9. We propose that the initial decrease in collagen I can be explained by the upregulation of MMP13 and MMP9, generated by IL-1β. We hypothesize that this event is started by the cell infiltration observed in histological analysis. We are currently investigating the involvement of macrophages. The synthesis of collagen III protein, the decrease in collagen I/III protein ratio and the increase in TGFβ1 suggests an induction of scar tissue or remodeling. To address whether molecular changes in tissue structure will also affect its functional mechanics, the tensile force and deformation were measured. Overall, our findings showed that ultimate force and stiffness were reduced at 14 days post burn compared to controls. These biomechanical results correlate with histological findings of less organized fibers. Although previous reports have shown that Achilles tendon could react to an inflammatory event produced in surrounding tissue (*8*), this study is the first to report that remote systemic inflammation is capable of inducing a local inflammatory response and remodeling in the tendon.

Abstract #14

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

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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;

Abstract #15

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

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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.

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Abstract # 16

Vascular Smooth Muscle Dysfunction After Burn

K. DeSpain BS, J. Song MD, CR Rosenfeld MD, S. Wolf MD

Introduction: Hypotension is a major complication after large surface area burns. Increased systemic vascular permeability causes hypovolemic shock in server burn patients. Historically the volumetric loss of fluid through leaking capillaries has been treated by replacement with fluids. However important, this is not the sole answer and often times the patient remains hypotensive. We proposed to determine if this hypotensive condition was solely due to fluid loss during the leaky vessel condition seen after burn, or if there were other contributing factors. The purpose of study is to investigate the physiological profile of vascular smooth muscle in response to severe burn.

Methods: Adults Sprague-Dawley male rats were enrolled in the study. 34 rats received a 40% total body surface area (TBSA) scald burn and fluid resuscitation using the Parkland formula (V = 4mL x TBSA % x kg. At time points of 6 hrs, 24 hrs, 3 days, 7 days and 14 days post burn, animals were euthanized and endothelial intact carotid arteries were dissected for ex vivo test in organ baths containing 37°C PBS. The force measurement of artery ring were collected under the Optimal length (Lo) as the vessels were exposed to the vasoconstrictors Norepinephrine (NE) and Angiotensin II (AngII), and vasodilator Acetylcholine (ACh) consequently. Force was measured in grams and converted to Stress (N/m^2) taking into account Lo and the weight of the vessel (grams).

Results: A cumulative dose response was used for NE ($10^{-8}M$ – $10^{-5}M$) and on average at each dose there was a 50% decrease in stress generated by the vascular smooth muscle (VSM) at 6hrs (P=0.012). We saw a very similar response to a single dose of AngII ($10^{-7}M$) in that there was a 70% decrease in stress generated at 6hrs (P<0.001). The arteries were preconstricted with NE $10^{-5}M$, allowed to reach a steady state and then were exposed to a cumulative dose response of ACh ($10^{-7}M$ – $10^{-4}M$). The 3 day, 7 day and 14 day arteries relaxed (specify for the force change) significantly more than the 6hr post burn arteries (P=0.001).

Conclusion: In this study we have shown that at 6 hrs after burn there is a significant decrease in responsiveness to both Norepinephrine, which works through adrenergic receptor, and Angiotensin II, which works through the angiotensin type II receptor. At 24 hrs after burn, these responses have returned to control levels.

In this study we showed that ACh does not change in dilatory effect at 6 and 24 hours post burn but at 3 days, 7 days and 14 days post burn there was increased relaxation.

Acetylcholine binds to a receptor on the cell membrane of the vascular smooth muscle endothelial cell. This causes the endothelial cell to generate nitric oxide (NO) through the activation of the nitric oxide synthase (NOS) pathway. The newly created NO diffuses into the VSM and initiates dilation. We administered ACh into the baths to determine if this NOS pathway also may contribute to a hypotensive state after burn. This could explain why one may see persistent hypotension days after burn possibly through an increase in NOS activity.

Applicability of Research to Practice: We believe that this study will help physicians understand the mechanism related to the hypotensive phenomena seen in patients after burn and how to treat that patient through the administration of pressors to attain a normotensive status.

Abstract #17

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

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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. 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.

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Abstract #18

Muscle Homeostasis is Disrupted in Burned Adults

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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.

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Abstract #19

Serum Level of Musclin Is Elevated Following Severe Burn

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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.

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