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PRINCIPAL INVESTIGATOR: Jarrod A Call

CONTRACTING ORGANIZATION: University of Georgia

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14. ABSTRACT

Battlefield casualty and trauma often results in major injury to the extremities, one example of this is volumetric muscle loss (VML) injuries. While advances in prolonged field care have saved many Servicemembers lives, those with VML injures are left with long-term functional complications. Unlike more simple muscle injuries, VML injuries are not capable of undergoing significant self-repair. One factor limiting muscle function recovery is a lack of current treatments and rehabilitation techniques and lack of understanding of the secondary effects due to VML such as physical inactivity. We are investigating how early rehabilitation may improve muscle function following VML injury. We will test two specific aims: 1) to determine if early rehabilitation approaches are sufficient to improve the function and quality of the remaining tissue after VML injury. And 2) to understand if injury-induced physical inactivity significantly impairs the quality of remaining tissue after VML injury and the responsiveness to rehabilitation. By studying and understanding early rehabilitation following injury we hope to improve healing of the muscle and effective rehabilitation. Additionally, we hope to elevate the wounded Servicemember's long-term quality of life. To date, we have identified mitochondrial dysfunction as a primary pathology of the remaining muscle after VML with long-term consequences, and the remaining muscle is resilient to traditional rehabilitation to correct this condition. Moving forward we are investigating new approaches and mitochondrial biogenesis targets.

15. SUBJECT TERMS

NONE LISTED

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1. Introduction

Battlefield casualty and trauma often results in major injury to the extremities, one example of this is volumetric muscle loss (VML) injuries. While advances in prolonged field care have saved many Servicemembers lives, those with VML injures are left with long-term functional complications. Unlike more simple muscle injuries, VML injuries are not capable of undergoing significant self-repair. One factor limiting muscle function recovery is a lack of current treatments and rehabilitation techniques and lack of understanding of the secondary effects due to VML such as physical inactivity. We are investigating how early rehabilitation may improve muscle function following VML injury. We will test two specific aims: 1) to determine if early rehabilitation approaches are sufficient to improve the function and quality of the remaining tissue after VML injury. And 2) to understand if injury-induced physical inactivity significantly impairs the quality of remaining tissue after VML injury and the responsiveness to rehabilitation. By studying and understanding early rehabilitation following injury we hope to improve healing of the muscle and effective rehabilitation. Additionally, we hope to elevate the wounded Servicemember's long-term quality of life.

2. Keywords

Regenerative rehabilitation, regenerative medicine, skeletal muscle physiology, mitochondrial physiology, volumetric muscle loss, rehabilitation, oxygen consumption, PGC1, physical inactivity, neuromuscular electrical stimulation
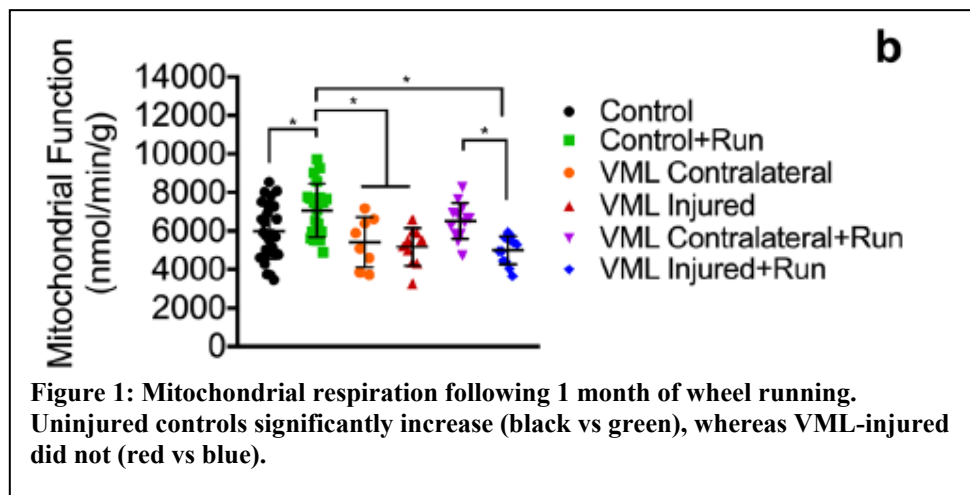
3. Accomplishments
- **What were the major goals of the project?**

Specific Aim 1: *To determine if early rehabilitation approaches are sufficient to improve the function and quality of the remaining tissue after VML injury*	Timeline (months)	UGA (Call)	Minnesota (Greising)
Major Task 1: To determine if approaches to enhance metabolic capacity in healthy tissue are feasible after VML injury			
Subtask 1: Obtain IACUC & ACURO approvals	1-3	Completed	N/A
Subtask 2: Determine optimal rehabilitation to improve metabolic function of muscle following VML	4-14	Completed	N/A
Major Task 2: To investigate the effectiveness of combined therapies for VML to improve the function of the remaining tissue after VML injury			
Subtask 3: Perform combined rehabilitation techniques following VML injury	12-27	In progress	N/A
Subtask 4: Perform muscle contractile/metabolic assessments, test mitochondrial function	14-32	In progress	N/A
Milestone Achieved: Local IACUC Approval	4	Completed	N/A
Milestone(s) Achieved: Quantification and understanding of optimal rehabilitation to improve metabolic function of muscle following VML injury.	14	In progress	*N/A*
Milestone(s) Achieved: Quantification and understanding of optimal rehabilitation and timing to improve contractile and metabolic function of muscle following VML injury.	14-33	In progress	N/A
Milestone Achieved: Final reporting	36	No progress	No progress

Specific Aim 2: *To understand if injury-induced physical inactivity significantly impairs the quality of remaining tissue after VML injury and the responsiveness to rehabilitation.*			
Major Task 3: To characterize physical activity levels following a multi-muscle VML injury in a rodent model.			
Subtask 1: Obtain IACUC & ACURO approvals	1-3	N/A	Completed
Subtask 2: Perform physical activity measures following VML injury	12-19	N/A	Completed
Major Task 4: To investigate how VML-induced and limited physical activity impairs the responsiveness of the remaining muscle to rehabilitation specifically range of motion, neuromuscular electrical stimulation, and mitochondrial therapies.			
Subtask 3: Model inactivity following injury with restricted caging	16-20	N/A	In progress
Subtask 4: Test various early rehabilitation with inactivity model for functional muscle improvements and histology outcomes	17-25	N/A	In progress
Milestone Achieved: Local IACUC Approval	4	N/A	Completed
Milestone(s) Achieved: Understanding of how VML-induced and limited physical activity impairs the responsiveness of the remaining muscle to rehabilitation.	32	N/A	In progress
Milestone Achieved: Final reporting	36	No progress	No progress

- **What was accomplished under these goals?**

Major Task 1-Subtask 2: determine optimal rehabilitation to improve metabolic function of muscle following VML

Figure 1: Mitochondrial respiration following 1 month of wheel running. Uninjured controls significantly increase (black vs green), whereas VML-injured did not (red vs blue).

We previously reported that skeletal muscle fibers from VML-injured mice had significantly less mitochondrial respiration compared to muscle fibers from uninjured mice. This was determined by assessing the oxygen consumption rates of permeabilized muscle fibers from VML-injured and uninjured mice using a high-resolution Oroboros O2K respirometer. We then tested the extent to which voluntary wheel running could enhance mitochondrial function in muscle after

VML injury. The results from this study were published in 2019 and suggested wheel running was not sufficient alone as a therapy to bolster mitochondrial function in injury muscle (Figure 1). We explored this unique pathology in VML-injured mice and determined that VML injury disrupted an importantly cellular signaling pathway to engaged mitochondrial genes necessary to elicit mitochondrial adaptations. Specifically, the mitochondrial transcription factor PGC1α was not activated properly in VML-injured muscle, and this ultimately limits the capacity to use wheel running as a rehabilitation therapy.

We decided to next test different pharmaceutical adjuvants that could bolster mitochondrial function in VML-injured muscle. These adjuvants were selected to stimulate a variety of pathways (AMPK- AICAR, G-coupled receptors- formoterol, PPARα- pioglitazone, cGMP-sildenafil) that increase expression of PGC1-α, a transcription factor that regulates mitochondrial biogenesis. We hypothesize that increasing mitochondrial biogenesis will improve overall muscle function and make tissue receptive to future physical rehabilitation. The pathways they stimulate all eventually enhance the expression of PGC1α and induce mitochondrial biogenesis, which, in turn, could enhance oxidative metabolism. We have shown that the treatment of VML-injured muscles with formoterol leads to greater muscle mass and peak isometric torque. Although formoterol treatment did not influence mitochondrial content, the muscle's metabolic function was significantly enhanced, suggesting an improvement in mitochondrial efficiency (Figure 2).
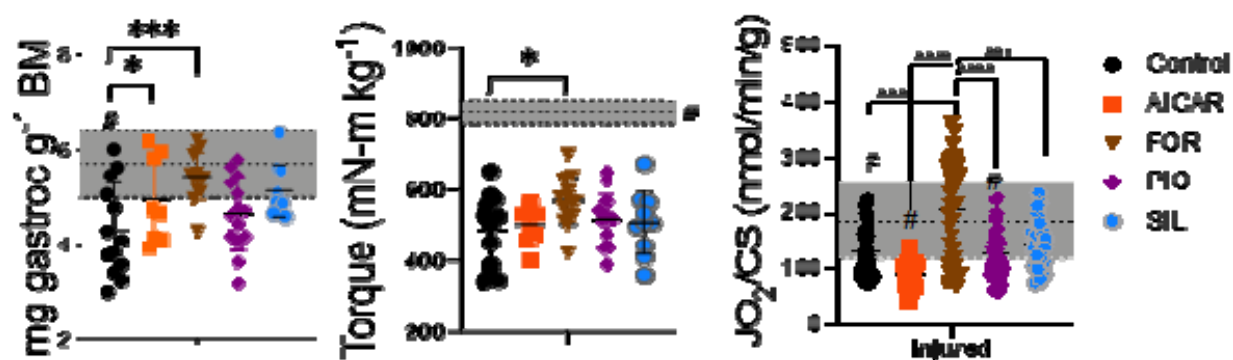

Figure 2: muscle mass normalized to body mass (left), muscle strength (middle), and mitochondrial respiration normalized to content (right) for injury untreated (control), AICAR-treated, formoterol-treated (FOR), pioglitazone-treated (PIO), and sildenafil-treated (SIL) VML mice. Significance indicated by asterisks and bars between groups

To summarize Major Task 1: we identified that passive range of motion decreased joint stiffness post-VML, electrical stimulation improved muscle strength but not metabolic function post-VML, voluntary wheel running improved strength but not metabolic function post-VML, and formoterol improved both muscle strength and metabolic function post-VML.

Major Task 2-Subtask 3: perform combined rehabilitation techniques following VML injury

We have our first cohort of subjects enrolled in a combined formoterol and voluntary wheel running strategy. This cohort started therapy 3 days post-VML injury. A second cohort of combined formoterol and wheel running will start 1-month post-VML injury to determine if early vs. late rehabilitation initiation impacts muscle adaptation. Our contingency plan for is to test combined formoterol and electrical stimulation if the first study does not result in a combined effect.

Major Task 2-Subtask 4: perform muscle contractile/metabolic assessments, test mitochondrial function

Our outcome measurements will include muscle strength, treadmill running endurance, and an exhaustive metabolic functional testing complete with fat vs. carbohydrate metabolism and mitochondrial membrane potential.

Major Task 3-Subtask 2: perform physical activity measures following VML injury

We enrolled subjects into a study assessment physical activity, metabolic function, and fuel utilization following VML injury. Whole body metabolism was evaluated for the 24-hour period and isolated by the 12 hours of active and inactive time. Respiratory exchange ratio (RER, physiologic range of 0.7-1.0), an indirect measure of muscle oxidative capacity, was not different across experimental groups over 24-hour periods. However, the metabolic flexibility of RER between active and inactive phased was significantly impaired following VML such that injury naïve mice had changes ~0.10 in RER while VML injury mice had only had changes of ~0.06 in their RER throughout the 24-hrs, suggesting following VML injury there is an impairment in the body's ability to switch between fuel sources (Figure 3). On average, mice ambulated ~1.2 km per 24-hour period and contrary to the hypothesis, there was no difference in activity levels between sham and VML injured mice. VML-injured gastrocnemius muscle masses were 22% less compared to uninjured. Additionally, plantarflexion torque normalized to muscle mass was 9% less in VML-injured compared to injury naïve mice, showing the disproportionate loss of muscle strength compared to muscle mass. Mitochondrial respiration rates normalized to mitochondrial content were 28% less in VML-injured compared to injury naïve mice.
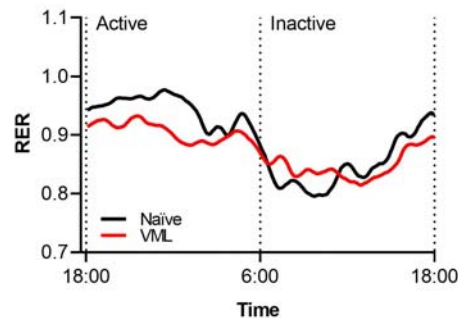

Figure 3: representative data from 24-hour continuous analysis of oxygen consumption and carbon dioxide production in uninjured (Naïve) and VML-injured mice during active and inactive phases of the day.

These findings indicate limited changes in physical activity levels in a model of VML injury. The ability to maintain physiologic homeostasis after VML injury is chronically impaired, metabolic flexibility is an indicator for overall health and the inability to moderate physiologic need for fuel sources after VML may limit other whole body challenges such as nutritional input, energetic demand, or environmental oxygen limitation.

Major Task 4-Subtask 3: model inactivity following injury with restricted caging

In order to test the extent to which reduced mobility may influence muscle function following VML injury, mimicking ICU scenarios, we created small cage systems. We enrolled a cohort of mice into this study in a cross-over design. We assessed RER, metabolic rate, and ambulation in the same subjects in a standard vs. small cage environment. Ambulation decreases from 1.7km to 0.7km in the small cage setting. There was a 20% reduction in metabolic rate and an increase in

RER during small cage system exposure. Importantly, an increase in RER indicates a stronger reliance on carbohydrates as a fuel source instead of fats. This is in agreement with RER analysis of human in bedrest scenarios. Together, the small cage system successfully models a reduced mobility setting and we will next assess the implications of this system on mice with VML injury.
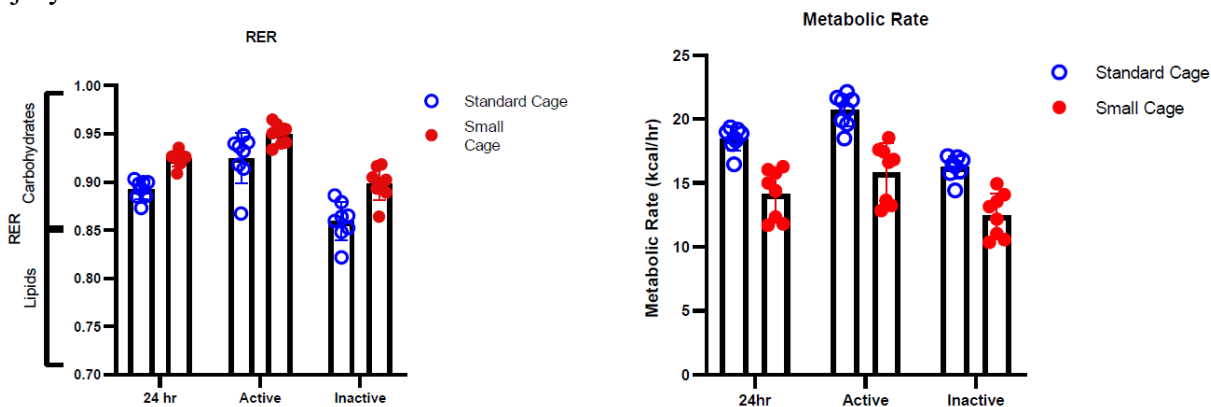

Figure 4: summary of continuous evaluation of oxygen consumption and carbon dioxide production (left) and total metabolic rate (right) in standard vs. small cage systems, a cross-over study design.

Finally, evidence-based approaches for VML rehabilitation are also limited by a predominant focus in the field on tissue regeneration that neglects the remaining muscle and overwhelming fails to include clinically-relevant outcomes measurements. We conducted a systematic review and meta-analysis (attached as a supplement) of the VML field and identified 2,312 studies (search ended January 2019). Screening of these studies for those that included an outcome measurement of muscle function left 44 studies for our meta-analysis. The findings of our meta-analysis[42] indicate that, compared to leaving the injury untreated, various treatments for VML can modestly (~16%) improve function (Figure 5); however, beneficial effect size is small, and on average, there was still a functional capacity deficit of 36%, suggesting that current regenerative treatment paradigms require further maturation to achieve clinically meaningful functional improvements. **We believe that these findings support our overarching goals to identify rehabilitation strategies to use in concert with regenerative medicine to maximize function recovery of the remaining muscle after VML injury.**
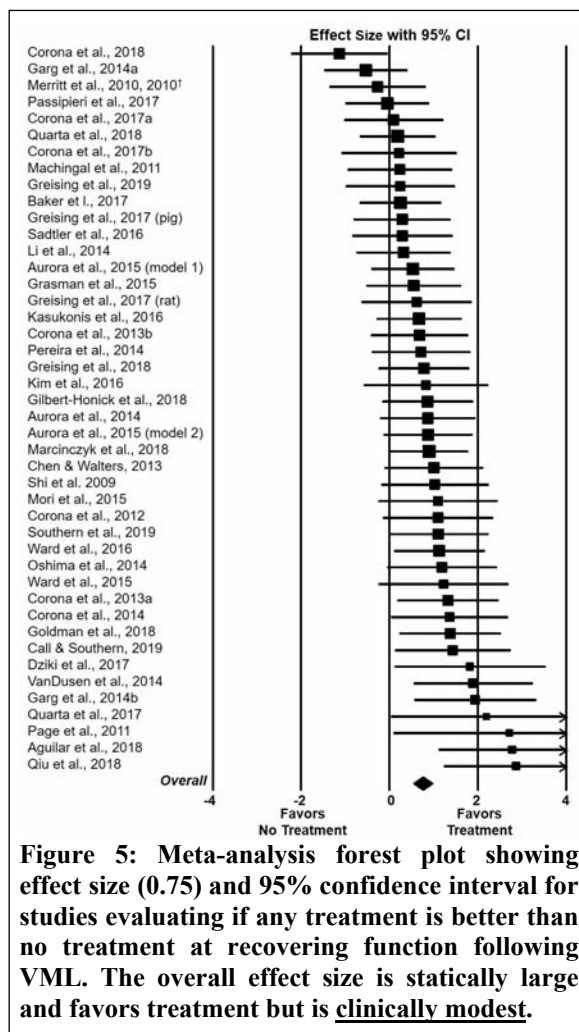

Figure 5: Meta-analysis forest plot showing effect size (0.75) and 95% confidence interval for studies evaluating if any treatment is better than no treatment at recovering function following VML. The overall effect size is statically large and favors treatment but is <u>clinically modest</u>.

- **What opportunities for training and professional development has the project provided?**

Jennifer McFaline-Figueroa, Anna Nichenko, and Albino Schifino at the University of Georgia have all be participating in the research efforts as graduate students. Additionally, undergraduate student-researchers Elizabeth Winders and Tate Hunda. They have submitted abstracts to international conferences based on data collected from this project. We have weekly journal clubs and lab meetings to discuss advances in the field and evaluate our own data, respectively. Graduate students interact frequently with Dr. Greising at the University of Minnesota. Jennifer is using this study to partially fulfill her dissertation work.

Kyle Dalske a graduate student, Alec Basten an undergraduate student, and Dr. Christiana Raymond-Pope, a postdoc at the University of Minnesota have all contributed to this work. They have received mentorship from Drs. Greising and Call regarding animal models of regenerative and rehabilitative medicine. Kyle and Alec's work on Aim 2 of this award will be a component of their Masters and Honors undergraduate theses, respectively. This work is expected to be submitted to various scientific meetings in the next period of performance. Christiana has recently submitted her work on metabolic cage activity to the conference Integrative Physiology of Exercise hosted by the American Physiological Society.

- **How were the results disseminated to communities of interest?**

1) Our primary findings were published in peer-reviewed journals, manuscripts and abstracts.
2) Our primary findings were discussed during invited talks at scientific meetings
3) Our primary findings were disseminated via collegial dialogue at scientific meetings.

- **What do you plan to do during the next reporting period to accomplish the goals?**

During the next reporting period we plan to complete Major Tasks 2 & 4. As discussed above, cohorts are already enrolled for the completion of Major Task 2 and 4. We are excited about our progress of creating a bedrest model to mimic to loss of mobility that can accompany VML. This will be critical to evaluating the potential long-term ramifications of VML injury and a lack of physical therapy provided early.

4. Impact
- **What was the impact of the development of the principal discipline(s) of the project?**

Regenerative Rehabilitation/Medicine: The primary impact of our work to date is advanced knowledge of the pathophysiology of the remaining muscle after VML, limitations of traditional rehabilitation strategies such as aerobic exercises, and the potential benefit of formoterol to complement rehabilitation strategies. We also showed VML injury is associated with a whole-body impairment, specifically, metabolic inflexibility that could associate with risk of cardiovascular disease and diabetes. If this is confirmed at the conclusion of our study, it may warrant epidemiological studies of patients with traumatic VML injuries and their risks for comorbidities. Finally, our meta-analysis highlighted some challenges for the field: first, a lack

of reliance physiological outcomes to assess validity of VML therapies; second, a significant although minor benefit of therapies for VML injuries that indicate a maturation of techniques for the field; and third, the few regenerative rehabilitation studies that have been done indicate no overall benefit indicating additional strategies are necessary. These findings provide a roadmap for future VML studies to follow.

- **What was the impact on other disciplines?**

Physiology: A tenant of physiology is that the function of an organ will adapt to a change in stimuli. Skeletal muscle is a highly adaptive organ, and an increase in oxidative capacity is the most robust adaptation in skeletal muscle to endurance exercise training. However, VML injury clearly attenuates the adaptative capacity of skeletal muscle and this may be due to motor unit recruitment and/or impaired molecular signaling. The etiology of poor motor unit recruitment and/or impaired molecular signaling in VML-injured muscle is still unclear but likely contributors include oxidative stress, inflammation, and fibrotic tissue deposition. Our research to date suggests that the cellular environment of skeletal muscle is influenced by these factors and can slow adaptive potential.

A new model: the small cage system produces outcomes in terms of metabolic rate and RER that mimics bedrest scenarios in humans. This model could be used by multiple disciplines to study muscle atrophy and wasting with bedrest in ICU settings.

- **What was the impact on technology transfer?**

Nothing to report

- **What was the impact on society beyond science and technology?**

Nothing to report

5. Changes/Problems
- **Changes in approach and reasons for change**

Nothing to report

- **Actual or anticipated problems or delays and actions or plans to resolve them**

The University of Georgia and University of Minnesota experienced shutdowns in April and May due to COVID-19. Reopening is ~75% at the University of Georgia and ~50% at the University of Minnesota resulting in delayed starts to second phase animal experiments. We have approval to work in close proximity in limited settings such as small animal surgery and tissue harvest as necessary for the completion of the planned experiments. Cohorts of mice have now been enrolled in the second phases and progressing.

- **Changes that had a significant impact on expenditures**

Similar to above, COVID-19 campus research shutdowns have slowed expenditures related to research for this project.

- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

Human subjects: Not applicable
Vertebrate animals: Nothing to report
Biohazards: Nothing to report

6. Products
- **Publications, conference papers, and presentations**

Journal publications
1. Therapeutic Approaches for Volumetric Muscle Loss Injury: A Systematic Review and Meta-Analysis. Tissue Eng Part B Rev 25(6):510-525. Greising SM, Corona BT, McGann C, Frankum JK, Warren GL PMID: 31578930 DOI: 10.1089/ten.TEB.2019.0207
2. Musculoskeletal Regeneration, Rehabilitation, and Plasticity Following Traumatic Injury. Greising SM, Corona BT, Call JA. Int J Sports Med. 2020 Apr 2. doi: 10.1055/a-1128-7128. [Epub ahead of print] PMID: 32242332

- **Books or other non-periodical, one-time publications**

Published abstracts
1) McFaline-Figueroa J, Nichenko AS, Schifino AG, Call JA. Ca2+induced complex I inactivity: a model for early mitochondrial dysfunction following volumetric muscle loss injury. The FASEB Journal 34(S1):1-1

2) Dalske KA, Basten AM, Raymond-Pope CJ, Call JA, Greising SM. Interplay between whole body metabolism, physical activity, and muscle function following volumetric muscle loss injury. The FASEB Journal 34(S1):1-1

3) Basten AM, Raymond-Pope CJ, Dalske KA, Call JA, Greising SM. Metabolic and contractile pathophysiology following volumetric muscle loss injury. The FASEB Journal 34(S1):1-1

4) Hoffman DB, Sorensen JR, Call JA, Corona BT, Greising SM. Temporal changes in pathologic fibrosis following volumetric muscle loss injury. The FASEB Journal 34(S1):1-1

5) Greising SM, Warren GL, Call JA. Bone deterioration and metabolic deficiency after volumetric muscle loss injury: targets for regenerative rehabilitation. Journal of Bone and Mineral Research Plus 3(S4):23

- **Other publications, conference papers, and presentations**

Oral presentations
1) Stranger things: the upside-down of muscle, mitochondrial, and bone plasticity after volumetric muscle loss injury. Symposium on Regenerative Rehabilitation. Jarrod Call on October 24, 2019.
2) Metabolic and contractile consequences of skeletal muscle injury and aging: targets for rehabilitation. Duke University Molecular Physiology Institute. Jarrod Call on December 10, 2019

3) Optimizing skeletal muscle function after volumetric muscle loss injury by leveraging the pathophysiology. Tissue Engineering and Regenerative Medicine International Society – Americas (TERMIS_AM), Orlando, FL. Sarah Greising on December 5, 2019
4) Molecular metabolism as a nexus for regenerative rehabilitation. Experimental Biology, San Diego, CA. Scheduled for April 5, 2020. Meeting Cancelled

Poster presentations
1) Abstract Accepted/Meeting Cancelled: Call JA, Dalske KA, McFaline-Figueroa J, Schifino AG, Raymond-Pope C, Greising SM. Are we overlooking the metabolic function of skeletal muscle following volumetric muscle loss injury? MHSRS 2020
2) McFaline-Figueroa J, Nichenko AS, Schifino AG, Call JA. Early mitochondrial dysfunction after VML may be explained by calcium-induced complex I inactivity. Orthopaedic Research Society 2020 Annual Meeting, Phoenix, AZ, February 9, 2020.

Online news report

- **Website(s) or other internet site(s)**
- **Technologies or techniques**
- **Inventions, patent applications, and/or licenses**
- **Other products**

Organized Symposium
- Regenerative Rehabilitation, specifically "Regenerative Rehabilitation: Optimizing the Functional Recovery of Muscle. Experimental Biology, San Diego, CA. Scheduled for April 5, 2020. Meeting Cancelled

7. Participants & other collaborating organizations
- **What individuals have worked on the project?**

Name:	Jarrod Call
Project Role:	PI
Researcher ID:	0000-0002-1094-4940
Nearest person month worked:	4.8
Contribution to Project:	Creation of AUP and submission at Georgia. Purchasing of necessary equipment and supplies at Georgia. Communication with Sarah Greising on upcoming study enrollment. Preparation for invited talk, purchasing of animals for first cohort enrollment, purchasing of surgical supplies and supplements for first cohort, communication with Sarah Greising on study execution. Tissue harvest and analysis for first cohort. Writing ACURO amendment. Identifying alternative drug targets for metabolic adaptation. Overseeing Major Task 2 studies and mentoring graduate students.
Funding Support	Summer salary support from NIH project unrelated to DoD.

Name:	Sarah Greising
Project Role:	PI
Researcher ID:	0000-0001-9285-4908
Nearest person month worked:	4.8
Contribution to Project:	Creation of AUP and submission at Minnesota. Purchasing of necessary equipment and supplies at Minnesota. Communication with Jarrod Call on upcoming study enrollment. Finalization of animal use documents. Purchasing of necessary equipment and supplies at Minnesota. Recruiting

	personnel for study enrollment. Ordering of first mouse cohort and planning for in vivo metabolic testing of this cohort over the next quarter.
Funding Support	

Name:	Jennifer McFaline-Figueroa
Project Role:	Graduate student
Researcher ID:	
Nearest person month worked:	4.0
Contribution to Project:	Responsible for day-to-day actions related to Major Task 2 identifying best practice to enhance oxidative capacity in remaining muscle, daily animal care and surgery, data acquisition and analysis, organization of study execution, writing reports and data summaries
Funding Support	

Name:	Albino Schifino
Project Role:	Graduate student
Researcher ID:	
Nearest person month worked:	3.0
Contribution to Project:	Facilitating study execution, performing physiological testing of muscle contractile function and metabolic capacity. Data acquisition and analysis
Funding Support	

Name:	Anna Nichenko
Project Role:	Graduate student
Researcher ID:	
Nearest person month worked:	3.0
Contribution to Project:	Facilitating study execution, performing physiological testing of muscle contractile function and metabolic capacity. Data acquisition and analysis
Funding Support	Graduate School Dissertation Completion Award provides salary for Anna's final year of graduate school.

Name:	Kyle Dalske
Project Role:	Graduate student
Researcher ID:	0000-0003-0268-0882
Nearest person month worked:	4.0
Contribution to Project:	Facilitating study execution, performing activity measure testing and metabolic capacity. Data acquisition and analysis
Funding Support	Kyle's graduate school stipend is covered by the UMN School of Kinesiology during the academic year and this award during the summer months.

Name:	Christiana Raymond-Pope
Project Role:	Postdoctoral Fellow
Researcher ID:	0000-0003-3930-5904
Nearest person month worked:	3.0
Contribution to Project:	Facilitating study execution, performing activity measure testing and metabolic capacity and developing small cage/ICU model. Data acquisition and analysis
Funding Support	Christiana is supported by a NIH T32 training grant through the department of orthopedics at UMN.

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

New Award

- **What other organizations were involved as partners?**

University of Minnesota
Minneapolis, MN
Sub-award to Sarah Greising at University of Minnesota

8. Special reporting requirements
- **Collaborative awards**
- **Quad charts**

9. Appendices
Published works attached

termis.

Tissue Engineering
& Regenerative Medicine
International Society

REVIEW ARTICLE

Therapeutic Approaches for Volumetric Muscle Loss Injury: A Systematic Review and Meta-Analysis

Sarah M. Greising, PhD,[1] Benjamin T. Corona, PhD,[2] Christopher McGann, DPT,[3] Jeremy K. Frankum, DPT,[3] and Gordon L. Warren, PhD[3]

Our goal was to understand the impact of regenerative therapies on the functional capacity of skeletal muscle following volumetric muscle loss (VML) injury. An extensive database search (e.g., PubMed, Cochrane Library, and ClinicalTrials.gov) was conducted up through January 2019 to evaluate the following: ''In humans or animals with VML injury, is treatment better than no treatment at recovering functional capacity?'' Study eligibility criteria required studies to have both an untreated and at least one treated VML injury group. From 2312 study reports, 44 studies met the inclusion criteria. Quantitative functional capacity data (absolute and/or normalized strength) or proportional measures (histological analysis quantifying viable muscle tissue, mitochondrial function, and/or exhaustive treadmill running) were extracted for use. While both human and animal studies were included in the searches, only animal studies met the eligibility criteria. Using a random-effects model, Hedges' g was used as the effect size (ES) and calculated such that a positive ES indicated treatment efficacy. The overall ES was 0.75 (95% confidence interval: 0.53–0.96; $p < 0.0000001$), indicating that the treatments, on average, resulted in a significant improvement in functional capacity. From network meta-analyses, it was determined that an acellular biomaterial combined with stem and/or progenitor cells had the greatest treatment effectiveness. The findings indicate that various treatments in animal models of VML improve the functional capacity of muscle compared to leaving the injury untreated; however, the ∼16% beneficial effect is small. Our results suggest that current regenerative therapy paradigms require further maturation to achieve clinically meaningful improvements in the functional capacity of the muscle.

Keywords: biomaterial, extracellular matrix, network meta-analysis, orthopedic trauma, regenerative medicine, satellite cell

Impact Statement

Our most salient findings are that (1) various treatment approaches used in animal models of volumetric muscle loss (VML) injury improve functional capacity compared to leaving the injury untreated and (2) an acellular biomaterial in combination with cellular components was the most effective treatment to improve functional capacity following VML injury to date. The nature of our findings has substantial implications for regenerative medicine, biomedical engineering, and rehabilitative techniques currently being evaluated and developed for VML injury repair, and are pivotal to the progression of the regenerative medicine effort aimed at restoring maximal function to traumatized and disabled limbs.

Introduction

SKELETAL MUSCLE INJURY is common in orthopedic trauma and can have a profound impact on a variety of treatment outcomes, including fracture healing,[1,2] muscle and limb function,[3] and disability.[4,5] Of particular signifi-

cance are injuries in which a relatively large piece of muscle tissue is abruptly removed, such as the orthopedic wound pattern observed in soldiers following explosive blast trauma.[6–8] This form of muscle trauma is referred to as volumetric muscle loss (VML) injury and differs in etiology from progressive conditions of muscle loss associated with

[1]School of Kinesiology, University of Minnesota, Minneapolis, Minnesota.
[2]School of Medicine, Wake Forest University, Winston-Salem, North Carolina.
[3]Department of Physical Therapy, Georgia State University, Atlanta, Georgia.

aging or disease, such as disuse atrophy and cachexia.[9] Another key distinction of VML injuries is that endogenous mechanisms of repair and regeneration are unable to fully restore muscle function, as is typically observed in other types of injury models (e.g., eccentric contraction-,[10] ischemia reperfusion-,[11] toxin-,[12] crush-,[13] and freeze-[14] induced injury). The acute and chronic functional deficits following VML injury are primarily attributed to the frank and chronic loss of muscle fibers, that is, the muscle fibers ablated do not regenerate.[15]

The little to no endogenous muscle fiber regeneration is due to three primary factors. First, the space from which the original muscle fibers were ablated is no longer resided by the satellite cells that are required to orchestrate canonical muscle fiber repair.[16,17] Second, the native extracellular matrix that organizes the tissue and informs regenerative processes is lost.[16,18] Third, the immune response to VML injury, which is characterized by a prolonged upregulation of proinflammatory gene programs, promotes insidious accumulation of fibrous tissue throughout the traumatized muscle compartment.[19,20] Teleologically, this natural response appears to be directed at minimizing systemic infection, but predisposes to chronic dysfunction.

Traditional rehabilitative therapies (i.e., physical therapy) are the mainstay of care for VML injury, but have demonstrated limited benefit toward functional recovery in available clinical reports.[3] The high incidence of VML injury among battlefield-wounded soldiers in recent wars prompted a focused regenerative research effort supported by the United States Department of Defense and Veterans Administration.[7,21] In response, a broad range of regenerative therapeutic approaches (i.e., treatments) have been developed for the explicit purpose of improving the functional capacity of the injured musculature principally by restoration of contractile tissue.[22]

Generally, treatments tested have comprised biological extracellular matrices, or acellular biomaterials, with and without stem and progenitor cells and growth factors. In addition, physical therapy strategies have been implemented in isolation and in coordination with regenerative therapies. However, the benefit of treatment and if a single clinically significant treatment option for VML injury exists are unclear, primarily because similar treatments across studies have resulted in negative, modest, and positive effects on functional capacity compared to leaving the injury untreated (*see for review*[22–25]). Also, few studies comparatively evaluate various treatment approaches, limiting direct comparisons under identical experimental conditions. Therefore, a unique methodology to evaluate both the direct and indirect effect of treatments is necessary to determine treatment effectiveness.

The diverse experimental conditions used across studies of VML injury restrict straightforward comparisons among treatments and thereby prevent a clear understanding of the impact of current regenerative treatment approaches on the recovery from VML. Studies investigating therapeutics for VML injury have primarily used rodent models, with only a few published[3,26–28] clinical reports. Other VML models have been developed in large animals, such as in pigs[20] or dogs.[29] Across species, numerous isolated muscles have been used, including the tibialis anterior, gastrocnemius, rectus abdominis, and latissimus dorsi muscles; additionally,

muscle groups, including the ankle dorsiflexors, knee flexors, and knee extensors, have been evaluated. Muscle performance has been assessed throughout the literature by electrically stimulated tetanic force or torque in *in vitro*, *in situ*, and *in vivo* testing preparations. Maximal exhaustive treadmill testing, mitochondrial function, and histological assessment of contractile muscle tissue content have also been measured as indicators of muscle performance. This diversity of experimental conditions warrants a rigorous and quantitative analysis of the literature.

The objective of this study was twofold. First, we sought to answer the following question: ''In humans or animals with VML injury, is treatment better than no treatment at recovering functional capacity?.'' Upon finding a benefit of treatment on VML injury, we sought to determine which treatment was most effective at improving functional capacity. These objectives were performed using a systematic review, traditional pairwise meta-analysis, and network meta-analysis. We are unaware of any previous attempts to address these objectives using these methodologies.

Methods

Systematic review

This systematic review and meta-analysis were conducted under the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA)-specified statement.[30] Functional capacity was operationally defined as direct muscle strength or measures that should be proportional to it. The preference for functional capacity data for this analysis was skeletal muscle strength, both absolute and/or normalized. Measures of functional capacity expected to be proportional to muscle strength, including maximal treadmill testing to exhaustion, histologic analysis of viable muscle tissue, and mitochondrial density, were also considered. For studies analyzed in this meta-analysis, functional capacity measures are noted in Table 1. Studies were required to have both a VML injury group that received no treatment and at least one VML injury group that did.

Our search was conducted between July 2016 and January 2019 using PubMed, Cochrane Library, EBSCO, ProQuest, EMBASE, and ClinicalTrials.gov databases. There were no limitations on the time frame in which a study was completed. The search terms and strategy used for PubMed were as follows: ''acellular biologic scaffold'' AND (''volumetric muscle loss'' OR ''decellularized extracellular matrix'' OR ''laceration'' OR ''tissue regeneration'' OR ''muscle tissue engineering'' OR ''biological scaffold'' OR ''orthopedic trauma'' OR ''rehabilitation'' OR ''volumetric muscle graft'' OR ''muscle'') OR ''volumetric muscle loss'' AND (''decellularized extracellular matrix'' OR ''laceration'' OR ''tissue regeneration'' OR ''muscle tissue engineering'' OR ''biological scaffold'' OR ''orthopedic trauma'' OR ''rehabilitation'' OR ''volumetric muscle graft'' OR ''muscle'') OR ''decellularized extracellular matrix'' AND (''laceration'' OR ''tissue regeneration'' OR ''muscle tissue engineering'' OR ''muscle regeneration medicine'' OR ''orthopedic trauma'' OR ''biologic scaffold'' OR ''rehabilitation'' OR ''volumetric muscle graft'' OR ''muscle'') OR ''laceration'': AND (''muscle regeneration medicine'' OR ''tissue regeneration'' OR ''orthopedic trauma'' OR ''neuromuscular strength'' OR ''volumetric muscle graft''

TABLE 1. STUDY CHARACTERISTIC AND DEMOGRAPHICS

Study	Species	Sex	Muscle or muscle group injured	Thickness of VML injury	Portion of tissue removed (reported or estimated)	Treatment type(s)[a]	Outcomes assessed (days post-injury)	Outcome measure of functional capacity
Aguilar et al.[19]	Rat	Male	Tibialis anterior	Full	20%	AB+C	56	Absolute strength
Aurora et al.[59]	Rat	Male	Tibialis anterior	Partial	20%	R	56	Absolute strength and quantitative histology
Aurora et al. (model 1)[35]	Rat	Male	Gastrocnemius[b]	Full	20%	AB	28	Absolute strength
Aurora et al. (model 2)[35]	Rat	Male	Tibialis anterior	Partial	20%	AB / AB+C / AB+R	14, 56, 112	Absolute strength
Baker et al.[52]	Mouse	Female	Latissimus dorsi	Partial	50%	AB / AB+C / AB+GF / AB+GF+C	60	Absolute strength and normalized strength
Call and Southern[69]	Mouse	Male	Gastrocnemius, soleus, plantaris	Full	15%	R	28	Absolute strength
Chen and Walters[63]	Rat	Male	Latissimus dorsi	Partial	10%	AB	60	Absolute strength and normalized strength
Corona et al.[70]	Mouse	Female	Latissimus dorsi	Partial	50%	AB+C	30, 60	Absolute strength and normalized strength
Corona et al.[71]	Rat	Male	Tibialis anterior	Partial	20%	AB+C / AB+C+R	56, 112	Absolute strength
Corona et al.[62]	Rat	Male	Tibialis anterior[c]	Partial	20%	AB / AB+C	60, 120, 180	Absolute strength
Corona et al.[72]	Rat	Female	Tibialis anterior	Partial	20%	AB / AB+C	30, 60, 90	Absolute strength
Corona et al.[61]	Mouse	Male	Tibialis anterior	Full	20%	AB+C	28	Absolute strength and quantitative histology
Corona et al.[73]	Pig	Female	Peroneus tertius	Partial	20%	AB+C / AB+C+D	14, 28, 42, 56, 70, 84	Absolute strength and normalized strength
Corona et al.[65]	Pig	Female	Peroneus tertius	Partial	20%	D	7, 14, 28	Absolute strength and normalized strength
Dziki et al.[74]	Mouse	Female	Gastrocnemius[b]	Full	—	AB / AB+C	180	Absolute strength
Garg et al.[64]	Rat	Male	Tibialis anterior	Full	20%	D / AB	28, 56	Absolute strength
Garg et al.[75]	Rat	Male	Tibialis anterior	Partial	20%	AB+C	56	Absolute strength

(continued)

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TABLE 1. (CONTINUED)

Study	Species	Sex	Muscle or muscle group injured	Thickness of VML injury	Portion of tissue removed (reported or estimated)	Treatment type(s)[a]	Outcomes assessed (days post-injury)	Outcome measure of functional capacity
Gilbert-Honick et al.[76]	Mouse	Female	Tibialis anterior	Partial	40%	AB, AB+C	14, 28	Absolute strength
Goldman et al.[56]	Rat	Male	Tibialis anterior	Full	20%	AB+C	56	Absolute strength
Grasman et al.[54]	Mouse	Female	Tibialis anterior	Partial	50%	AB+C	60	Absolute strength
Greising et al. (pig)[20]	Pig	Female	Peroneus tertius	Partial	20%	AB, AB+GF	56, 70, 84	Absolute strength and normalized strength
Greising et al. (rat)[20]	Rat	Male	Tibialis anterior	Full	20%	AB	56	Absolute strength
Greising et al.[57]	Mouse	Male	Gastrocnemius, soleus, plantaris	Full	20%	R, D+R	30, 60, 120	Absolute strength and mitochondrial function
Greising et al.[77]	Rat	Male	Tibialis anterior	Full	20%	R	28, 56	Absolute strength
Kasukonis et al.[78]	Rat	—	Tibialis anterior	Partial	20%	AB, AB+C	84	Absolute strength
Kim et al.[79]	Rat	Male	Tibialis anterior[c]	Partial	30%	AB, AB+C	28	Absolute strength
Li et al.[80]	Rat	Female	Knee extensor	Full	10%	AB+C	14, 28	Absolute strength
Machingal et al.[81]	Mouse	Female	Latissimus dorsi	Partial	50%	AB, AB+C	30, 60	Absolute strength and normalized strength
Marcinczyk et al.[82]	Mouse	Male	Gastrocnemius, soleus	Full	10%	AB	28	Absolute strength
Merritt et al.[d,36,37]	Rat	Male	Lateral gastrocnemius	Partial	20%	AB, AB+C	7, 14, 28, 42	Absolute strength and normalized strength
Mori et al.[47]	Rat	Female	Tibialis anterior	Partial	20%	C	7, 28	Absolute strength
Oshima et al.[48]	Rat	Female	Tibialis anterior	Partial	20%	C	7, 21	Absolute strength
Page et al.[53]	Mouse	Female	Tibialis anterior	Partial	50%	AB+C	30, 60, 90, 120	Absolute strength
Passipieri et al.[55]	Rat	Female	Tibialis anterior[c]	Partial	30%	AB, AB+C, AB+GF, AB+GF+C	28, 56, 84	Absolute strength and normalized strength
Pereira et al.[83]	Rat	Male	Tibialis anterior	Full	10%	AB, AB+C, AB+GF	35	Absolute strength
Qiu et al.[84]	Rat	—	Tibialis anterior	Partial	20%	AB, AB+C, C	14, 56	Absolute strength

(continued)

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TABLE 1. (CONTINUED)

Study	Species	Sex	Muscle or muscle group injured	Thickness of VML injury	Portion of tissue removed (reported or estimated)	Treatment type(s)[a]	Outcomes assessed (days post-injury)	Outcome measure of functional capacity
Quarta et al.[67]	Mouse	Female	Tibialis anterior	Partial	40%	AB AB+C R AB+R	30	Absolute strength
Quarta et al.[85]	Mouse	—	Tibialis anterior	Partial	40%	AB AB+C	30	Absolute strength and normalized strength
Sadtler et al.[86]	Mouse	Female	Knee extensor	Partial	—	AB D	21, 42	Distance
Shi et al.[49]	Rat	Female	Tibialis anterior	Partial	15%	C	7, 28	Absolute strength
Southern et al.[87]	Mouse	Male	Gastrocnemius, soleus, plantaris	Full	15%	R	30	Absolute strength and normalized strength and mitochondrial function
VanDusen et al.[88]	Rat	Female	Tibialis anterior	Partial	30%	AB+C	28	Absolute strength and normalized strength
Ward et al.[60]	Rat	Male	Tibialis anterior	Full	20%	AB AB+C	56	Absolute strength and quantitative histology
Ward et al.[89]	Pig	Female	Peroneus tertius	Partial	20%	AB+C	14, 28, 42, 56, 70, 84	Absolute strength

"—," indicates information not provided or unable to be appropriately estimated (magnitude of VML injury).
[a]Single treatment groups are as follows: acellular biomaterial (AB); cells (C); rehabilitation (R); and adjunction drug (D).
[b]Injury model also included Achilles tendon.
[c]Injury model also included extensor digitorum longus and extensor hallucis longus muscle ablation.
[d]Two published articles resulting from one study.
    In addition, treatments were considered if they fit into multiple categories: acellular biomaterial+cells (AB+C); acellular biomaterial+cells+drug (AB+C+D); acellular biomaterial+growth factors (AB+GF); acellular biomaterial+cells+growth factors (AB+C+GF); acellular biomaterial+rehabilitation (AB+R); acellular biomaterial+cells+rehabilitation (AB+C+R); and drug+rehabilitation (D+R).
    VML, volumetric muscle loss.

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OR "muscle"). These search terms and strategy were modified as needed for the other databases. Reference lists of relevant review articles[9,22,24,25,31–33] and the 44 included studies were also screened.

Study inclusion and exclusion criteria

The accepted clinical definition for VML injury is "the traumatic or surgical loss of skeletal muscle with resultant functional impairment."[34] Studies conducted on humans with VML injury or animal models of VML injury were included in our search criteria. Specific inclusion criteria for review were functional quantitative data, including muscle strength (absolute and/or normalized to an indicator of muscle size), maximal treadmill test to exhaustion, histological analysis quantifying viable and/or fibrotic muscle tissue, and/or mitochondrial density and/or function. Studies were required to have at least one treatment approach and a group that received no treatment. Studies on patients were required to be conducted as randomized controlled clinical trials, with a standard of care control group. Studies were excluded for the following reasons: (1) no untreated or standard of care VML control groups; (2) use of adjunctive immunomodulation in all experimental groups; (3) case study; (4) VML combined with another type of injury, such as bone injury; (5) qualitative studies; and (6) insufficient data to calculate effect size (ES).

Selection of studies

In total, we identified 2321 relevant publications and of those, 2176 were excluded based on the review of titles and abstracts (Supplementary Fig. S1). Subsequently, 136 relevant full text publications were evaluated and 92 were eliminated for not meeting the *a priori* established study eligibility criteria. One publication provided evidence for two studies, with data for two different species.[20] A second publication also provided evidence for two studies, as it had two independent injury models, one VML injury to the tibialis anterior muscle and one to the gastrocnemius muscle.[35] Two studies were conducted with a shared control group and those were combined into one study for the analysis.[36,37] All steps of the study selection process were conducted by two authors independently. In the event of disagreements, another author was used to mediate the disagreement.

While both human and animal studies were included in the database searches, only animal studies met the study eligibility criteria. To date, human studies have neither included both a standard of care control group and additional treatment group, nor been designed as randomized controlled trials. While clinical case studies have been published and provide important information, they did not fit the inclusion criteria. Even more, expanded case series[28] of VML-injured patients ($n = 13$) were not able to be included due to unique and individual data presented by patient. Notably, these (and other[3]) clinical case studies provide extremely useful information on a relatively rare patient population that provides necessary information for this multidisciplinary field.

Primary data, including muscle strength values reported without (i.e., absolute strength) and with (i.e., normalized strength) normalization to animal body weight, muscle weight, muscle volume, or estimate of physiologic cross-sectional area, were extracted. In addition, quantitative his-tology of total muscle fiber number within the muscle belly region, as well as volitional run to exhaustion and mitochondrial function were also extracted and considered indices of functional capacity for this analysis. Histological analyses from isolated regions of interest were not considered indices of functional capacity, as the regions of interest are not universally applied to assess the whole musculature, but rather the defect area. If data for a measure of functional capacity were available for an uninjured control or sham group, those were extracted as well. The lack of control/uninjured data did not exclude a study. These data for uninjured groups were used to calculate the percent deficit (i.e., percent functional capacity difference) among treated and untreated groups. In all cases of insufficient data for study inclusion, multiple attempts were made to contact the corresponding author to recover these data.

Data extraction and assessment of study risk of bias

For each study, data were extracted as means, standard deviations, and sample size for the untreated and treated VML injury groups, when available p-values between paired samples were also extracted. Data were extracted for all post-VML injury and treatment time points. All data extraction was completed by two authors independently; disagreements were mediated by another author as necessary. In the nine studies where data were extracted directly from graphical form, means and standard deviations were extracted using the image analysis software, ImageJ.[38] Data extraction was completed by two authors independently and averaged. A Cochrane[39] risk of bias assessment was conducted by two authors independently on all studies based on six categories: random sequence generation, allocation concealment, blinding of personnel (participant blinding is not applicable since only animal studies were included), blinding of outcome assessment, incomplete data, and selective reporting bias. Information on funding sources and any noted disclosures or conflicts of interest were noted.

Meta-analysis

ESs for the extracted data were calculated as Hedges' g as previously described,[40] and the meta-analysis was conducted using a random-effects model to account for experimental variability across the included studies. Treatment types were then compared in a subgroup meta-analysis, which uses Q tests on the basis of analysis of variance. The extent of heterogeneity was assessed using both the I^2 value and a chi-square test of the Q value.[40] The smallest group of variables analyzed was three studies with the same variable. Moderator variables specifically examined included the species, sex, animal age (i.e., mature vs. immature),[41–43] muscle injured, weight bearing capacity of muscle, thickness (full or partial) of VML defect, muscle injury complexity (single or multiple muscle injury), outcome tool used, identified study bias, and if the study disclosed industry funding or authorship. To evaluate if an individual research group conducting studies could explain any between-study variation in ES, we designated research groups as a moderator variable. Collaborative research groups were identified by evaluating authors of the studies included in this meta-analysis, who were authors on multiple studies; authors were cross-referenced with each other to identify collaborative groups identified by senior

authorship. Each collaborative group was required to have three or more studies in this analysis. We identified five research groups that met these criteria; studies not belonging to a research group ($n = 15$) were not used for this moderator. Meta-regression analyses were conducted using a method-of-moments model for the continuous moderator variables of time since VML injury induction, the percentage of tissue removed at the time of VML injury, and the functional capacity deficit induced by the VML injury.

Network meta-analysis

A network meta-analysis was conducted on absolute strength measures from the final time points of studies. Treatment subgroups were analyzed only if four or more studies that used the treatment and if three or more collaborative groups conducted these studies, to align with criteria of the network meta-analysis comparing different treatment approaches treatment in pairwise meta-analyses, used these same criteria. The network meta-analysis was conducted using frequentist framework and under the following three assumptions: similarity, transitivity, and consistency. Analyses were first evaluated assuming consistency and that was subsequently tested. When appropriate analyses were rerun assuming inconsistency, the summary of ranking effectiveness was evaluated and presented as probability of superiority.

Meta-analysis procedures were conducted using Comprehensive Meta-Analysis (version 3.3; Biostat, Inc.) and the network meta-analysis was conducted using Stata (version 14; StataCorp LLC) and the network package.[44,45] An α level of 0.05 was used in all analyses, except when a nominal moderator variable with more than two levels was being probed in a subgroup meta-analysis. ESs of 0.2, 0.5, and 0.8 were considered to be small, moderate, and large, respectively. I^2 values of 25%, 50%, and 75% were considered to indicate low, medium, and large degrees, respectively, of heterogeneity. Publication bias in the primary meta-analysis was assessed using a funnel plot of study ES versus standard error, and Duval & Tweedie trim-and-fill correction to the overall ES was evaluated.

Results

In total, this analysis included 44 studies (Supplementary Fig. S1), published between 2009 and 2019; these studies assessed the effectiveness of treatments for VML-injured skeletal muscle. Studies included unpublished work, conference proceedings, or work published in peer-reviewed journals, and characteristics of the studies are provided in Table 1. To determine if treatment is better than no treatment at recovering functional capacity following VML injury, Hedges' g ESs were calculated from the 44 studies with ~ 7 ESs per study (range 1–37 per study; total 292 ESs). The Hedges' g ES is a standardized mean difference adjusted for study sample size. The adjustment is greater in studies with smaller sample sizes. Each study's ESs were combined into a single ES. The range of study ESs was from -1.14 to 2.87. A positive ES indicates that the treatment approach was effective in improving functional capacity over leaving the injury untreated. The meta-analysis was conducted using a random-effects model to account for experimental variability across the included studies. The

overall ES was calculated to be 0.75 (95% confidence interval = 0.53–0.96; $p < 0.0000001$; Fig. 1), indicating that the treatments employed typically resulted in a significant improvement in functional capacity. Based on benchmarks from Cohen,[46] the overall ES would be considered large in magnitude, although we estimate it to equate to $\sim 16\%$ smaller functional capacity deficit for treated VML injuries compared to those left untreated.

Treatment approaches used in the 44 studies varied considerably. Acellular biomaterial-type treatments ranged from laboratory derived to commercially available and were used solely in 25 of the 44 studies. Cell treatment approaches alone (i.e., intramuscular injection of cells) were used in only four studies, three of which came from the same research group.[47–49] Combinations of acellular biomaterials and cells were used in 27 studies. Drugs administered were immunomodulatory or antifibrotic agents. Rehabilitation approaches (i.e., models of physical therapy) were implemented using chronic intermittent electrical stimulation, passive range of motion exercises, eccentric and concentric contraction training, voluntary wheel running, and/or forced treadmill running.

To determine if differences among treatment types existed, ESs were calculated for each treatment as if it originated in a separate study in studies that employed more than one treatment type. Treatment subgroups were only evaluated statistically if four or more studies used the treatment and if three or more collaborative groups conducted these studies. There was no difference among acellular biomaterial, acellular biomaterial and cells combined, acellular biomaterial and growth factors combined, or rehabilitation treatment subgroups when analyzed using a pairwise subgroup meta-analysis ($p = 0.53$; Fig. 2). Since the overwhelming majority of studies evaluated herein, and in the literature, used acellular biomaterial or acellular biomaterial and cells combined, those were evaluated separately for differences across treatments using the same methodology. Again, there was no statistical difference in ESs between the two treatment types when evaluated by the traditional pairwise meta-analysis ($p = 0.28$).

Across the 44 included studies, heterogeneity was moderate and statistically significant ($Q\text{-}df = 19.7$, $p = 0.03$; $I^2 = 31.4\%$). It is possible that the differences in treatment approaches used could explain this between-study variance. Using a network meta-analysis, the magnitude of treatment options evaluated to date is shown by the network geometry of included studies (Fig. 3A). Visibly, the overwhelming majority of treatments were acellular biomaterial or acellular biomaterial and cells combined. To align with the pairwise meta-analyses, treatments were statistically evaluated by network meta-analysis using the same criteria noted above (Fig. 3B). Using both the direct comparisons (*connecting lines in* Fig. 3B) and logical inference to determine indirect comparisons, a treatment of acellular biomaterial seeded with cells was determined to have the greatest effectiveness (Fig. 3D). The next two most effective treatments were acellular biomaterial by itself and acellular biomaterial with growth factors. Rehabilitation was found to be detrimental because it was ranked lower than untreated VML injury (*ranking of 5 vs. 4, respectively, in* Fig. 3D). Notably, ranking probabilities were similar for acellular biomaterial seeded with cells, acellular biomaterial by itself,
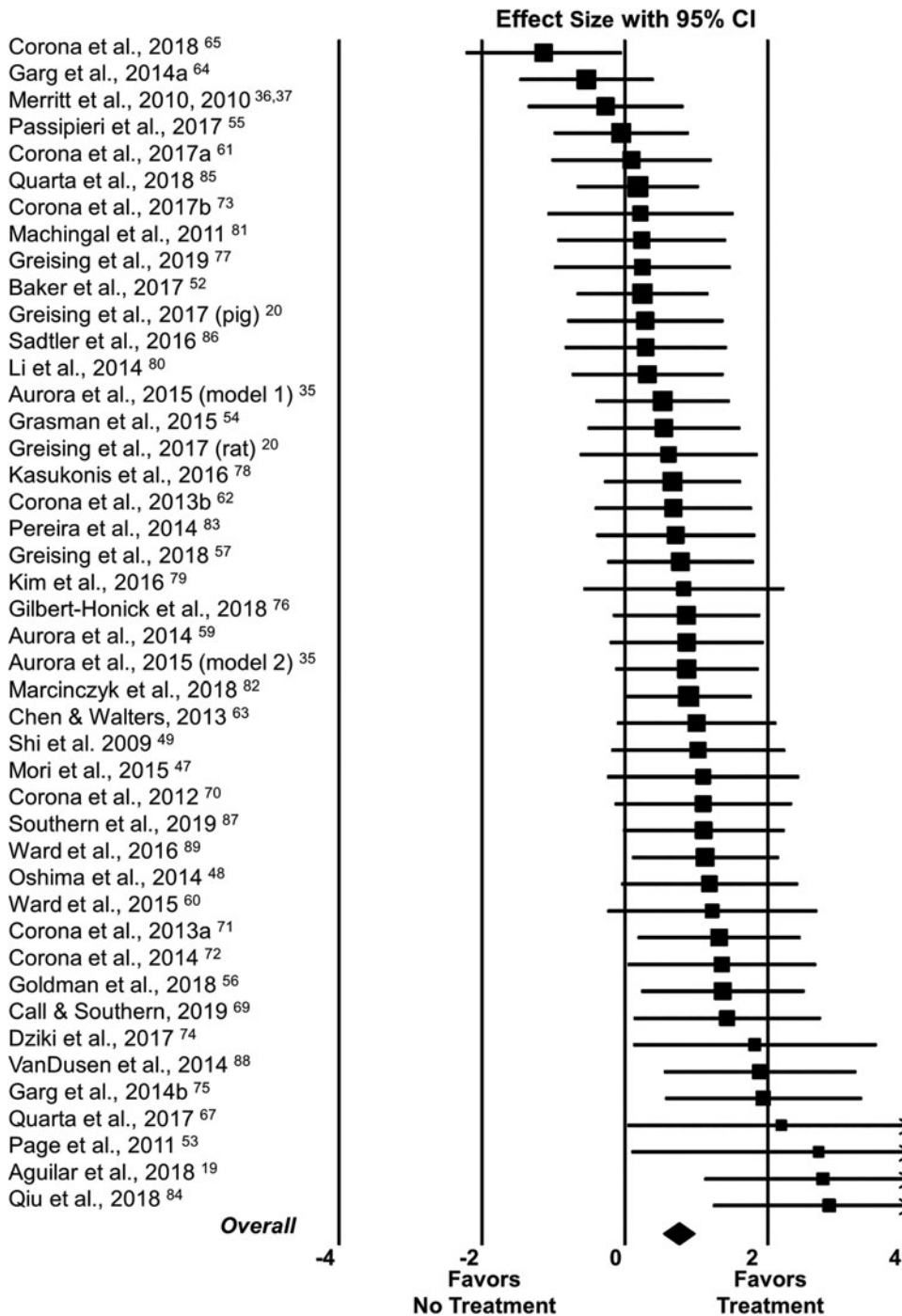
FIG. 1. Forest plot of study ESs and 95% CI of the 44 included studies evaluating if any treatment is better than no treatment at recovering functional capacity following VML injury. Study and overall ESs were calculated using Hedges' g and a random-effects model. For each study, the superscripted number after the publication year is the reference number (see Table 1 for expanded study information). For each study, the *square* represents its ES and the square's size is proportional to the weighting of the study in the meta-analysis. Studies are organized in ascending order of ES. A study's 95% CI is indicated by the horizontal line running through the square. The diamond at the *bottom* represents the overall ES, with the diamond width representing the 95% CI. The overall ES was 0.75 (95% CI = 0.53–0.96; $p < 0.0000001$). CI, confidence interval; ES, effect size; VML, volumetric muscle loss.

and acellular biomaterial with growth factors. Again, since the majority of studies evaluated used acellular biomaterial or acellular biomaterial and cells combined, a separate network meta-analysis was performed (Fig. 3C), which determined that an acellular biomaterial combined with cells has the greatest probability of treatment effectiveness, followed by an acellular biomaterial. In this case, both treatments evaluated were more effective than leaving the VML injury untreated (Fig. 3E).

Heterogeneity was moderate and statistically significant ($Q\text{-}df \geq 13.6$, $p \leq 0.04$; $I^2 \geq 36.2\%$) in both the acellular biomaterial compared to untreated and acellular biomaterial

and cells combined compared to untreated groups. This heterogeneity was similar to the overall analysis; thus, the diverse experimental conditions used in the 44 included studies (Table 1) were evaluated as moderator variables that could potentially explain between-study variance. All studies were conducted using animals, with 15, 25, and 4 studies using mice, rats, and pigs, respectively. There were 21 studies that used male animals and 20 that used female; 3 studies did not note animal sex. Animals were characterized as immature (14 studies) or mature (27 studies) at the time of VML injury; 3 studies did not report animal age. The VML injury either fully or partially encompassed the entire
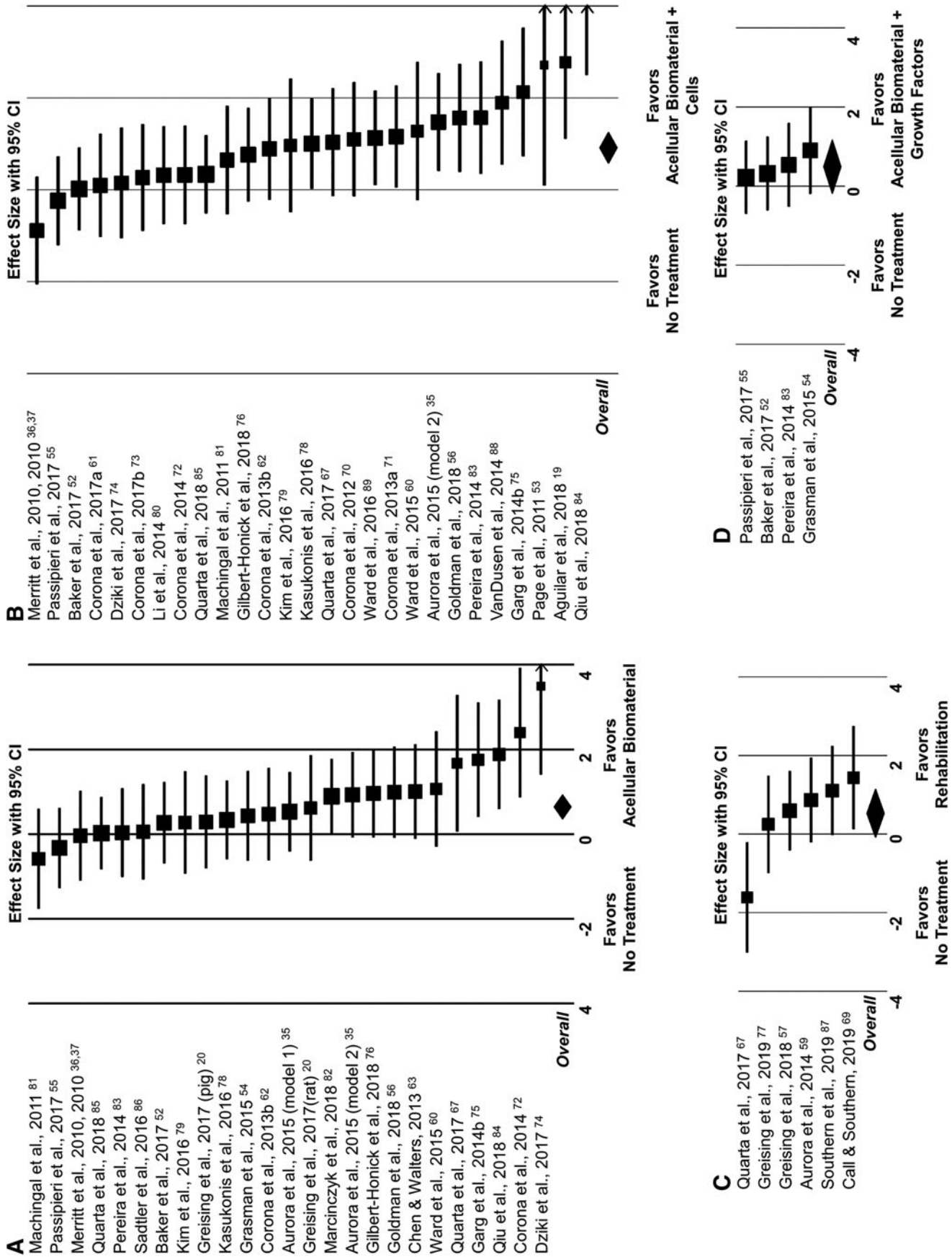
FIG. 2. Forest plot of study ESs and 95% CI of the studies analyzed in subgroup analysis of different treatment types; (**A**) acellular biomaterial, (**B**) acellular biomaterial and cells combined, (**C**) acellular biomaterial and growth factors combined, or (**D**) rehabilitation approaches. There was no difference among the four treatments ($p = 0.53$).
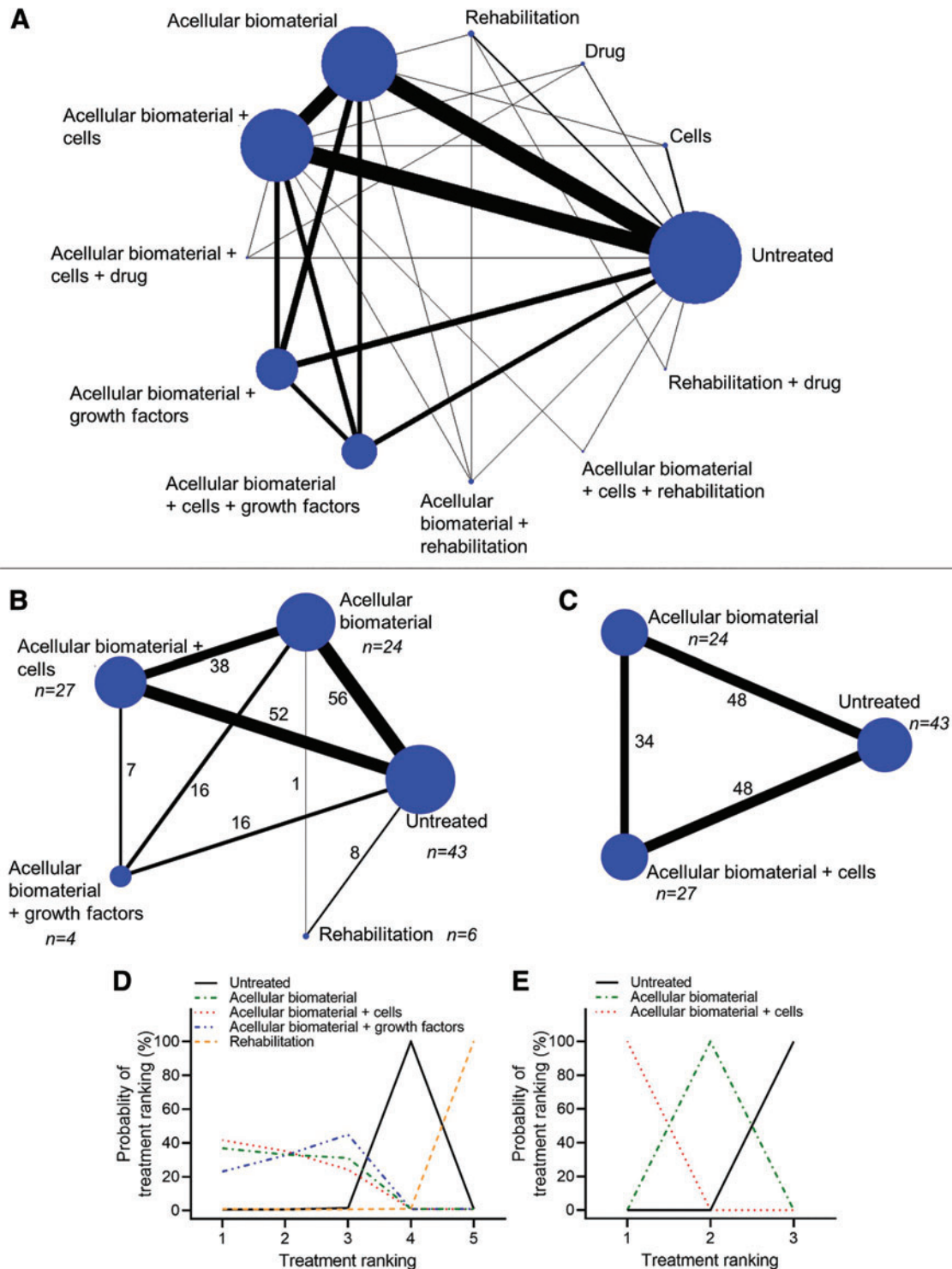
FIG. 3. Data evaluated by network meta-analysis were completed at the terminal time point of evaluation for the absolute strength outcome measure, which represented data available in 43 of the 44 included studies. Network plot of direct effects of leaving the VML injury untreated or treating with various approaches. Each *circle size* is proportional to the number of observations (*n of number of studies*) within a given treatment approach. The thickness of the interconnecting lines is proportional to the number of direct comparison between any two treatments (noted in **B** and **C**). **(A)** Network plot for all treatments is displayed to visualize the magnitude of treatment options evaluated to date. Treatments were statistically evaluated by network meta-analysis if more than four studies from three or more research groups used the same treatment, and this was evaluated in two ways. **(B)** The probability of having the best effect was specifically evaluated if the VML injury was left untreated or treated with an acellular biomaterial alone, an acellular biomaterial with cells combined, acellular biomaterial and growth factors combined, or rehabilitation. **(C)** In addition, since the majority of studies evaluated used acellular biomaterial or acellular biomaterial and cells combined, those were evaluated by network meta-analysis separately. Network plots show each intervention and the direct comparison between each treatment is noted at each line. **(D, E)** Probability for each treatment at each ranking is indicated graphically, where treatment ranking of 1 suggests greater functional improvement and 5 or 3 (in panel **D** or **E**, respectively) suggests lesser functional improvements. Color images are available online.

thickness of the muscle in 15 and 29 studies, respectively. Studies either noted the approximate percentage of tissue removed or it was estimated; the percentage of tissue loss at the time of VML injury ranged from 10% to 50% of the muscle volume and most (38 studies) of the injuries were to a single muscle. Functional capacity assessments were conducted as early as 1 week and as late as 4 months postinjury. The injuries were to muscles of the anterior (31 studies) or posterior (7 studies) compartments of the hind leg, latissimus dorsi muscle (4 studies), or knee extensor muscle group (2 studies). These were categorized as weight bearing (i.e., ankle plantar flexors and knee extensors) or non-weight bearing (i.e., ankle dorsiflexors and latissimus dorsi muscle) for analysis of moderator variables. Neither the time since injury ($p = 0.08$) nor percent of tissue removed ($p = 0.82$) at the time of VML injury was significantly related to study ES. The range of functional capacity defects between uninjured control and the untreated VML injuries (range: $\sim 1\%$ to 80%) was not significantly related to study ES ($p = 0.06$). For the nearly significant findings of time since injury ($p = 0.08$) and range of functional capacity deficit ($p = 0.06$), there was no change in significance if analyzed within the acellular biomaterial or acellular biomaterial and cell-treated groups individually. Collectively, moderator variables, including outcome measure type, animal sex, age, species, thickness of injury, injury complexity (i.e., single or multiple muscles), muscle or muscle group injured, and weight bearing nature of the muscle, could not statistically explain any between-study variance (Table 2).

Potential limitations of these analyses include the following: (1) studies with high risk of bias, (2) publication bias, and (3) the likelihood of this review missing published or unpublished studies. First, Cochrane risk of bias assessment[39] was conducted for all studies; overall, risk of bias was apparent, particularly in two categories. Identification of random group assignment was only noted in 39% of the studies and blinding of study personnel was only noted in 23%. It is possible this is a limitation in study reporting and not a limitation in how the studies were conducted. Eight included studies were identified as having a high risk for bias, specifically for selective reporting, meaning that they did not report all of the study's *a priori* specified outcomes. There is no method to directly determine the effect the omitted data may have on the overall ES; however, if those eight studies were removed, the overall ES would decrease slightly, but would still be highly significant. Second, publication bias occurs when published literature is systematically unrepresentative of the population of completed research. There is a tendency for studies with nonsignificant and/or negative findings to not be published,[50,51] and therefore meta-analyses tend to be biased toward published studies. Assessment of publication bias was conducted by evaluating asymmetry of the funnel plot (Supplementary Fig. S2). Publication bias was observed within the included studies of this meta-analysis; some asymmetry was present and thus the Duval & Tweedie trim-and-fill adjustment was applied. The adjustment reduced the overall ES from 0.75 (Fig. 1) to 0.49. The adjusted overall ES was still highly significant and its magnitude would be considered moderate. Potential conflicts of interest were evaluated by involvement of the company developing the treatment tested, noted in one study[35] that disclosed funding, support, or gift in kind for the treatment tested (this study had two independent injury models) directly, and four studies[52–55] noted authors with roles in various corporations. Five collaborative research groups were identified across the included studies.

TABLE 2. SUMMARY OF SUBGROUP META-ANALYSES ASSESSING THE EFFECT OF NOMINAL POTENTIAL MODERATOR VARIABLES

Moderator variable	Comparison	p
Outcome measure	Quantitative histology ($n = 3$, ES = 0.96 [0.02 to 1.90]) vs. Absolute strength ($n = 43$, ES = 0.80 [0.57 to 1.04]) vs. Normalized strength ($n = 12$, ES = 0.27 [−0.15 to 0.69])	0.07
Species	Mouse ($n = 15$, ES = 0.74 [0.38 to 1.10]) vs. Rat ($n = 25$, ES = 0.85 [0.57 to 1.13]) vs. Pig ($n = 4$, ES = 0.14 [−0.54 to 0.82])	0.17
Sex	Male ($n = 21$, ES = 0.79 [0.50 to 1.08]) vs. Female ($n = 20$, ES = 0.66 [0.35 to 0.98])	0.57
Age	Immature ($n = 14$, ES = 0.67 [0.27 to 1.07]) vs. Mature ($n = 27$, ES = 0.76 [0.48 to 1.03])	0.73
Muscle or muscle group involved	Ankle plantar flexors ($n = 7$, ES = 0.81 [0.27 to 1.35]) vs. Ankle dorsiflexors ($n = 31$, ES = 0.79 [0.53 to 1.06]) vs. Latissimus dorsi ($n = 4$, ES = 0.62 [−0.09 to 1.33])	0.89
Thickness of VML injury	Partial thickness ($n = 29$, ES = 0.74 [0.48 to 1.01]) vs. Full thickness ($n = 15$, ES = 0.76 [0.40 to 1.13])	0.93
Weight bearing muscle	Non-weight bearing ($n = 35$, ES = 0.77 [0.52 to 1.01]) vs. Weight bearing ($n = 9$, ES = 0.69 [0.23 to 1.15])	0.78
Injury complexity	Single muscle ($n = 38$, ES = 0.74 [0.51 to 0.98]) vs. Multiple muscles ($n = 6$, ES = 0.79 [0.23 to 1.34])	0.89

ES, effect size calculated as Hedges' g.
Values inside square brackets represent the 95% CI for the overall ES.
CI, confidence interval.

Collectively, noted publication bias, disclosed company funding, and work from collaborative research groups could not explain any between-study variance ($p \geq 0.29$). Finally, while possible, we do not expect that our search strategy missed any published work on VML injury. Unpublished studies and studies from conference proceedings were included in this analysis, two of which were subsequently published[56,57] during the interpretation of this analysis.

Discussion

The main finding of this systematic review and meta-analysis is that various treatment approaches used in animal models of VML injury improve functional capacity compared to leaving the injury untreated. Subsequent findings of this network meta-analysis determined that an acellular biomaterial combined with cellular components was the most effective treatment to improve functional capacity following VML injury to date. Analyses were conducted on outcome measure evaluated, animal sex, age, species, thickness of injury, injury complexity, muscle or muscle group injured, weight bearing of the muscle, research group, study bias, time since injury to evaluation, the percentage of tissue removed at the time of VML injury, and percentage of functional capacity difference between uninjured control and the untreated VML injury; and none of these variables could explain the between-study variance in ES.

The observed overall improvement in functional capacity with treatment versus no treatment reflects an incremental improvement ($\sim 16\%$) in functional deficits. The beneficial effect of treatment, in animal models, equates to functional capacity deficit of $\sim 36\%$, compared to those VML injures that were left untreated and had a functional capacity deficit of $\sim 43\%$. This magnitude of treatment efficacy is in line with published clinical case reports using acellular biomaterial repair of quadriceps VML injuries in two wounded service members.[26,27] In one report, the patient presented with a 72% strength deficit before surgical acellular biomaterial repair and ~ 4 months posttreatment, a 68% deficit compared to the presurgical contralateral limb remained.[27] The second patient presented an 89% strength deficit to the uninjured contralateral limb and ~ 6 months posttreatment, an 87% deficit remained.[26] Overall, these results highlight a consistent positive effect of current therapies on functional capacity that elaborates statistical, although potentially equivocal clinical significance.

Two separate analyses were performed to identify differences in efficacy among acellular biomaterial, acellular biomaterial and cells combined, acellular biomaterial and growth factors combined, or rehabilitation approaches. The first strategy was to evaluate between-study variance in treatment ESs by a subgroup meta-analysis. Treatments were evaluated to determine if an individual treatment had a greater effect on improving functional capacity compared to leaving the VML injury untreated, and there was no indication of differences in the efficacy of treatments. However, traditional meta-analyses are not able to evaluate comparative effectiveness of more than two interventions. Thus, a second strategy was used to evaluate treatment efficacy by a network meta-analysis, allowing for direct and indirect comparison of multiple interventions simultaneously. Probability of effectiveness of treatment superiority ranking

induced a slightly better effect for acellular biomaterial combined with cells than an acellular biomaterial alone, and both were superior to leaving the injury untreated. Evaluation of two additional treatments indicated that an acellular biomaterial and growth factor-combined treatment was slightly worse than an acellular biomaterial alone, but still superior to leaving the injury untreated. Rehabilitation was found to result in worse outcomes than leaving the VML injury untreated in this comparison, which is in line with clinical observations using the Army Physical Evaluation Board, indicating that disability ratings following VML-related injuries do not improve even if given temporary retirement status for additional rehabilitation time.[5]

The findings of the network meta-analyses suggest that the efficacy of biomaterial approaches is currently optimized when co-delivered with stem cells, but do not elaborate mechanism, or optimal stem cell(s) or biomaterial technologies.[58] Treatment approaches combined of acellular biomaterial and cells could have improved effectiveness when combined with other approaches, such as rehabilitation; however due to inadequate numbers of studies for inclusion, this and many combinations of treatment options could not be evaluated with these methods. Since the regulatory pathways for the various approaches to repair VML injury may carry varying levels of complexity, clinical translation plans may require a balance of efficacy and regulatory burden.

The observed improvement in functional capacity with treatment does not necessarily reflect regeneration of functional skeletal muscle tissue. The vast majority of studies evaluated herein used physiological functional outcomes (absolute strength $n = 43$; normalized strength $n = 12$; running distance $n = 1$; and mitochondrial function $n = 2$) to assess treatment efficacy, while only three studies were identified that reported a quantitative analysis of the entire cross-section of the muscle (e.g., total fiber number) that would allow inference of functional capacity.[59–61] Prior studies have demonstrated that functional capacity can be improved following VML injury repair in the absence of skeletal muscle regeneration, potentially secondary to augmentation of force transmission across the tissue defect.[35,62,63] In support, antifibrotic therapies have been shown to decrease isometric torque, expression of genes related to extracellular matrix metabolism, and fibrous tissue deposition in rodent and porcine models of VML injury.[64,65] These data therefore indicate consistent benefit of repairing the VML defect with a space-occupying therapy, which may impart improvement of functional capacity through multiple mechanisms (e.g., force production and transmission).[66]

The studies included in the meta-analysis involved the immediate repair of isolated, sterile VML models, which reflects the design of the vast majority of current VML studies, but may not reflect clinical scenarios of VML injury repair. For instance, surgical management of concomitant fracture and infectious or neoplastic disease processes is of greater priority than definitive soft tissue repair, and may therefore delay treatment of VML injuries with regenerative therapies. A recent study by Quarta *et al.*[67] reported a similar treatment effect between immediate and delayed repair using an acellular biomaterial with a stem cell approach. The conserved efficacy observed may be related to the relatively similar wound responses in muscle tissue following primary and secondary (surgical debridement of

primary) VML injuries observed in a porcine model, and thus similar environments that a therapy encounters.[68] Likewise, the impact of concurrent infection, fracture, denervation or neuropathy, and vascular disruption on the improvement of functional capacity in isolated VML injury was not included in the current analysis, and reflects important considerations that may impact the effectiveness of regenerative therapies.

In conclusion, VML injury, especially among battlefield-wounded soldiers, is a continuing problem with an unmet clinical need. Findings herein indicate that in animal models of VML injury, current treatment approaches to date result in an improvement in functional capacity after injury, and that therapies that include biomaterials combined with stem cells currently achieve the greatest improvement in functional capacity among the therapeutic approaches investigated. It has been stated that, ''the ultimate goal of regenerative medicine is to completely restore missing or damaged tissues to a level functionally and aesthetically indistinguishable from the preinjury/diseased state''[21]; the modest magnitude of functional improvement observed to date with therapies for VML injury marks significant progress and invites continued innovation.

Data Availability

The datasets used and/or analyzed during this study are primarily presented in this article and are available from the corresponding author on reasonable request.

Acknowledgments

Authors' Contributions

All authors (S.M.G., B.T.C., C.M., J.K.F., and G.L.W.) contributed to the design, data extraction, and analyses for this article. S.M.G. and B.T.C. drafted the article. G.L.W. critically reviewed and edited the article. All authors (S.M.G., B.T.C., C.M., J.K.F., and G.L.W.) have read and approved the final submitted article.

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Supplementary Material

Supplementary Figure S1
Supplementary Figure S2

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Address correspondence to:
Sarah M. Greising, PhD
School of Kinesiology
University of Minnesota
1900 University Ave SE
220A Cooke Hall
Minneapolis, MN 55455

E-mail: grei0064@umn.edu

Musculoskeletal Regeneration, Rehabilitation, and Plasticity Following Traumatic Injury

Authors
Sarah M. Greising[1] ⓘ, Benjamin T. Corona[2], Jarrod A. Call[3, 4]

Affiliations
1 School of Kinesiology, University of Minnesota, Minneapolis, United States
2 School of Medicine, Wake Forest University, Winston-Salem, United States
3 Department of Kinesiology, University of Georgia, Athens, United States
4 Regenerative Bioscience Center, University of Georgia, Athens, United States

Correspondence
Dr. Sarah M. Greising
School of Kinesiology, University of Minnesota
1900 University Ave SE
55455 Minneapolis
United States
Tel.: +1 612 626 7890, Fax : +1 612 626 7700
grei0064@umn.edu

ABSTRACT

The musculoskeletal system has an integral role throughout life, including structural support to the body, protection, and allowing a range of fine to complex movements for daily living to elite sporting events. At various times, injuries to the musculoskeletal system occur resulting in varying levels of impact to the person both acutely and chronically. Specifically, there is a spectrum of complexity in orthopedic injuries, with some such as common muscle strains, that while burdensome will have no impact on life-long functional ability, and others that can result in long lasting disability. Focusing on extremity injuries, this review highlights: i) the current impact of orthopedic injuries in sport and daily life; ii) the foundation of bone and skeletal muscle repair and regeneration; and iii) the disruptions in regenerative healing due to traumatic orthopedic injuries. This review seeks to maximize the broad and collective research impact on sport and traumatic orthopedic injuries in search of promoting ongoing innovation for treatment and rehabilitation approaches aimed to improve musculoskeletal health throughout life.

Introduction

Musculoskeletal rehabilitation is based on the principle of tissue plasticity, the ability of tissues to adapt to mechanical and/or chemical cues in order to improve functional capacity or efficiently recover from injury. Effective evidence-based rehabilitation approaches (i. e. actions to enhance functional outcomes) have existed for almost two decades for common musculoskeletal injuries that can occur frequently during sports and daily life, such as a strain or contraction-induced muscle injury [1, 2]. However, severely injured musculoskeletal tissue from, for example, high-energy orthopedic trauma, may have diminished tissue plasticity and can therefore be unresponsive to rehabilitation efforts [3]. For the patient, this manifests in the form of long-term functional limitations,

disability, co-morbidities, and decreased quality of life. For instance, a college athlete who suffers an open fracture of the tibia could have initial resistance to rehabilitation and lifelong limitations due to the lack of plasticity in the muscle after injury. In fact, former National Collegiate Athletic Association (NCAA) Division I college athletes who sustained injuries during their college sport years (~30 years prior) have lower health-related quality of life scores and ~2.5 times more limitations than non-athletes [4]. The long term consequences of traumatic musculoskeletal injuries is also evident in civilian and military populations, as about half of those who sustained injuries still have significant disability at 7 years after the initial incident, according to the Sickness Impact Profile (or SIP) [5]. It is possible that overall quality of life, as well as

the ability to maintain physical activity levels later in life, may also be limited by prior traumatic orthopedic injuries, and specifically the lack of functional plasticity (i. e. contractility, oxygen consumption, ultimate load) in the musculoskeletal system. With particular focus on extremity injuries, this study focuses on the current impact of musculoskeletal and traumatic orthopedic injuries across the life span, the physiology of normal repair and regeneration, and the current understanding and limitations of functional musculoskeletal plasticity spanning pre-clinical to clinical investigations.

Traumatic musculoskeletal injuries

Traumatic injury is often indiscriminate and crosses various physiologic systems such as bone, skeletal muscle, vascular, tendinous, ligamentous, and/or cartilaginous structures; primarily due to blunt force, penetrating injury (e. g. high-energy injuries or collisions), or controlled (i. e. surgical) trauma [6, 7]. Of traumatic injuries treated at United States trauma centers, two-thirds occur to extremities with ~32 % and 40 % to the upper and lower extremities, respectively [8]. Of injuries that are of primary interest here are those musculoskeletal injuries that commonly are reported as fractures, sprains, strains, contusions, dislocation/derangements, crushing and open wounds, or amputations. According to the United States Bone and Joint Initiative [9], fractures and open wounds account for ~26.5 million injuries a year. While there is a range of injury severity and complexity, and functional impact imposed by these injuries, they collectively result in significant health care costs, functional limitations and pain.

Etiology

The acute cause of all traumatic musculoskeletal-related injuries generally falls into one of two categories: blunt or penetrating trauma. Blunt force trauma occurs as an object (or person contact) strikes the body, while penetrating trauma occurs when an object pierces the body often resulting in open wounds. Within the general population, about one-third of all traumatic injuries are due to falls [9]. Various injury mechanisms account for the remaining two-thirds such as motor vehicle accidents, machinery, or moving objects. Injuries within the NCAA span player contact, other contact, and non-contact, with the majority occurring from blunt force trauma due to contact with other players [10]. In active duty military populations, traumatic musculoskeletal injuries encountered on the battlefield were primarily due to high-energy, explosive mechanism [11, 12].

Epidemiology

With particular focus on sports-related injuries, the United States Bone and Joint Initiative estimates that ~2.8 million sports-related injuries are treated annually [9]. Using the NCAA Injury Surveillance Program Database [13], ~48 000 injuries of any type occur per ~5 million athlete-exposures (i. e. one athlete's participation in one competition or practice). For musculoskeletal-related injuries specifically, the incidence is ~63 per 1000 NCAA athlete-exposures [14]. Injuries that occur specifically in the skeletal muscle can range from strains, contusions and tears. Supported by the abundance of evidence-based rehabilitation approaches for injuries such as muscle strains [1, 2], these injury types are common [15] and account for ~17.1 million injuries annually [9]. In NCAA athletes, for

example, strains of the quadriceps muscle group occur at a rate of ~2 per 10 000 NCAA athlete-exposures overall, with higher rates in specific sports, such as soccer (up to ~6 per 10 000 exposures) [16]. Similarly, in this athletic population hamstring muscle group strains occur at a rate of ~3 per 10 000 NCAA athlete-exposures [17]. Relatedly, in a similar highly active military population, musculoskeletal injuries account for ~77 % of the 14 500 battle field evacuations [7].

Specific to skeletal fracture, the most common fractures (>60 % of cases) are of the distal radius, metacarpus, proximal femur, finger phalanges and ankle. Overwhelmingly though the literature presents data and reports on femoral diaphysis, distal femur, proximal tibia, tibial diaphysis, tibial plafond, talus and calcaneus that make up only ~6.6 % of cases [18]. Collectively the estimated ~18.3 million fractures that occur annually in the in US represent a common injury that can require expensive and complicated care. Any type of fracture in the NCAA population accounts for about 6–7 % of all injuries seen in college athletes. In the general population, any type of fracture is expected to occur in ~11 per 1000 persons in adulthood [18]. More complex fractures, such as open fracture of the tibia, invariably result in severe bone and surrounding soft-tissue injury, including bone comminution, disruption of the periosteum, damage to surrounding skeletal muscle, and global injury contamination, which frequently result in segmental bone defects and volumetric muscle loss (VML). Open fracture involving segmental bone defects with VML is prevalent in both civilian and military trauma populations and contributes to the greater than $400 billion yearly economic impact (~$86 billion and $326 billion in medical treatment and lost productivity, respectively) of traumas in the US [19]. Collectively, traumatic musculoskeletal-related injuries are common and present across a broad range of severity that directly influences short-term care and associates with long-term clinical outcomes.

Basic science of musculoskeletal healing and plasticity

The capacity for the musculoskeletal system to repair, regenerate and adapt is directly related to mortality and morbidity. Throughout daily life, the tissues that bear and generate force so that we may naturally withstand gravity, ambulate, eat and communicate are continuously injured and constantly 'rebuilding'. Moreover, musculoskeletal tissues adapt in specific ways to their daily use, to both improve the desired function of the tissue and/or to reduce whole-body metabolic burden. Since antiquity, physical activity and planned physical activity, i. e. exercise, sports or physical therapy, have been known to promote health and prevent disease. The benefits of exercise are made possible by the adaptive nature of the musculoskeletal tissues, a process commonly referred to as tissue plasticity. In the following section, the foundations of normal physiologic repair, regeneration and plasticity are discussed.

Skeletal muscle and bone regeneration

Tissue regeneration is considered a form of plasticity, as there are acute changes in cellular signaling that lead to tissue remodeling and repair. Bone and skeletal muscle plasticity following injury have common stages; and most importantly, both tissues have a robust regenerative and repair process that concludes with tissue indis-

▶ **Table 1** The phases of bone and muscle regeneration.

	Bone	Muscle
Injury Phase	Hematoma & callus formation	Loss of $[Ca^{2+}]_i$ homeostasis and disruption of force-generating proteins
Inflammation	TNFα, IL-1,-6,-11: peak within 72 hours, resolved by 7–10 days	TNF-α, MIP-1, MCP-1: peak within 72 hours, resolved by 7–10 days
Regeneration	Recapitulates phases of development; prior inflammatory phase implicated in response, callus mineralization	Recapitulates phases of development; prior inflammatory phase implicated in response, myotube formation bridges between uninjured muscle fiber sections
Remodeling	Resorption of soft callus tissue, portions of hard callus tissue broken down to form medullary cavity	Myotube receiving activation patterns from alpha-motoneuron, protein synthesis increase the amount of contractile protein content
Primary Regenerative Cell	Mesenchymal Stem Cell	Satellite Cell
Default healing mode (Severe Injury)	Regeneration	Repair (fibrosis)
1* response increase use	Mechanical (Wolfes law)	Metabolic

tinguishable from pre-injury. The phases of bone and muscle regeneration are briefly highlighted here and in ▶ **Table 1**.

Injury

Initial skeletal muscle injury is marked by a loss of intracellular calcium homeostasis within damaged muscle fibers (i. e. myofibers) [20, 21]. The loss of calcium homeostasis activates a number of degradative processes such as calcium-activated proteases. These proteases begin to degrade damaged proteins and the first phase of skeletal muscle regeneration is therefore referred to as the degradative phase [22]. Initial bone injury is marked by a hematoma, or a bleeding as a result of the bone damage or damage to the surrounding tissues. The hematoma will eventually form a clot between the fragmented areas of the damaged bone and serve as the template for eventual new bone formation, the callus [23]. Both early phases of injury in the muscle and bone are reported to give rise to a subsequent inflammatory phase critical for functional recovery.

Inflammation

While chronic inflammation negatively affects bone mineral density and skeletal muscle function, an acute inflammatory response that resolves in a timely manner is absolutely necessary for bone and muscle repair. Skeletal muscle inflammation can begin as early as 6 h post-injury with marked increases in the expression of inflammatory cytokines and chemokines such as tumor necrosis factor alpha (TNF-α), macrophage inflammatory protein-1 (MIP-1), and monocyte chemoattractant protein-1 (MCP-1) [24]. Subsequently, neutrophil populations and macrophage populations peak at 24 h and 72 h post-injury, respectively, and the inflammatory response is largely resolved between 7–10 days post-injury. TNF-α, and interleukins -1, -6, and -11 (IL-) are rapidly responding cytokines to bone injury that recruit neutrophils and macrophages to the site of injury. Similar to skeletal muscle, the inflammatory response to bone injury is largely resolved by seven days post-injury [23, 25]. Numerous studies have provided valuable insight into the necessity of the inflammatory process in both muscle and bone for functional healing. In muscle, two articles by Warren and colleagues demonstrate that neutralizing the TNF-α cytokine or

knocking out the chemokine receptor CCR2 prolongs the recovery of muscle strength after traumatic freeze injury [26, 27]. Similarly, neutralizing TNF-α and CCR2 after a mouse tibia fracture also impaired mineralization of the callous, a critical step for ultimate functional recovery of the bone [28]. It is clear that disruption of the inflammatory phase extends the timeframe for muscle and bone recovery indicating that the inflammatory response is critical for timely regeneration.

Regeneration

The critical role of the inflammatory response in muscle and bone healing can be explained in part by the evidence indicating inflammatory markers are responsible for signaling to resident stem cells to exit quiescence and participate in regeneration of the tissue. Muscle and bone owe their robust regenerative capacity to the resident stem cells, satellite cells and mesenchymal stem cells, respectively, that are capable of proliferating and differentiating to form new muscle and bone tissue. In skeletal muscle, low doses of TNF-α, as well as other cytokines and chemokines, increase satellite cells differentiation *in vitro* and *in vivo* [29, 30]. Additionally, Glass et al. reported that a low-dose TNF-α strategy was able to increase mesenchymal stem cells migration *in vitro* and enhance callus mineralization *in vivo* suggesting a strong relationship between the inflammatory response and mesenchymal stem cell dynamics in bone [31]. Notably, the muscle fiber developmental steps and the ossification steps of skeletogenesis are recapitulated during the regenerative phase, and the satellite and mesenchymal stem cells are necessary for myofiber regeneration and generation of the callus tissue in muscle and bone, respectively.

Remodeling

The final phase of tissue regeneration in both muscle and bone is remodeling. During this phase in skeletal muscle, the satellite cells have migrated and differentiated to form myotubes spanning the portion of muscle fiber damaged during the initial injury. At the beginning of the remodeling phase, the newly formed myotubes are distinguishable from uninjured fibers by a centralized nuclei, a visible representation of decreased muscle fiber density and less contractile protein material. During remodeling, increases in protein

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synthesis will generate more contractile protein content eventually increasing the density of the myofiber and pushing the nuclei to the periphery of the muscle cell. This process coincides with a full functional recovery of strength and damaged myofibers are now indistinguishable from uninjured fibers. The remodeling phase of bone healing involves the reabsorption of the soft tissue callus that bridges the fractured bone ends and mineralization leaving a hard tissue callus in its place. Finally, this hard bony callus is broken down by osteoclasts and then osteoblasts will help form a medullary cavity with a lamellar bone structure. Like skeletal muscle, when this is complete the newly formed bone will be indistinguishable from the uninjured regions.

Tissue plasticity

Tissue plasticity also serves as a foundation for exercise, rehabilitation, and physical therapy. The fundamental physiologic responses to exercise are briefly highlighted here and in ▶ **Table 2**. As advances in molecular biology and genetics improve the precision by which tissue plasticity is defined, a new frontier emerges for exploration of the mechanisms of poor tissue plasticity in disease and injury.

Exercise is known to improve physiological capacities of skeletal muscle such as strength and endurance, with the adaptations dependent on the specific physiological system being stressed. Exercise is a physiological stressor and stimulates various signaling pathways to increase expression of genes and their protein products that represent adaptation to the stress and yields changes in physiological function. For example, each muscle contraction results in a calcium transient as calcium is released and sequestered back into the sarcoplasmic reticulum. Each muscle contraction also is energetically demanding and results in an accumulation of AMP as well as a shift in redox homeostasis as reactive oxygen species (ROS) are generated during the synthesis of ATP for sustained muscle performance. As such, muscles that are frequently activated, as would happen during a 90 min training run, i. e. endurance training, are going to experience more frequent calcium transients and greater accumulation of AMP and reactive oxygen species. These products of endurance exercise-induced muscle stress are responsible in part for activating signaling cascades that will change the physiology of the muscle fiber. Specifically, AMP activates AMPK which subsequently phosphorylates the transcription factor PGC1α

that is responsible for regulating some 2000 genes [32, 33], many of which are related to metabolism and improving vascularization of muscle fibers [34]. Calcium activates calcineurin that is responsible for regulating slow-twitch contractile genes such as slower isoforms of myosin heavy chain and the sarcoplasmic reticulum calcium ATPase [35]. Reactive oxygen species can stimulate the transcription factor NF-kappa B that coordinates antioxidant gene responses [36, 37]. Thus, endurance-trained muscles physiologically have greater oxidative capacity and oxygen saturation, slower contractile phenotypes, and greater antioxidant protein expression.

In contrast, a bout of resistance training may only involve 60–90 s of actual muscle contractile activity, while those contractions are of greater intensity. The total muscle contraction time (i. e. 60–90s) is simply insufficient to elicit the calcium, AMP, and reactive oxygen species response as in endurance-trained muscle; however, resistance training is associated with an increase in circulating growth factors such as insulin-like growth factor 1 (IGF-1) [38]. IGF-1 initiates the mammalian target of rapamycin (mTOR) signaling cascade that leads to an increase in protein synthesis and decrease in protein degradation [39]. Overtime, contractile protein content will accumulate in the muscle fiber leading to an increase in physiological cross-sectional area, or hypertrophy. The greater amount of contractile protein essentially means the greater number of myosin-actin cross-bridges and greater muscular force production. IGF-1 accomplishes this feat by stimulating the intracellular phosphorylation of AKT that has two important roles: i) AKT will turn "off" the TSC2/TSC1 complex that effectively acts as a brake on mTOR-dependent protein synthesis [40, 41], ii) AKT will also phosphorylate the FOXO transcription factor that turns "on" genes associated with protein degradation thus prevent FOXO nuclear translocation [42]. Endurance-trained muscle simply does not experience a similar rise in circulating IGF to have a similar physiological response.

The most robust physiological adaptation to endurance type training is an increase in oxidative capacity (▶ **Table 2**). The significance of this adaptation is that muscle with greater oxidative capacity has greater endurance, or less fatigue. An example of this is such: 1 mole of glucose can yield 2 moles of ATP during anaerobic metabolism in less mitochondrial-rich muscle fibers, while 1 mole of glucose can yield 36 moles of ATP during aerobic metabolism in mitochondrial-rich muscle fibers. The 16-fold greater production

▶ **Table 2** Physiologic adaptations to training.

Stimulus	1° Response	2° Response	Muscle plasticity	Physiological change
Endurance training				
Calcium transients	Calcineurin	Increase slow-contractile genes	Slow-contractile phenotypes	Greater endurance
AMP	AMPK:PGC1α	Increase metabolic genes	Greater oxidative capacity	
	AMPK:Ulk1	Increase autophagy initiation	Greater quality of mitochondria	
ROS	NF-kappa B	Increase antioxidant genes	Less ROS-induced damage	
Resistance training				
Neural adaptations			Greater motor unit recruitment	Greater muscle strength
Circulating IGF-1	AKT	Increase protein synthesis by turning "off" TSC2/TSC1	Hypertrophy	
		Decreased protein degradation by preventing FOXO nuclear translocation	Hypertrophy	

of ATP from a similar fuel source provides endurance-trained muscle more ATP supply to meet sustained ATP demand. The most robust physiological adaption to resistance-type training is an increase in strength (▸ **Table 2**). While the above paragraph highlighted molecular pathways leading to muscle hypertrophy, it is well-established that early adaptations to resistance training are neural, leading to greater motor unit recruitment [43, 44]. Over the long-term, muscle hypertrophy will play a larger role and primarily be responsible for continued gains in muscle strength.

Trauma-driven disruption of healing

Despite the significant potential for plasticity and regeneration of both bone and skeletal muscle tissues, severe trauma can disrupt these processes leading to poor healing outcomes. The following section discusses conditions under which endogenous musculoskeletal regeneration and plasticity are impaired, and elaborates on potential mechanisms of impairment.

Fracture healing

Tibia fractures generally have a low incidence of non-union (<2 %). However, the incidence of non-union can increase to as much as ~23 % when the fracture pattern is multi-fragmentary or wedge-shaped, versus simple [45]. Moreover, fractures that cause segmental bone loss and extensive injury to the surrounding soft tissue place an even greater risk of non-union compared to less severe open fractures with an observed incidence of 67 % [45]. The causes for the impairment of fracture healing is mostly contributed to by the degree of damage of the surrounding soft tissue, disruption of vascular supply, and contamination of the wound. Basic science studies have supported the notion of a multifaceted communication between bone and muscle and other surrounding soft tissue that is critical to timely fracture healing. Under various experimental conditions, the importance of an adequate vascular supply, intact periosteum, and/or skeletal muscle coverage has been repeatedly demonstrated. In particular, studies have observed that skeletal muscle aids fracture healing and subsequent remodeling through the provision of vascular derived mesenchymal stem cells, muscle stem cells, osteogenic myokines, and mechanical stimulation (see for review [46–48]), which manifest impaired simple fracture healing as well as rhBMP-2 mediated osteogenesis in a critical size segmental bone defect [49–57]. Supporting the clinical relevance of these basic science findings, severe open fractures can require more advanced fixation, soft tissue grafts or flaps, or multistep operative techniques (e. g. Masquelet technique [58]) to achieve union.

Another significant factor playing a role in impaired fracture healing in the severely traumatized extremity is the heightened and prolonged inflammatory response that ensues. As noted above, the immune response to musculoskeletal injury is of critical importance to regeneration, wherein abolition of the immune response and inflammatory signaling impairs isolated fracture healing. However, the inflammatory response following severe extremity trauma appears excessive and detrimental to the signal required for regeneration. For instance, wound effluent from severely traumatized extremities has been shown to have extremely heightened levels of inflammatory cytokines that associated with poor healing outcomes to include heterotopic ossification [59]. Similarly, patients

with inflammatory comorbidities, such as diabetes, have demonstrated impaired healing of fragility fractures compared to age-matched controls that associates with systemic levels of inflammatory cytokines [60]. These findings are further supported in animal models of polytrauma and open fracture in which heightened and prolonged systemic and local immune responses are associated with impairment of fracture healing; the attenuation thereof using either systemic pharmacological agents (e. g. FK506) or muscle tissue replacement successfully restored the rate of fracture healing [49, 61–64].

Skeletal muscle regeneration

Investigations of severe skeletal muscle injury that results in muscle tissue removal from either iatrogenic (e. g. debridement or sarcoma resection) or traumatic (i. e. high-energy type mechanisms, such as improvised explosive device or motor vehicle accidents) causes also illustrates gross impairment of endogenous regenerative mechanisms. This type of muscle injury has been termed VML and operationally defined as the traumatic or surgical removal of a portion of muscle or muscle unit that results in chronic functional deficits [65]. There are relatively limited clinical data specifically describing VML injury. The reports stemmed from the high incidence of soft tissue loss secondary to blast trauma and to a lesser extent gunshot wounds among US service members injured on the battlefield in recent wars [6, 7]. Available clinical investigations from the military population demonstrate that VML injuries occurred mostly to the lower extremities [7] and resulted in chronic loss of limb function and strength [66, 67]. Volumetric muscle loss injury was associated with separation from the military with disability rating levels directly proportional to the time post-injury, raising concern of progressive degeneration secondary to the initial trauma [68].

Volumetric muscle loss injury is recognized as a primary barrier to functional recovery of the severely traumatized extremity. Due to recognition of the increased incidence and loss of function in battlefield injured service members, the Department of Defense initiated a regenerative medicine research program to develop novel therapeutics for muscle tissue restoration [69]. As a result, much of what is currently understood of the natural pathophysiology of VML injury has been learned in animal models. The key characteristics of this etiology of muscle injury is perhaps best demonstrated in rodents, in which eccentric contractions, crush injury, ischemia reperfusion, or freeze-injuries impart acutely severe injury from which full functional recovery is achieved over the ensuing ~4–6 weeks [27, 70–73]. In contrast, rodent models of VML injury present losses of strength and muscle fibers chronically post-injury (see for review [74]). The permanent loss of muscle fibers is due to a fundamental loss of native regenerative elements required for skeletal muscle regeneration, such as the basal lamina and satellite cells [75–77]. Additionally, rodent and porcine animal models of VML injury have observed a heightened and prolonged inflammatory response [78, 79] that significantly deviates from that observed in recoverable injury models such as ischemia reperfusion injury [80]. The prolonged inflammatory response after VML injury appears to drive extracellular matrix protein production and deposition, resulting in extensive compartmental fibrosis [81, 82]. It is currently unknown at this time what specific effect protracted in-

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flammation has on satellite cell viability and function within the remaining portion of the muscle; however, given the necessity of local satellite cells for muscle regeneration and their importance to plasticity, research investigating the quality of chronically injured muscle after VML, is highly needed. Other salient observations following VML injury include motor neuron axotomy, loss of neuromuscular junctions, heightened oxidative stress, devascularization, and mitochondrial dysfunction [83–85]; all of these may be deleterious to regenerative healing and the capacity to respond to tissue level physical therapies.

General efficacy of physical therapy

The specific characteristics of the therapy employed will widely vary depending on the type and severity of injury and the patient's deficits, goals of therapy, and resources, as well as the clinician's expertise. Generally, clinical data have demonstrated benefit of physical therapy to improve functional outcomes following most musculoskeletal injuries. For instance, early weight bearing is recommended for simple mid-shaft tibia fractures operatively managed with open reduction internal fixation with an intramedullary nail [86]; corresponding with retrospective evidence that delayed initial weight bearing following open and closed tibia diaphyseal fractures associates with increased risk of non-union [87]. As another example, a prospective randomized control trial demonstrated benefit of using an active controlled motion device in addition to a standard physical therapy targeted at early partial weight-bearing for isolated unstable ankle fractures (i. e. Weber type B- or C-Fracture) [88]. In this study, physical therapy began on the first post-operative day in hospital and progressed to 2–3 times per week for 20 min as tolerated out of hospital for a total six weeks. Active controlled motion was implemented in hospital 2–5 days post-surgery and consisted of 20-minute daily sessions continued at home for a total of six weeks. Active controlled motion was shown to improve recovery of ankle range of motion out to 12 weeks, as well as significantly shorten time to return to work [88]. Naturally, there exists considerable variability among clinical studies that may additionally suffer from low sample sizes. To that end, systematic reviews, meta-analyses, and expert panels have distilled the existing data for rehabilitation of many common musculoskeletal injuries to help guide clinical practice (see, e. g. [89–91]).

Because primary outcome measures used in clinical trials assessing physical therapies typically involve standardized functional assessments and validated clinical assessment tools, delineating specific tissue level effects may require inference from smaller clinical studies investigating a similar patient population and using similar therapeutic methodology, if available. For example, a randomized control trial that investigated the benefit of a 6-month extended outpatient rehabilitation program involving whole-body resistance exercise versus flexibility-based physical therapy on disability and function in elderly patients suffering hip fracture demonstrated significantly greater performance of instrumental activities of daily living and basic activities of daily living, as well as improved muscle strength across most major muscle groups and functional indices with the extended resistance exercise program [92]. However, no evaluation of putative mechanism of physical therapy was evaluated. Interestingly, a clinical study of patients with end-stage osteoarthritis electing for total hip arthroplasty identified discrete

inflammatory phenotypes based on tumor necrosis factor-like weak inducer of apoptosis (TWEAK) expression within the surrounding muscle tissue, which were indirectly associated with muscle protein synthesis levels. The authors proposed that the findings suggested an inflammatory-based prediction of muscle regeneration, the corollary of which is early identification of patients at risk for prolonged muscle weakness and resulting worse post-arthroplasty functional outcome [93]. In support of this idea, a recent pilot study of elderly patients with recent hip fracture reported prolonged up-regulated gene expression or inflammatory genes in ipsilateral quadriceps muscle biopsies compared to matched control subjects [94]. Notably, following the completion a 3-month high-intensity, resistance-based training program gene expression of inflammatory mediators (*NFKB1 & IL6*) and some toll-like receptor signaling molecules (e. g. *MYD88*) were significantly reduced in a pre-post analysis. An inverse relationship between inflammation (MYD88) and quadriceps isometric strength ($r = -0.42$, $p = 0.05$) and cross-sectional area (-0.60, $p = 0.01$) measured with MRI was observed, further suggesting a pathological role of prolonged muscle inflammation on functional recovery after hip fracture.

Tissue level resistance to physical therapy in VML injury

Rehabilitation-focused investigations are sparse for the VML injured population. The only clinical trial of traumatic VML-injured patients described a cohort of 13 patients, 7–120 months removed from the time of injury, who were initially treated with extensive physical therapy and still had significant functional deficits remaining. Interpretation of rehabilitation effectiveness is limited by the exclusion of a sufficient control group and pre-intervention muscle function not being thoroughly assessed prior to intervention [95, 96]. A 2010 case report of a 19-year-old patient with a right femur fracture and associated large VML quadriceps muscle injury noted long-term disability and ineffectiveness of physical therapy to fully restore function of the remaining muscle [66]. More recently, a retrospective evaluation of 17 patients that had a component of VML secondary to soft tissue sarcoma, revealed the long-term consequences of unmet rehabilitation needs on quality of life [97]. The common outcome among patients was knee flexion weakness that had a high predictive value on a reduction in activities of daily living (Toronto Extremity Salvage Score, R = 0.66) and quality of life (European Quality of life-5 Dimensions score, R = 0.54).

Clinical data of tissue level pathology and unresponsiveness to physical therapies is not currently available in this patient population. Available data from animal models of VML indicate that the remaining portion of muscle does not demonstrate a robust increase in oxidative capacity with endurance exercise type training due to inadequate activation of the necessary cellular signaling cascades (e. g. transcription factor PGC1α) [85]. Alarmingly, a recent systematic review, meta-analysis, and network meta-analysis of VML injury studies that included quantitative functional analyses [98] determined that in animal models, rehabilitation approaches for VML injury resulted in worse functional outcomes than if the injury was left to its natural sequela. This work specifically focused on studies testing rehabilitation in animal models in the form of voluntary wheel running, chronic-intermittent electrical nerve stimulation and/or passive range of motion exercises resulting in

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only modest functional improvements [84, 99–103]. Collectively, these limited clinical and pre-clinical studies indicate that the remaining muscle does not fully recover strength, may be resistant to rehabilitation, and results in long-term disability. Which is to say that it is possible that after traumatic injuries, such as VML, the remaining muscle is inhospitable to plastic changes and rehabilitation efforts, further worsening functional limitations.

Closing perspectives and future directions

Lifelong considerations following injury

The impact of traumatic musculoskeletal injuries can affect those injured far beyond the initial injury and regenerative phase. For those who have sustained traumatic VML injuries, for example, the lack of response to rehabilitation and normal repair processes can have lifelong impacts. For instance, military service members who sustained traumatic orthopedic injuries have disability ratings that did not improve when given temporary status to allow additional recovery and rehabilitation time and, in fact, their function continued to deteriorate over time [68]. Health-related quality of life [104], which often represents a compilation of physical, psychological, and social domains of health, and has been used often as a benchmark for health and can provide information on the long-term impact of injuries. As an example, one year after tibial fracture a significant impairment in general health (determined by health state utility values) may still be present despite successful fracture healing, such that patients had improved since the time of initial injury but not to the level of a healthy population [105]. Similarly, for those with sports-related injuries sustained during college athletics their quality of life scores have been shown to worsen over time, well past their time of healing [4].

It is well appreciated that there is a relationship between lack of physical activity, inactivity, and sedentary lifestyles with all-cause mortality [106]. Broadly, following a range of injuries physical activity levels are shown to significantly decline just three months after the initial injury [107]. With this, the decrease in physical activity was noted independent of injury severity and return to sports/work, and there was an association in low physical activity levels with poor health, greater disability, and pain/discomfort. In more identified traumatic orthopedic conditions such as fracture, patients with both upper and lower extremity fractures also had decreases in physical activity and increases in sedentary activity following injury [108]. These early changes in physical activity appear to be extrapolated into later life, too. Again, data from NCAA athletes supports that athletes have lower health-related quality of life scores and more limitations than non-athletes [4]. Additionally, it has been proposed that injury during sports into adulthood can impact long-term risk of osteoarthritis [109, 110], and bone quality [111], which in turn likely will limit physical activity. In fact, if extrapolated, former college athletes who became physically inactive in later life have greater risks of cardiovascular disease [112]; while not directly investigated in those with previous injuries, it is possible to posit that long-term consequences could have stemmed from the initial injury.

Innovative evidence-based treatment and rehabilitation approaches aimed at improving musculoskeletal function both acutely following injury and throughout life are still needed, especially for the most severe sport and traumatic orthopedic injuries. We posit that future work needs to evaluate functional deficits, progressive and worsening pathophysiology and comorbidities due to injury. With this, any long-term limitations due to injury-induced inactivity, quality of life, and long-term co-morbidities should be considered. Future work [113] should strive to understand the span of traumatic orthopedic injuries from prevention strategies, acute care, rehabilitation, long-term health, and physiologic limitations. Additionally, the multidisciplinary use of combined approaches such as regenerative rehabilitation [114] should be explored, in the hopes that multiple approaches could work together in synergy to promote long-term functional gains and health.

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SMG, BTC, and JAC all drafted, edited, and revised the manuscript. All authors approved the final version of this manuscript.

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Conflict of Interest

The authors declare that they have no potential or actual conflicts of interest.

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