The Role of Non-Functional Overreaching and Neuromuscular Fatigue in Traumatic Injuries in NCAA Division-I Football

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The Role of Non-Functional Overreaching and Neuromuscular Fatigue in Traumatic Injuries in NCAA Division-I Football

Patrick Adam Peterson, MA University of Pittsburgh, 2024

This series of studies explored the relationship between neuromuscular fatigue (NMF) and countermovement jump (CMJ) performance in NCAA Division I football athletes. Understanding NMF's impact on performance is crucial for reducing injury risk and optimizing performance. We used Exploratory Factor Analysis (EFA) to simplify CMJ data, uncovering performance constructs. Multi-Group Confirmatory Factor Analysis (MGCFA) tested these factors' stability across different fatigue states, challenging assumptions about fatigue's effect on CMJ performance. Further analysis focused on the relationship between salivary testosterone and cortisol ratio (TC ratio) and NMF throughout a season. Despite changes in self-reported fatigue and soreness, no significant alterations were found in the TC ratio or CMJ factor scores. Linear mixed models (LMMs) indicated that CMJ measures might not fully capture NMF nuances. Although there were significant changes in self-reported fatigue and salivary biomarkers over time, no significant associations with NMF were detected. These findings suggest the need for more comprehensive assessments to detect Non-Functional Overreaching (NFOR), as NMF alone may not be sufficient. Additionally, we examined the relationship between NFOR-induced NMF and traumatic Lower Extremity Injuries (LEIs). Analyzing CMJ data over four seasons, we aimed to construct a model for traumatic LEIs, considering various covariates. Results showed differences in baseline CMJ performances between injured and uninjured athletes, with those later suffering LEIs demonstrating greater baseline CMJ performances in certain groups. Our analysis revealed insights, including reduced odds of traumatic LEI over time and increased odds associated with

NFOR. In conclusion, these studies highlight the importance of monitoring NFOR-induced changes in neuromuscular performance to assess injury risk. The findings underscore the need for standardized assessment protocols and larger, more diverse samples to better understand the longitudinal association between NFOR and traumatic LEIs in this population.

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Preface

In my four-year journey at the University of Pittsburgh I have been so lucky to engage in both the academic and professional ventures of serving the football athletes at this fine institution. It was not without the support and guidance of many great mentors that this was made possible for me. As I reflect on this time I am reminded of the many joys and difficulties of pursuing a doctorate whilst serving the young men of the University of Pittsburgh Panthers football team. The countless agonies of defeat evaporate when reminded of the spoils of victory. In keeping my own sense of proportion, I have been truly blessed to be aided in this journey by my loving family and friends, my fiancée Jenna Mola, my mother Joanne and my father Paul. I would be remiss if I did not take the opportunity to thank Coach Narduzzi, Coach Stacchiotti, Dr. Elizabeth Herrman, and the rest of the Pitt Iron Works staff. Undoubtedly these feats would not have been possible without the patience and mentorship of my committee chair Dr. James J. Irrgang. I cannot begin to think about what my experience would have been without his willingness to help. To my committee; Dr.Lauren Terhorst, Dr. Patrick Sparto, Dr. Shawn M. Arent, and Dr. Volker Musahl, words cannot express my gratitude to you all for your willingness to serve on my committee. I am truly honored to have had you all be a part of this journey.

1.0 Introduction

NCAA DI football is a sport characterized by intermittent high intensity bouts of athletic tasks and bodily collisions.^{1–4} Chronic exposure to these demands results in neuromuscular fatigue (NMF), tissue damage, and hormonal disruptions among a myriad of other maladaptive responses.5–8 The National Collegiate Athletics Association Division I (NCAA DI) regular season football typically lasts thirteen weeks. The need for sustained performance places a premium on physical preparation and recruitment of athletes that are stronger and more powerful than their predecessors.9,10 Coaches are tasked with prescribing training loads to maximize and sustain physical qualities associated with on-field performance.¹¹ In doing so, consideration must be placed on prescribing a training load that is not excessive and is coupled with adequate recovery. Functional overreaching (FOR) is the process in which the prescribed training load results in acute decrements in performance preceding positive adaptation.^{12–14}

In the presence of excessive training loads and inadequate recovery athletes can experience performance decrements due to NMF lasting between several days to weeks (non-functional overreaching [NFOR]). More transient responses during the training process however are crucial to creating positive adaptations which and improving performance, these responses are referred to throughout as Functional Overreaching (FOR).¹⁵ In more severe cases, these decrements can last several weeks to months which results in overtraining syndrome.^{13,14} The ability to identify athletes experiencing such decrements can be advantageous to practitioners prescribing training to combat the stressors of the competitive season. As outlined in the American College of Sports Medicine and European College of Sport Science joint consensus statement on overtraining, evidence of lasting NMF is crucial for proper diagnosis of NFOR.¹⁴ Previous studies have shown the countermovement jump (CMJ) is a viable means to assess NMF and recovery across a competitive season.^{5,16,17} Force-time characteristics of the CMJ provide coaches with highly dimensional data that is confounded by intrinsic factors such as position, training history, and playing status.18,19 At current there exists a dearth of knowledge regarding the differentiation of CMJ performance variables most susceptible to NMF, thus obfuscating the ability to properly detect the phenomenon in athletes.

Modeling the acute endocrine response to training and competition is another key to differentiating NFOR.^{14,15} Such responses have been documented in athletes by assessing the ratio of testosterone to cortisol (T:C), an indicator of hormonal function.¹⁵ Several studies have demonstrated adrenal-testicular axis disruptions following games in similar athletic populations but the current literature on this topic in NCAA DI Football athletes lacks consensus.^{8,16,20–22} It has been posited that the training effect of stress is mediated by intrinsic factors in these athletes, and thus the T:C response to games is highly individual. $21,22$ To understand the nuances of NMF research more adequately should aim to investigate the longitudinal associations of changes in the T:C ratio across the competitive season and performance decrements due to NFOR, or NMF.

A 16-year summary of injury data from 15 NCAA sports concluded that NCAA DI football had the highest rates of injury per 1000 athlete exposures (A-E) for both games and practices (35.9 per 1000 A-Es & 9.6 per 1000 A-Es, respectively), the closest corresponding game and practice injury rates by sport were approximately 26% and 36% lower, respectively.^{23,24} On the individual level, injuries can affect performance, length of career, earning potential, and place a high orthopedic burden on athletes.25,26 At the team level, injuries affect game performance outcomes and postseason incentives.^{27,28} Though NFOR has been shown to elevate risk of injury to athletes of other sporting populations, to date these findings have yet to be replicated in NCAA DI football.11,29,30

1.1 Statement of the Problem

The present body of literature in the sports sciences does not adequately address the plethora of challenges faced by NCAA Division I (DI) football athletes in enduring the rigorous demands of the sport, including neuromuscular fatigue (NMF), hormonal disruptions, and the risk of injury. With a regular season lasting thirteen weeks, athletes must sustain peak performance levels while managing physical strain and recovery. The following series of studies aims to make discernable the results of countermovement jump (CMJ) testing by reducing the dimensionality of the data and subsequently to examine the recovery time course of countermovement jump (CMJ) variables known to be susceptible to NMF. Additionally, we seek to elucidate the acute endocrine response to training and competition through the testosterone to cortisol (T:C) ratio, considering individualized stress responses among athletes. Lastly, after differentiating between functional overreaching (FOR) and non-functional overreaching (NFOR) we aim to explore the potential impact of NMF induced by these alterations to training status and the effect on traumatic lower extremity injury (LEI) risk during the competitive phase. By addressing these aspects comprehensively, our research aims to inform training protocols and injury prevention strategies for better athlete management and performance outcomes.

1.2 Purpose

The collective objective of this series of studies is to describe and identify CMJ variables are most affected NMF in a sample of NCAA DI football athletes. Additionally, we seek to enhance understanding as to how perturbations to the hormonal milieu as highlighted by changes in the TC ratio relate to NMF over a competitive season. Further analysis of longitudinal CMJ performance in this cohort is intended to investigate the impact of NFOR on the odds of experiencing traumatic LEI injury NCAA DI football athletes during the competitive season.

1.3 Specific Aims and Hypotheses

Specific Aim 1: To enhance the understanding of CMJ data in response to NMF in NCAA DI football.

Hypothesis 1A: CMJ variables reflecting rapid force production with respect to time are most susceptive to NMF across position groups.

Hypothesis 1B: The CMJ latent factor structure is invariant across time points and thus inferences on mean changes can be made.

Specific Aim 2: To examine the relationship between hormonal and neuromuscular responses during a competitive NCAA DI season.

Hypothesis 2A: CMJ variables reflecting rapid force production with respect to time are most susceptive to NMF across position groups.

Hypothesis 2B: The CMJ latent factor structure is invariant across time points and thus inferences on mean changes in factor scores can be made.

Specific Aim 3: To determine the longitudinal relationship of NMF as described by decrements in CMJ performance with the development of traumatic LEI.

Hypothesis 3: The odds of experiencing a traumatic LEI is greater in those experiencing NFOR induced NMF after controlling for playing position, snap count, and time of seasom.

1.4 Study Significance

1.4.1 Traumatic Injury in NCAA DI Football

In recent years many have called into question the safety of participation in NCAA DI football as it is known to expose athletes to high force collisions often resulting in injury. $1-4,23,31$ Repeated exposure to these demands results in a higher risk of injury when compared to other NCAA sport profiles.²⁴ The most common musculoskeletal injuries are traumatic in nature and occur to the lower limbs.²³ The NCAA Injury Surveillance System (ISS) estimated more than 50% of all injuries to NCAA DI football athletes are LEIs.²³ At the team level injury can negatively impact game performance, player availability, and post-season incentive though the real burden of injury is imparted onto the athlete.^{28,32} Such injuries can effect earning potential, cause detriment to quality of life, early mortality, and greater whole-body impairments for these athletes.^{25,26,33}

The preparatory training phases for competition in this sport places a premium on robustness yet often subject athletes to inordinately higher intensities than competition demands

resulting in NFOR, overtraining, and in rare cases death.^{15,34} Developing strategies to reduce the likelihood of overtraining and mitigate injury is among the foremost responsibilities of NCAA DI football coaches and support staffs.³⁵ The lengthy competition phase of NCAA DI football can also be toilsome resulting in accumulation of fatigue that disrupt neuromuscular competencies and hormonal balances necessary for maintaining performance and avoiding injury.^{20,21} Several intrinsic factors have been previously reported as having mediating effects on NMF, hormonal status, and risk of injury in this population.^{5,8,21,22,31,36–39} Therefore, more work in this space during the competitive season on fatigue and recovery and how those indices interact with risk of injury is warranted.

1.4.2 Assessing Neuromuscular Fatigue in Sport

The term "fatigue" is attributed to a state of tiredness or the action of causing something to degrade.⁴⁰ In human subjects research, fatigue refers to any number of instances in which a bodily system fails or diminishes in function, eliciting a sensed or perceived cascade of physiological, physical, biomechanical, or cognitive events.⁴¹ In recent years, sports science researchers and practitioners have made efforts to understand and quantify post-game fatigue as decrements in motor performance, or NMF.42,43 NMF is a complex phenomenon occurring at various sites along the neural pathway which results in decreased voluntary activation and/or contractile force. $44,45$ NMF has been shown to be sensitive to both acute bouts and chronic exposure to arduous competition with the absence of adequate recovery.5,16,17,46,47 Monitoring post-competition NMF is difficult in heterogenous populations like NCAA DI football as neuromuscular performance and fatigue resistance is mediated by a number of intrinsic factors such as playing position and training history.^{9,19}

Currently, the most widely used assessment of NMF in sport is the CMJ. $42,43$ Significant reductions in CMJ performance (i.e., NMF) can persist for up to 72 hours in athletes following intense training or competition.^{16,43,47} Though jump height (JH) is the most used variable derived from the CMJ to assess neuromuscular fatigue in athletes, recent studies have shown that other kinetic and kinematic outputs (i.e. peak velocity, mean and peak force, mean and peak power, flight time, contact time, and rate of force development) may be more sensitive to the effects of NMF, however further research is needed.^{18,43,48} One benefit of the use of force plates to quantify the CMF is that it provides coaches with the ability to draw inferences from the different loading phases of the CMJ as they are predicated on differing muscle actions.18,19,42,49 Studies of similar athletic populations have indicated these phase-specific indices of neuromuscular fatigue may be more sensitive than simple metrics such as JH though this has not been confirmed in NCAA DI football athletes. $42,47,50$ Moreover these data are highly dimensional further complicating the process of identifying key performance indicators from the CMJ.18,19 Though recent efforts have been made to reduce the dimensionality of CMJ data in the context of performance, these efforts have not been replicated with respect to time or recovery status.

1.4.3 Assessing Non-Functional Overreaching in Sport

Proper diagnosis of overtraining and delineation from NFOR is often challenging due to many confounding influences.¹⁴ Non-functional overreaching as a construct is not defined exclusively by alterations in motor function and neuromuscular performance, but commonly includes hormonal dysregulation. Therefore the presence of NMF alone may not be sufficient for the diagnosis of NFOR unless presented concomitantly with neuroendocrine disruptions (i.e. hormonal imbalance).^{14,15,50} Instances of NMF following competition often coincide with

disruptions to the hormonal milieu in response to stress.^{5,16} This response is tightly regulated by the hypothalamic–pituitary–adrenal (HPA) axis and results in the secretion of both the catecholamine C and the corticosteroid T.51,52 Monitoring the interplay of CMJ with both T and C by way of the T:C ratio provides a snapshot of the functional state of the athlete.⁵ This is due to catabolic tendency of C and anabolic nature of $T_{0.52,53}$ Following prolonged exposure to training stress NCAA DI football athletes may become more resilient to stress as indexed by minimal disruption or an unwavering of the T:C ratio.^{8,22} Research in similar contact sports has shown these disruptions often occur in parallel with NMF, though conflicting results have been reported in studies conducted in NCAA DI football.^{8,16,20,22} It is likely that NCAA DI football athletes' neuromuscular and hormonal response to competition is highly individualized and thus difficult to classify. 21,22

1.4.4 Neuromuscular Fatigue and Lower Extremity Injury

The injury-workload relationship in NCAA DI football posits that risk of non-contact LEI is higher with acute increases in training load.⁵⁴ However high intensity contact drills and games beget greater incidence of traumatic LEI.^{31,55,56} NMF induced impairments in voluntary activation, neuromuscular control, proprioception, and stability are evident following arduous physical activity such as NCAA DI football games.⁵⁷ These degradations in motor function coupled with augmented sensorimotor delay, prediction errors, erroneous movement tracking and time-tocontact estimation may negatively alter an athlete's ability to evade and initiate contact potentially heightening risk of injury.^{58–65} Over the course of the competitive season the potential for accumulated NMF and subsequent NFOR is high which may result in elevated LEI risk, though this has yet to be confirmed in NCAA DI football athletes. $66-68$

We propose that the use of a mixed modeling approach on longitudinal NMF in this sample of NCAA DI football athletes as the ability to model individual variances and responses via random slopes and intercepts may best reflect the nuances in capturing NMF. Differences in the effects of playing position and game exposures as covariates may provide new insight to intrinsic factors that mediate the effect of NMF on injury in this population.23,24,69,56,36 The implications of which would aid in the practice of pre-emptively identifying at risk athletes in this population. This work would provide a foundation for future investigations into enhancing training models to address modifiable risk factors in these athletes with the aim of ultimately making participation in NCAA DI football safer for all.

2.0 Literature Review

2.1 Theoretical Frameworks and Underpinnings

American football is a complex team sport in which high force bodily collisions are a key component. At the turn of the century President Theodore Roosevelt spearheaded a concerted effort of universities to reform the rules making the sport safer for athletes.²³ This effort helped lead to the formation of the National Collegiate Athletic Association (NCAA). More than a century later, football athletes are at the highest risk of injury when compared to any other NCAA sport.^{24,24} Acute decrements in neuromuscular function limit performance and influence the risk of injury to athletes.54,56,70 President Roosevelt alluded to this in a 1906 letter to his son, a football athlete. He implored him to use a "sense of proportion," so that he may, "keep in training the faculties which would make you, if the need arose, able to put your last ounce of pluck and strength into a contest."⁷¹ Herein we propose a theoretical framework which underpins the following investigations into how NMF and NFOR may influence injury and aims to inform coaches and practitioners as to how to best detect these phenomena.

2.2 Overreaching

Graded exposure to strenuous physical activity and performance dates as far back as ancient Greek literature on 6-time Olympic champion Milo of Croton. Milo regularly carried a bull over his shoulder.⁷² Milo first attempted the feat as a child when the bull was only a calf. Size and stature of the bull increased and so did Milo's strength and athleticism. Milo's bull is the earliest documented instance of progressive overload, the principle that force producing capabilities of the neuromuscular systems can augment in training given the proper progression of intensity and volume.72–74

Two millenniums later endocrinologist Dr. Hans Selye provided the theoretical framework from which current models of stress and adaptation to training are derived.73,75 Selye posited that the general and nonspecific neuroendocrine response to stress occurs in three stages. The theory is aptly named General Adaptation Syndrome (GAS).^{76–78} In the GAS model of stress organisms first enter an "Alarm Reaction" stage of catabolic processes eliciting acute decline in function. The "Resistance" stage follows thereafter where the organism responds to stress through several adaptive processes so that function returns to baseline levels.^{76,77} The third stage, known as "Exhaustion", mimics the Alarm stage wherein the duration or magnitude of continuous stress is so great that adaptive resources are exhausted in the Resistance stage and performance again declines below baseline. An important distinction made in the GAS theory is that if the application of the stressor is not prolonged and is of an appropriate magnitude then recovery will be prompt.⁷⁶ The theory of cyclic application of such stressors, or "periodicity" is the impetus for what is known as today as "periodization."⁷⁶ Selye (1974) later stated adequate stress brings about positive adaptations in performance and should regarded as "eustress" where "distress" prolongs the exhaustion stage.⁷⁸ Supercompensation is a phenomena where performance elevates above baseline in the 36-72h following the cessation of stress.⁷⁹ In applying this theory to sport it is paramount then that the individual prescribing training discern between eustress and distress, adhering to the idea of periodicity, and allotting for rest and recovery so that supercompensation may occur.

Other models of stress either confirm or deny the principles of GAS dependent on the context. GAS was challenged by W.B. Cannon, who is credited with the popularizing the theory of homeostasis which states that in response to changing external conditions the body selfregulates through compensatory processes to maintain a consistent internal environment.⁸⁰ Homeostasis was adapted from Bernard's theory of internal consistency of the human body *milieu intérieur*. ⁸¹ The primary difference is that GAS defines stress as non-specific, focusing on chronic adaptations of the adrenal cortex to secrete stress hormones, or catecholamines. Homeostasis focuses on hemodynamic and cardiovascular responses to acute stress where the adrenal medulla secretes epinephrine. These theories complement one another. $82,83$ Recent stress theory provides evidence for the role of "stress history," a non-associative learning effect resulting in either a decreased magnitude of response after repeated exposure to low- or moderate- intensity stressor (habituation), exposure to a novel stressor and increased response to the original stressor (dishabituation), or an increased magnitude following repeated exposure to a high intensity stressor (sensitization).⁸³ Habituated responses are implicated in studies of overreached athletes where anaerobic sport athletes experience autonomic imbalance of the sympathetic nervous system as opposed to parasympathetic dysfunction in endurance sports athletes.⁸⁴

Training load is the operational definition used for the quantity or magnitude of nonspecific stressors an athlete endures in sport.⁸⁵ Periodicity of stress in sport is quantified with training load. Periodized recovery from training load enables positive adaptation, or supercompensation. Exposure to training and competition where progressive overload is not considered results in dishabituation due to novelty of the stimulus or sensitization where the stimulus intensity is too great relative to the athlete's stress history. Periodization refers to the science of training prescription with respect to the cyclic application and is underpinned by the GAS theory.^{86–88} Periodization with respect to intrinsic factors that mediate stress responses enhances performance and mitigates injury through supercompensation.⁸⁷

Stress history, an intrinsic factor influencing overreaching, can be expressed using retrospective training load.¹⁴ Retrospective training load has been implicated in the literature for its relevance to overreaching and injury.^{89–91} Current models draw from the Fitness-Fatigue model, an adapted version of GAS specific to sport.^{92,93} Fitness-Fatigue posits that for each stressor there are negative (fatigue) and positive (fitness) training effects. The difference between the two equates to realized performance.⁹² The Fitness-Fatigue model applies an exponential time decay model to reflect the antagonistic fatiguing and fitness effects on performance relative to training load magnitude and the additive nature of time to recovery from a stressful stimulus.^{94,95} Recently, retrospective measures of training load expressed relative to stress history have been shown to be associated with increased risk of injury in NCAA DI football athletes.^{54,96}

In addition to these measures of training load magnitude there is supporting evidence for the variance of weekly training load as a protective measure for overreaching and injury in NCAA DI football athletes.⁹⁷ Invariance is a marked feature of improperly periodized training loads as the athlete is not allotted time to recover between stressful bouts.12,98 Hence, monotony, a measure of training load variance correlated with injury risk, is used as a criterion for diagnosis of overtraining and overreaching.14,99 Therefore in preparing NCAA DI football athletes for the stressors of game exposure throughout a competitive season it is imperative that coaches take into consideration the periodization of training loads to limit the presentation of overreaching and its related downstream effects on performance and injury risk.

The goal of periodization is to minimize exhaustion phases so much as necessary to reduce maladaptation potential.^{100,101} Per GAS theory, transient performance decrements, or FOR, predate for supercompensation.¹⁴ With excessive training load and prolonged exhaustion phases more pronounced chronic performance decrements with effects of several days to weeks NFOR occur.¹⁴ The constitutive definition of overreaching has evolved with etiological evidence. Currently accepted definitions of overreaching and overtraining point to training and non-training stress accumulation resulting in performance decrement in the absence of other explanatory psychological and physiological factors. The presence of chronically high and invariant training loads can often manifest in the presentation of NFOR and related decrements in performance or disruptions to the health and well-being of athletes.

2.3 Hormonal Perturbations

Diagnosis of overreaching is difficult as mechanistic research suggests a complex cascade of metabolic and neuroendocrine responses including but not limited to substrate depletion, immunosuppression, autonomic imbalance, oxidative stress, cytokine release, inflammation, hypothalamic dysregulation, and fatigue.^{13,84,99,102} The European College of Sport Science and the American College of Sports Medicine published a consensus on the prevention and diagnosis of overreaching to help rule out confounding intrinsic and extrinsic factors.¹⁴ The neuroendocrine system is modulated by the HPA-axis and can be modeled using the T:C ratio.

The T:C ratio has been previously used to examine instances of NFOR during offseason training in American Football athletes.¹⁵ At current, there are numerous studies to support the notion that NMF in athletes is often associated with acute decreases in the T:C ratio.5,16 This response is induced by acute exposure to game demands but may be modifiable with chronic exposure in what has been deemed "contact adaptation."7,8,20,22 These findings have been replicated

across several athletic populations particularly those with an element of bodily contacts or collisions.46,47,103 However there is conflicting evidence around the relevance of T:C in monitoring in-season hormonal disruptions of American Football athletes.8,20,22 Therefore analysis of the hormonal response to training and competition as evidenced by changes to the T:C ratio across the competitive season in American football is warranted to more properly detect instances of NFOR induced NMF.

2.4 Neuromuscular Fatigue

Traditionally the term "fatigue" is used to describe a state of tiredness or the action of causing something to break down.⁴⁰ Fatigue may refer to any non-specific decrement in function which can give rise to a sensations of muscle weakness or decreased vigor.⁴¹ Decreases in motor performance observed post exercise or following sport participation are operationally defined as NMF.^{42,43} The detection and subsequent reporting of NMF can be difficult as access to laboratory grade equipment with which gold-standard assessments of NMF can be conducted is uncommon in sporting environments. This can be troublesome for coaches and athletes as NMF is a complex phenomenon and the prognosis for treating NMF can be vastly different depending upon which part of the neural pathway has been most affected.^{44,45,104}

Neuromuscular fatigue is of particular interest to For those working with team sport accurately detecting NMF may be of interest as it often brought on by instances of overreaching.5,16,17,46,47 In the literature, NMF tends to bear definitions pertinent to the discipline or area of study from which the means employed to measure the phenomena originate. $41,105$ In laboratory studies, decrements in peak force as derived from a maximal voluntary isometric

contraction (MVIC) is considered the "gold standard" assessment.¹⁰⁶ As it is often not feasible to conduct MVIC testing in team settings researchers in sport science have used instead used CMJ performance as a proxy measure for detecting NMF.

When exploring the mechanisms and treatment interventions for NMF, it is imperative to first distinguish between central and peripheral fatigue as there are distinct differences in the expected response to training stimuli of the two.⁴⁵ First, central fatigue refers a decrease in output or "drive" from the structures of the nervous system preceding the neuromuscular junction (spinal and supraspinal).^{107,108} It has been previously reported that decreases in excitability of the motorneuron pool following fatiguing exercise may be consequent of increased membrane potential of the motorneurons, inadequate secretion of neurotransmitters, or the binding of 5 hydroxytryptamine (serotonin) to receptors in the brain.^{109–111} The revised central fatigue hypothesis posits an increase in the ratio of the neurotransmitters serotonin to dopamine accelerates the onset of fatigue.¹¹² Where it has been shown that increased serotonin synthesis can result in lethargy, depression, and decreased motor neuron excitability it has also been hypothesized that chronic exposure to elevated levels of serotonin due to high volumes of training in athletes presents as symptoms of NFOR and overtraining.^{113–115} The corticospinal pathway works to transmit neural outputs from the brain to the muscle, the efficacy of which is indicated by corticospinal excitability.¹⁰⁹ Decreases in corticospinal excitability following intense bouts of training are well documented though recent evidence suggests that the time course of recovery from such indices of central fatigue (6h post) may conflict with previously proposed models of super-compensation (24-48h post).73,76,110,116

Peripheral fatigue refers to decrements in performance attributable to disruption of the structures at or distal to the neuromuscular junction.⁴⁵ Peripheral fatigue presents in the presence of depleted energy stores, metabolite accumulation, and damage to the contractile proteins.117,118 This notion was first conceived by Angelo Mosso in his 1906 book titled *"Fatigue"*, where he in one experiment found that the rate of decline in performance of successive muscular contractions was similar between voluntary and electrically stimulated conditions.¹¹⁹ Mosso's early works have helped to shape modern day understanding of the role of peripheral fatigue in acute and chronic decrements of muscular performance. Enoka & Duchateau (2016) proposed a comprehensive model highlighting the mechanisms of fatigue, focusing on identifying "rate- limiting adjustments" of either central or peripheral processes that damper human performance. In this model, disruption of structures and processes ascribed to peripheral fatigue include skeletal muscle metabolism, hemodynamics, intracellular milieu, contractile apparatuses, excitation- contraction coupling and action potential propagation.

In deducing the role of central fatigue in voluntary activation the use of peripheral nerve stimulation or transcranial magnetic stimulation to generate motor evoked potentials is needed. To properly distinguish peripheral fatigue as the primary cause for reductions in motor performance contractile force must be expressed relative to a given neural output that is held constant through stimulation.¹²⁰ Given the often arduous and invasive nature of collecting such data there has been a dearth of studies have investigating central fatigue in vivo with team sport athletes. Though the two are not mutually exclusive commonly used field- based measures of neuromuscular fatigue lack the ability to differentiate between central and peripheral fatigue.¹²⁰ Therefore, it cannot be ruled out that observed decrements in performance used in sport science to categorize neuromuscular fatigue may be the gross response and amalgamation of both central and peripheral fatigue. The distinction between central and peripheral fatigue in this sense is often arbitrary and leaves much to be desired, the outcome of interest should drive interpretation provided the protocol

maintains ecological validity as has been outlined in proposed models monitoring fatigue in athletes across the training cycle.48,104

After fatiguing exercise corticospinal inhibition presents in both the active and inactive muscles suggesting a broader non-specific effect of central fatigue on the motor pathway and descending drive.¹⁰⁵ In cases of trauma transient decreases in force producing capabilities of muscle are related to elevated levels of ATP, inorganic phosphate, and H+ ions causing a drop in tissue pH suggesting a disruption of central nociceptive processing.¹²¹ Elevations in serum creatine kinase levels, an indirect marker of muscle damage, are also associated with impaired strength and reduced ATP synthesis following exercise.^{20,122} Per the Central Governor model, the central nervous system receives information about peripheral fatigue via Group III afferents and Group IV afferents.117,123 Group III afferents are myelinated and transmit mechanosensory information from the muscle to the central nervous system.^{124,125} Unmyelinated Group IV afferents are sensitive to biochemical disruptions of skeletal muscle arising from increases in metabolite concentrations. 124,125 Following training and competition athletes exhibit compromised stiffness of the muscle-tendon junction this altered mechanical stimuli is transmitted to the central nervous system via Group III afferents while metabolite build up, another byproduct of sport induced fatigue, is communicated via Group IV afferents.^{126–128} These coupled with nociceptive stimuli from pain or trauma coalesce as neuromuscular fatigue where the brain decreases power output by inhibiting motor unit recruitment to prevent physiological failure such as rigor or injury at the muscular level.121,129

During voluntary activation the brain self organizes to recruit motor units relative to the desired outcome and sensory prediction of fatigue through continuous visuomotor feedback. Induced temporal delay in this visuomotor feedback loop increases perception of fatigue.⁵⁹ This sensory prediction is subject to error where perceived fatigue Is greater than actualized and vice versa. The Central Governor model has been criticized for neglecting to consider the subjective experience in perception of fatigue as well as evidence presented that incentive and motivation can preserve performance outcomes independent of fatigue.^{129–131} This challenge in measuring neuromuscular fatigue in sport is especially noteworthy as disruptions in self-reported mood and motivation often arise from the trials and tribulations of winning and losing and are also seen in the development of overtraining symptoms.^{13,14,16}

A more recent paradigm shift in the study of NMF has prompted the sport science community to look more closely at athletically relevant tasks such as the CMJ to monitor decrements in performance.¹³² The recovery time course of CMJ following competition in fieldsport athletes closely mimics that of both voluntary activation and MVIC irrespective of changes in corticospinal excitability.¹³³ These results support the notion that neuromuscular fatigue is taskspecific and tests to deduce its presence must be reflective of the imposed exercise demands. As opposed to MVIC, the kinetic and temporal features of the CMJ require contribution of the stretch shortening cycle and are more representative of sporting actions in NCAA DI football.^{48,134} Additionally, the high intensity ultra-short duration (>250ms) of the CMJ indicates greater reliance on the phosphagen system, the primary energy system utilized in NCAA DI football.^{21,134–136} Recent evidence supports that in the 24-48 hours following fatiguing exercise individuals selforganize to produce alterations in CMJ force waveforms with respect to time whilst maintaining gross task performance (i.e., jump height). ¹³⁷ Therefore force- time characteristics derived from the CMJ offer a valid and field-expedient measure of NMF in athletes where other means are not accessible whilst also bolstering ecological validity through sport-specific biomechanics and bioenergetics.18,40,48,106,132

The use of CMJ to assess neuromuscular fatigue is common practice in the world of highperformance sport.40,43,138 In 2012 a survey of practitioners from elite sporting organizations found that 80% of respondents allocated time to assessing fatigue in their athletes, of those 61% administered tests of neuromuscular performance, the most common of which being the CMJ.⁴² The literature on which CMJ metrics to report for NMF lacks consensus and though jump height may be the most commonly used variable derived from the CMJ to assess NMF it is not always shown to be the most sensitive.^{18,43,48,139}

Acute neuromuscular fatigue is modulated by several factors including but not limited to; age, sex, modality, intensity, frequency, and duration of exercise, contraction type, environmental climatic conditions, nutrient availability, circadian cycle, hydration, health status, travel, and individual physical fitness.17,45,118,140 Over the course of a competitive season an athlete may be repeatedly subjected to neuromuscular fatigue of varying degrees. Studies have shown that upwards of 60% of competitive events can result in significant declines in the CMJ performance where successive events of which might be indicative of overreaching or overtraining.⁴⁶ In some instances, athletes may never attain pre-season levels of performance.¹⁷ It has also been shown that both chronic exposure and abrupt changes in training volumes negatively affect excitability in experienced athletes.¹⁴¹ These data suggest that although maintenance of physical activities relative to expected training volumes may enhance resilience to acute neuromuscular fatigue, even highly trained athletes may not be impervious to the effects of or chronic exposure to such stressors. 17,46

Sports science research governing bodies have presented a consensus statement on NFOR which postulates that barring confounders the presence of NMF where performance decrements equate to $\geq 10\%$ is sufficient for diagnosis.¹⁴ The expected time course neuromuscular fatigue

imparted on an athlete by way of functional overreaching is 24-72h and is dependent upon extrinsic factors such as the mode, duration, and intensity of stress and moderated by intrinsic factors such as fitness, hydration, nutrient availability, and lifestyle.17,43,45,118,140 In athletes observed motor performance decrements in the subacute (0.5-24h) phases are attributed to central fatigue and the acute phases $(24-72h)$ are predominated by peripheral fatigue.^{120,133} Following competition athletes demonstrate both central fatigue and peripheral fatigue the summation of which can present as CMJ performance decrements.66,126,128,142 With inadequate recovery neuromuscular fatigue prolongs beyond the subacute time course to that of NFOR, increasing person-time exposures at risk.

2.5 Perception-Action Coupling

In response to fatigue the central nervous system downregulates motor unit recruitment, decreasing power output to preserve homeostasis and reduce the perceived threat of injury.¹¹⁷ This Central Governor model is subconscious in nature suggesting myelinated group III afferents provide mechanoreceptive feedback where unmyelinated group IV afferents provide metabosensitive feedback at the spinal and supraspinal levels, modulating motor command.121,129,130 In contrast, classic feedforward models of human movement generate environmental representations from which pre-planned motor outputs dictate task performance, as these occur prior to sensory and proprioceptive feedback.^{143,144} In NCAA DI football however athletes are coupled with fast changing environments.¹⁴⁵ The sport requires rapid perception of constraints providing opportunities for movement, or affordances, to which the athlete selforganizes to producing desired action.¹⁴⁶ Perception-Action Coupling matches task performance

to affordances.147,148 In stable environments, movement and coordination adapt to reduce movement variability.149,150

Three domains of constraints constantly interact: environmental, task, and organismic.¹⁴⁷ Environmental constraints in American football are extrinsic to the athlete including weather, crowd noise, and field conditions. Performance dependent task constraints include rules of the game, proximity, number of opposing players, relative position, and approach speed.^{146,151} Organismic constraints are intrinsic to the athlete, subdivide into structural and functional. Structural constraints are relatively stable over time, in athletes these include anthropometrics and muscle architecture.¹⁵² Playing position (ex. wide receiver, defensive back) has a significant effect on anthropometrics and body composition and thus must be accounted for in statistical models.¹⁵³ Functional constraints attribute to behavioral characteristics.¹⁴⁷ These intrinsic constraints are more sensitive to NFOR and include NMF, cognition, and effort perception.^{146,152,154,155}

Neuromuscular fatigue alters perception-action coupling by augmenting sensorimotor delay and prediction error with concomitant decreases in voluntary force production, one or any combination of which prompt real time motor adaptations to preserve successful task outcomes.58– ⁶¹ Continuous processing of sensorimotor inputs and feedforward representations of movement are essential in interceptive tasks where elite athletes demonstrate improved capabilities in perceiving an objects velocity and self-organizing movement to initiate contact. $63,65,156$ This is especially relevant in American football as evasion and initiation of contact are required in tackling. Fatigued athletes are less capable of spatiotemporal perception of environmental constraints such as player positioning.⁶² Such information is crucial in interceptive tasks like tackling to estimate time-tocontact.64,65 Neuromuscular fatigue in competition impairs the ability to perceive perturbations to the center of mass to maintain balance and coordination, heightening the risk of injury due to contact.¹⁵⁷ Constraints in American football however are unstable and thus athletes must continuously adapt to new information with movement variability.^{97,146} These intrinsic constraints result erroneous movement tracking and time-to-contact estimation which are crucial to withstanding contact and evading bodily positions susceptible to traumatic injury.^{62–65,158}

2.6 Theoretical Framework

This research aims to develop a comprehensive theoretical framework for understanding how NMF and NFOR may influence the risk of traumatic LEIs in NCAA Division I football athletes. Drawing from historical perspectives, such as President Theodore Roosevelt's advocacy for safer sports practices, and contemporary physiological models like the GAS and the Fitness-Fatigue models, the study seeks to elucidate the interplay between training stressors, hormonal responses, and neuromuscular fatigue. The theoretical framework will address the principles of overreaching, the hormonal perturbations associated with NMF and NFOR, and the impact of NMF on perception-action coupling, particularly in the dynamic and unpredictable context of American football. By synthesizing these concepts, the study aims to provide coaches and practitioners with insights into detecting and mitigating the risks associated with NMF and overreaching, thereby enhancing injury prevention strategies and optimizing athlete performance throughout the competitive season.

Given the body of evidence we propose conceptual model of maladaptive responses to physical stress brought on by American football and exacerbated by potentially inadequate periods of recovery, which present as NFOR induced NMF and subsequently increase risk of injury.68,93,159,160 These studies will focus on NMF and traumatic LEI through the lens of the Central Governor Model and Perception-Action Coupling. To account for the dynamic nature of competition where the athletes adapt to a myriad of stressors and recovery is inconstant, timevarying covariates and individual variances will be accounted for wherever possible.⁸⁴ Intrinsic factors such as position and stress history, measured by game exposures, may make selected athletes resilient to stress generating a phenotypic plasticity as adaptation responses are learned at the individual level.¹⁶¹ This theoretical framework is presented graphically below in Figure 1.

Figure 1. Theoretical Framework

The theoretical framework proposes that the responses to training load elicit central and peripheral fatigue disrupting sensorimotor feedback and corticospinal excitability and presenting as Neuromuscular Fatigue per the Central Governor Model. This maladaptation to training load imparts functional constraints onto the athlete perturbing Perception-Action Coupling resulting in affordances for emergent motor behavior that induces a greater risk of injury. Person-time at risk is increased where these deleterious effects are prolonged in cases of Non-Functional Overreaching and Overtraining.
3.0 Manuscript 1: Structural Invariance of Countermovement Jump Testing in NCAA DI Football Athletes

BACKGROUND: The American football season is physically arduous and results in a high prevalence of physical ailments and sport-related injury in athletes. Coaches place a premium on maintaining performance during this period to prepare athletes for competition and limit the occurrence of injury. Neuromuscular Fatigue (NMF) is operationally defined as a clinically meaningful reduction in neuromuscular performance in response to sport-related stressors. The Countermovement Jump (CMJ) is a reliable and ecologically valid means of assessing NMF yet the data regarding performance is highly dimensional., which may hinder the ability to identify NMF in athletes. **PURPOSE:** The primary aim is to reduce the dimensionality of CMJ data in a sample of American Football athletes to determine which constructs may be sensitive to NMF. **METHODS:** Baseline CMJ performance data were attained from 176 collegiate athletes in a nonfatigued state following a scheduled break from training at the beginning of the season. A subset of CMJ performance variables were selected based on previous literature related to NMF and CMJ performance. Athletes were grouped by playing position into either the BIG, MID, or SKILL groups. Force and power variables were normalized to body mass to adjust for between-group anthropometric differences. The data were reduced using Exploratory Factor Analysis (EFA). Multi-Group Confirmatory Factor Analysis (MGCFA) was then used to test for metric invariance on a sub sample of athletes with repeated measures under recovered and fatigued conditions. Thereafter two-way ANOVA was used to test the effects of time and position group on CMJ performance using factor scores derived from the EFA model. Where significant main effects were found, post hoc Tukey's HSD tests were used for pairwise comparisons $(\alpha < 0.05)$. **RESULTS:** A two-factor EFA with weighted least squares (WLS) estimators and oblique rotation explained 84% of the total variance. Factor 1 was labeled Stretch Shortening Cycle (SSC) as it was loaded predominately by variables indicating brief amortization and vertical displacement; Jump Height (JH)(factor loading $\lambda=0.97$), Peak Relative Propulsive Power (PPP)($\lambda=0.80$), and Reactive Strength Index-Modified (mRSI)(λ =0.72). Factor 2 was labeled Maximal Dynamic Strength (MDS) as the strongest factor loadings, Average Propulsive Force (APF) $(\lambda=0.83)$ and Propulsive Phase Duration (PPD)(λ =-0.87), represent the ability to produce concentric forces rapidly. Both factors demonstrated excellent internal consistency as assessed by Cronbach's alpha (SSC α =0.94; MDS α =0.93). Metric invariance was confirmed with MGCFA fit on a sample of athletes under recovered and fatigued conditions. Significant main effects of position group were found in both SSC (F(2, 315)=91.20, p<0.001), and MDS (F(2, 315)=32.08, p<0.001). Significant group x time interactions for were reported for SSC only $(F(4, 315)=14.62, p<0.001)$. Pairwise group comparisons revealed significant differences between all position groups $(p<0.05, \text{ all})$ while comparisons of group x time interactions revealed differences in time-varying responses between SKILL and MID groups relative to BIGS, with no significant differences between MIDs and SKILLs at any timepoint. **CONCLUSION:** The findings of this study show that 84% of the variance in CMJ performance in this sample of NCAA DI football athletes can be attributed to two latent factors. The structure and loadings of these factors are also time-invariant allowing for the comparison of latent value means across time. Although no main effect of time was found, significant group and group x time differences are prevalent for both factors. This reflects the contextual nature of CMJ performance as it pertains to position groupings. Future studies should aim to investigate the utility of identifying NMF in these athletes by way of CMJ performance by validating against criterion measures of fatigue, controlling for mediators such as position.

3.1 Introduction

American football is a sport characterized by intermittent high intensity bouts of athletic tasks and bodily collisions.^{1-4,162} Chronic exposure to these demands results in neuromuscular fatigue (NMF), tissue damage, and hormonal disruptions among a myriad of other maladaptive responses.5–8 The National Collegiate Athletics Association Division I (NCAA DI) regular season football typically lasts about thirteen weeks. The need for sustained performance places a premium on physical preparation and recruitment of athletes that are stronger and more powerful than their predecessors.9,10 Coaches are tasked with prescribing training loads to maximize and sustain physical qualities associated with on-field performance.¹⁶³ In doing so, consideration must be placed on prescribing a training load that is not excessive and is coupled with adequate recovery.

Previous work has shown the CMJ is a viable means to assess NMF and recovery across a competitive season.5,16,17 Traditionally Jump Height (JH) and peak power are the most commonly reported CMJ measures for monitoring fatigue in athletes. Though other authors have suggested the need for including more task relevant metrics such as the Reactive Strength Index-modified (mRSI), which is the JH divided by time to takeoff.⁴² The data derived from CMJ is highly dimensional leaving interpretation and feature selection up to the practitioner.^{19,164} Merrigan et al., (2022) found through Principal Components Analysis (PCA) that 89.5% of the variance in CMJ performance in NCAA DI football athletes was explained by four dimensions predominated by mRSI, Braking to Propulsive Power Ratio, Countermovement Depth, and Braking Rate of Force Development. The same study showed that the factor loadings differed between position groups though this factor structure has not been tested for measurement invariance with respect to time.¹⁹ Between-position differences may exist not only under fatigued conditions following games but as well as during presumed non-fatigued conditions such as baseline testing. Moreover, the direction of these changes across the competitive cycle may also be dependent on the dimension being measured. Therefore, a more comprehensive analysis of the underlying factor structure and the stability of its constructs, or latent factors, is warranted. Herein we employ the use of Exploratory Factor Analysis (EFA) on a subsample of CMJ metrics previously cited as relevant to performance, injury, and fatigue.

Whereby NMF is known to effect post game CMJ performance we aim to test the measurement invariance using Multiple-group Confirmatory Factor Analysis (MGCFA) on the CMJ variables with pre- and post- game data. We hypothesize that the factor structure of CMJ performance variables is invariable when compared across these timepoints and mean factor loadings may be used to express the performance of groups in longitudinal designs. MGCFA is an extension of structural equation modeling used for testing measurement and structural invariance in longitudinal data.¹⁶⁵ By testing the difference in fit indices between the constrained and free models for time we can determine whether the factor structure is maintained over time. Confirming measurement invariance provides support for the between-group or longitudinal comparison of mean values of the latent factor loadings (factor scores). These factor scores might then provide additional information where univariable analysis of individual CMJ variables leaves much to be desired when detecting changes in performance induced by NMF. Therefore, the purposes of this research are to further elucidate the ability of CMJ performance testing for detection of NMF in NCAA DI football athletes by reducing the dimensionality of this data and subsequently examining the stability of different performance constructs under fatigued and recovered conditions.

3.2 Methods

3.2.1 Participants

Countermovement Jump (CMJ) data were extracted from the University of Pittsburgh football team records for the $2020 - 2023$ seasons. A total of 176 athletes (age 19.8 \pm 1.5y) completed the CMJ test prior to the first in-season practice as part of standard of training procedures. Data from the most recent season for each athlete at the time of writing were retained as not to introduce any repeated measures into the baseline model. The athletes were familiar with the testing protocols at the time of data collection. Athletes with known pre-existing injury as delineated on the team injury reports provided by licensed athletic trainers were excluded from analysis. The positions were grouped by similar sporting demands and anthropometrics as is in accordance with previously conducted studies in this population. These groupings are denoted as BIG (offensive and defensive linemen), MID (running backs, tight ends, quarterbacks, and linebackers) and SKILL (wide receivers and defensive backs). Subject demographics are listed below in Table 1. The University of Pittsburgh Institutional Review Board (STUDY20070389) approved the procurement of deidentified team data for retrospective analysis.

Table 1. Sample Demographics

3.2.2 Countermovement Jump

Following a general dynamic warm up each athlete performed three bilateral CMJs with hands on hips on dual force platforms sampling at 1000 Hz (Hawkin Dynamics, Maine, USA). Force-time data were analyzed and exported from a previously validated commercially available software (Hawkin Dynamics, Maine, USA).¹⁶⁶ Athletes were required to "stand completely still" for ≥ 1 s to acquire a mean system weight from which the software used a -5SD threshold to determine the initiation of the CMJ. 167 The kinetic phases of the CMJ where then categorized using the taxonomy described by McMahon et al. (2018).¹⁶⁸ Phase specific peak force asymmetry indices were then calculated using the bilateral strength asymmetry equation.^{169–171}

As part of the testing procedures athletes were instructed to "jump as high and as fast as you can." The performance staff provided verbal encouragement and supervised test administration to ensure proper technique. Tests were separated by ~30s rest. Samples in which the athletes recorded the highest Jump Height (JH) were kept for analysis. Variable selection was informed by a recent review of the literature on CMJ outcomes relevant to NMF and injury listed in Table 2^{139} To reduce the potential for influence of between-group anthropometric differences, kinetic variables were normalized to body mass.

Table 2. Countermovement Jump (CMJ) force-time variables

3.2.3 Statistical Analysis

To account for the highly dimensional and interdependent kinetic data associated with CMJ performance, exploratory factor analysis (EFA) was used to uncover the latent variables or unobserved constructs within the sample (using the fa function of the "psych" package in R version 4.0.3).¹⁷² Observations were restricted to the baseline timepoint prior to the start of the season to control for the potential effects of fatigue in subsequent tests. Prior to performing EFA, a Pearson's correlation matrix was produced to assess for univariate relationships. The variables were arranged by the angular order of eigenvectors to visualize the factor structure. Variables in closest proximity to one another are most similar in their shared variance in dimension space. Those listed furthest away are most orthogonal. The assumptions of EFA sampling adequacy was tested with the Kaiser-Meyer-Olkin (KMO) test (*kmo* function in the "psych" package in R version 4.0.3).¹⁷² The KMO test offers a rule of thumb for the overall measure of sampling adequacy (MSA) which posits that an MSA<0.50 is unacceptable for factor analysis and an MSA closer to 1.0 is desirable.^{173–175}

Univariate MSA values are then inspected and those with low factor loadings and MSA<0.50 were discarded to reduce noise and redundancy in the final EFA model.¹⁷⁶ Bartlett's test of sphericity was then used to determine if correlations among the retained variables were sufficient for factor analysis. Multivariate normality was assessed using Mardia's test of skewness and kurtosis and the Henze-Zirkler's test along with inspection of $Q-Q$ plots.^{177–180}

Upon confirming the data were fit for factor analysis, a parallel analysis then determined the optimal number of factors to retain in the EFA model. Parallel analysis is a Monte Carlo simulation technique shown to consistently outperform traditional model fit indices for determining the optimum number of factors for EFA.^{181,182} Parallel analysis simulates datasets parallel to the real data with a large number of iterations (in this case n=1000) from which eigenvalues are calculated and compared to the real data. The greatest number of factors which maintained eigenvalues greater than the mean eigenvalues of the simulations was selected. To reduce Type I error, the eigenvalues of the real data were compared to the 95% confidence interval for those of the correlation matrices of the simulations.183,184

After fitting the EFA model, oblique rotation was used allowing for latent factors to be correlated given that this work is underpinned by the evidence for high collinearity in kinetic variables derived from CMJ data. The rotation yielding the most parsimonious and interpretable model, absent of substantial cross-factor loadings and most influential factor loadings was selected.^{185,186} Items with factor loadings ≥ 0.45 were deemed influential, which is a more conservative approach for smaller sample sizes.¹⁸⁷ The internal consistency of the identified factors was assessed using Cronbach's alpha.

A multi-group confirmatory factor analysis (MGCFA) was used to test for metric invariance of the latent factors derived from the preceding EFA. Complete cases were used from a sub-sample of n=108 athlete's CMJ data from the 2021-2023 seasons was queried for this analysis. In this sample, CMJ tests were performed and subsequently analyzed as previously described. The testing however was conducted at baseline, prior to the start of the season, during the "bye week" where training volume was significantly reduced at the approximate midpoint of competitive season, and again following the first game after the bye week. At each time point ~48h rest was allotted and 7d since the previous competition. The subsequent timepoint occurred 7d later and ~24h after the following competition under fatigued conditions.

Metric invariance, or the stability of the theoretical factor structure with respect to time, was tested using MGCFA models within the *lavaan* R package. The configural model was comprised of the structure revealed in the EFA where factors were free to be correlated to test whether factor structure was stable across timepoints. The weak invariance model then was tested with factor loadings constrained to be equal across both time points which tests whether the relationship between the observed variables and the latent factors is consistent across timepoints. Strong invariance was tested by constraining both factor loadings. Strong invariance then would indicate that not only the relationship between observed variables and the latent factors is invariant but that the mean levels of the latent factors were invariable at each timepoint is as well. The Tucker Lewis Index (TLI), the Comparative Fit Index (CFI), and Standardized Root Mean Square Residual (SRMR) were used to assess model fit and models were compared using chi-squared difference tests.

Two-way analyses of variance (ANOVAs) were conducted on factor scores extrapolated from the baseline EFA model to investigate the effects of time and position group on in-season CMJ performance under both recovered and fatigued conditions. A subsample of CMJ data from 108 athletes across three seasons (2021, 2022, and 2023) were used to investigate the effects of fatigue and recovery on CMJ performance. In the 2021 and 2023 season, the bye week occurred after the fifth game whilst in 2022 the bye occurred after game four. Only the 2020 season was omitted from the dataset where there was no testing conducted during the bye week. Baseline CMJ performances were assessed prior to the start of the first week of the competitive season. The presumed 'recovered' condition consisted of results from CMJ tests conducted on the Sunday following the 'bye' week, 8 days after the preceding game. The 'fatigued' conditions then were comprised of results from the subsequent week's CMJ tests 24h post-game. Where significant main effects were found, post hoc Tukey's HSD tests were used for pairwise comparisons. All data processing, visualization, and statistical analyses were completed in R version 4.0.3.

3.3 Results

3.3.1 Exploratory Factor Analysis

Summary statistics (means and standard deviations) were calculated for baseline univariate CMJ performance metrics by position groupings in Table 3. Univariate relationships among CMJ variables were computed and visualized in Figure 2 Of the items with MSA>0.50, PNI was not retained in the final dataset despite having an MSA=0.96 as no moderate-strong univariate relationships were found with this variable when inspecting the correlation matrix and PNI did not substantially load on any latent factor. The results of the KMO are listed in Table 4. Moreover, the inclusion of PNI, PPD, and APF would have added redundancy to the model as PNI is the integral of net force (Newtons - system weight) with respect to time in the propulsive phase of the CMJ. Lastly, after comparing overall the MSA, the interpretation of sampling adequacy was unchanged from the reduced dataset to the final dataset used for EFA.

Bartlett's test revealed sufficient correlations among features in the final dataset for factor analysis $(\chi^2_{10}) = 1276.062$, $p > 0.001$. The results of both Mardia's and Henze-Zirkler's tests of multivariate normality failed to reject the null hypothesis and indicated joint skewness and kurtosis, deviating from normality (*p*>0.05). Therefore a Weighted Least Squares (WLS) method was used for factor extraction as it is more robust to non-normal data than other extraction methods such as maximum likelihood estimators.^{188,189} The WLS method is preferred for less complex models with small samples (n<200) as weighting observations reduces model bias toward outliers and influential points, improving model fit.¹⁹⁰ Parallel analysis using the WLS extraction method and n=1000 simulations determined a two-factor model to be optimal, the results of which are visualized in Figure 3.

	$BIG(n=62)$	MID (n=60)	$SKILL$ (n=54)
JH(m)	0.37 ± 0.06	0.45 ± 0.05	0.48 ± 0.05
PPP (W/kg)	$54.11 + 7.12$	62.97 ± 5.99	$66.78 + 7.06$
TTO (S)	$0.750+0.12$	$0.723 + 0.08$	$0.730 + 0.10$
mRSI (AU)	0.50 ± 0.11	0.63 ± 0.09	$0.66 + 0.12$
APF(N/kg)	208.86±19.13	227.51 ± 16.96	236.75±21.41
PPD(s)	$0.256 + 0.03$	$0.241 + 0.03$	0.232 ± 0.03
$PNI(N*s)$	353.25+34.17	300.90±27.29	260.00 ± 24.35
PPFA $(%)$	6.75 ± 5.51	$7.09{\pm}5.89$	$6.62{\pm}5.07$
PBFA $(%)$	$9.51 + 7.08$	$9.59 + 6.87$	$9.13 + 6.88$

Table 3. Baseline Countermovement Jump (CMJ) Performances

Figure 2. Countermovement Jump Correlation Matrix

The countermovement jump (CMJ) variables were arranged by the angular order of eigenvectors. Variables listed closest to one another are most similar in their shared variance in dimension space.

Table 4. Kaiser-Meyer-Olkin (KMO) Test Results

Results of Kaiser-Meyer-Olkin (KMO) test for overall Measure of Sampling Adequacy (MSA) and for each iteration of the CMJ dataset. Overall MSA was interpreted using the following benchmarks: <0.50, unacceptable; 0.50–0.60, miserable; 0.60–0.70, mediocre; 0.70–0.80, middling; 0.80–0.90, meritorious; and .0.90, marvelous. Features with MSA<0.50, were systematically removed from the dataset and only those with large factor loadings and moderate-strong correlations with other features were retained in the final dataset.

Figure 3. Parallel Analysis

Eigenvalues from the number of extracted factors using the Weighted Least Squares (WLS) method for observed data (red) and the upper limit of the 95% confidence interval from n=1000 simulations (blue).

The two-factor WLS model was fit to the data using the oblimin rotation method. Factor structure and loading patterns are visualized in Figure 4. The results showed the two factors accounted for 84% of the total variance with a moderate positive correlation of 0.64. Factor loadings and communalities are summarized in Table 5.

Factor 1 accounted for 48% of the total variance with excellent internal consistency (Cronbach's α =0.94). Factor 1 thus was labeled as Stretch Shortening Cycle (SSC) as the three variables that had high factor loadings (JH, PPP, and mRSI) are commonly associated with elastic capabilities of athletes.18,19 Factor 2, which accounted for 36% of the total variance was predominated by APF and PPD with excellent internal consistency (Cronbach's α =0.93). As PPD loaded negatively on Factor 2 and APF positively, this factor was labelled as Maximal Dynamic Strength (MDS), a construct described previously as maximal force produced against no external load in a short amount of time $(\leq 0.300s).^{191}$

Table 5. Countermovement Jump (CMJ) Factor Loadings

Factor loadings representing the standardized coefficients indicating the strength and direction of the relationship between each observed variable and the underlying latent factors Stretch Shortening Cycle (SSC) and Maximal Dynamic Strength (MDS). Loadings 0.45 are considered influential and highlighted in

bold.

Figure 4. Factor Loading Plot

Results of a two-factor Weighted Least Squares (WLS) Factor Analysis with Oblimin rotation. Observed variables are represented at left by rectangles, and arrows indicate the loading of variables on the respective latent factors delineated by circles at right. Factor loadings greater than 0.45 are considered influential. Negative loadings are indicated by the red dashed line. The weights of factor loadings are listed on the corresponding paths. The correlation of latent factors SSC and MDS is also listed.

3.3.2 Multi-Group Confirmatory Factor Analysis

The results of the MGCFA using the WLS estimator indicated acceptable model fits for the configural model with freely estimated factor loadings (CFI = 0.98 , TLI = 0.96 , SRMR = 0.04), weak invariance model (CFI = 0.99 , TLI = 0.98 , RMSEA = 0.05), and strong invariance model $(CFI = 0.99, TLI = 0.98, RMSEA = 0.07)$. Factor loadings were constrained to be equal in both rested and fatigued conditions in the weak invariance model. Both factor loadings and intercepts were constrained to be equal in the strong invariance model. The chi-squared difference tests revealed no significant differences between the configural model and the weak invariance model or the weak and strong invariance model (*p*>0.05, both). The results of the MGCFA confirm the evidence for metric invariance of the latent constructs under both fatigued and non-fatigued conditions in this sample.

3.3.3 Effect of Fatigue on Countermovement Jump Latent Factors

Descriptive statistics for each group and time point with respect to both SSC and MDS are reported in Table 6 and presented graphically in corresponding boxplots in Figure 5. Results of the two-way ANOVA conducted to examine the effects of position group (BIG vs MID vs SKILL) and time (BASELINE vs RECOVERED vs FATIGUED) on SSC revealed a significant main effect of group (F(2, 315)=91.20, p<0.001). Post-hoc pairwise comparisons of SSC using Tukey's HSD test revealed significant group differences between SKILL and BIG (meandiff=2.74, p<0.001), BIG and MID and BIG (mean $_{diff}$ =2.09, p<0.001), as well as SKILL and MID (meand $m = 0.65$, p=0.008). The main effect of time did not reach significance (p>0.05). Similarly, the two-way ANOVA employed to test the effects of position group and time on MDS also

indicated a significant main effect of group $(F(2, 315)=32.08, p<0.001)$, and no corresponding main effect of time (p>0.05). Post-hoc analyses of MDS indicated significant group differences in SKILL and BIG (mean_{diff}=1.60, p<0.001), MID and BIG (mean_{diff}=0.96, p<0.001), and SKILL and MID (meandiff= 0.63 , p= 0.006).

The two-way ANOVAs revealed a significant group x time interaction for SSC only (F(4, 315)=14.62, p<0.001). For SSC at baseline, the SKILL group (0.62 ± 0.74) exhibited significantly greater scores when compared to BIGs (-0.74±0.88, *p*=0.005), but did not significantly differ from MIDs (0.30±0.67, *p*=0.994). Baseline SSC scores also did not significantly differ between MIDs and BIGs $(p=0.107)$. Under recovered conditions following the bye week, both SKILLs (1.61) \pm 1.39), and MIDs (0.95 \pm 1.68) exhibited a significantly greater SSC scores when compared to BIGs (-1.71±2.27; *p*<0.001, both). Similarly, under fatigued conditions~24h post-game significantly greater SSC scores were again observed in SKILLs (1.50 ± 1.43) , and MIDs (0.52 ± 1.49) relative to BIGs $(-2.03\pm2.19; p<0.001, both)$. No significant between group differences were observed for the SKILL and MID groups relative any timepoint (*p*>0.050, all).

Table 6. Countermovement Jump (CMJ) Factor Scores

Means and standard deviations for factor scores () derived from latent factors extracted by the EFA model. Stretch Shortening Cycle (SSC) and Maximal Dynamic Strength (MDS) expressed relative to position group for preseason (BASELINE) and both timepoints after the 'bye' week (RECOVERED) and 24h post the

Factor 2. Maximal Dynamic Strength (MDS)

Figure 5. Longitudinal Countermovement Jump (CMJ) Factor Scores Boxplots depicting distribution of SSC and MDS factor scores from baseline, recovered conditions (bye week) followed by subsequent fatigued conditions (24h post-game) for BIG, MID, and SKILL position groups.

3.4 Discussion

The present study found that of the selected CMJ variables shown to be relevant to performance, fatigue, and injury status two latent factors comprised of five variables (JH, PPP, mRSI, APF, PPD) can be deduced. The first factor, SSC, was comprised of variables most commonly reported for the testing and diagnoses of NMF in athletes (JH, PPP, mRSI).^{18,42,139,192}

Additionally the SSC factor accounted for 48% of the total variance in the data. Interestingly the second factor, MDS accounted for an additional 36% of the total variance and was predominately loaded on by APF (λ =0.83) and PPD (λ =-0.87). This factor was labeled MDS as a recent review would classify these metrics as being telling of an athlete's ability to produce large quantities of force in short intervals of time.¹⁹¹ As such PPD, a surrogate measure of concentric duration, was highly negatively loaded on the MDS construct meaning that when APF was held constant a subsequent increase in the contractile time resulted in lower MDS_n . Conceptually this aligns with the notion that to enhance performance, athletes must generate large forces in short periods of time.^{193,194} More recent literature has proposed that temporal measures such as impulse, rate of force development, or peak forces at specific time domains may be more sensitive to NMF as athletes may adopt compensatory movement strategies to attain performance in SSC based outcomes.18,139,195

In this sample, configural invariance was confirmed indicating the underlying factor structure was shown to be similar across time points under both presumed recovered and fatigued conditions. Most interestingly, strong invariance was also confirmed indicating that not only were factor loadings consistent across time points but the mean level of the latent constructs was also maintained in the MGCFA model. When testing for the differences in factor score (η) means between position group and fatigue state (time) no significant main effects of time were reported. The results then would contradict the notion that athletes adopt compensatory movement strategies to attain performance in SSC based outcomes in acutely fatigued conditions (24h post-game).¹⁹⁵

As depicted in Table 6 the mean values for both SSC_n and MDS_n and results of the twoway ANOVAs indicate non-significant decreases in both latent factors of CMJ performance with respect to time. However, the significant group x time interaction $(F(4, 315)=14.62, p<0.001)$ for

SSC and the direction of change agrees with the hypothesis that under fatigued conditions both constructs may be liable to decrements in performance relative to differences in position group. The absence of any significant interaction effect for MDS may also support for the need for standardization of NMF assessment given that both univariate factors in MDS have been previously suggested as relevant CMJ fatigue indicators.^{18,139,196} As displayed graphically in the boxplots in Figure 5 and indicated by the standard deviations in Table 6, there are large variances in responses. It is plausible that such mediating factors as participation, acute physical activity exposure, chronic training adaptation, fitness, anthropometrics, and injury history may be contributing to the group-level responses seen in this study.70,197,198 To further understand the effect of NMF in this population, future investigations on the topic should aim to account for such variables as game and practice participation, workload derived from wearables, and physical fitness. Moreover, in the two-way design athletes were only tested for a singular fatiguing instance or game week while previous literature in contact sports shows that the cumulative effect of NMF is much more pronounced than acute or low-frequency incidences.^{16,46,195,199}

One of the difficulties in analyzing data from American football athletes are the large differences in athletic phenotypes and anthropometrics that often give way to skewed distributions and limited interpretations. Body mass in this population is shown to range from as low as 65kg to as high as 172kg.¹⁵³ Recent studies in this population have posited that although linemen or, BIGs, produce larger absolute forces and possess far greater lean mass than other position groups (SKILL and MID) the parallel increased fat mass and non-contractile tissue masses cause moments of inertia that are much greater to overcome in dynamic movements such as CMJ thus elongating movement duration and decreasing vertical displacement or JH.¹⁹ In the present study an attempt was made to account for these positional differences and anthropometric biases by selecting

temporal variables and normalizing kinetic variables to body mass, with the exception of JH. The results however align with those of previous studies into this population where features of the CMJ that are related to the viscoelastic components of the skeletomuscular system are much higher in SKILL positions.

These findings illustrate that when deploying a multidimensional approach to monitoring CMJ as a surrogate measure of NMF the mean differences in the theoretical constructs that underpin performance are not significantly diminished with time. Though this notion contradicts much of the available literature on CMJ in response to NMF it is important to note that monitoring univariate changes in CMJ kinetic variables may subsequently obfuscate the understanding of how NMF presents in athletes. Without gold standard criterion measures of fatigue measured alongside the CMJ in the training environment it is nearly impossible to select features that may be indicative of NMF. This is because Newtonian physics govern the equations used to parameterize kinetic features of the CMJ. The equations are algebraically transitive and thus sports medicine professionals and coaches must bear in mind that acute changes in performance due to fatigue may not be detectable with univariate analysis alone.

Thus, future studies should aim to longitudinally examine SSC and MDS capabilities in this population to further elucidate relationship between cumulative or chronic NMF to CMJ performance. In addition, practitioners must weigh other intrinsic mediators such as anthropometric differences, fatigue resistance, and body composition as well as environmental factors such as travel or scheduling demands, preceding physical activity volume and intensity, injury history and more. To evaluate wholly the effectiveness of monitoring CMJ to detect NMF, adjusted models using information from self-reported wellness questionnaires and/or objective indices of stress and fatigue may be of use.

3.5 Conclusion

In conclusion, the present study investigated key variables relevant to performance, fatigue, and injury status in athletes performing countermovement jumps (CMJ). Through a multidimensional approach, two latent factors, namely SSC (comprised of jump height, peak power production, and modified reactive strength index) and MDS (associated with metrics indicating an athlete's ability to generate large forces in short intervals), were identified. These factors accounted for a significant portion of the variance in CMJ performance. Configural and strong invariance across different time points and fatigue states were confirmed, suggesting consistency in factor structure and mean scores. Contrary to previous literature, no significant main effects of time on CMJ performance were observed, challenging the notion of compensatory movement strategies in fatigued conditions. However, while mean decreases in SSC and MDS were not found under fatigued conditions, group x time interactions suggest potential mediation by position. Future research should longitudinally examine CMJ capabilities to elucidate the relationship between fatigue and performance, considering position and other relevant factors. Additionally, adjusted models incorporating self-reported measures and objective indices of stress and fatigue may enhance the effectiveness of CMJ monitoring in detecting NMF. Overall, this study underscores the complexity of interpreting CMJ data and emphasizes the need for comprehensive approaches to understand its implications for athlete performance and well-being.

4.0 Manuscript 2: The Relationship Between Hormonal Perturbations and Neuromuscular Fatigue in NCAA DI Football Athletes

BACKGROUND: In athletes high training stress and inadequate recovery can contribute to performance stagnation or decline, or Neuromuscular Fatigue (NMF). This acute response is known as Non-Functional Overreaching (NFOR). Efforts to standardize the diagnosis of NFOR call for the inclusion of valid and reliable tests of NMF such as the Countermovement Jump (CMJ). As NMF alone is not sufficient for diagnosis, the inclusion of person-reported fatigue questionnaires may aid in the detection of NFOR. Salivary biomarkers of testosterone and cortisol and their ratio (TC ratio) are often touted for their sensitivity to detect NFOR. To date, there are no longitudinal studies investigating the relationship between measures of NMF to the TC ratio in NCAA DI football athletes. **PURPOSE:** The aim of this study is to examine the relationship between the TC ratio and NMF during an NCAA DI football season. **METHODS:** Data were attained from a sample of 47 NCAA DI athletes \sim 72 hours after the final preseason practice (T1) and post-game at weeks 1 (T2), 6 (T3), and 11 (T4). Salivary biomarkers were attained using ELISA kits (Salimetrics, LLC, State College, PA). The Hooper Index was calculated from a fouritem fatigue questionnaire. CMJ factor scores were extrapolated from two latent factors: Stretch Shortening Cycle (SSC) and Maximal Dynamic Strength (MDS). Longitudinal changes were assessed using Kruskal-Wallis tests with $(\alpha<0.050)$. Bonferroni corrected Conover-Iman tests were used to make post-hoc pairwise comparisons. Linear mixed models (LMM) were constructed to model the longitudinal relationships with TC ratio. Time was maintained as a fixed effect in each candidate model. Covariates added to each LMM included SSC, MDS, and the Hooper Index.

Interaction effects were used to reduce Type-II error. Random slopes and intercepts were entered into each model. Likelihood ratio tests (LRT) were used to compare covariate adjusted models to an unconditional model. Model selection was performed using corrected Akaike Information Criteria (AICc) and marginal and conditional R². RESULTS: Longitudinal changes were revealed for Hooper Index scores (χ 2(3) = 9.0448, p = 0.029), soreness (χ 2(3)=8.8037, p =0.032), testosterone (χ 2(3)=18.7686, p<0.001) and cortisol (χ 2(3)=7.9538, p=0.047). Post-hoc analyses indicated increased fatigue at T2 and T3 as demonstrated by increased Hooper Index $(T3vST1,p=0.016)$ and reduced T $(T2vST1,p=0.033; T3vST1,p=0.001)$. Self-reported recovery at T4 was noted by decreases in the Hooper Index (T4vsT3,p=0.040) and T (T4vsT2,p=0.004; T4vsT3,p=0.001). Soreness decreased (T4vsT3, p=0.015). Elevations in salivary C were observed at T4 (T4vsT2,p=0.023). Covariate adjusted LMMs improved model fit compared to the unconditional model ($p>0.050$, all). The AICc values decreased after covariates were added to the models though no covariates or interactions influenced the TC ratio (p>0.10, all). **CONCLUSION:** This study supports the need for more robust detection of NFOR in team sport athletes. Though C and T were significantly altered at various timepoints throughout the season, no significant changes were detected in the TC ratios or CMJ factor scores. Despite significant group level effects of time, detriments in the Hooper Index were not related to the TC ratio. This further obfuscates detection NFOR in athletes. Though adding covariates to the LMMs improved model fit, the data should be cautiously interpreted as no fixed effects were significant. Due to the high complexity of LMMs larger samples may be needed to test the relationship between NMF and TC ratios over time.

4.1 Introduction

Functional Overreaching (FOR) is the process in which exposure to prescribed training volumes and intensities result in transient maladaptation marked by acute decrements in sport and motor performance, or Neuromuscular Fatigue (NMF). When instances of FOR are followed by a period of recovery, desired performance adaptations may be attained.^{12–14} However, in cases where the training stimulus exceeds the functional adaptation reserve or fitness level of the individual, performance decrements may persist for days or even several weeks. This is a maladaptive process known as Non-Functional Overreaching (NFOR).^{13,14}

The process of accurate differential diagnosis of FOR or NFOR may be difficult for sport coaches and athletes. The American College of Sports Medicine and European College of Sport Science joint consensus statement on diagnosis of NFOR should be based on disruption to selfreported perceptions of well-being, and elevated stress hormone levels.¹⁴ Despite numerous studies on these unidimensional assessments of fatigue and readiness across different team sport populations, to date there has been a dearth of data in NCAA DI football athletes.^{5,8,15,20,22,39}

The NCAA DI football season is an arduous 13-week period of intermittent competitions and practices marked by violent collisions registering gravitational forces that rival those seen in motor vehicle accidents and sprint speeds and volumes that are seldom seen in other team sports.^{1–} ^{4,162} The season is thought to elicit prolonged NMF, psychological disturbances, and maladaptive hormonal responses.^{5–8,20} Chronic exposure to the sporting demands described above coupled with the cascading maladaptive responses may manifest in performance decline or stagnation and ultimately harm team performance. Like other NCAA athletes, football athletes also experience stressors that are external to sport such as those imposed by periods of high academic requirements.³⁹ External psychological stressors can enhance perceptions of fatigue in athletes and manifest as disturbances to the hormonal milieu relevant to performance and recovery.^{200–202} Therefore periodized rest and recovery are paramount for not only maintenance of performance but also management of fatigue symptoms. With only one designated week for rest and recovery known as the "bye week" an enhanced understanding on the longitudinal accruement of fatigue during the season may benefit coaches and athletes alike in preparing for the demands of the sport.

The Countermovement Jump (CMJ) is widely accepted as the standard for longitudinally assessing NMF in team sport athletes across competitive seasons.^{5,16,17} This is due to its task relevance to sport, reliability, validity, and expediency in the field.^{18,104,120,133,166} Previous studies have shown metrics derived from CMJ tests to be sensitive to a multitude of criterion assessments for fatigue, both objective and perceived.^{139,199} Among the most common variables assessed for their relevance to fatigue are Jump Height (JH), Peak Propulsive Power (PPP), and the modified Reactive Strength Index (mRSI).^{18,42,139,203} However, there is also data to support Average Propulsive Force (APF) and Propulsive Phase Duration (PPD) as being sensitive to NMF.139,196,199,204 Dimensionality reduction techniques such as principal components analysis and exploratory factor analysis have been used in sports science literature to ease the communication of such data where CMJ variables are highly collinear.^{205–207} These methods have also proven fruitful for simplifying prediction models for indices of fatigue derived from the CMJ.²⁰⁸

Self-reported questionnaires also play a crucial role in monitoring for NFOR in team sport athletes. These questionnaires allow athletes to provide their perceived feedback on various aspects of their physical and mental well-being, such as fatigue levels, mood disturbances, sleep quality, and perceived stress.^{14,209–211} By regularly completing these questionnaires, athletes can track changes in their internal state over time, providing valuable insights into their readiness to train and compete. Moreover, self-reported questionnaires serve as a practical and cost-effective method for assessing NFOR, as they can be easily administered and interpreted by athletes and coaches alike.42,67 While the utility of objective measures for fatigue monitoring cannot be understated, in many cases self-reported questionnaires often outperform objective measures such as the CMJ as they can offer a more holistic understanding of the athlete's condition and perceptions of fatitgue.16,209 These questionnaires are also cost effective and reduce the burden of testing on the athlete. The Hooper Index is a commonly used questionnaire in clinical practice and sports science research as it has demonstrated construct validity against laboratory grade measures of fatigue and practitioners can benefit from its ease of use.^{210,212–215}

The NFOR construct is not exclusively defined by NMF or elevated perceptions of fatigue as it is also commonly associated with hormonal dysregulation, or perturbations.^{14,15,102,216} In instances of high sport related stress and fatigue, the hypothalamic–pituitary–adrenal (HPA) axis engages in a feedback loop and results in the secretion of both the catecholamine cortisol and the corticosteroid testosterone.51,52 Instances of sport-related NMF following competition have been reported alongside disruptions to perceived well-being and elevated markers of stress as measured by increases salivary cortisol and decreases in testosterone.16,122,217 The ratio of testosterone to cortisol (TC ratio) is often used to report the adaptive state of the athlete in training and monitor for overtraining.⁵ Cortisol in particular has catabolic tendencies and is related to a number of health complications that present with NFOR, while testosterone seems to work in the opposite direction to maintain anabolism.52,53 Recent literature in NCAA DI football has suggested that as the season progresses, these athletes can become increasingly resilient to the stressors of competition and demonstrate more stable TC ratios.8,22 However, these studies have not aimed to account for concomitant decrements in performance or perceived feelings of fatigue. As such, both are crucial for drawing inferences on NFOR. In light of conflicting previous reports it may be posited that the

HPA axis responses to the stressors of competition in NCAAA DI football are highly individual.^{21,22}

The construct of NFOR and its complex relationship with measures of NMF may have been lost on simpler models reported in previous studies. A more complex multilevel approach may be needed to accurately represent the cumulative effects of performance decline on overreaching status as indicated by the TC ratio. Therefore, the purpose of this study is to examine the relationship between the TC ratio and NMF during an NCAA DI football season utilizing a mixed modeling approach accounting for within-individual variance of with respect to time.

4.2 Methods

4.2.1 Participants

A sample of 47 NCAA DI football athletes (age= 21.3 ± 1.5 y, height= 1.9 ± 0.1 m, mass=108.0±22.2) provided written informed consent to participate. Football athletes with known pre-existing injury at the time of consent which would prevent an athlete from physical activity (lower extremity time-loss injury or mild-traumatic brain injury) as delineated on the team injury reports provided by licensed athletic trainers were excluded from participation. Inclusion criteria included listed current travel roster or game eligible roster at the time of recruitment (i.e. nonredshirt athletes). The study was approved by the University of Pittsburgh Institutional Review Board (STUDY23070101).

4.2.2 Countermovement Jump

Data for all measures were attained concomitantly across four timepoints, ~72 hours after the final preseason practice (T1), \sim 24h post-game 1 (T2), post-game 5 (T3), and \sim 72h post-game 11 (T4). These timepoints were selected as relative to the schedule where T3 occurred following the bye week and T4 followed a short game week with enhanced recovery time prior to testing. Subjects performed three bilateral CMJs on dual force platforms sampling at 1000 Hz (Hawkin Dynamics, Maine, USA). All tests were performed with hands on hips as to reduce the influence of added momentum from the upper limbs on center mass kinetics and temporal measures.²¹⁸ Prior to each test subjects were required to complete $a \ge 1$ s quiet standing phase to accurately detect movement initiation via center of mass displacement.¹⁶⁷ The CMJ tests were administered by the athletic performance coaches as part of routine standard practice and training procedures and thus no familiarization attempts were necessary. Verbal encouragement was provided, and performance coaches supervised CMJ testing to assure subjects performed the CMJ as described. A validated commercially available software was used to process the raw force-time data.¹⁶⁶ CMJ tests where within-subject maximum Jump Height (JH) at each time point was recorded were kept for analysis. In addition to JH, Peak Propulsive Power (PPP), modified Reactive Strength Index (mRSI), Average Propulsive Force (APF), and Propulsive Phase Duration (PPD) were recorded. Variable selection was informed by previous literature on NMF in team sport populations.^{16,46,139,219} Factor scores were then extracted from the dataset using a previously reported weighted least-squares model with oblique rotations. The first factor score, deemed Stretch Shortening Cycle (SSC) was comprised of JH, PPP, and mRSI whilst the second factor score, Maximal Dynamic Strength (MDS) was extrapolated from APF and PPD.

4.2.3 Self-Reported Readiness Questionnaire

Responses to a self-reported well-being questionnaire were administered to each subject via a tablet. The Hooper questionnaire was developed to be used with team sports athletes to monitor for overtraining induced fatigue and demonstrates construct validity and reliability in team sport populations.^{67,212–214} The questionnaire is comprised of four items: perceived stress, soreness, fatigue, and sleep quality. Each item was measured on a 7-point scale where a value of 1 is very, very low and a value of 7 is very, very high. The Hooper Index was calculated by summing the four items to create a composite score of athlete readiness.

4.2.4 Salivary Biomarkers

Upon arrival to the facility and prior to meals, participants provided salivary samples via the passive drool method. The passive drool method entails allowing for saliva to pool at the bottom of the mouth for 30s prior to passing the sample through a straw into a collection device. Salivary samples were collected at the same approximate time of day between 12:00 and 2:00 PM prior to team workouts and CMJ to control for diurnal responses and activity. Salivary samples were then stored at -80C before being analyzed for T and C using commercially available ELISA kits (Salimetrics, LLC, State College, PA). This method allows for the measurement of the hormones in their bioavailable or free state, providing a more relevant picture of fatigue state and adaptability than binded hormones levels such as those deduced from serum. The detection limit for the testosterone assays ranged 6.1-600 pg/mL, with inter-assays coefficient of variation (CV) of <10%. The cortisol assay had a detection limit of 0.12 μg/dL with inter-assays CV of <7%.

4.2.5 Statistical Analysis

In attempts to reduce model complexity and adjust for correlated features, scores from two latent factors Shortening Cycle (SSC) and Maximal Dynamic Strength (MDS) were extracted from the dataset using a weighted Least-squares factor analysis with oblique rotations with the "fa" function of the "psych" package in R version $4.3.0$ ¹⁷² Factor scores were produced from five CMJ variables commonly used to assess NMF including Jump Height (JH), modified Reactive Strength Index (mRSI), Peak Relative Propulsive Power (PPP), Average Propulsive Force (APF) and Propulsive Phase Duration (PPD).

Linear mixed models (LMMs) were then constructed to test the associations of NMF to the TC ratio. Prior to model construction an exploratory data analysis was undertaken to inform covariate selection. First, a correlation matrix was used to investigate univariate associations for items of the Hooper Index and CMJ factor scores with the TC ratio at baseline. Descriptive statistics and Kruskal-Wallis tests were then used to deduce longitudinal mean rank changes in assessments of NMF in this sample $(\alpha<0.050)$.

Finally, candidate models were constructed using covariates for NMF assessments shown to be correlated with TC ratio at baseline and sensitive to change with respect to time. Based on these criteria, the Hooper Index, SSC, and MDS were included as fixed effects, or covariates, in separate candidate models. Covariates were grand mean centered to capture the relationship between each covariate and initial TC. The relationship between the covariates and the TC ratio over time was modeled with a time*covariate interaction term. To reduce the likelihood of committing a Type II error statistical significance was inferred at $p<0.100$ for fixed effects. Likelihood ratio tests (LRTs) were used to determine whether the addition of covariates as fixed effects improved model fit when compared to the unconditional model. Candidate models were

then compared using both the marginal and conditional \mathbb{R}^2 for relative goodness of fit as well as the corrected Akaike information criteria (AICc) for parsimony.

4.3 Results

The correlation matrix revealed two significant associations of the univariate predictors with the TC ratio at baseline, these included Sleep Quality ($r=-0.14$, $p=0.079$) and Stress ($r=-0.13$, p=0.096). Despite reaching significance at the exploratory alpha level, these correlations were very weak and warranted caution. However, being that the individual items of the Hooper Index were all substantially associated (all r values > 0.650) with the overall score Hooper Index, this predictor was included as a fixed effect in a covariate adjusted growth model. Both CMJ factor scores, SSC and MDS, were included as fixed effects in separate covariate adjusted growth models. These findings are visualized below in Figure 6.

		TC Ratio	MDS			Honor Katigue _{Soreness} Street		Sleep Quality	
TC Ratio	1			-0.25 -0.05 -0.13 -0.1			$-0.01 -0.13 -0.14$		$\mathbf 1$ 0.8
SSC	-0.25	$\mathbf{1}$	0.7	-0.11	0.01		$-0.14 - 0.28$	0.1	0.6
MDS	-0.05	0.7	\blacktriangleleft	-0.11	0.03	$-0.17 - 0.23$		0.06	0.4
Hooper Index -0.13 -0.11 -0.11				$\mathbf{1}$	0.77	0.66	0.84	0.69	0.2
Fatigue	-0.1	0.01	0.03	0.77	$\mathbf{1}$	0.46	0.48	0.3	0 -0.2
Soreness			$-0.01 -0.14 -0.17$	0.66	0.46	$\mathbf{1}$	0.38	0.18	-0.4
Stress			$-0.13 - 0.28 - 0.23$	0.84	0.48	0.38	$\overline{\mathbf{1}}$	0.6	-0.6
Sleep Quality	-0.14	0.1	0.06	0.69	0.3	0.18	0.6	$\mathbf{1}$	-0.8 -1

Figure 6. Correlation Matrix of Objective and Self-Reported Fatigue Measures

Pearson's correlation matrix depicting univariate associations between hormonal stress balance as indicated by the testosterone to cortisol ratio (TC ratio) and both objective measures of neuromuscular fatigue (Stretch Shortening Cycle (SSC) and Maximal Dynamic Strength (MDS)) and self-reported or self-reported measures

(the Hooper Index its scale items; Fatigue, Soreness, Stress, and Sleep quality).

Results from the Kruskal-Wallis Test are summarized in Table 7. Kruskal-Wallis tests revealed statistically significant differences across time in mean rank Hooper Index scores ($χ2(3)$) = 9.0448, p = 0.029), Soreness (χ 2(3)=8.8037, p = 0.032), and both salivary biomarkers Testosterone (χ 2(3)=18.7686, p<0.001) and cortisol (χ 2(3)=7.9538, p=0.047). The Conover-Iman tests with Bonferroni corrections indicated significant increases in mean rank Hooper Index scores at T3 when compared to T1 (p=0.016) followed by decreases at T4 when compared to T3 (p=0.040). Self-reported ratings of soreness were also significantly improved at T4 when compared to T3 ($p=0.015$). Testosterone was significantly reduced at T2 ($p=0.033$) and T3 (p=0.001) when compared to T1. Testosterone was significantly elevated at T4 when compared to both T2 (p=0.004) and T3 (p=0.001). Cortisol was significantly elevated at T4 compared to T2 $(p=0.023)$.

Table 7. Descriptive Statistics of Objective and Self-Reported Measures of Fatigue Descriptive statistics (Median, IQR) for CMJ variables (SSC, MDS, JH, PPP, mRSI, APF, PPD), Hooper Questionnaire items, and salivary biomarkers (T, C, TC) respective to Baseline (T1), 24h post-game 1 (T2),

24h post-game 5 (T3), and 72h post-game 11 (T4).

	T1	T2	T3	T4	\boldsymbol{p}
SSC	0.43 (-0.71 , 1.66)	$0.38(-1.17, 2.01)$	0.62 (-1.49 , 1.33)	$0.22(-1.87, 1.32)$	0.818
MDS	0.09 (-0.92 , 1.23)	$0.12(-1.09, 1.62)$	$-0.06(-0.91, 0.98)$	-0.42 $(-1.36, 0.68)$	0.427
JH(m)	0.45(0.41, 0.48)	0.44(0.39, 0.47)	0.44(0.38, 0.47)	0.44(0.38, 0.47)	0.742
PPP (W/kg)	62(57, 66)	62(56, 67)	62(55, 65)	60(54, 66)	0.816
mRSI	0.64(0.56, 0.68)	0.62(0.54, 0.69)	0.60(0.53, 0.69)	0.57(0.51, 0.67)	0.423
APF(N/kg)	224 (211, 235)	225 (210, 240)	222 (213, 234)	221 (209, 229)	0.572
PPD(s)	0.24(0.22, 0.26)	0.24(0.22, 0.26)	0.24(0.23, 0.26)	0.25(0.23, 0.27)	0.531
Hooper Index	$12.0 (10.0, 15.0)^3$	13.0(11.0, 15.0)	$15.0 (12.0, 17.0)^{1,4}$	$13.0 (10.0, 15.0)^3$	0.029
Fatigue $(1-7)$	3.00(2.00, 4.00)	3.00(3.00, 4.00)	4.00(3.00, 4.00)	3.00(2.00, 4.00)	0.185
Soreness $(1-7)$	4.00(3.00, 4.00)	4.00(3.00, 4.00)	4.00(4.00, 5.00)	$3.00 (2.00, 4.00)^3$	0.032
Stress $(1-7)$	3.00(2.00, 4.00)	3.00(2.00, 4.00)	4.00(2.00, 4.00)	3.00(2.00, 4.00)	0.401
Sleep Quality (1-7)	3.00(2.00, 3.50)	3.00(2.00, 4.00)	3.00(3.00, 4.00)	3.00(3.00, 4.00)	0.089
TC ratio	932 (735, 1,224)	980 (728, 1,232)	716 (606, 1,003)	927 (673, 1,161)	0.164
Testosterone (pg/mL)	$210 (163, 300)^{2,3}$	$175(141, 226)^{1,4}$	161 $(128, 227)^{1,4}$	$239(193, 278)^{2,3}$	< 0.001
Cortisol (µg/dL)	0.24(0.19, 0.33)	$0.19(0.13, 0.26)^4$	0.23(0.17, 0.31)	$0.27(0.18, 0.35)^2$	0.047

Figure 7. Longitudinal Observations of Objective and Subjective Measures of Fatigue Mean and standard deviation values for univariate assessments of fatigue and performance across four timepoints (72h post camp = T1, 24h post game 1 = T2, 24h post game 5 = T3, 72h post game 11 = T4). Significant differences in mean rank between time points as indicated by post-hoc Conover-Iman tests with Bonferroni corrections are indicated by brackets. Observations in red are labeled as low per previously reported normative values (Testosterone<100 pg/mL).²²⁰*p<0.050, **p<0.010,*p<0.001**

Results of the LRTs indicated that model fit was significantly improved for LMMs with the covariates: SSC (χ 2(2)=16.440, p>0.001), MDS (χ 2(2)=16.571, p>0.001), and Hooper Index $(\chi^2(2)=13.975, p=0.001)$. The proportion of variance in TC ratio explained by the fixed effects in all models was very, very, weak (marginal R^2 < 0.10, all). Conditional R^2 , or the explained variance in TC ratio explained both random and fixed effects in the covariate adjusted ranged from 31.2%

to 35.4% indicated a weak fit for all models. The AICc values decreased in the covariate adjusted models compared to the unconditional model. The MDS model yielded the lowest AICc (AICc=1353.5) and thus the highest relative likelihood suggesting the most parsimonious fit to TC ratio of the three candidate models. However, none of the fixed effects included in the more complex, covariate adjusted models reached significance. These findings are summarized below in Table 8.

Table 8. Salivary Testosterone to Cortisol (TC) Ratio Linear Mixed Models

Linear mixed models (LMMs) fitted for the outcome variable testosterone to cortisol (TC) ratio with random slopes and intercepts per subject. Time and Time x covariate interactions were entered as fixed effects. Separate covariate-adjusted models were constructed across all assessment domains to include SSC, MDS, and the Hooper Index. Model fit was compared against using marginal R² , conditional R² , and the corrected

	Unconditional Model		Stretch Shortening Cycle (SSC)			Maximal Dynamic Strength (MDS)			Hooper Index			
Predictors	Estimates	90%CI	p-value	Estimates	90%CI		p-value Estimates	90%CI	p-value	Estimates	90%CI	p-value
Time	-18.66	$-60.28 - 22.97$	0.458	-17.15	$-59.75 - 25.46$	0.505	-15.76	$-57.81 - 26.30$	0.535	-21.63	$-64.63 - 21.37$	0.405
SSC				-11.69	$-58.89 - 35.50 \quad 0.681$							
Time x SSC				-7.76	$-26.85 - 11.34$ 0.501							
MDS							27.73	$-33.24 - 88.69$	0.452			
Time x MDS							-15.09	$-39.43 - 9.25$	0.306			
Hooper Index										3.03	$-21.18 - 27.24$	0.836
Time x Hooper Index										-7.4	$-19.11 - 4.31$	0.296
Conditional R^2	0.339			0.312			0.353			0.354		
Marginal R^2	0.004			0.033			0.011			0.02		
AICc	1365.601			1353.666			1353.534			1356.13		

Akaike Information Criteria (AICc) and relative likelihood.

5.0 Discussion

The results of this study demonstrate that despite longitudinal changes in self-reported fatigue ($χ2(3) = 9.0448$, $p = 0.029$), Soreness ($χ2(3)=8.8037$, $p =0.032$), and both salivary biomarkers Testosterone (χ 2(3)=18.7686, p<0.001) and Cortisol (χ 2(3)=7.9538, p=0.047) at the team level, the longitudinal relationship with the TC ratio cannot be adequately explained by measures of NMF. This is evidenced by a lack of significance for each of the centered covariates SSC (B=-11.69, 95%CI [-68.11, 44.72], p=0.681), MDS (B=27.73, 95%CI [-45.15, 100.60], $p=0.452$), and the Hooper Index (B=3.03, 95%CI [-25.91, 31.9], $p=0.836$) as well as interactions for SSC x Time (B=-7.76, 95%CI [-30.58, 15.07], p=0.501), MDS x Time (B=-15.09, 95%CI [- 44.18, 14.00], p=0.306), and Hooper Index x Time (B=-7.40, 95%CI [-21.40, 6.60], p=0.296). Thus, the null hypotheses were accepted for each model, indicating that measures of objective and self-reported NMF were not associated with changes to the TC ratio in this sample.

The use of the TC ratio to monitor NFOR in American football athletes has been previously supported by its effectiveness in assessing the balance between anabolic and catabolic processes in response to stress and physical activity. For team sport athletes this provides insight into their functional capacities.²¹ As NCAA DI football involves high levels of physical trauma and psychological stress, monitoring hormonal responses may be crucial to understanding athletes' adaptation to training loads and identifying NFOR. Previous studies have noted that contextual factors, namely playing position, significantly influence hormonal concentration in this population during the competitive season.^{7,8,20} Most notably in this sample however, the TC ratio remained unchanged at the team level when tested for longitudinal differences despite significant
fluctuations of both testosterone and cortisol between timepoints. This may be due to a group training effect or pooled statistics.⁸ Salivary biomarkers are useful in providing information about the functional state of the athlete where free hormones are biologically active and available. Serum testosterone, though perhaps more reliable, does not adequately identify androgen deficiencies such as those often seen in fatigue states.

The strength in mixed modeling is the ability to account for individual variance within group designs. However, a limitation in utilizing LMMs is that the addition of contextual factors complicates interpretation of fixed effects and increases model complexity. With greater model complexity large samples are needed to allow for model convergence and there is an increased chance that models do not converge or are overfit to the data. Future studies may aim to replicate these findings in larger samples with more serial measures of NMF adjusted for contextual factors such as playing position, snap counts or participation.

An exploratory approach was adopted for model selection in the analysis of LMMS. Given the complexity of the data, relatively small samples, and the need to balance model fit and parsimony, an iterative process was undertaken to identify the most suitable model specification. A range of candidate models were considered encompassing different constructs used to diagnose NFOR. Model evaluation was guided by multiple criteria, including the corrected AICc, relative likelihoods, conditional and marginal R2. Specifically, AICc was used to assess model fit while accounting for model complexity and sample size. Relative likelihoods were computed based on differences in AICc values to compare the models' likelihoods relative to the best-fitting model. Conditional R2 was employed to gauge the proportion of variance explained by both fixed and random effects, providing insight into the overall predictive power of the model. Marginal R2 was utilized to quantify the proportion of variance explained by the fixed effects alone, offering insight into the contribution of individual predictors. Additionally, diagnostic checks, such as examination of residuals were conducted to assess the adequacy of the chosen model. This approach allowed for flexibility in exploring various modeling strategies and facilitated the selection of the most appropriate and interpretable model for further analysis underpinned by the theory of NFOR in team sport.

In the present study it should be noted that when adjusting for time and random slopes and intercepts univariate assessments of NMF may not adequately describe the functional state of the athlete when measured against more objective criterion such as the TC ratio. The exploratory approach and a-priori hypothesis in this study called for SSC, MDS, and the Hooper Index to be entered into candidate models despite not reaching significance where a more stringent approach may have discarded these covariates and elected for simpler models. Moreover, despite the MDS adjusted model achieving the most parsimonious AICc and relative likelihood, the marginal and conditional R2 values in the simplest model with only covariates for playing position and snap count performed nearly as well on the data. Future studies should investigate the notion that with larger samples and the standard of care assessments of fatigue may be outperformed by more intuitive categorical covariates such as position or simple continuous counts like snaps.

Ultimately the limitation in the complete cases analysis was that a large proportion of the sample were not included due to missingness from lower extremity time loss injury $(n=11)$, or mild-traumatic brain injury (n=4). The frequency and timing of data collection was constrained by the team competition and training schedule. Best efforts were made to standardize testing procedures and make for repeatable testing methods. What could not be accounted for however was the omission of a timepoint ~24h following week 12, or the final game of the season. Due to the team's performance and elimination from playoff contention no organized activities were held that day, thus the investigators opted to collect data at week 11 instead. This may have been problematic for the integrity of the data given that the preceding game was played on an irregular schedule (Thursday evening). Most notably however, each data collection was held following a team win where snaps, or participation, was more evenly distributed amongst the team roster. Disruptions in mood and subsequent stress responses may have been hindered in these cases as previously shown in a similar study of CMJ performance and TC ratio recovery time courses following a positive team performance in elite Rugby.¹⁶

It may be said that much like any of the other assessments or screens for NMF and functional state of the athlete, the use of univariate measures SSC, MDS, and the Hooper Index alone may not be sensitive enough to detect the presence of NFOR evidenced by changes in the TC ratio. Additionally, the TC ratio itself may not be an adequate diagnostic for NFOR where findings of previous studies indicating longitudinal univariate changes in testosterone and cortisol may not be reflected in the ratio of the two. These were confirmed in this sample during the exploratory data analysis prior to constructing the LMMs. With larger samples and more frequent longitudinal measurements more insight into NFOR may be gleaned from the relationship between measures of NMF and salivary biomarkers testosterone and cortisol adjusted for contextual factors. At current there are no gold-standard or criterion measurements for NFOR and for instances of NMF to be tested against. Ultimately the intuition and trained knowledge of coaches and athletes must be taken into consideration as fatigue is a largely subjective experience. Future studies should aim to explore further the factors in training and competition that may enhance fatigue resistance and increase robustness. The contextual influence of sporting environment and within-subject variances must be taken into consideration.

6.0 Conclusion

In this sample, measures of objective and self-reported NMF were not associated with changes to the TC ratio. The findings of this study highlight the need for more comprehensive multifactorial assessment protocols for detecting NFOR in team sport athletes. A multifactorial plan should not only include valid and reliable objective and self-reported measures but should also adjust for contextual factors such as playing position and status. This point is illustrated by the evidence that in this sample of NCAA DI football athletes, detriments in perceived well-being and fatigue were not related to the TC ratio when adjusted for within-subject variances. This may further obfuscate the process of detecting NFOR. Future investigations should aim to further validate clinical measures of NFOR and deduce its longitudinal relationship of NMF, enhancing the practitioner's ability to detect meaningful changes in athletic populations.

7.0 Manuscript 3: Non-Functional Overreaching and External Workloads are Not Associated with Traumatic Lower Extremity Injury Risk in NCAA DI Football Athletes

BACKGROUND: In NCAA DI football lower extremity injuries (LEIs) or those injuries occurring to the musculoskeletal system distal to the spine, account for the large majority.^{23,221,222} These injuries occur either due to an excessive chronic load placed on the tissue in overuse or a singular instance of collision with either a surface or another athlete, known as traumatic injury.23,222 When sport related physical activity, or workload, is too great relative to previous training or applied chronically over long periods with inadequate recovery, neuromuscular fatigue (NMF) results. Sensorimotor function perturbations brought on by NMF may also increase the risk of injury in athletes.^{68,223} Prolonged NMF $(>7d)$ is referred to Non-Functional Overreaching (NFOR), a consequence of inadequate recovery relative to training stress known to increase risk of injury.¹⁴ The ability to adapt to a high volume of workload and avoid NFOR is highly individual and influenced by various factors. Though previous studies have investigated its relevance for overuse injury, no such model has been constructed for traumatic LEI. Nor has an attempt been made to do so in NCAA DI football whilst adjusting for multiple covariate variables. **PURPOSE:** The primary aim of this study is to determine whether instances of NFOR induced NMF are associated with an increased likelihood of sustaining traumatic LEIs in NCAA DI football athletes. **METHODS:** Data were compiled from a sample of 129 NCAA DI football athletes across four seasons (age= 20.4 ± 1.5 y, height=1.9 \pm 0.1m, mass=108.7 \pm 21.5). Biweekly countermovement jump (CMJ) tests were performed on force plates sampling at 1000Hz (Hawkin Dynamics, Maine, USA)

to assess for NFOR. Commonly reported indices of NMF, including jump height (JH), peak

propulsive power (PPP), modified reactive strength index-(mRSI), average propulsive force (APF), and propulsive phase duration (PPD) were entered into a confirmatory factor analysis to derive factor scores. The duration of reductions of >10% in factor scores for stretch shortening cycle (SSC) and maximal dynamic strength (MDS) was used to categorize instances of NMF as either functional overreaching (FOR) or NFOR. Athlete positions were grouped as previously reported in other NCAA DI football papers (BIGS=linemen, MIDS=linebackers, tight ends, running backs, and quarterbacks, SKILL=wide receivers and defensive backs). Frequency of injury were reported by location. Baseline descriptive statistics and within-position mean differences for CMJ factor scores between injured and uninjured athletes were calculated using Cohen's *d*. Generalized linear mixed models (GLMMs) were fit to the dataset with a binomial outcome for the occurrence of a traumatic LEI. Models were compared using the corrected Akaike information criteria (AICc), as well as marginal and conditional \mathbb{R}^2 . All processing and analysis were completed in R (Version 4.3.0.) with statistical significance set at α <0.10, reducing the likelihood of Type-II error. **RESULTS:** Traumatic LEIs were most frequently reported in the ankle joint (n=13, 32.5%), followed by the knee (n=9, 22.5%) and thigh regions (n=6, 15%). Baseline SSC and MDS scores were higher in injured BIGs when compared to uninjured BIGS (*d*=0.83; *d*=0.33, respectively). Inversely for both SKILLs and MIDs lower baseline SSC were noted for injured athletes (*d*=-0.67; *d*=-2.07, respectively) though MDS was greater in injured SKILLs (*d*=0.55) and lower in uninjured MIDs (*d*=-1.46). No traumatic LEIs were reported in athletes who did not experience NFOR during the season indicated by either a reduction in SSC (n=26) or MDS (n=32). The full model explained 56.3% of the variance in traumatic LEI odds, with added covariates of explaining 29.1% of the variance alone. The odds of experiencing traumatic LEIs were reduced across Weeks (OR=0.67 [0.46-0.96], *p*=0.071). Reduced odds of traumatic LEI were reported in the SKILL group (OR=0.24 [0.06-0.98], *p*=0.096). Detection of NFOR as indicated by greater that a 10% reduction from baseline SSC increased odds of traumatic LEI in-season (OR=6.49 [1.22-34.38], *p*=0.065). **CONCLUSION:** The present study explored the relationship between NFOR induced NMF and traumatic LEI in NCAA DI football athletes. The data presents distinct differences between injured and uninjured athletes across position groups with respect to specific facets of CMJ performance. Mixed modelling revealed protective effects of time and decreased odds of traumatic LEI in SKILL position players, while detection of NFOR via CMJ factor scores emerged as a significant predictor of increased odds of sustaining traumatic LEI during the season. These findings emphasize the importance of monitoring for NFOR induced changes to neuromuscular performance in this population to assess risk of injury.

7.1 Introduction

In recent years, concerns have been raised regarding the safety of American football participation due to the high frequency of forceful collisions, often leading to traumatic injuries and lasting ailments. $1-4,23,31$ Football participation poses a greater injury risk compared to other NCAA sports, with over 50% of injuries being lower extremity injuries (LEIs).^{23,24} These instances are not only of concern to the athlete's immediate health or the team and how overall performance may be impacted but each injury can impose a significant burden on athletes by affecting their subsequent earning potential, quality of life, future development of arthritis and overall well-being. With the increasing knowledge of the short- and long-term impact of traumatic injuries, youth participation in football in the United States has decreased substantially, yet it remains the most

popular sport.²²⁴ An estimated 5 million youth enroll to play football annually making the incidence of LEI a public health concern.

Enhancing the robustness to injury and fatigue resistance has always been a hallmark of preparation in NCAA DI football and has only become more prominent with time. ^{9,10} A key component of physical preparation, practice, and training for football is the capacity for adaptation of the athlete so that they can experience transient decrements in neuromuscular performance (NMF) only to realize higher levels of enhanced performance through a process known as Functional Overreaching (FOR) .^{12–14} Unfortunately, in preparation and training it is not uncommon to see these athletes subjected to intensities exceeding those of actual competition, leading to non-functional overreaching (NFOR), overtraining, and, in rare cases, fatalities. ^{9,34} Thus it is crucial for football coaches and support staff to develop strategies to identify and prevent overtraining to mitigate injury risks.

The American College of Sports Medicine (ACSM) and European College of Sports Science (ECSS) produced a joint statement on the diagnoses of both FOR and NFOR as time dependent, where NMF lasting <7d in general may be due to FOR while the longer time course is more often indicative of NFOR.¹⁴ The difficulty in assessing the validity of FOR and NFOR as risk enhancing instances of training maladaptation is that to properly define the construct the instrument with which it is measured must also be valid and reliable. The Countermovement Jump (CMJ) is often touted as the best way to reliably assess NMF with field expedience as it is now commonplace in most elite team sport environments.^{17,42,67} However, feature selection is cumbersome as the data itself is wrought with multicollinearity and correlations between variables obtained from instrumented measures of the CJM with force plates. Use of factor analysis to reduce the dimensionality of the CMJ data has proven beneficial for explaining between subject differences across a suite of variables.^{19,205,206,208} To date, only one other study has used multivariate assessment to longitudinally assess fatigue in athletes.²⁰⁸ By extrapolating factor scores and using those to explain within-subject changes in CMJ performance indicative of NMF, establishing the validity of testing and diagnosis of the construct of overreaching becomes more feasible.

The proposed workload-injury relationship in sport asserts that risk of non-contact LEI is higher during periods where athletes experience relative increases in workload far greater than what they have been previously exposed to.⁵⁴ This relationship has been tested in NCAA DI football athletes for overuse injury but further research is needed to elucidate the effects of fatigue induced alterations in motor performance on the incidence of traumatic LEI in high intensity contact drills or game situations. This research should seek to determine the relationship between NFOR induced NMF and traumatic LEI.^{31,55,56} In NCAA DI football the ability to perceive and act to evolving sensory inputs stimuli such as bodily position and impending contact is paramount for performance and withstanding forces to avoid injury.^{97,146,225} Following strenuous athletic tasks such as an NCAA DI football game or practice there is evidence of gross motor impairments, decreased neuromuscular control, proprioception, and stability.⁵⁷ As these NMF induced impairments often present alongside increases in sensorimotor delay, prediction and tracking error, and time-to-contact estimation it is warranted to hypothesize that NMF may negatively alter an athlete's ability to evade and initiate contact potentially heightening risk of injury.58–65 During progression of the NCAA DI football season the likelihood for experiencing NMF and potentially NFOR is high due to the cumulative nature of fatigue.^{20,22,226,227} Thus the primary aim of this study is to examine the longitudinal association of the occurrence of traumatic LEI and NFOR as

determined by NMF when adjusted for position and playing status across the NCAA DI football season.

7.2 Methods

7.2.1 Participants

Team records from across four competitive seasons were accessed and data compiled from a sample of 129 NCAA DI football athletes (age= $20.4\pm1.5y$, height=1.9 $\pm0.1m$, mass=108.7 ±21.5). Athletes with known pre-existing injuries which would have barred them from physical activity at the commencement of the season were excluded from the analysis. The present study was conducted as part of a larger investigation into the effects of regularly collected performance and wellbeing outcomes on athlete health and safety that was approved by the University of Pittsburgh Institutional Review Board (STUDY20070389). The testing and recording of data were completed by the performance and sports medicine staff as part of standard team protocols to monitor athletic performance. No additional experimental procedures were undertaken for the completion of this study.

As previous literature in football has supported the notion of between-subject and position group differences of both CMJ performance and external workloads, subjects were grouped by "positions that mirror each other" to control for the influence of intrinsic differences such as anthropometrics and imposed demands of play at each respective position.^{10,19,228} The positions groups consisted of quarterbacks, running backs, tight ends, and linebackers in the MIDS group; offensive and defensive linemen in the BIGS group; and wide receivers and defensive backs in the SKILL group.

7.2.2 Countermovement Jump

Bilateral Countermovement Jump (CMJ) tests were administered biweekly in season, \sim 24h post-game and \sim 48h pre-game. The tests were performed on dual force platforms sampling at 1000 Hz (Hawkin Dynamics, Maine, USA). Athletes were instructed to "jump as high and as fast as you can." The athletes were also instructed to keep both hands placed firmly on the hips with the purpose of reducing the influence of the upper limbs moments of inertia on center of mass kinetics and temporal measures.²¹⁸ A quiet standing phase of \geq 1s was required to adequately detect center of mass displacement and initiation of the CMJ.¹⁶⁷ Trained performance coaches conducted the data collection and recording of CMJ tests. Additional verbal encouragement, coaching, and feedback was provided wherever necessary. With added supervision from coaches, athletes who did not properly perform the CMJ test were instructed to repeat the procedures until a minimum of two adequate tests could be recorded.

The commercially available software used to parametrize the raw force-time data has recently been validated against other gold-standard and laboratory grade devices (Hawkin Dynamics, Maine, USA).¹⁶⁶ Testing summary data was exported from the cloud software where it was cleaned and analyzed in R version 4.3.0. Data from each testing session was filtered for the within-subject maximum jump height (JH). Subsequent CMJ metrics included in the dataset were peak propulsive power (PPP), modified reactive strength index (mRSI), average propulsive force (APF), and propulsive phase duration (PPD). The CMJ variables were selected based on a search

of the previous literature on relevant CMJ measures of performance, fatigue, and injury in team sport athletes.16,46,139,219

Chosen CMJ metrics were then processed using a confirmatory factor analysis model that was produced in previous work by the study team. The corresponding factor scores were then added to the dataset. The first of the two latent factors or constructs are referred to throughout as stretch shortening cycle (SSC) as it has primary loadings from JH, PPP, and mRSI. The loadings are those primarily used to describe the elastic potential or stretch shortening capabilities of the lower limb ambulatory muscles.^{229,230} The second latent factor, maximal dynamic strength (MDS), is loaded primarily by APF and negatively loaded by PPD. Therefore factor scores derived from MDS reflect the athlete's ability to concentrically produce force relative to their body mass in short periods of time. ²³¹

The classification and of incidence of FOR or NFOR was informed by the joint position statement of the American College of Sports Medicine (ACSM) and European College of Sports Sciences (ECSS) which deem a performance reduction of greater than or equal to 10% as meaningful for detecting overreaching.¹⁴ This threshold was then longitudinally applied to both SSC and MDS using within-subject maximums from baseline CMJ testing occurring within 28 days prior to the start of the season. Individual feedforward rolling maxima were used for the within-season repeated measures design as to account for instances in which athletes record new personal bests. A custom R script (Version 4.3.0) was used to extrapolate the length of time where instances overreaching occurred and a classification system was developed to delineate between FOR and NFOR based on the repeated measures design. A lag model was then applied to the dataset to allow for the analysis of overreaching as a time-varying categorical predictor. A schematic for the

classification of overreaching relative to the standard schedule of organized team activities within the season is depicted below in Figure 8.

Figure 8. Study Design

Fatigue state will be categorized as a factor with thresholds based on the American College of Sports Medicine (ACSM) and European College of Sports Sciences (ECSS) definition of Neuromuscular Fatigue (NMF) and diagnoses of overreaching; (Functional Overreaching (FOR), NMF > 10% of baseline resolving between 5-7 days; NFOR, NMF > 10% of baseline present for >7 days).

7.2.3 Injury Data

Historical injury records and corresponding reports were provided and reviewed by team physicians and sports medicine staff. The injury classification system employed by the team was translated using the NCAA Injury Surveillance System (ISS) guidelines.^{23,24,232} For the purposes of investigating the effects of NFOR induced NMF on injury, overuse injuries were not included in the dataset. Instead, only injuries that were deemed traumatic injuries were kept for analysis. The definition of which included those that could be attributed to a singular event resulting in damage to the tissues or structures of the skeletomuscular system.233,234 Additionally, to be recorded as a traumatic injury, the injury must have resulted in time loss of greater than or equal to one day or training session. As the classification of NFOR and assessments of NMF rely primarily on the ability to generate force through use of the lower extremities, only injuries to the lower extremities were included in the dataset.

7.2.4 Statistical Analysis

Factor scores for both SSC and MDS were calculated using a weighted least-squares factor analysis with oblique rotations via the "psych" package in R version 4.3.0.¹⁷² Descriptive statistics were calculated for baseline factor scores (SSC and MDS) for each of the position groups (BIG, MID, SKILL). Within-group Cohen's *d* effect sizes were used to compare baseline CMJ factor scores between injured and uninjured athletes.

Generalized linear mixed models (GLMM) were constructed to predict the risk of traumatic LEI (coded 1,0) with random intercepts per subject and position group (BIG, MID, SKILL) as a fixed effect using the "lme4" package in R version 4.3.0.²³⁵ Covariates entered in the models as fixed effects included Cumulative Snaps, time (week), overreaching status (FOR or NFOR) as classified by >10% reduction in both MDS and SSC respectively. Time-varying covariates for both SSC and MDS were modeled as interactions with Week (1-13). An exploratory growth modeling approach with stepwise deletion of covariates was used where fixed effects produced Wald test p-values of <0.10. Exponentiated betas were reported as odds ratios and 95% confidence intervals.

The likelihood ratio test (LRT) was used to compare covariate-adjusted candidate models against an unconditional growth model only time (Week) were entered as a fixed effect with random slopes and intercepts per subject. The LRT calculated the difference in log-likelihoods between models where under the null hypothesis the test statistic approximately follows a chisquared distribution with degrees of freedom equal to the difference in the number of parameters estimated. Significant chi-squared differences (χ^2) were inferred using an alpha of 0.05, where rejecting the null hypothesis asserts that a more complex model provides a better fit.

Additionally, candidate models were compared for goodness of fit and parsimony using the Akaike information criteria (AICc). Marginal R-squared (R^2) assessed the variance explained by the fixed effects alone across all possible levels of the random effects (subjects). While conditional \mathbb{R}^2 was used to measures the proportion of variance explained by both fixed and random effects in the model, considering all covariates and their interactions. For this matter, conditional \mathbb{R}^2 reflects the goodness of fit within the observed data.^{236,237}

7.3 Results

A summary of injury frequencies included in this dataset by location and description is summarized in Table 9. All injuries were recorded in during weeks 1-13 of the in-season periods from 2020-2023. In total, 40 traumatic LEIs were reported across 12,050 observations (3.31/1000 athlete exposures). The most common location of injury was the ankle joint $(n=13)$ while injuries to the knee $(n=9)$ and thigh (both posterior and anterior) $(n=6)$, were the next most common. A total of n=3 subjects suffered a recurrent injury to the same location as such these injuries were not included in the analysis. No traumatic LEIs were reported in athletes who did not experience NFOR during the season indicated by either a reduction in SSC (n=26) or MDS (n=32). Of those injured in this sample, a larger proportion were found to be non-functionally overreached via a reduction in SSC (n=20, 50%) than MDS (n=11, 27.5%).

Mechanism of injury (contact vs. non-contact) was also not consistently defined and thus the data were restricted only to injuries traditionally defined as "traumatic" rather than "overuse" unless otherwise noted in the injury report. The average time to resolve or return to full participation from injury was 68.6 days. Descriptive statistics for baseline factor scores and comparisons between injured and uninjured positions using Cohen's *d* effect sizes are depicted below in Table 10.

Location of Injury	N
Foot/Toes	4
Ankle	13
Lower Leg	5
Knee	9
Thigh	6
Hip/Groin	3
Total	40

Table 9. Traumatic Lower Extremity Injury (LEI) Frequencies by Location

Table 10. Within-Position Effects of Baseline Factor Scores and Injury

Baseline factor scores derived from countermovement jump (CMJ) performance across position groups (BIG,

MID, SKILL) for latent factors; stretch shortening cycle (SSC) and maximal dynamic strength (MDS).

Descriptive statistics are presented as meanstandard deviation and between-group effect sizes for injured

		SSC	Cohen's d	MDS	Cohen's d
BIG	Injury	-0.97 ± 1.31	0.83	-0.58 ± 1.95	0.33
	No Injury	-2.09 ± 1.39		$-1.13+1.34$	
MID	Injury	$-0.40 + 1.76$	-0.67	$-1.57+1.46$	-1.46
	No Injury	$0.80 + 1.81$		$0.59 + 1.49$	
SKILL	Injury	$-2.25+2.34$	-2.07	$1.92 + 2.31$	0.55
	No Injury	$2.08 + 1.81$		$0.81 + 1.73$	

and uninjured athletes were reported as Cohen's d effect sizes.

Within the BIGs position group greater baseline CMJ performances were observed in athletes who went on to sustain a traumatic LEI injured when compared to those who did not for both SSC (Mean_{diff}=1.12, $d=0.83$) and MDS (Mean_{diff}=0.55, $d=0.33$). Conversely, within the MIDs group greater baseline CMJ performances were observed in uninjured athletes for both SSC (Mean_{diff}=-1.20, d =-0.67) and MDS (Meandiff=-2.16, d =-1.46). In the SKILL group, athletes who went on to sustain a traumatic LEI during the season demonstrated lower SSC (Mean_{diff}=-4.33, *d*=-2.07) at baseline when compared to their counterparts despite demonstrating greater MDS scores (Meandiff=1.11, *d*=0.55).

GLMM specifications and results of the LRT are summarized below in Tables 11 and 12, respectively. The LRT revealed non-significant differences in model fit between the unconditional growth model and the more complex covariate adjusted models (*p*>0.05, all). Though the full model yielded the best fit of the observed data (conditional $R^2=0.563$, marginal $R^2=0.291$), the increased complexity came at the cost of model parsimony as indicated by the LRT (χ^2 =0.095, p=0.758). No significant effects of the time-varying covariates for classifications of NFOR as evidenced by either a $>10\%$ reduction in SSC (SSC[NFOR] x Week, OR=0.82 [0.54-1.24], *p*=0.427) or MDS (MDS[NFOR] x Week, OR=1.28 [0.79-2.06], *p*=0.400) were noted in this sample. However, after adjusting for all other covariates in the full model each unit increase in Week resulted in a reduced odds of experiencing traumatic LEI (OR=0.67 [0.46-0.96], *p*=0.071). Decreased odds of experiencing traumatic LEI were also observed in the SKILL position group (OR=0.24 [0.06-0.98], *p*=0.096). Additionally, a significant increase in odds of traumatic LEI was observed in these athletes at baseline for reductions in SSC (SSC[NFOR], OR=6.49 [1.22-34.38], *p*=0.065). Model parameter estimates are depicted using a forest plot in Figure 9.

Table 11. Generalized Linear Mixed Model (GLMM) Specifications

Specifications for generalized linear mixed models (GLMMs) produced to predict traumatic LEI are depicted

alongside model fit comparisons to the unconditional growth model made using the likelihood ratio tests

(LRT) reported as χ^2

Table 12. Generalized Linear Mixed Model (GLMM) Results

Generalized linear mixed models (GLMMs) fitted with random slopes and intercepts per subject. The unconditional model was fit with time or Week (1-13) as a fixed effect. Covariates entered into the models included overreaching status (Non-functional Overreaching [NFOR], as determined by >10% reductions in either stretch shortening cycle (SSC) or maximal dynamic strength (MDS) scores). Both covariates were then tested for their interaction with time (Week). Cumulative snaps, and position group were also tested in the full model. The model fits were assessed using corrected Akaike information criteria (AICc), marginal R² ,

and condition R² .

Figure 9. Full Model Forest Plot

Forest plot displaying exponentiated beta coefficients (odds ratios) and corresponding 90% confidence intervals representing the association between each of the model covariates and traumatic lower extremity injury (LEI) after adjusting other fixed effects in the model.

7.4 Discussion

The purpose of the present study was to examine the longitudinal association of the occurrence of traumatic LEI and NFOR as determined by NMF. The analysis revealed several impactful findings regarding injury characteristics and their potential predictors. First, a

summary of injury frequencies indicated that traumatic LEIs were most frequently reported in the ankle joint (n=13, 32.5%), followed by the knee $(22.5\%, n=9)$ and thigh regions (15%, n=6). Recurrent injuries to the same location within the same season were observed in a subset of subjects, although these were not included in the analysis due to a limited sample size. Mechanism of injury, particularly distinguishing between contact and non-contact injuries, posed challenges due to inconsistent definitions, leading to a focus on injuries linked to a singular event, or traumatic LEI injuries by the common definition.²⁰⁰ Interestingly, no traumatic LEIs were reported among athletes who were not found to be non-functionally overreached at any point in the season. However, in those that did record an instance of NFOR it would appear the rate of injury was much higher for SSC (50%, n=20) than MDS (27.5%, n=11). Additional caution is warranted in this interpretation however as the frequency of NMF measurements and temporal proximities to practice may not have captured instances of recovery and these rates were not adjusted for game exposures, workload, previous injury or even age.

Further analysis of baseline factors derived from CMJ performance across position groups unveiled intriguing patterns. Within the BIGs group, athletes who subsequently sustained traumatic LEIs exhibited greater baseline CMJ performances consistent with elastic properties of the lower extremities (SSC) and rapid force production (MDS). This finding is noteworthy as previous reviews and investigations into CMJ performance as a prospective injury risk indicator in athletes have proposed the univariate measures used to construct the factor scores as potential moderators of injury risk. At the time of writing, no such investigations have been conducted in offensive and defensive linemen (BIGs). It has been previously shown however that over the course of a competitive season this subgroup of NCAA DI football athletes is particularly susceptible to fatigue and thus may be at an increased risk of such injuries.^{6,238,239} In this sample it begs to mention that the frequency of injury across all position groups was lower than in previous studies of this population.^{23,54,96,97} This is a potential limitation in the data as decreased prevalence of traumatic LEI may skew model interpretations and negatively affect the generalizations of these findings to the population. The inclusion of more detailed information about mechanisms of injury and standardization of the definition of traumatic LEI may aid in future investigations aimed at deducing the relationship between traumatic LEI and NFOR.

To the contrary, while only the injured group in the MIDs demonstrated lower MDS scores relative to the uninjured MIDs, both the SKILL and MIDs groups uninjured athletes demonstrated higher baseline SSC. The former was also corroborated by the full model. In the model interpretation it was found that lower SSC was associated with increased odds of subsequent traumatic LEIs when controlling for other covariates in the model (SSC[NFOR], OR=6.49 [1.22- 34.38], *p*=0.065). As an individual growth modeling approach was taken to constructing the GLMM it may then be deduced that after constraining Week (or time) and cumulative snaps to zero (i.e. baseline following preseason camp), if NFOR is detected in SSC factor scores, the odds of aa subsequent traumatic LEI are increased by a factor of 6.49 (p=0.065). Interestingly, the interaction effect of SSC and Week did not reach significance suggesting then that the relationship between NFOR as determined by a reduction in SSC and traumatic LEI does not increase relative to time during the season (SSC[NFOR] x Week, OR=0.82 [0.54-1.24], *p*=0.427). This finding may be particularly useful for coaches in periodizing preseason practices to allow for adequate recovery prior to the start of the season.

One of the most reported measures of the CMJ used to assess NMF, mRSI is the ratio of JH to total time. This variable along with jump height accounted for two thirds of the metrics used to create the SSC factor score. In theory this ratio represents the ability of the athlete to rapidly

express force through both the transition of eccentric to isometric loading of the lower limbs in amortization phase but as well as into the concentric portion through terminal hip and knee extension and eventual plantarflexion.^{240–243} There is potential confounding from the way the test is cued by coaches to "jump as high and as fast as you can" as the added constraints of height often result in longer amortization to produce greater forces but concomitantly adding the environmental constraint to increase the overall speed of movement provides a rate limiting factor to these capabilities.244,245

Given that fatigue and movement related pain are known to functionally constraint or influence motor preparation processes it may be that this measure is reflective of ailments to the elastic properties of the lower limbs brought about by NMF.^{60,195,246} One compensatory strategy or emergent motor behavior employed for generating a higher JH in the face of such ailments is to increase contractile time, reducing mRSI and thus conflating the reductions in the SSC factor score. This assertion is more speculatory of course as the scope and aim of the present study being exploratory in nature limits the ability to test such hypotheses to draw these conclusions. Moreover the operational definition supplied for NFOR used in this study has also recently been challenged which in tandem with these findings highlights the need for a unified definition and testing battery to make such assumptions about training status of athletes.¹⁰² All the while, these data may inform practitioners when looking at measures to properly detect NFOR induced NMF.

Interpretation of the GLMM analyses provided further insights into the relationship between time-varying covariates and the occurrence of traumatic LEIs. Contrary to the hypothesis, the absence of any significant interaction effects of SSC or MDS would indicate that the longitudinal rate of change in these parameters was not associated with increased odds of traumatic LEIs. However, when controlling for the other covariates in the full model there seemed to be a

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protective effect of time as each unit increase in Week was associated with reduced odds of experiencing traumatic LEIs (OR=0.67 [0.46-0.96], *p*=0.071), suggesting a potential protective effect over time. Additionally, there seemed to be a reduced odds of traumatic LEI in the SKILL group (OR=0.24 [0.06-0.98], *p*=0.096) when adjusted for the model parameters. These findings should be interpreted with caution however as the LRT indicated non-significant differences in model fit between the unconditional growth model and more complex covariate-adjusted models. Although the full model showed the best fit to the data, its increased complexity compromised model parsimony. It is worth mentioning here that in this sample, the frequency of injury across all position groups was lower than in previously reported findings.^{23,39,54,96,97} With a low prevalence of traumatic injuries relative to the population at risk, the findings should be interpreted with caution as a complex modeling approach was undertaken which poses potential for overfitting. Future studies should aim to recruit a larger, more diverse sample from multiple sites or teams to better represent the longitudinal association of NFOR with the occurrence of traumatic LEI.

Cumulative snaps were adjusted for in the model as a proxy measure of physical activity and to deduce whether an effect of exposure to game demands may be a predictor of increased risk for injury over time. It was also deemed necessary to account for game exposure in the model as the increased risk of traumatic injury in NCAA DI football games when compared to other organized activities such as training and practice is well known.23,24 However as the cumulative snap counts did not reflect the relative volumes or intensities of physical activity experienced by each during plays it may be more pertinent to use an objective measure of physical workload to account for exposures to game demands. More recently, investigations into injury risk during the in-season periods in this population have found that external workloads monitored by wearable devices can prove useful for identifying at-risk athletes potentially due to increasing volumes of physical activity.54,96 Therefore, future investigations into this topic with similar populations should aim to account for such metrics in their models to better understand the association of the volume of physical activity on NFOR and injury risk in NCAA DI football athletes.

7.5 Conclusion

The present study was able to describe covariates associated with traumatic LEI in NCAA DI football athletes through the theoretical lens of overreaching as defined by reductions in CMJ performance. However, the overall prediction of this outcome was made difficult by a limited sample and complex modelling techniques. Though the GLMM analyses revealed significant predictors of injury risk adjusted for individual growth rates using random slopes and intercepts, addition of these covariates to the model did not significantly improve model fit relative to the number of predictors. While the full model exhibited higher explanatory power, unconditional growth model featuring only random slopes and intercepts per individual and time as a fixed effect fit the data nearly as well suggesting a highly inter-individual nature to NFOR induced NMF and traumatic LEI.

8.0 Conclusion

This collection of research provides a comprehensive picture of the CMJ test as a means of detecting NMF in NCAA DI football with insights into how the data can best be collected and reported, meaningful associations of changes in CMJ performance to criterion measures of fatigue, and the use of CMJ for detecting NFOR and its effects on risk of traumatic LEI. Through a multidimensional analysis, two distinct latent factors emerged: SSC and MDS. These factors accounted for a substantial portion of the variance in CMJ performance. Importantly, the study confirmed configural and strong invariance across different time points and fatigue states, suggesting consistency in factor structure.

The above findings would support the comparison of longitudinal mean differences in factor scores between groups. Surprisingly, contrary to prevailing literature, no significant main effects of time on CMJ performance were detected. These findings challenge pre-existing evidence of altered CMJ performance under fatigued conditions in team sport athletes. This may be because previous investigations have predominantly used univariate measures (i.e. JH, PPP, mRSI) rather than composite measures such as factor scores to reflect CMJ performance constructs (i.e. stretch shortening capabilities). Future studies should aim to further investigate the latent factors to deduce how changes in emergent motor behavior and performance over time may be attained given the affordances provided by fatigue. Additionally, group x time interactions hinted at potential mediation by position, indicating a nuanced relationship between fatigue and CMJ performance, particularly in specific player roles.

These results highlighted the need for longitudinal studies to further unravel the intricate interplay between NMF and CMJ performance, whilst considering positional differences and other relevant factors. Furthermore, the study highlighted the complexity of interpreting CMJ data and emphasizes the necessity for comprehensive approaches to understand its implications for athlete well-being. Covariate adjusted models incorporating self-reported measures and objective indices of stress and fatigue were presented to enhance the effectiveness of CMJ monitoring in detecting NMF. Overall, this research emphasized the importance of considering multiple dimensions when assessing NMF and via the CMJ, providing valuable insights for coaches, practitioners, and sports medicine professionals aiming to optimize athlete performance and health.

Expanding on the prior findings, in the second study we aimed to explore the associations between salivary biomarkers of stress and recovery, represented by the TC ratio, and NMF over the course of a competitive season. Despite notable fluctuations in self-reported fatigue levels and hormonal concentrations over time, no significant decreases were detected in the TC ratio or CMJ factor scores at the team level. This suggests that while athletes experienced varying degrees of fatigue and hormonal changes, these factors did not consistently correlate with objective measures of NMF. The findings highlight the complexity of assessing NFOR in team sport athletes and may indicate the need for the development and validation of more comprehensive criteria for the detection of NFOR that encompass both objective biomarkers and subjective assessments, while also considering contextual factors like playing position and training status.

Currently accepted best practices for identifying NFOR in athletes may not adequately account for the highly individualized nature of fatigue. Moreover, it would appear that univariate measures previously deemed sufficient for detection of the which may need to be further investigated for their sensitivity to fatigue induced changes relative to other laboratory grade measures in this population. For these reasons coaches and practitioners should exercise caution when integrating multifactorial assessment protocols such as the one employed in this study. An enhanced protocol may be one that incorporates reliable measures of both objective and selfreported measures of fatigue, while also adjusting for contextual factors specific to each athlete, such as their playing position and individual training loads from wearables. Moreover, the development and validation of clinical guidelines for measures of NFOR is warranted to aid practitioners in their practice. Further extrapolation of the longitudinal relationship between NMF and hormonal responses such as those from salivary biomarkers as may enhance the understanding of fatigue-related issues in athletic populations and facilitate the development of targeted intervention strategies to optimize athlete performance and well-being.

Lastly, we investigated the link between NMF induced by NFOR and traumatic LEIs in NCAA DI football athletes. Using biweekly countermovement jump (CMJ) tests to assess NMF, the research analyzed data from 129 athletes over four seasons. The findings revealed distinct differences in baseline CMJ performance between injured and uninjured athletes across position groups, with NFOR detection via CMJ factor scores emerging as a significant predictor of increased odds of sustaining traumatic LEIs during the season. Specifically, NFOR as indicated by reductions in SSC factor scores when time was controlled for were associated with higher odds of subsequent LEIs. This finding was noteworthy as coaches and practitioners may benefit from targeted interventions to build resiliency of the elastic properties of the lower limbs prior to preseason camp. Additionally, the analysis indicated reduced odds of sustaining a traumatic LEI across weeks as well as in SKILL position players, suggesting a potential cumulative training effect for enhanced resiliency.

Monitoring for NFOR-induced changes in neuromuscular performance, particularly through CMJ assessments, can serve as a valuable tool in identifying athletes at risk of traumatic LEIs. The utility of CMJ assessments can be enhanced by concomitantly tracking self-reported fatigue to aid in detecting NFOR. Furthermore, understanding the positional differences in injury susceptibility revealed by the study can inform position-specific training and injury prevention protocols. Overall, the findings illuminate the importance of comprehensive monitoring protocols and individualized interventions to optimize athlete health and performance in NCAA DI football.

8.1 Limitations

This collection of studies identified key factors in CMJ performance (SSC and MDS) and modeled longitudinal fatigue and recovery trends in this sample yet several limitations in the data must be acknowledged. First, the frequency of NMF measurements did not allow for precise determination of the time to injury or NFOR status at the time of injury, necessitating the use of a time lag model. Therefore, it may be plausible that not all instances of NMF were accounted for in this sample. Additionally, multiple unforeseen circumstances, including scheduling conflicts and changes in team policies, posed significant challenges to follow-up. These challenges are not unique to this study however and are often those that may be faced in practice. The 2020 season's data were potentially compromised by the global pandemic, with undetected active cases and irregular schedules affecting the results. Moreover, not all time-loss injuries were included in the dataset, as some injuries did not involve the lower limbs or were beyond the study's scope. The inconsistencies in the definition of injury across literature and practice further limit the generalizability of these findings. Lastly, self-reported assessments are inherently subject to bias and often fail to account for lifestyle factors that might influence the interpretation of the data.

8.2 Future Directions

Future research should undertake longitudinal approaches to deepening the understanding of the relationship between NMF and CMJ performance, with special attention being paid to positional differences among other relevant factors. Expanding the scope and aims of these studies to include larger and more diverse samples from multiple teams or universities would enhance the robustness of the findings. With adequate power, it may be feasible to develop more complex models that account for internal and external workload from wearables, injury history, and other pertinent factors such as team performance and style of play. An increased frequency of testing could provide better person-time at risk models regarding instances of NFOR and/or NMF. Finally, the establishment of a standardized definition of NFOR, validated against laboratory-grade assessments of NMF, would greatly benefit researchers and coaches in making more precise and reliable evaluations.

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