THE INFLUENCE OF LOWER EXTREMITY BIOMECHANICS ON BIOCHEMICAL MARKERS OF BONE TURNOVER DURING ARMY CADET BASIC TRAINING

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ABSTRACT

Timothy C. Mauntel: The Influence of Lower Extremity Biomechanics on Biochemical Markers of Skeletal Stress During Army Cadet Basic Training
(Under the direction of Darin A. Padua)

Lower extremity stress fracture rates are high among military personnel, result in substantial lost duty time, and inhibit military readiness. Stress fracture risk factors include aberrant biomechanics, previous musculoskeletal injury, physical fitness, and anthropometric measurements. It is unknown how these risk factors influence bone formation and resorption (turnover) biomarkers. Elucidating the relationships between stress fracture risk factors and bone turnover biomarkers will provide insight into how these factors influence bone health. Our primary aim was to characterize the effects of stress fracture risk factors on bone turnover biomarkers. Our secondary aim was to validate an automated markerless motion capture system. We hypothesized the presence of stress fracture risk factors would result in bone biomarker profiles indicative of high turnover rates. We also hypothesized the markerless motion capture system would provide valid kinematic measurements.

Army cadets completing Cadet Basic Training (CBT) were assessed via a jump-landing assessment and other stress fracture risk factors were recorded. Bone turnover biomarkers were measured post-CBT. Linear regression models were used to determine the extent to which stress fracture risk factors influenced bone turnover biomarkers. Kinematic measures calculated by the markerless motion capture system during a jump-landing assessment were compared against a

stereophotogrammetric motion capture system.

Lower extremity stress fracture risk factors predicted post-CBT bone turnover biomarkers. Overall movement quality was not predictive, but variables associated with sagittal plane displacement and foot position at initial ground contact did predict post-CBT bone turnover biomarkers. Injury during CBT, physical fitness test performance, and mass also predicted post-CBT bone turnover biomarkers.

Moderate agreement was observed between the markerless and stereophotogrammetric motion capture systems. Better agreement was observed for sagittal than frontal plane joint angles and for maximum and displacement angles than initial ground contact joint angles.

Our findings provide important information regarding how stress fracture risk factors affect bone health. The markerless motion capture system was limited in identifying minute changes in trunk and lower extremity joint angles but can accurately identify gross movement patterns. These findings will guide interventions to reduce stress fracture risks and guide the use of automated movement assessments for identifying injury risks.

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LIST OF ABBREVIATIONS

APFT	Army Physical Fitness Test
BALP	Bone-specific alkaline phosphate
BLQ	Baseline questionnaire
BMI	Body mass index
CTx-1	Cross-linked collagen telopeptide
DPD	Deoxypyridinoline
ELISA	Enzyme-linked immunosorbent assays
LESS	Landing Error Scoring System
PABAK	Prevalence and Bias Adjusted Kappa statistic
PICP	Procollagen type I carboxy-terminal propeptide
PINP	Procollagen type I aminoterminal propeptide
TRAP5b	Tartrate resistant acid phosphate
USMA	United States Military Academy

CHAPTER I

INTRODUCTION

1.1 – Background and Introduction

Musculoskeletal injuries affect 63% of non-deployed military personnel[1] and are the most significant medical issue limiting military readiness.[2] Lower extremity injuries account for 39% of non-deployed military personnel injuries, with 82% of these injuries resulting from overuse mechanisms.[1] The direct and indirect costs associated with musculoskeletal injuries are estimated at $3.7 billion annually for the Department of Defense.[3] One of the most common injuries affecting military personnel is lower extremity stress fractures,[1,4] which affect nearly 1 in 3 male service members.[5] These injuries result in significant lost duty time, medical costs, and attrition.[4] Given the high prevalence of musculoskeletal injuries and their substantial physical and financial costs it is critical to understand the factors that contribute to individuals sustaining injury during military training.[3]

Military training is highly repetitive but also involves bouts of high intensity exercise, this training regimen results in high training loads that are associated with increased lower extremity injury rates.[6-8] This is especially true for overuse bone injuries (e.g. stress fractures).[1,4] Musculoskeletal stress occurring during military training may be amplified by aberrant biomechanics which are associated with traumatic and overuse musculoskeletal injuries.[4,9,10] Individually, both physical training and aberrant biomechanics increase musculoskeletal stress, but when occurring simultaneously these factors may interact and result in injury.

Aberrant biomechanical patterns can be easily identified with common clinical movement assessments (e.g. jump-landing tasks).[11,12] The jump-landing task has been developed into a validated clinical movement assessment that is scored on visual observation of aberrant movement patterns (the Landing Error Scoring System or LESS). The LESS is capable of discriminating between individuals at increased lower extremity injury risk from those who are not.[11-13] Individuals who score high on the LESS (>6) and individuals who score low on the LESS (≤4) display different three-dimensional lower extremity biomechanical patterns.[11] The aberrant biomechanical patterns observed among individuals with high LESS scores have been associated with traumatic and overuse musculoskeletal injuries.[4,9,10,12,13]

The LESS is a movement assessment that meets many of the requirements put forth by a consortium of civilian and military experts on injury risks and prevention.[11] Primarily, it is valid, reliable, and can be implemented quickly across a large number of individuals. However, the LESS does have its limitations.[14] The LESS requires video replay and manual scoring of jump-landing trials, which is time consuming and therefore prohibitive for clinicians to implement.[11,14] Thus, there has been a call for automated systems that accurately and quickly identify individuals at increased injury risk.[14,15]

Automated injury risk assessments have been implemented with military personnel, and substantially reduced the time required to screen individuals for injury risks.[15] The major pitfall of these screening systems was that they did not automate the movement assessment, which is a key component of injury risk screenings.[14,15] A new markerless motion capture system reliably automates the LESS scoring process.[16] However, the joint angles and displacements reported by this system have yet to be validated against the gold-standard of movement assessments, marker based stereophotogrammetric motion capture systems. Thus, validation of this markerless motion

capture system is required before wide-spread implementation can occur to aid clinicians in identifying lower extremity injury risks.

Biochemical markers (biomarkers) associated with musculoskeletal system stress may be useful in identifying individuals who are overstressing their musculoskeletal systems, prior to them sustaining an injury.[5,17,18] Biomarkers indicative of skeletal stress ("bone turnover") change with alterations in physical activity, thus they may be able to identify individuals prior to injury.[5,17,19-27] Biomarkers indicative of bone formation (procollagen type I aminoterminal propeptide [PINP]) and resorption (cross-linked collagen telopeptide [CTx-1]) (i.e. turnover) are altered by military training.[5,17,25,27] Bone turnover biomarkers also increase following traumatic lower extremity joint injuries,[28,29] which are common amongst military personnel.[1-3] Examining serum biomarkers representative of bone turnover will provide insight into the extent to which lower extremity biomechanics influence skeletal stress during military training.

Bone turnover biomarkers may also be influenced by other known stress fracture risk factors. These factors include modifiable and non-modifiable factors. Modifiable risk factors include training load,[6,30-32] aerobic and anaerobic fitness,[14,33-40] physical activity preceding military training,[33,35,38,41-43] body composition,[17,33,44] and lifestyle choices.[14,33,37,45] Non-modifiable risk factors include previous history of musculoskeletal injury,[37,45] age,[37,38] race,[34,37,44] and sex.[44,46] It is therefore important to consider the aforementioned factors when assessing bone turnover biomarkers.

Given the high prevalence of musculoskeletal injuries during military training, especially overuse bone injuries, and their substantial short- and long-term consequences, it is critical to understand the factors that increase injury risk. Therefore the purpose of this study was to identify how lower extremity biomechanical patterns influence biochemical markers of bone

turnover. Understanding the influence of biomechanics on bone turnover biomarkers will allow for the development of intervention strategies to reduce injury risk and optimize performance during military training.

1.2 – Operational Definitions

1) Cadet Basic Training: A 6-week course completed by new cadets at the United States Military Academy (West Point) the summer prior to the start of their first academic year. The course is designed to improve physical fitness, teach basic military skills (e.g. marksman-ship, first aid, land navigation), and improve confidence.
2) Jump-Landing Movement Assessment: A clinical movement assessment in which the study participant jumps from a 30cm tall box to a target area located a standardized 0.9m away from the front of the box. Participants complete a vertical jump for maximal height immediately following landing in the target area. Biomechanical patterns are identified during the landing phase of the initial jump (initial ground contact ➔ peak knee flexion).[11]
    a. Initial Ground Contact: The video frame immediately preceding the video frame in which the entire foot is in contact in the ground, or when the ground reaction force is ≥10N.
    b. Peak Knee Flexion: The maximum knee flexion angle the participant reaches following initial ground contact.
3) Landing Error Scoring System (LESS): A valid and reliable clinical movement assessment during which lower extremity movement patterns are visually observed during a jump-landing movement assessment.[11]
4) Biochemical Markers of Bone Turnover (biomarker): A characteristic that is objectively

measured and evaluated as an indicator of normal or pathogenic biologic processes[47] (skeletal response to stress induced by biomechanical patterns and military basic training), that is measured through blood serum.

   a. Procollagen type I aminoterminal propeptide (PINP): A biochemical marker indicative of type I collagen neogenesis, representative of bone formation.
   b. Cross-linked collagen telopeptide (CTx-1): A biochemical marker indicative of type I collagen breakdown, representative of bone resorption.

5) Biochemical Marker Turnover: The ratio between biochemical markers indicative of tissue neogenesis and tissue breakdown (type I collagen, bone).
6) Baseline Questionnaire (BLQ): A comprehensive questionnaire that is designed to assess previous and current physical activity levels, previous and current injury history, and overall current physical well-being
7) Army Physical Fitness Test (APFT): A test of physical fitness administered by the United States Army to determine the muscular strength, muscular endurance, and cardiorespiratory fitness of each cadet. The APFT includes 2 minutes of push-ups, 2 minutes of sit-ups, and a timed 2-mile run. The raw score and standardized score (0 – 100 points) for each event and a cumulative score (0 – 300 points) are recorded.
8) Previous Physical Activity: The physical activity the cadet participated in prior to beginning Cadet Basic Training.
   a. Previous Physical Activity Level: The number of seasons (*season* = participation in a physical activity ≥3 times a week for ≥3 months) an individual completed structured physical activity.
   b. Previous Physical Activity Volume: The product of the average frequency of physical

activity multiplied by the average duration of physical activity**.**

c. Previous Physical Activity Type: Physical activity that either directly loads (weight bearing) or does not directly load the lower extremity completed prior to beginning Cadet Basic Training.

9) Body Mass Index (BMI): An index of mass-to-height used to classify individuals into categories of underweight, normal, overweight, and obese. This value is obtained with the following equation: BMI = mass (kg) / height $(cm)^2$.[48]

1.3 – Assumptions and Limitations

The following assumptions and limitations will apply to this study:

1) The PhysiMax^{TM} LESS Scoring Platform is a valid measure of trunk and lower extremity movement patterns.
2) Participants will jump for maximal effort during the jump-landing assessments.
3) Participants will give maximal effort throughout Cadet Basic Training.
4) The enzyme-linked immunosorbent assay (ELISA) kits for measurement of bone turnover biomarkers will be reliable within <10% inter and intra-assay coefficients of variation.
5) Circulating serum concentrations of bone biomarkers (PINP and CTx-1) measured within 2 weeks of completing Cadet Basic Training accurately and reliably reflect bone turnover rates.
6) The rates of bone turnover of military cadets completing Cadet Basic Training at the United States Military Academy (West Point) are generalizable to other military populations that complete similar training.

1.4 – Delimitations

The following delimitations were made for this study.

1) 45 male cadets were recruited from the United States Military Academy (West Point).
2) All participants were injury-free at the time of the jump-landing movement assessment testing.
3) All participants were healthy with no history of neurological or metabolic disorders.
4) All serum biomarker concentrations were measured using enzyme-linked immunosorbent assays (ELISA) and spectrophotometry.

1.5a – Independent (Predictor) Variables

1) Lower Extremity Movement Quality
    a. LESS total score
    b. LESS individual items
    c. Average frontal and sagittal plane trunk, hip, knee, and ankle joint angles at initial ground contact, maximum values, and displacements
2) Previous Physical Activity Levels
    a. Volume of physical activity prior to Cadet Basic Training
    b. Total number of previous physical activity seasons
3) Previous Physical Activity Type
    a. Total number of previous non-weight bearing physical activity seasons
    b. Total number of previous low impact weight bearing physical activity seasons
    c. Total number of previous high impact weight bearing physical activity seasons
    d. Pre-Cadet Basic Training Marx lower extremity activity rating score

e. History of jump/movement training

4) Physical Fitness Levels
    a. APFT standardized composite score
    b. APFT individual event scores
        i. Raw scores
        ii. Standardized scores
5) Musculoskeletal Injury History (dichotomous)
    a. Previous history of lower extremity stress fracture
    b. Previous history of lower extremity acute fracture
    c. Previous history of lower extremity musculoskeletal injury (e.g. ligamentous sprain, meniscal injury)
        i. Any history
        ii. Injury within 6 months preceding Cadet Basic Training
        iii. History of musculoskeletal injury during Cadet Basic Training
            a. Duration of time loss from Cadet Basic Training following musculoskeletal injury
    d. Previous history of orthopaedic surgery
6) Body Compositions Measurements
    a. Height
        i. Pre-Cadet Basic Training
        ii. Post-Cadet Basic Training
    b. Mass
        iii. Pre-Cadet Basic Training

iv. Post-Cadet Basic Training

v. Change from Pre-to-Post-Cadet Basic Training measurements

c. Body Mass Index (BMI)

vi. Pre-Cadet Basic Training

vii. Post-Cadet Basic Training

viii. Change from Pre-to-Post-Cadet Basic Training measurements

7) Post-Cadet Basic Training blood draw preceding 12 hours physical activity and food consumption

a. Food Consumption

i. Time

ii. Protein vs Non-Protein rich foods

b. Exercise

i. Time

ii. Weight bearing vs Non-weight bearing

1.5b – Dependent Variables

1) Biomarkers Representative of Bone Turnover – Individual

a. Procollagen type I aminoterminal propeptide (PINP) at post-Cadet Basic Training

b. Cross-linked collagen telopeptide (CTx-1) at post-Cadet Basic Training

2) Biomarkers Representative of Bone Turnover – Turnover Ratio

a. PINP : CTx-1 at post-Cadet Basic Training

1.6 – Specific Aims and Research Hypotheses

The following specific aims were addressed by this project.

1.6a – Specific Aim 1

Characterize the effects of lower extremity biomechanics on biomarker profiles representing bone turnover through predictive models incorporating serum biomarker measures collected following military basic training (post-Cadet Basic Training).

Hypothesis 1a: Qualitative measures of lower extremity movement quality (LESS total score and individual LESS items) will be predictive of post-Cadet Basic Training PINP, CTx-1, and PINP : CTx-1 serum concentration levels.

Hypothesis 1a.1: Higher LESS scores (poorer movement quality) will result in higher serum concentrations of PINP and CTx-1. Higher LESS scores will also result in smaller PINP : CTx-1 ratios.

Hypothesis 1a.2: Positive findings of sagittal plane LESS items (trunk, hip, knee, and ankle items at initial ground contact and displacements) will result in higher serum concentrations of PINP and CTx-1. Positive findings of sagittal plane LESS items will also result in smaller PINP : CTx-1 ratios.

Hypothesis 1a.3: Positive findings of frontal plane LESS items (hip and knee alignments at initial ground contact and displacement) will result in higher serum concentrations of PINP and CTx-1. Positive findings of frontal plane LESS items will also result in smaller PINP : CTx-1 ratios. Frontal plane trunk items will not be predictive of serum concentrations of PINP, CTx-1, or PINP : CTx-1 ratios.

Hypothesis 1a.4: Positive findings of transverse plane LESS items (foot internal and external rotation) will not be predictive of serum concentrations of PINP, CTx-1, or PINP : CTx-1 ratios.

Hypothesis 1b: Quantitative measures of lower extremity sagittal and frontal plane movement

quality (average trunk, hip, knee, and ankle angles) will be predictive of PINP, CTx-1, and PINP : CTx-1 serum concentration levels.

Hypothesis 1b.1: Smaller trunk, hip, and knee sagittal plane joint angles at initial ground contact, maximum values, and displacements will result in higher serum concentrations of PINP and CTx-1. Smaller trunk, hip, and knee sagittal plane joint angles will also result in smaller PINP : CTx-1 ratios.

Hypothesis 1b.2: Larger hip and knee frontal plane joint angles at initial ground contact, maximum values, and displacements will result in higher serum concentrations of PINP and CTx-1. Larger hip and knee frontal plane joint angles will also result in smaller PINP : CTx-1 ratios. Frontal plane trunk angles will not be predictive of serum concentrations of PINP, CTx-1, or PINP : CTx-1 ratios.

1.6b – Specific Aim 2

Characterize the effects of known stress fracture risk factors on biomarker profiles representing bone turnover through predictive models incorporating serum biomarker measures collected following military basic training (post-Cadet Basic Training).

***Hypothesis 2a*:** Previous physical activity volume will be predictive of PINP, CTx-1, and PINP : CTx-1 serum concentration levels.

Hypothesis 2a.1: Smaller volumes of previous physical activity will result in higher serum concentrations of PINP and CTx-1. Smaller volumes of previous physical activity will also result in smaller PINP : CTx-1 ratios.

Hypothesis 2a.2: Fewer previous physical activity seasons will result in higher serum concentrations of PINP and CTx-1. Fewer previous physical activity seasons will also result in smaller PINP : CTx-1 ratios.

***Hypothesis 2b*:** Previous physical activity type will be predictive of PINP, CTx-1, and PINP : CTx-1 serum concentration levels.

Hypothesis 2b.1: Fewer seasons of previous weight bearing physical activity will result in higher serum concentrations of PINP and CTx-1. Fewer seasons of previous weight bearing physical activity will also result in smaller PINP : CTx-1 ratios.

Hypothesis 2b.2: Smaller Marx lower extremity activity rating scores will result in higher serum concentrations of PINP and CTx-1. Smaller Marx lower extremity activity rating scores will also result in smaller PINP : CTx-1 ratios.

Hypothesis 2b.3: Previous history of jump or movement training will result in smaller serum concentrations of CTx-1, but not PINP. Previous history of jump or movement training will also result in larger PINP : CTx-1 ratios.

***Hypothesis 2c*:** Physical fitness levels will be predictive of PINP, CTx-1, and PINP : CTx-1 serum concentration levels.

Hypothesis 2c.1: Lower composite APFT scores will result in higher serum concentrations of PINP and CTx-1. Lower composite APFT scores will also result in smaller PINP : CTx-1 ratios.

Hypothesis 2c.2: Lower APFT push-ups and sit-ups raw and standardized scores will result in higher serum concentrations of PINP and CTx-1. Lower APFT push-ups and sit-ups raw and standardized scores will also result in smaller PINP : CTx-1 ratios.

Hypothesis 2c.3: Higher APFT raw run time and lower standardized score will result in higher serum concentrations of PINP and CTx-1. Higher APFT raw run time and lower standardized score will also result in smaller PINP : CTx-1 ratios.

***Hypothesis 2d*:** Musculoskeletal injury history will be predictive of PINP, CTx-1, and PINP :

CTx-1 serum concentration levels.

Hypothesis 2d.1: History of lower extremity fracture (acute and stress) will result in higher serum concentrations of PINP and CTx-1. History of lower extremity fracture will also result in smaller PINP : CTx-1 ratios.

Hypothesis 2d.2: History of lower extremity musculoskeletal injury (any history and within 6 months preceding Cadet Basic Training) will result in higher serum concentrations of CTx-1, but not PINP. History of lower extremity fracture (acute and stress) will also result in smaller PINP : CTx-1 ratios.

Hypothesis 2d.3: History of orthopaedic surgery will result in higher serum concentrations of PINP and CTx-1. History of orthopaedic surgery will also result in smaller PINP : CTx-1 ratios.

Hypothesis 2d.4: History of musculoskeletal injury during Cadet Basic Training will result in higher serum concentrations of PINP and CTx-1. History of musculoskeletal injury during Cadet Basic Training will also result in smaller PINP : CTx-1 ratios.

Hypothesis 2d.5: Longer duration of time loss from Cadet Basic Training training as the result of a musculoskeletal injury will result in higher serum concentrations of PINP and CTx-1. Longer duration of time loss from Cadet Basic Training training as the result of a musculoskeletal injury will also result in smaller PINP : CTx-1 ratios.

***Hypothesis 2e*:** Anthropometric measurements will be predictive of PINP, CTx-1, and PINP : CTx-1 serum concentration levels.

Hypothesis 2e.1: Pre- and Post-Cadet Basic Training height will not be predictive of serum concentrations of PINP or CTx-1. Pre- and Post-Cadet Basic Training height will also not be predictive of PINP : CTx-1 ratios.

Hypothesis 2e.2: Lower Pre- and Post-Cadet Basic Training mass will result in higher serum concentrations of PINP and CTx-1. Lower Pre- and Post-Cadet Basic Training mass will also result in smaller PINP : CTx-1 ratios.

Hypothesis 2e.3: Greater Pre-to-Post-Cadet Basic Training changes in mass will result in higher serum concentrations of PINP and CTx-1. Greater Pre-to-Post-Cadet Basic Training changes in mass will also result in smaller PINP : CTx-1 ratios.

Hypothesis 2e.4: Lower Pre- and Post-Cadet Basic Training BMI will result in higher serum concentrations of PINP and CTx-1. Lower Pre- and Post-Cadet Basic Training BMI will also result in smaller PINP : CTx-1 ratios.

Hypothesis 2e.5: Greater Pre-to-Post-Cadet Basic Training changes in BMI will result in higher serum concentrations of PINP and CTx-1. Greater Pre-to-Post-Cadet Basic Training changes in BMI will also result in smaller PINP : CTx-1 ratios.

***Hypothesis 2f*:** Food consumption and physical activity within 12 hours preceding the post-Cadet Basic Training blood draw will be predictive of CTx-1 and PINP : CTx-1 serum concentration levels, but not PINP.

Hypothesis 2f.1: Protein rich food consumption within 12 hours of the post-Cadet Basic Training blood draw will result in higher serum concentrations of CTx-1, but not PINP. Protein rich food consumption within 12 hours of the post-Cadet Basic Training blood draw will also result in smaller PINP : CTx-1 ratios.

Hypothesis 2f.2: Weight bearing physical activity within 12 hours of the post-Cadet Basic Training blood draw will result in higher serum concentrations of CTx-1, but not PINP. Weight bearing physical activity within 12 hours of the post-Cadet Basic Training blood draw will also result in smaller PINP : CTx-1 ratios.

1.6c – Specific Aim 3

Characterize how each significant predictor variable in specific aim 2 modifies the effects of lower extremity biomechanics on biomarker profiles representing bone turnover through predictive models incorporating serum biomarker measures collected following military basic training (post-Cadet Basic Training).

Hypothesis 3a: Previous physical activity exposure will interact with lower extremity biomechanics and significantly alter the effects of lower extremity biomechanics on serum biomarker measures. Smaller volumes of previous physical activity and fewer seasons will exacerbate the effects of lower extremity biomechanics and result in higher PINP and CTx-1 serum concentrations and smaller PINP : CTx-1 ratios.

Hypothesis 3b: Previous physical activity type will interact with lower extremity biomechanics and significantly alter the effects of lower extremity biomechanics on serum biomarker measures. Fewer seasons of previous weight bearing physical activity and smaller Marx lower extremity activity rating scores will exacerbate the effects of lower extremity biomechanics and result in higher PINP and CTx-1 serum concentrations and smaller PINP : CTx-1 ratios. Previous history of jump or movement training will not significantly interact with lower extremity biomechanics.

Hypothesis 3c: Physical fitness levels will interact with lower extremity biomechanics and significantly alter the effects of lower extremity biomechanics on serum biomarker measures. Worse composite and individual APFT scores will exacerbate the effects of lower extremity biomechanics and result in higher PINP and CTx-1 serum concentrations and smaller PINP : CTx-1 ratios.

Hypothesis 3d: History of lower extremity injury will interact with lower extremity

biomechanics and significantly alter the effects of lower extremity biomechanics on serum biomarker measures. A history of fracture or lower extremity musculoskeletal injury will exacerbate the effects of lower extremity biomechanics and result in higher PINP and CTx-1 serum concentrations and smaller PINP : CTx-1 ratios.

Hypothesis 3e: History of orthopaedic surgery will interact with lower extremity biomechanics and significantly alter the effects of lower extremity biomechanics on serum biomarker measures. A history of orthopaedic surgery will exacerbate the effects of lower extremity biomechanics and result in higher PINP and CTx-1 serum concentrations and smaller PINP : CTx-1 ratios.

Hypothesis 3f: Sustaining a musculoskeletal injury during Cadet Basic Training will interact with lower extremity biomechanics and significantly alter the effects of lower extremity biomechanics on serum biomarker measures. Sustaining a musculoskeletal injury during Cadet Basic Training will exacerbate the effects of lower extremity biomechanics and result in higher PINP and CTx-1 serum concentrations and smaller PINP : CTx-1 ratios. The longer duration of time loss from Cadet Basic Training as a result of the musculoskeletal injury will result in higher PINP and CTx-1 serum concentrations and smaller PINP : CTx-1 ratios.

Hypothesis 3g: Pre- and Post-Cadet Basic Training mass will interact with lower extremity biomechanics and significantly alter the effects of lower extremity biomechanics on serum biomarker measures. Lower Pre- and Post-Cadet Basic Training mass will exacerbate the effects of lower extremity biomechanics and result in higher PINP and CTx-1 serum concentrations and smaller PINP : CTx-1 ratios.

Hypothesis 3h: Pre-to-Post-Cadet Basic Training changes in mass will interact with lower extremity biomechanics and significantly alter the effects of lower extremity biomechanics on

serum biomarker measures. Greater Pre-to-Post-Cadet Basic Training changes in mass will exacerbate the effects of lower extremity biomechanics and result in higher PINP and CTx-1 serum concentrations and smaller PINP : CTx-1 ratios.

Hypothesis 3i: Pre- and Post-Cadet Basic Training BMI will interact with lower extremity biomechanics and significantly alter the effects of lower extremity biomechanics on serum biomarker measures. Lower Pre- and Post-Cadet Basic Training BMI will exacerbate the effects of lower extremity biomechanics and result in higher PINP and CTx-1 serum concentrations and smaller PINP : CTx-1 ratios.

Hypothesis 3j: Pre-to-Post-Cadet Basic Training changes in BMI will interact with lower extremity biomechanics and significantly alter the effects of lower extremity biomechanics on serum biomarker measures. Greater Pre-to-Post-Cadet Basic Training changes in BMI will exacerbate the effects of lower extremity biomechanics and result in higher PINP and CTx-1 serum concentrations and smaller PINP : CTx-1 ratios.

Hypothesis 3k: Protein rich food consumption and weight bearing physical activity within 12 hours of the post-Cadet Basic Training blood draw will not significantly alter the effects of lower extremity biomechanics on PINP or CTx-1 serum biomarker concentrations or PINP : CTx-1 ratios.

1.6d – Specific Aim 4

Validate the trunk and lower extremity angles calculated by the PhysiMax™ markerless motion capture system against the current gold-standard (marker based stereophotogrammetry system [Vicon]) of motion capture systems.

***Hypothesis 4a*:** Frontal and sagittal plane trunk angles calculated by the PhysiMax™ markerless motion capture system will be valid measures of trunk kinematics as compared to the current

gold-standard of motion capture systems. Maximum joint angles will demonstrate the best agreement between motion capture systems, followed by joint angle displacements, and then joint angles at initial ground contact.

***Hypothesis 4b*:** Frontal and sagittal plane hip angles calculated by the PhysiMax^{TM} markerless motion capture system will be valid measures of hip kinematics as compared to the current gold-standard of motion capture systems. Maximum joint angles will demonstrate the best agreement between motion capture systems, followed by joint angle displacements, and then joint angles at initial ground contact.

***Hypothesis 4c*:** Frontal and sagittal plane knee angles calculated by the PhysiMax^{TM} markerless motion capture system will be valid measures of knee kinematics as compared to the current gold-standard of motion capture systems. Maximum joint angles will demonstrate the best agreement between motion capture systems, followed by joint angle displacements, and then joint angles at initial ground contact.

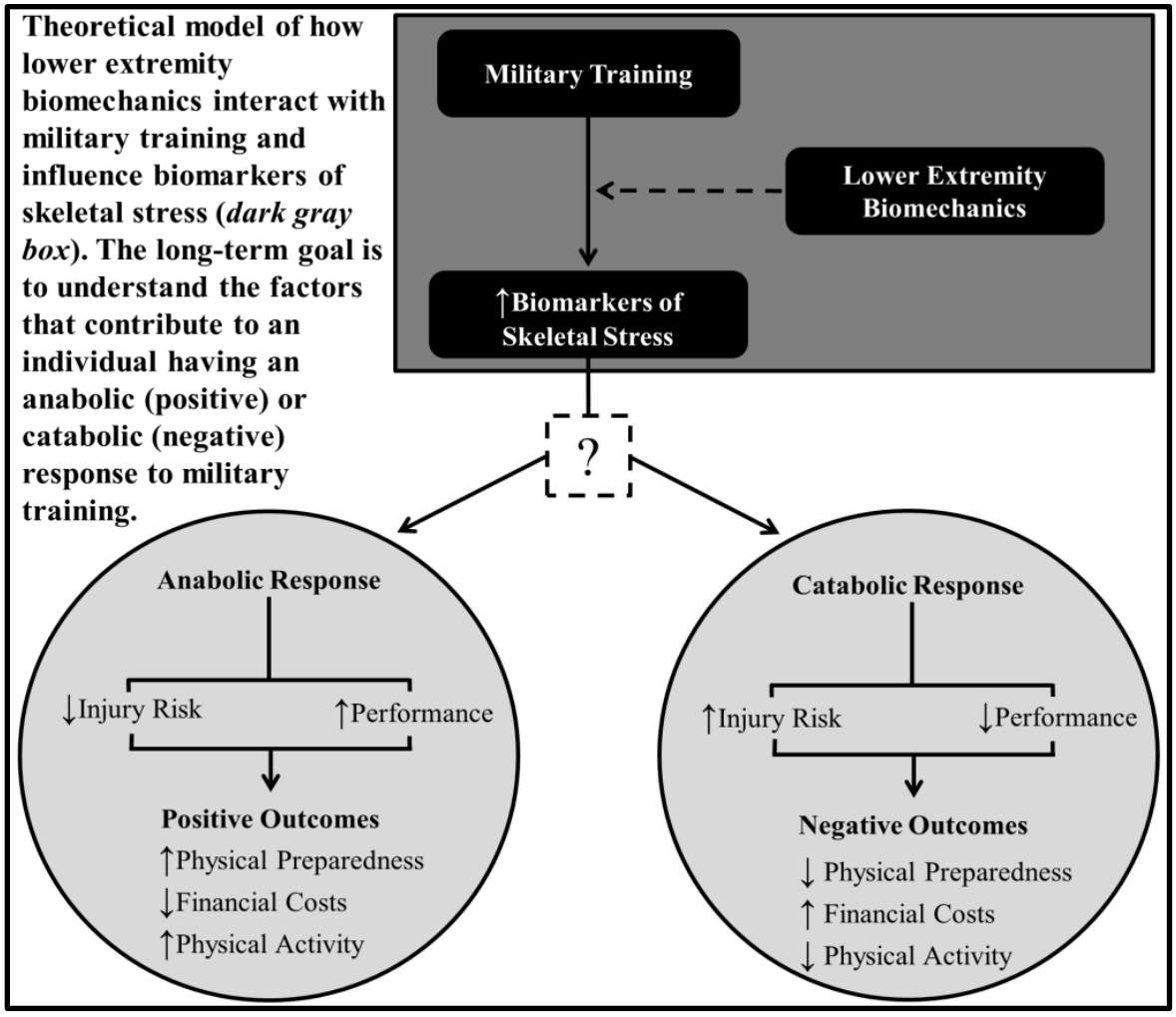
***Hypothesis 4d*:** Sagittal plane ankle angles calculated by the PhysiMax^{TM} markerless motion capture system will be valid measures of ankle kinematics as compared to the current gold-standard of motion capture systems. Maximum joint angles will demonstrate the best agreement between motion capture systems, followed by joint angle displacements, and then joint angles at initial ground contact.

1.7 – Significance

Given the high prevalence of stress fractures during military training and their substantial short- and long-term consequences, it is critical to understand the factors that increase injury risk during military training. Thus, the contribution of this study is it determined how known stress

fracture risk factors influence bone turnover biomarkers during military training. This contribution is significant because it is a major step towards understanding why some individuals have an anabolic (positive) response while others have a catabolic (negative) response to military training (**Figure 1.1 – Theoretical Model**). Understanding the influence of biomechanics on biomarkers of skeletal stress will allow for the development of intervention strategies to reduce injury risk and optimize performance during military training. These intervention strategies will positively impact the physical readiness of our military and reduce the enormous costs of musculoskeletal injuries.[3,4]

Figure 1.1 – Theoretical Model



CHAPTER II

REVIEW OF THE LITERATURE

2.1 – General Information and Introduction

Lower extremity musculoskeletal injuries significantly affect military personnel.[2,49] These injuries result in substantial medical costs, forced attrition from physical activity,[3,50,51] and long-term physical[52,53] and financial consequences.[54] The direct and indirect costs associated with musculoskeletal injuries cost the Department of Defense $3.7 billion annually.[3] Training related injuries not only affect non-deployed military personnel, but are among the top reasons why individuals are medically evacuated from war zones.[55-57] Thus, they are a primary concern for military commanders[58] and healthcare professionals.[14] Of particular importance are lower extremity fractures that result in the greatest amount of lost duty time.[34] Many lower extremity fractures result from overuse mechanisms, and thus are preventable.[1,4,59] It is therefore essential to identify the factors that increase lower extremity stress fractures risk so that targeted injury intervention strategies may be implemented.[58]

Lower extremity non-contact injury risks are multifactorial in nature.[14,33,35,37,38,40-43,45] 1 primary predictor of non-contact injury is lower extremity biomechanical patterns.[9,10,12,14,35,36] Laboratory based movement assessments effectively identify high-risk biomechanical patterns,[9,10,60] but these assessments are largely inaccessible to sports medicine clinicians. Therefore, clinicians use field-expedient movement assessments to identify individuals at increased injury risk.[11,12,14] The Landing Error Scoring System (LESS) is an example of such a movement assessment.[11,12] LESS scoring has recently been automated with a markerless motion

capture system; however, the kinematic measures calculated by this system have yet to be validated.[16]

Biochemical markers (biomarkers) of bone turnover may be beneficial in identifying individuals at high-risk of stress fracture, prior to them becoming injured.[5,17,18] Bones are dynamic tissues that are constantly remodeling.[61] As boney tissue remodels, proteins are cleaved off the ends of procollagen and collagen fibers during the formation and resorption processes. These proteins can be measured in the blood.[62-66] Previous research has found changes in bone turnover concentrations resulting from military training,[5,17,27] and that aberrant lower extremity biomechanics can increase stress fracture risk.[9] However, it is still unknown how lower extremity biomechanics and other stress fracture risk factors influence bone turnover biomarker concentrations during military training.

2.2 – Military Training Related Injuries

2.2a – Military Training Related Injuries: Military Training

Military training is highly repetitive but also involves episodes of high intensity exercise. Military training results in high training loads[8] that are associated with high lower extremity injury rates.[1,4,6-8] Military training involves planned events designed to challagne the human body and improve aerobic and anaerobic fitness. In addition to the planned physical training events, military personnel further stress their bodies through running and marching between training events. This additional running can add an additional 18 miles a week of weight-bearing activity, further stressing the musculoskeletal system.[39]

The average physical activity of military recruits has been tracked during United States Army basic training.[67] On average, recruits were sedentary 419 minutes/day, completed light

physical activity 219.5 minutes/day, moderate physical activity for 74.5 minutes/day, moderately intense physical activity 97 minutes/day, and vigorous physical activity 22.1 minutes/day. These same recruits stood for 522 minutes/day, sat for 271 minutes/day, walked for 103.5 minutes/day, completed menial chores 119 minutes/day, participated in calisthenics for 44 minutes/day, and engaged in load carriage for 449 minutes/day.[67] The cumulative effects of military training increase musculoskeletal injury risk.[1,4,6-8]

Military personnel are carrying heavier loads than they ever have historically.[68] External load carriage increases stress on the musculoskeletal system. The increased stress occurs rapidly and the musculoskeletal system may not have sufficient time to adapt and withstand the greater loads. External loads change trunk and lower extremity biomechanical patterns that can increase injury risk.[68] Brown et al.[69] showed greater external loads negatively affect jump-landing biomechanical patterns. Medium (20kg) and heavy (40kg) loads resulted in more stiff landings with less hip but not knee flexion, compared to a light (6kg) load. Normalized vertical ground reaction forces also increased with each increase in external load.[69] Collectively the increased stress placed on the musculoskeletal system by external loads and the aforementioned changes in lower extremity and trunk biomechanics increase injury risk.[68,69]

A number of different military occupational specialties (MOS) exist that require unique training, but the vast majority of basic training is similar across military branches. One of the most common MOS is the United States Army infantryman.[70] Infantrymen commonly carry loads in excess of 65 pounds while walking and running up to 25 miles a day.[40] The large internal and external forces placed on the musculoskeletal system during infantry training can result in training "overload" which occurs when training stress is not balanced with adequate recovery.[31,32,71] Training overload results in increased injury risks with no subsequent gains in

physical fitness and potentially a loss of physical performance.[3]

2.2b – Military Training Related Injuries: Epidemiology

Musculoskeletal injury is the primary medical issue limiting military physical readiness.[2] During Army basic training over 45% of male recruits sustain at least one musculoskeletal injuries of which nearly half are overuse lower extremity injuries.[37] Similar injury rates (58.5%) are observed among British infantrymen, during pre-deployment training; 30-35% of these injuries are directly related to soldier specific physical training.[38,72] Among the most commonly injured non-deployed military personnel are United States Army infantrymen.[40] Injury rates among these individuals are as high as 1.42 injuries per infantryman.[34] The peak incidence of musculoskeletal injuries occurs between weeks 4 and 6 of training.[5,26]

Lower extremity and low back injuries are the most commonly reported injuries amongst individuals completing military training.[34,37,38,40,72] The most common lower extremity injury sites include the knee (18.5%),[40,41,72] ankle (16%),[41,72] and lower leg (8%).[41,72] Lower extremity stress fractures, effect 2-32% of military trainees.[4,5,17,41,55,59,73] Nearly half (46%) of these stress fractures occur within the first 4 weeks of training.[5]

Acute lower extremity injuries are also problematic for militaries and share many of the same risk factors as chronic lower extremity injuries.[38,40,72,74] Wilkinson et al.[38] showed 83% of British infantry training related injuries were acute while only 13% of injuries were chronic.[38] Ankle sprains account for the vast majority of acute lower extremity injuries during military training,[72,75] and because of their high prevalence (35 injuries per 1000 person-years) their cumulative effects are problematic.[72,75] Acute anterior cruciate ligament (ACL) injuries occur less frequently (3.3 injuries per 1000 person-years), but because of their resulting care and extensive rehabilitation they are also problematic.[74,76] ACL injuries are the leading cause of

training and sport related hospitalizations in the United States Army.[76] The rates of ACL injuries among military personnel are nearly 10 times greater than general civilian population ACL injury rates.[77,78]

Musculoskeletal injuries are the primary medical issue limiting military physical readiness.[2,38] Musculoskeletal injuries largely contribute to the United States Army's deployment readiness being at only 85%[79] because they result in substantial lost duty time.[3,34,38,41,76] Lost duty time is the total number of days a soldier is unable to perform regular duties; this is a combination of days spent in the hospital, days on convalescent leave (time to recover), and days in a medical holding company.[76] Musculoskeletal injuries result in total limited duty days equivalent to 68,000 service members annually.[3] Lost duty days reduce training and operational effectiveness and increase demands on medical care providers.[38]

Training and sport related injuries account for 11% of all military hospitalizations. Males miss 13 days per musculoskeletal injury requiring hospitalization[76] and miss approximately 27 days per 100 person-weeks due to injury during United States Army basic training.[41] Fractures account for the largest amount of lost duty days.[34] Specifically, the average stress fracture rehabilitation requires 63 days to complete.[59]

There are long-term consequences associated with musculoskeletal injuries. Musculoskeletal disorders account for 51% of all United States Army disability cases.[40] These cases require long-term medical care and result in high financial costs.[3] Significant links have been observed between acute joint injuries and post-traumatic osteoarthritis[52-54] and early biochemical cartilage metabolism changes following acute joint injury.[28,29] Post-traumatic osteoarthritis likely contributes to the substantially higher rates of arthritis among United States military veterans (1 in 3) compared to the general population (1 in 5).[80]

2.2c – Military Training Related Injuries: Risk Factors

A number of factors have been identified that increase military training related musculoskeletal injury risk.[14,33,35,37,38,40-43,45] Typically these factors are classified into those that are modifiable in nature, and those that are non-modifiable in nature. Modifiable risk factors include biomechanical patterns,[9,11,35,36,45,81] training load,[6,30-32] aerobic and anaerobic fitness,[14,33-40] physical activity preceding military training,[33,35,38,41-43] body composition,[17,33,44] and lifestyle choices.[14,33,37,45] Non-modifiable risk factors include previous history of musculoskeletal injury,[37,45] age,[37,38] race,[34,37,44] and sex.[44,46]

2.2c.1 – Military Training Related Injuries: Risk Factors – Biomechanical Patterns

Aberrant movement patterns are a primary predictor of acute and chronic lower extremity injuries in military and civilian populations.[9,10,12,14,35,36] Aberrant biomechanical patterns can result from static skeletal malalignments[61] but are more commonly the result of neuromuscular control deficiencies.[10,11] Aberrant biomechanics increase the forces acting on normally aligned lower extremity segments or may cause normal forces to act on abnormally aligned lower extremity segments. Both of these examples can occur simultaneously, which results in further abnormal musculoskeletal loading and increased injury risk.[61]

Laboratory based[9,10,60] and field-expedient[11,12,14] movement assessments effectively identify aberrant, high-risk biomechanical patterns. Jump-landing,[9-12] squatting,[82,83] and lunge[35,36,45,81] movement assessments are commonly employed to identify individuals at increased musculoskeletal injury risk.

The Landing Error Scoring System (LESS) is a valid and reliable field-expedient jump-landing movement assessment.[11] The LESS has been utilized with military units to assess individual movement quality, en masse.[9,11] The LESS requires individuals to complete a jump-

landing movement assessment while being videotaped from frontal and sagittal plane views. The videos are replayed and scored by trained raters using a standardized rubric to identify lower extremity and trunk movement errors. Items on the LESS are evaluated at initial ground contact, peak knee flexion, and the time between initial ground contact and peak knee flexion (landing phase). A larger LESS score is indicative of more aberrant biomechanical patterns than a smaller LESS score.[11] The LESS is able to discriminate between individuals with high-risk (i.e. aberrant) biomechanics and individuals with low-risk biomechanics.[11,12] Individuals who score ≥5 on the LESS have a greater risk of non-contact anterior cruciate ligament injury.[12]

The LESS has excellent intra-rater ($ICC_{2,k}$=0.84, SEM=0.42) and good inter-rater reliability ($ICC_{2,1}$=0.91, SEM=0.71).[11] The originally validated LESS scoring rubric has been expanded from the original 17-item LESS rubric to a 22-item LESS rubric. The 5 additional LESS items include: further clarification of asymmetrical foot contact (timing and plantar flexion, 1 item each); excessive trunk flexion displacement; asymmetrical weight shift; and knee "wobble."[84]

Females display significantly different trunk and hip biomechanical patterns during landing tasks, compared to males.[11,85] Specifically, during jump-landing assessments, females have higher LESS scores, indicating greater injury risk. Females also display greater hip flexion and greater knee valgus at initial ground contact during stop-jump and drop-landing movement assessments. Females also have significantly more knee flexion at initial ground contact during the drop-landing, compared to males. [85] Because of these differences in biomechanical patterns during landing assessments, females were not included in this study.

The Functional Movement Screen (FMS) is commonly used among military personnel to assess lower extremity injury risks.[35,36,45,81] The FMS incorporates 7 unique movement

assessments that examine upper extremity, trunk, and lower extremity movement quality. The FMS incorporates the deep squat and forward lunge movements to assess lower extremity and trunk biomechanics. Each test is visually sored real-time by a trained rater on a 4-level ordinal scale (0-3). Individual test scores are summed to provide a total score ranging from 0-21. Contrary to the LESS, lower FMS scores indicate poor movement quality while larger FMS scores indicate better movement quality.[86,87] The FMS has similar inter-rater reliability (κ_{range}=0.31-1.00; κ_{avg}=0.74±0.18) as the LESS.[88-90]

The FMS can identify military personnel at increased musculoskeletal injury risk.[35,36,81] Male United States Marine Corps officer candidates who score ≤14 on the FMS are at 2 times greater acute musculoskeletal injury risk during training than individuals who score >14. 45.8% of individuals with a cumulative FMS score ≤14 sustain an injury while only and 30.6% of individuals with FMS >14 sustain an injury. [36] Similar findings have been reported with similar cohorts of Marine Corps officer candidates and male United States Coast Guard cadets that go on to sustain a training related injury compared to those who do not (injured = ≤11, uninjured = ≥12).[35,81] Finally, United Starts Army Rangers who have pain with an FMS clearing test are at greater risk of sustaining a musculoskeletal injury.[45] FMS performance and subsequent musculoskeletal injuries demonstrate how biomechanical factors influence injury risks.

2.2c.2 – Military Training Related Injuries: Risk Factors – Training Load

External training load is a key determinant of injury risk in both military[3,30,33,38,39] and civilian[6,31,32,91] populations. Large increases in 1-week and 2-week cumulative training loads are associated with greater injury risk in civilian populations.[6,31,32,91] Similar trends are observed in military personnel as they enter into new training regimens.[39] Drastic increases in week-to-week totals of physical activity may result in muscle fatigue. As muscles become fatigued they are less

capable of attenuating forces and thus more force is transmitted to underlying bone, increasing skeletal stress and bone injury risk.[61] Acute muscle fatigue alters lower extremity biomechanical patterns that can further increase injury risk.[92-94] However, it is still unknown how repetitive, chronic bouts of fatiguing exercise alter biomechanics and effect injury risks.

Individuals entering the military likely have poor physical fitness levels prior to beginning military training. Military personnel come from the general American population, of which only 22% adhere to the American College of Sports Medicine guidelines for physical activity.[39] However, individuals entering military basic training self-report that they complete significant physical activity prior to training.[37] 14.9% of trainees report running 4+ days/week and 49.3% report running 1-3 days/week; 28.1% report participating in physical activity other than running 4+ days/week and 49.5% report participating in physical activity other than running 1-3 days/week. 60.6% of individuals report being more active than "average" and only 9% report being "inactive."[37] The potential exists that individuals are unaware of how physically active they actually are or how active they should be. Thus, they believe they are more physically active and fit than they actually are. Regardless, as individuals enter military basic training they have large increases in physical activity which increases injury risk.[39]

One issue with military training is that it employs a "one size fits all" training format. All military personnel in the same unit complete the same physical training, regardless of their current physical fitness levels or past experiences with physical activity.[95] When soldiers are deployed, unit level required physical training decreases but personal physical training increases. Individualized personal training results in substantial gains in physical fitness, compared to unit based training. Furthermore, training related musculoskeletal injury rates drop from 36.2 to 19.0 injuries per 1000 soldiers when soldiers are deployed.[95] These findings suggest that when

individuals train on their own they decrease their injury risk.

However, other studies show that when individuals have the option to engage in physical fitness outside of required military training they increase their injury risk.[33,38,39] Individuals who run the most, additional to the running required by the military, have greater lower extremity injury risk, but no additional gains in physical fitness.[33,39] Conversely, individuals who minimally participate in physical fitness training in addition to what is required by the military are at greater injury risk.[38] Collectively, these studies suggest that a minimum level of physical training is needed to stay fit and minimize the risk of injury, but if excessive physical training occurs there is increased injury risk, with no subsequent gains in physical performance.[3]

The type of physical training is also an important determinant of injury risk.[7,30] Long-duration continual impact loading increases musculoskeletal injury risk. When the cumulative duration of training is reduced, and programs implementing variable training speeds and durations are implemented, United States Marines have substantial reductions in musculoskeletal injuries and improvements in physical fitness.[30] This is supported by Jones et al.[37] who showed military units that complete the greatest amount of running have a greater incidence of lower extremity injury (41.8%) compared to units that complete the least amount of running (32.5%, rate ratio = 1.3). These units have no differences in physical fitness levels.[37] Similar results are observed in civilian populations.[7]

2.2c.3 – Military Training Related Injuries: Risk Factors – Physical Fitness

Physical fitness levels prior to military training influence musculoskeletal injury risk during training.[33-37,43,45,96,97] This is especially true of aerobic fitness, which is a key component of military training. Poor aerobic fitness and aberrant biomechanics increase the work the body has to do and in-turn increase musculoskeletal stress and injury risk.[41] Individuals with slow run

times, an indicator of poor aerobic fitness, and a low cumulative FMS scores are 4.19 times as likely to sustain an injury as individuals who do not have poor aerobic fitness or movement quality.[35]

Performance on military standardized assessments of physical fitness is a key indicator of who goes on to sustain musculoskeletal injuries during military training and who does not. Overall low performance on standardized assessments of physical fitness increases injury risk.[33,36] Low performing Marine Corp officer candidates (<280 points out of 300 available points) were 2.2 times more likely to sustain an injury as high performing candidates (≥280).[36] Run assessment performance is most predictive of injury risks, especially lower extremity stress fractures.[34,35,37,45,97] Non-deployed United States Army infantrymen in the slowest 2-mile run time quartile are 1.6 times more likely to be injured than those in the fastest quartile.[34,37]

Muscular strength also plays an important role in injury risks.[4034,37,43,45,96,97] Military recruits who are ≥1 standard deviations below the mean for muscle strength, as measured by a 1-repetition max leg-press, are at greater lower extremity stress fracture risk.[43] Multiple studies show that low performance on the sit-up component of standardized military physical fitness assessments also increases injury risk.[34,37,45,97] United States infantrymen in the lowest quartile for number of sit-ups are 1.9 times more likely to be injured than those in the highest quartile.[34,37] Finally, upper extremity strength may be representative of total body strength as poor performance on push-up assessments is indicative of greater stress fracture risk.[40,96]

Other studies have found no differences in physical fitness assessment performance and injury risk.[34-37,43,96] Aerobic fitness,[43,96] sit-up performance,[35,36] pull-up performance,[35,36] and push-up performance[34,37] may not differ between individuals who go on to sustain and injury and individuals who do not. It is important to note, these are the minority of studies that examined

associations between physical fitness and lower extremity injury risk during military training and are not representative of the body of literature as a whole.

2.2c.4 – Military Training Related Injuries: Risk Factors – Previous Physical Activity

Previous experience with weight-bearing physical activity is protective against lower extremity musculoskeletal injuries during military training.[33,40,43,96] Male military recruits who are not physically active prior to starting military training have substantially more limited duty days, than recruits who participate in physical activity prior to training.[43] Individuals with low prior running and exercise frequency are at the greatest risk of injury. Also, individuals who rate themselves as less physically active than average and exercise less are at increased injury risk.[37] Finnish military conscripts who engage in brisk leisure time physical activity prior to military training experience fewer overuse musculoskeletal injuries during their initial military training than individuals who do not engage in weight-bearing physical activity.[40] Finally, military personnel who perform resistance and agility training prior to military training also have lower injury risk.[33]

Similar trends are observed for stress fracture risks among United States military trainees.[35,43,96] United States Naval recruits who do not participate in weight-bearing intensive sports prior to entering military training are at greater risk of sustaining a stress fracture.[96] Marine Corps officer candidates who participate in sports or physical activity <5 times a week are 1.81 times more likely to become injured than individuals who participate in sports or physical activity ≥5 or more times a week.[35] Other studies looking at similar populations found no differences in injury risks when looking at previous weight training frequency or duration and frequency of running.[35]

2.2c.5 – Military Training Related Injuries: Risk Factors – Demographic Measures

Height, mass, and body composition have all been identified as potential risk factors for musculoskeletal injury,[33,40] and specifically stress fractures during military training.[17,44,96,97] Individuals who are underweight,[17,40,44] overweight,[40] or obese[33] based on their body mass index (BMI = mass [kg] / (height [cm]^2)[48] are at increased musculoskeletal injury risk during military training. Similar findings are observed when looking at stress fracture risk specifically. Individuals with low body weight,[44,97] shorter individuals,[97] and taller individuals[17] are also at greater stress fracture risk. However, these findings are not consistent.[17,96]

Interactions exist between measures of body composition and physical fitness. As previously described, poor physical fitness is a primary predictor of future musculoskeletal injury during military training. Individuals who are either underweight or overweight and have poor performance on a Cooper's run test, a measure of aerobic physical fitness, are more likely to sustain an injury.[40]

Measures of skeletal length and width are also potential risk factors for lower extremity stress fractures among military personnel.[96] Male, United States Naval recruits who go on to sustain stress fractures have significantly longer tibias and near significantly smaller thigh girth.[96] The smaller thigh girth may be an indication of less muscle mass. Muscle mass is an important factor as muscles absorb forces and help to attenuate forces that would otherwise act through the bone.[61] Similarly, United States Marines with smaller pelvic width are more likely to sustain a stress fracture than healthy controls.[97] Lower total body bone mineral content also increases stress fracture risk during military training.[96]

2.2c.6 – Military Training Related Injuries: Risk Factors – Unmodifiable Risks

There are a number of unmodifiable risk factors that impact lower extremity injury risk

during military training. These factors include previous history of musculoskeletal injury,[35,37,45] age,[37,38] race,[34,37,44] sex, [44,46] and history of smoking.[33,37,45] It is important to consider and assess these risk factors when determining lower extremity injury risks.

Previous history of musculoskeletal injuries increase overuse[37,45] and acute injury risk.[98,99] Military cadets at the three largest United States military academies completing Cadet Basic Training are at increased risk for medically treated lower extremity injuries if they have a history of previous injury. Importantly, this increased injury risk was observed specifically for lower extremity stress fractures.[99] Similarly, United States Army Rangers with a previous history of musculoskeletal surgery, history of recurrent musculoskeletal injury, or limited duty days in the preceding year as the result of injury are at increased risk of sustaining an overuse musculoskeletal injury during training.[45] Lisman et al.[35] reported there is no increase in overuse or acute lower extremity injury risk among United States Marine Corps officer candidates with a previous history of injury, but individuals with a previous history of lower extremity injury are at an overall greater risk of future injury. Similar trends in increased injury risk following initial musculoskeletal injuries have been observed in civilian populations.[98]

A multitude of studies have identified age as an injury predictor.[37,38,44,70] However, both younger[38,70] and older[37,44] age have been identified as risk factors. Civilian studies show individuals younger than 30 years are at increased risk of sports-related musculoskeletal injuries; this is important to note because 70% of active duty military personnel are <30 years old.[70] Younger British soldiers completing pre-deployment training were at increased risk of musculoskeletal injury risk. This is likely because the younger soldiers hold lower ranks and are engaged in more physically demanding jobs than older, more experienced, soldiers.[38] However, males that are older than 24 years completing United States Army basic training are at a greater

risk of any musculoskeletal injury than individuals younger than 19 years.[37] This is supported by Knapik et al.[44] who report high rates of lower extremity stress fractures among older individuals completing military training. Other studies report no associations between injury risk and age.[34,96] In studies that age is not a predictor of future injury, it is likely no difference was observed in injury rates between age groups because the study populations were very homogenous, with minimal differences in age between military trainees.[96]

An individual's race[44] and sex[44,46] also influence lower extremity injury risk. United States military personnel who are black have decreased stress fracture risk, compared to all other races.[44] However, this finding may only be relevant to stress fracture risk, and may not be pertinent when any musculoskeletal injury risk is evaluated.[37] Females, compared to males, are at increased risk of injury in both military[41,44,46,100] and civilian[51,101] populations. Because of the discrepancies in lower extremity injury rates between males and females, females were excluded from the study.

History of tobacco smoking is a strong predictor of musculoskeletal injury, especially stress fracture.[20,23,26] Individuals with a history of smoking have a greater injury risk than individuals who do not have a history of smoking.[20,23,26] Smoking impairs tissue healing[102] and negatively affects bone mineral density.[103] Collectively, impaired tissue healing and low bone mineral density increase stress fracture risk since the bones are weaker to begin with and require prolonged healing time as the result of smoking.

2.3 – Bone Tissue

Bone is a metabolically active tissue that continuously undergoes remodeling involving bone resorption and formation.[63,65,66] Bone matrix is 90% type I collagen and 10% non-

collagenous proteins.[62,66,104] Type I collagen is also found in skin, dentin, cornea, vessels, fibrocartilage, and tendons.[62,63] Type I collagen is formed by osteoblasts in the form of pre-procollagen. Pre-collagen molecules contain amino-terminal (procollagen type I aminoterminal propeptide [PINP]) and carboxy-terminal propeptides (procollagen type I carboxyterminal propeptide [PICP]). These propeptides are cleaved off of the end of the pre-collagen molecules as new type I collagen is formed.[66]

Bone tissue remodels throughout life in response to physical load (e.g. ground reaction and muscular forces) and the metabolic environment.[63,65,105] Bone remodeling helps maintain healthy bone density.[66] Bone remodeling takes place on the surface of the bone and is regulated by osteoblasts (formation), osteoclasts (resorption), and osteocytes (maintenance);[63,66] these cells all interact in tightly coupled processes.[39,41] Bone remodeling strongly influences bone properties, including collagen and bone-specific proteins.[106]

Bone remodeling is initiated by increased bone resorption.[27] Generally, bone resorption takes 7-10 days while formation takes 2-3 months.[39,41] The necessary substrates must be present for bone to remodel. If these substrates are not present it can result in bone resorption with limited bone formation, creating weakened bones.[63,65] During normal bone growth, bone formation exceeds resorption and bone tissue is gained. This process can be inhibited in pathologic populations and more bone tissue is lost as resorption exceeds formation. If bone tissue is lost, bone mineral density drops, there is a loss in trabecular integrity, and increased fracture risk.[63,65]

2.3a – Bone Tissue: Stress Fractures

Lower extremity stress fractures are a major concern for military administrators[34] as they affect a large portion of individuals completing military training.[4,5,17,41,55,59,73] Lower extremity

stress fractures result in significant lost duty time ranging from 13.1-23.6 weeks.[107] Lost duty time negatively impacts the military's readiness status.[2]

Lower extremity stress fracture risks are multifactorial in nature. These factors include bone composition, vascular supply, surrounding muscular attachments, systematic factors, and the type of physical activity an individual is engaged in.[108]

Bone remodeling is vital for bone health and maintaining "skeletal competence." This is especially true for "targeted remodeling" that occurs in response to internal and external loading factors.[61,63,65,105] Bone remodeling is dependent on a "feedforward" mechanism in which bone resorption precedes bone formation. This feedforward mechanism is largely controlled by the amount of bone deformation that occurs during weight-bearing activities.[61,105] Factors that influence the amount of bony deformation include: the number of bone strain cycles, strain magnitude, and the strain rate [61,105]

Strenuous exercise increases connective tissue matrix protein (e.g. collagen) turnover rate.[105,109] Torsion and bending stresses are concentrated in the bone cortex.[97] Repetitive torsional and bending forces increase cyclic hydrostatic pressures which are sensed by osteocytes within the bone matrix. These mechanical pressures stimulate osteoclasts to begin resorbing cortical bone, and initiate the bone remodeling process.[27] Initially osteoclastic activity outpaces osteoblast activity, resulting in greater bone resorption than formation[44,61] causing "microfatigue damage."[27,109,110] Accelerated bone remodeling may compromise bone strength at fracture prone sites because mineralization of new bone is inhibited.[111] This results in a vulnerable period when the bone is weakened and susceptible to stress fracture.[44,61] Thus, bone stress injuries result from the bone not withstanding repetitive mechanical loading that results in structural fatigue.[61]

Endurance athletes are at increased stress fracture risk. Endurance athletes commonly

engage in repetitive weight-bearing activities and also may have low testosterone levels.[108] Testosterone inhibits interleukin-6, which enhances osteoclast development. If interleukin-6 is not inhibited by testosterone it will enhance osteoclast development which will lead to increased bone resorption that may not be offset by bone formation.[108]

Stress fractures can occur on the compression ("low-risk") or tension ("high-risk") side of a bone's bending axis.[107] High-risk stress fractures require additional time to heal and are more likely to result in non-union and complete fractures, compared to low-risk stress fractures.[107] It is important to consider the fracture location within a bone when developing a rehabilitation plan.

2.3b – Bone Tissue: Biochemical Makers of Bone Turnover – General Information

Type I collagen synthesizes or resorption releases biochemical markers (biomarkers) in the form of enzymes and proteins into the bloodstream which can then be measured via laboratory analyses.[63,65,66] Bone formation and resorption also releases these biomarkers.[62,63] Biomarkers reflect the bone remodeling process and can reveal acute changes in bone turnover (formation vs resorption).[66] Many biomarkers representative of bone formation and resorption can also be found in other tissues. However, non-skeletal tissues have slower turnover rates than bone and contribute very little to the circulating serum concentration levels.[62,63] Biomarkers provide a more dynamic measure of bone turnover than more static measures including x-ray and dual-energy x-ray absorptiometry (DEXA).[26,65,106] Therefore, biomarkers can effectively evaluate bone quality.[18,64]

Pre-collagen molecules contain amino-terminal and carboxy-terminal propeptides; measurement of these pro-peptides are considered to be quantitative measures of new type I collagen synthesis.[66] Procollagen type I aminoterminal propeptide (PINP) and Procollagen type I carboxyterminal propeptide (PICP) have been identified as viable biomarkers of bone formation.

[18,62,63,106] Both PINP and PICP are specific products of proliferating osteoblasts and fibroblasts, and are cleaved off the ends of pre-collagen molecules as type I collagen is formed.[66] As PINP is cleaved off the ends of the pre-collagen molecules it enters the blood stream and circulates as 2 fragments in the serum (100-kDa and 30-kDa fragments) that are detected by immunoassays.[66] PINP and PICP concentrations are predominately associated with bone formation, but can also be released into the blood stream during other soft tissue formation, including skin.[35,38,39,92]

Serum concentrations of PINP and PICP can be effectively analyzed with commercially available enzyme-linked immunosorbent assays (ELISA). Assays evaluating PINP serum concentrations correlate better with bone formation and therefore have better diagnostic validity than assays evaluating PICP serum concentrations.[38,39] Furthermore, PINP assays have good performance in clinical trials, are easily available, have relatively low variability, and good stability; therefore serum PINP is recommended by the International Osteoporosis Foundation as the biomarker of choice for assessing bone formation.[18]

Carboxy-terminal crosslinking telopeptide of type I collagen (CTx-1) is specific to type I collagen. CTx-1 is found in all tissues containing type I collagen, but has the highest percentage coming from bone.[18,62,63,66] Free CTx-1 can be analyzed in either serum or urine, but similar to bone formation markers, serum concentrations appear to be more stable.[18,62,63,66] However, because of the biological variability in CTx-1 measures, the differences between 2 measures must vary by a minimum 54% to be considered clinically meaningful.[66]

Ratios of bone formation and resorption biomarkers are superior to looking at either makers of formation or resorption alone as measure of bone turnover and health.[65] Simultaneous measurement (ratios) of the 2 free forms of CTx-1, CTx-1α and CTx-1β may be representative of bone turnover.[62] However, CTx-1β can be measured with ELISA easier than CTx-1α, which may

require radioimmunoassay. Therefore, it is recommended that CTx-1β be analyzed in conjunction with a biomarker of bone formation to assess bone turnover.[63]

CTx-1 is considered to be better than other biomarkers to assess bone resorption[66] because of its performance in clinical trials, availability, relatively low variability, and good stability. Therefore, it has been recommended by the International Osteoporosis Foundation as the preeminent biomarker for assessing bone resorption.[18]

2.3c – Bone Tissue: Biochemical Makers of Bone Turnover – Response to Physical Activity

2.3c.1 – Bone Tissue: Biochemical Makers of Bone Turnover – General Physical Activity

Bone remodeling is essential for maintaining healthy levels of bone tissue. Bone remodeling is stimulated by weight-bearing activity.[61,63,65,105] There is an initial increase in bone resorption, followed by bone formation. Changes in bone resorption and formation, in response to physical activity, can be detected by biomarkers indicative of the bone remodeling process. [19-24,112]

A study of male high school students examined the effects of exercise on biomarkers of bone formation and resorption.[24] The participants were randomized into exercise and control groups. Both groups completed 2 hours of "activity" each day for 4 weeks. Individuals in the exercise intervention group completed aerobic and weight training activities while the control group completed computer work. [24] No significant differences were observed between groups for any biomarker at baseline testing. However, significant increases in biomarkers of bone formation (osteocalcin, bone-specific alkaline phosphate [BSAP], and PICP) were observed in the exercise group, but not the control group. There was also a significant decrease in N-terminal crosslinking telopeptide (NTx) but not CTx-1, biomarkers of resorption, in the exercise group, but not the control group.[24]

In contrast to the aforementioned study, high intensity, repetitive (3 week) non-weight-bearing cycling exercise resulted in an overall slowdown in bone turnover rate.[112] There were significant reductions in PINP, CTx-1, and NTx-1. These reductions were observed between day 1 and day 12 of the intervention, and PINP further decreased between day 12 and day 23.[112] This study is important because it indicates that muscle contraction alone is not sufficient in preserving bone strength. Muscle contraction must occur in conjunction with weight-bearing activity.[112]

Changes in bone biomarker concentrations have also been observed when physically active individuals stop participating in physical activity. Male professional soccer athletes were compared to healthy controls.[19] Immediately following the competitive soccer season the soccer players had significantly greater CTx-1 concentrations compared to controls. Following the cessation of activity, CTx-1 increased while PICP decreased within 2 weeks. Indicating that there was more bone resorption than formation during this period.[19] These same groups were also tracked as the soccer athletes returned to physical activity, and significate changes were observed after 10 days of increased activity. PICP significantly increased and CTx-1 decreased, suggesting that more bone formation was occurring in response to the physical activity.[19]

Acute changes in biomarkers of bone turnover also occur.[20-23] Following a long-distance running race there is a temporary inhibition in bone formation and stimulation of bone resorption in well trained men and women.[22] In males, bone resorption biomarkers are reduced following endurance[20,21] and strength training[20] activities, but no changes are observed in bone formation biomarkers for up to 32 hours following the bout of exercise.[21] Opposite changes were observed in regularly physically active females. PICP was reduced1 hour following 45 minutes of jogging, then significantly increased 24 and 72 hours later. CTx-1 significantly increased at 24 and 72

hours following activity.[23] Male and female bone biomarker concentrations respond differently to similar bouts of physical activity; for this reason this study limited its analyses of biomarkers to male participants.

Long-term human studies examining skeletal biomarkers and bone density measurements are needed to establish the net effect of exercise on bone metabolism.[23] This study is the first step in establishing such a long-term study with military personnel.

2.3c.2 – Bone Tissue: Biochemical Makers of Bone Turnover – Military Training

The high-intensity, repetitive nature of military training results in cyclic loading of the lower extremity.[8] This cyclic loading results in changes in musculoskeletal tissues that increase injury risk.[1,4,6-8] Many musculoskeletal tissue changes can be detected with biomarkers, prior to the onset of injury.[5,17,25-27,113]

Biomarkers of bone formation (PINP and bone-specific alkaline phosphate [BALP]) and resorption (CTx-1 and tartrate resistant acid phosphate [TRAP5b]) were tracked in male and female Israeli military trainees.[27] All biomarker concentrations were significantly higher in males than females at baseline and throughout the entire course of training.[27] Bone formation biomarkers significantly increased over time for both sexes. BALP increased from months 0 to 2, then did not change from 2 to 4 months. Females demonstrated a greater percent increase in PINP than males from 0 to 2 months. Bone resorption biomarkers changed similarly for males and females. CTx-1 increased from 0 to 2 months, then returned to baseline levels by 4 months. TRAP5b increased from 0 to 2 months, then did not change.[27]

No differences were observed in baseline measures (pre-basic training) of bone biomarkers (BALP, PINP, TRAP5b, CTx) between males who went on to sustain a stress fracture and those who did not.[5] Both groups displayed similar changes in bone formation

biomarkers throughout training. BALP did not significantly change for either group for the first 6 weeks (3% and 1% decrease for stress fracture and non-stress fracture groups, respectively) but significant changes occurred between weeks 0 and 18 (13% and 20% decrease for stress fracture and non-stress fracture groups, respectively). PINP did not significantly change between weeks 0 and 6 for either the stress fracture (2.9% decrease) or non-stress fracture (10.9% decrease) group; but significant decreases in PINP were observed for each group from week 0 to week 18 (stress fracture=22%, non-stress fracture=41%).[5] There were also similar changes in bone resorption markers between the 2 groups. TRAP5b did not change during the 18 week training period. CTx-1 levels significantly decreased between weeks 0 and 6 (stress fracture=18%, non-stress fracture=17%). There was a slight increase in CTx-1 levels at week 12 (non-significant), but then the CTx-1 levels returned to week 6 levels by week 18. At week 18 the stress fracture group had less change in CTx-1 than the non-stress fracture group; no other differences in changes of bone biomarkers were observed.[5]

Female soldiers completing similar military training to the previous study[5] had significant changes in bone formation biomarkers (PINP, BALP) and bone resorption biomarkers (CTx-1, TRAP5b). PINP and BALP significantly increased pre-to-post-basic training in individuals who went on to sustain a stress fracture and those who did not; there was no difference in the amount of change between groups.[17] Bone resorption biomarkers (CTx-1, TRAPb) did not significantly change from pre-to-post-basic training.[17] Both findings of this study directly contrast the changes observed in male military personnel completing similar training.[5]

Baseline concentrations of bone formation biomarkers (osteocalcin and BALP) were significantly lower in males completing basic military training who went on to sustain any musculoskeletal injury, not specific to stress fracture. Significant decreases were observed in

osteocalcin and BALP and a non-significant decrease in TRAP5b were observed among all males, regardless of future injury.[26] The overall decrease in biomarkers of formation and resorption indicates there was less bone turnover following military training. The potential exist that this training did not sufficiently load the musculoskeletal system and thus no changes in biomarker concentrations were observed.

Deoxypyridinoline (DPD), a cross-link of collagen fibers specific to bone (representative of bone resorption) was examined in male and female United States Marine recruits.[113] DPD levels were significantly higher at weeks 10 and 11 compared to baseline for males, and weeks 2, 8, 9, 10, and 11 were higher for females when compared to baseline. At week 6 the percent change decreased for females and increased for males, and at week 9 the percent change increased for females and decreased for males. Overall mean concentrations were greater in females (6.02) compared to males (5.42). There were no differences between DPD concentrations in females with stress fractures and healthy controls. This same analysis was not completed for males because of a low number of stress fractures in males.[113] In a similar cohort of females completing military training, females had significant increases in biomarkers of bone formation (PINP and BALP) and bone resorption (CTx-1 and TRAP5b) at the end of basic training.[25]

The previous studies highlight the changes that occur in biomarkers of bone formation and resorption during military training. These studies also highlight the differences in changes between males and females completing similar military training. Because of the different responses of males and females to similar military training, females were excluded from this study to eliminate the potential of sex confounding the study results.

2.3d – Biochemical Markers of Bone Turnover: Data Collection Considerations

Serum concentrations of bone turnover biomarkers are influenced by a number of external factors that should be controlled for as best as possible when examining these biomarkers. Serum concentrations of bone biomarkers are influenced by: diurnal variations,[5,17,22,24,27,63,66,106,109,114-119] food consumption,[5,17,22,25,27,63,119] and physical activity.[23,63,119120] Other key factors that have more chronic effects on bone biomarkers, include: renal function,[106,115 115,116] seasonal variations,[63,121] and the female menstrual cycle.[63,122]

Bone biomarker serum concentrations are influenced by the time of day during which the serum sample is collected. The majority of bone biomarkers show increases in formation[115] and resorption[115,116] during rest periods, even acutely. Bone biomarkers typically have the highest concentrations in the morning and the lowest concentrations in the afternoon and evening.[115-117] Diurnal variations for bone formation biomarkers are not as pronounced as variations in bone resorption biomarkers; bone formation biomarkers have longer half-lives than bone resorption biomarkers.[66] As such, diurnal variations do not appear to exist for PINP[63,114] but do exist for CTx-1.[118] In order to minimize the effects of diurnal variation on bone biomarkers, serum samples should be collected as early in the day as possible,[22,24,63,106,109,119] and ideally within the first hour of waking-up [5] or before 0800.[17,27]

While there are noted diurnal variations in serum concentrations of bone biomarkers, these variations are not as pronounced as they would be in urine samples.[35,39,41] Serum biomarker variability is between 5% and 10% while urine biomarker concentration variability is as high as 10% to 45%.[65] Therefore, serum biomarker concentrations appear to be more stable throughout the day and may be more representative of true bone turnover.[18,63,65]

Food consumption immediately preceding serum collections may influence some

biomarkers of bone turnover.[63] Protein rich foods (e.g. meat, eggs, milk) can alter the concentrations of collagen byproducts in the serum as the food is broken down and digested, which may be incorrectly identified as bone resorption byproducts, such as CTx-1.[63] PINP does not appear to be influenced by food consumption,[106] but this has not been definitively shown. Therefore, it is recommended serum samples are collected following a fasting period.[22,27] The typical fasting period is a minimum of 8-10 hours.[5,17,25,119] For these reasons, serum samples should be collected as early in the day as possible, ideally before the first meal of the day. Other studies have used non-fasting samples when the collection of fasting samples is impractical.[23,104] One example is a study that looked at overuse injury risks among Navy SEALS. The authors report that all study participants ate a "standard diet" so the risk of sample contamination from food consumption was equally likely for all study participants.[109] This study's participants are similar the West Point cadets examined in this study.

Episodes of acute exercise may also lead to artificially elevated levels of bone biomarker serum concentrations. [63,119] Previous studies have found increases in PICP following exercise.[23,120] Regularly physically active females had a significant reduction in PICP 1 hour following 45 minutes of jogging, then significant increases in PICP 24 and 72 hours later. There was no significant difference in CTx-1 concentrations 1 hour following activity, but significant increases at 24 and 72 hours.[23] However, these findings are not consistent across all studies. Kristoffersson et al.[123] found no changes in PICP or CTx-1 1 hour after short-term maximal exercise in male athletes, which suggest there is no pool of collagen biomarkers released following acute activity.

One proposed explanation for the observed changes in bone biomarker serum concentrations following acute bouts of physical activity is a plasma volume expansion.[23,124]

Fellmann et al.[124] observed plasma volume shifts following bouts of exercise. However, Thorsen et al.[23] did not find a change in plasma volume 1 hour following 45 minutes of jogging, but did find significant increases 24 and 72 hours following the bout of exercise. Until evidence is presented that definitely shows, or does not show, changes in skeletal biomarkers following acute activity and how potential plasma volume shifts affect these biomarkers, physical activity immediately preceding serum collections should be controlled.

Biomarker concentration variations resulting from seasonal[63,121] and hormonal [63,122] variations should be considered when designing long-term prospective studies. PICP concentrations are highest in the winter months.[63,121] Bone biomarker concentrations also differ across the menstrual cycle, in females.[63,122] It is suggested that osteoblastic activity is higher during the luteal phase, compared to the other phases of the menstrual cycle.[63,122] Compromised renal function can also alter serum concentrations of PINP and CTx-1.[106] This is likely the result of an imbalance in systemic plasma levels or potentially creatinine levels.[113]

2.4 – Automated Markerless Motion Capture Systems

A recent consortium of civilian and military experts on injury risks and prevention concluded there is a need for an automated system that accurately and quickly identifies individuals at increased injury risk.[14,15] A key component of this system must include an automated movement assessment that can identify aberrant movement patterns, an essential component of injury risk screenings.[14,15]

A new markerless motion capture system can reliably identify movement errors during a jump-landing movement assessment.[16] Overall the system has moderate reliability (κ_{avg}=0.48±0.40) compared to expert LESS raters. When the kappa statistics are adjusted to

address the prevalence of the movement errors and rater bias (PABAK) there is high reliability ($PABAK_{avg}$=0.81±0.27) between the markerless motion capture system and the expert raters. These levels of reliability are similar to the reliability of identifying movement errors between 2 expert LESS raters (κ_{avg}=0.45±0.35; $PABAK_{avg}$=0.67±0.34).[16] This automated movement assessment has the potential to remove a major obstacle to implementing movement assessments, en masse. However, the joint angles reported by the markerless motion capture system have yet to be validated against the current gold standard of three-dimensional (3D) motion assessment, marker based stereophotogrammetry. Validation of this markerless motion capture system is needed before wide-spread implementation can occur and aid clinicians in identifying lower extremity injury risks.

Similar markerless motion capture systems have been validated against marker based stereophotogrammetric systems.[125-130] A markerless motion capture system utilizing a Microsoft Kinect depth camera provided valid measures of sagittal (±0.5º) and frontal (±2.0º) plane angles, but it was unable to provide valid measures of transverse plane angles.[125] The major pitfalls of this study were that it only looked at static postures and did not use human participants. A similar study examined the ability of a markerless motion capture system using Kinect depth camera technology in human participants to examine cardinal movements (single plain: shoulder abduction, elbow flexion, hip abduction, knee flexion [squat]). The markerless system had good repeatability for all measures. However, the level of agreement between the systems varied from no agreement (hip abduction and knee flexion) to excellent agreement (shoulder abduction).[131]

Markerless motion capture systems utilizing Microsoft Kinect cameras have provided valid measures of lower extremity and trunk joint angles during functional tasks.[126-130] Microsoft Kinect markerless motion capture systems provide good validity during squatting

assessments.[126,130] Hip and knee sagittal plane kinematics demonstrate the best reliability.[130] However, the squatting assessments were highly standardized and controlled, and may not mimic real-world movement assessments. One study examined the validity of a markerless Kinect camera system and found good to excellent validity (ICC 95% confidence interval range 0.72-0.95) for frontal plane knee angles during a drop-vertical jump.[127] The drop-vertical jump is similar to the jump-landing movement assessment which can be used to accurately predict who is at increased risk of injury.[9-12] These findings have been refuted by Eltoukhy et al.[130] who reported good consistency between a Kinect system and a stereophotogrammetric system for sagittal plane joint angles, but only poor-to-fair consistency for hip and knee frontal plane joint angles. Overall, markerless motion capture system joint angles are within the acceptable range of 3-dimensional (3D) kinematic angles (2-5º),[132] however others disagree.[130]

CHAPTER III

EXPERIMENTAL DESIGN AND METHODS

3.1 – Experimental Design Overview

The overall goal of this study was to determine how lower extremity biomechanics influence biomarkers representative of bone turnover during military training. The study employed a cross-sectional study design. 45 male United States Army cadets completing a 6-week basic training course (Cadet Basic Training) at the United States Military Academy (USMA) were recruited to participate in this study. Lower extremity biomechanical, physical fitness, prior and present orthopedic injury history, and bone turnover biomarker data were collected following Cadet Basic Training. From these data, the extent to which an individual's lower extremity biomechanics influence bone biomarkers during military training (Cadet Basic Training) was determined. Our central hypothesis was individuals who displayed aberrant lower extremity biomechanics and other known stress fracture risk factors would have biomarker profiles indicative of high bone turnover rates following military training. This work aims to advance our understanding of how biomechanics affect the stresses placed on an individual's skeletal system and is a step towards understanding why some individuals have an anabolic (positive) response while others have a catabolic (negative) response to military training (**Figure 1 – Theoretical Model**). This study will provide military administrators with additional information that will help them in decision making to adapt training regimens to reduce overuse stress fracture risk and improve the military's physical fitness and performance.

3.2 – Participants

Participants were recruited from USMA who were completing a 6-week Cadet Basic Training course. A total of 45 participants who provided informed consent to have their lower extremity biomechanical profiles analyzed (*see section 3.3b – Biomechanical Assessment*) for a larger prospective study were recruited for this study. All male cadets who completed the lower extremity biomechanical assessment and completed Cadet Basic Training (n=800) were sent a standardized recruitment email. Cadets who responded to the email and volunteered to participate in this study were consented and further screened to ensure they met the inclusion criteria of this study.

An *a prior* power analysis based on previous literature was calculated. Between group effect sizes were determined for each biomarker of interest and the corresponding odds ratio was calculated. The odds ratio and desired power and significance level were entered into G*Power 3.1.9.2. The power analysis determined a sample of 45 participants would be sufficient for all outcome measures with an *a priori* alpha level of 0.05 and power of 0.85 (**Table 3.1 – Power Analysis**).[5] While more participants would have been ideal, the resource limitations of this dissertation precluded a larger sample size. As mentioned previously, women were excluded in order to create a more homogenous sample.

Table 3.1 – Power Analysis

Outcome Measure	Effect Size (d)	Odds Ratio	Subjects	Reference
PINP	0.64	3.19	43	Yanovich et al., 2013
CTx-1	1.00	6.13	24	Yanovich et al., 2013

Our participant pool is representative of individuals completing entry level military training. Military personnel are the ideal study population as they have homogenous physical training and recovery periods and diets.[26] Participants were not excluded based on race or ethnic

background.

3.2a – Inclusion Criteria

Male United States Army cadets who completed Cadet Basic Training the summer of 2015, and who also meet all of the following criteria:

1) Able and willing to give informed consent
2) Age 18-26 years
3) Baseline Questionnaire (BLQ) completed at the beginning of Cadet Basic Training
4) Completed the biomechanical assessment (jump-landing movement assessment) at the time of testing

3.2b – Exclusion Criteria

Male United States Army cadets who were unable to physically complete the lower extremity biomechanical assessment (jump-landing movement assessment) at the time of testing, or individuals who met 1 or more of the following criteria:

1) History of musculoskeletal injury during Cadet Basic Training that precluded the cadet from completing Cadet Basic Training
2) Neurological or metabolic disorder
3) History of inflammatory arthritis or gout
4) Females: females were excluded as we aimed to minimize the effect of sex on biochemical and biomechanical measures.

3.3 – Data Collection Procedures

3.3a – Post-Cadet Basic Training Serum Samples

Blood draws were completed on site at USMA. Post-Cadet Basic Training blood draws

occurred within 2 weeks of the post-Cadet Basic Training Army Physical Fitness Test (APFT). Blood draws were completed between 0600 and 0800 to minimize the effects of diurnal variations and regular physical activity on the biomarkers of interest. Immediately preceding the post-Cadet Basic Training blood draw each participant completed a questionnaire which assessed musculoskeletal injuries during Cadet Basic Training, physical activity immediately preceding the blood draw, and food and beverage consumption over the preceding 12 hours. Information regarding physical activity and diet were controlled for in the statistical models.

Standard blood draw procedures (e.g. cleaning the area with isopropyl alcohol prior to the insertion of the needle, using a new needle for each participant, bandaging the area with a clean bandage following the blood draw) were followed to minimize the risk of infection. Blood was collected in 1 (5ml) red top tube without additives. Upon collection, the serum tube was immediately inverted gently 3-5 times and allowed to clot at room temperature for at least 30 minutes but no longer than 60 minutes. Immediately after clotting, each sample was centrifuged at room temperature at 1300g for 10 minutes to separate the serum from the clot. This yielded approximately 2.5ml of serum, per tube. The serum was extracted from the collection tubes and aliquoted into cryotubes. Each cryotube contained ~125µl of serum; as many cryotubes as possible were created and stored at -80ºC until analyses were completed. Serum samples were batched and bioassayed at the end of all data collections to minimize inter-assay variability.

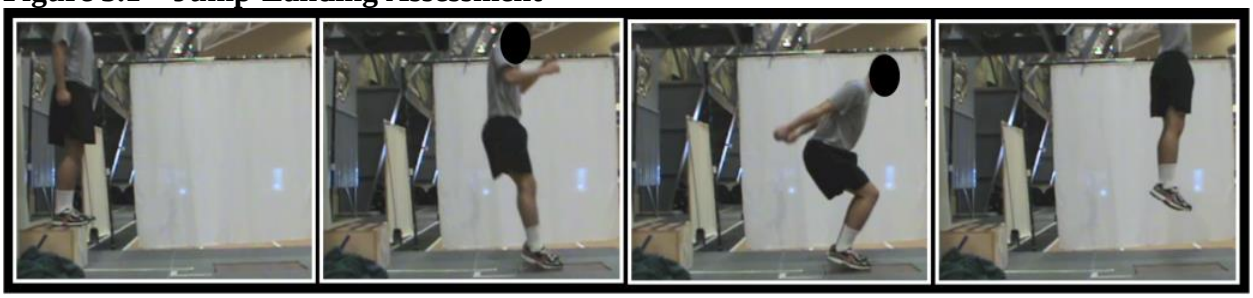
3.3b – Biomechanical Assessment

3.3b.1 – Biomechanical Assessment: 2-Dimensional (2D) Motion Analysis

All cadets completed the biomechanical assessment in the second-to-last week of Cadet Basic Training, this is standard practice at USMA and is completed during the APFT. This biomechanical assessment was part of a larger prospective study.

Participants completed a jump-landing movement assessment from a 30cm tall box to a target area located 0.9m in front of the box. Participants were instructed to complete a vertical jump for maximal height immediately following landing in the target area. Participants did not receive feedback or coaching concerning technique, other than what constituted a successful trial. A trial was deemed successful if the participant: 1) jumped off the box with both feet leaving the box at the same time; 2) jumped forward, and not vertically, to reach the target area; 3) landed with both feet in the target area; and 4) completed the task in a fluid motion (**Figure 3.1 – Jump-landing Assessment**).[11]

Figure 3.1 – Jump-Landing Assessment



Lower extremity movement patterns were evaluated during the jump-landing assessment using the Landing Error Scoring System (LESS). The LESS is a 22-item scoring rubric that is used to visually identify aberrant lower extremity and trunk movement patterns during a jump-landing assessment. Items on the LESS are evaluated at initial ground contact and the time between initial ground contact and peak knee flexion (**Appendix 3.1**).[11] A larger LESS score is indicative of more aberrant biomechanical patterns than a smaller LESS score. The original 17-item LESS rubric is a validated 2-dimensional (2D) assessment of lower extremity kinematics and has excellent intra-rater ($ICC_{2,k}$=0.84, SEM=0.42) and good inter-rater reliability ($ICC_{2,1}$=0.91, SEM=0.71).[11] The LESS is able to discriminate between individuals with high-risk (i.e. aberrant) movement patterns and individuals with low-risk movement patterns.

Traditionally, LESS scoring has involved recording the jump-landing assessment with standard 2-dimensional (2D) video cameras (frontal and sagittal views), loading these videos to a computer, and then manually scoring each set of videos by a trained rater for movement errors. A new, automated LESS testing platform is capable of automatically capturing and calculating full-body kinematics without the use of reflective markers or electromagnetic sensors and allows for accurate real-time scoring of the LESS via the use of an Xbox Kinect camera version 2 (Microsoft Co.; Redmond, WA) and a laptop running proprietary software (PhysiMax™ Technologies Ltd.; Tel-Aviv, Israel). The Kinect camera collects video depth data at 30Hz. This automated LESS scoring only requires 45-seconds of testing time per participant. Pilot work with USMA cadets the previous summer (2014) showed the reliability of the PhysiMax™ software against expert LESS raters ($Kappa_{avg}$ = 0.48±0.40; *adjusted* $Kappa_{avg}$ *(PABAK), = 0.71*±0.27; percent agreement = 0.85±0.14), with the majority of LESS items demonstrating almost perfect agreement.[16] The proprietary software automatically generates assessment reports for each participant including the total LESS score and each individual LESS item (*see section 3.4a.2 – Biomechanical Analyses*).

The Kinetic camera was aligned 3.4m in front of the participant on a tripod so that the camera was 0.84cm off of the ground.

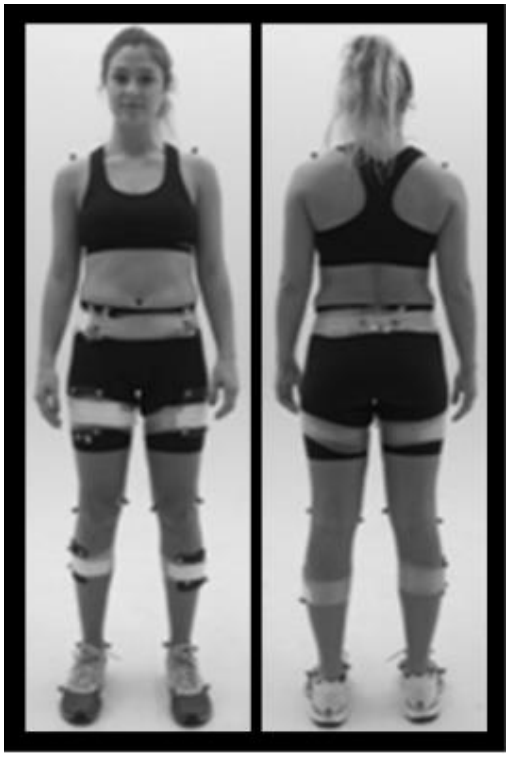
3.3b.2 – Biomechanical Assessment: 3-Dimensional (3D) Motion Analysis

Twenty (male = 10, female = 10) participants were recruited from the general student body population at the University of North Carolina at Chapel Hill. The primary investigator recruited participants in-person from Exercise and Sport Science classes using a standardized recruitment flyer and script. Participants were physically active a minimum of 30 minutes, 3 times a week, free of lower extremity injury that required 3 consecutive days of missed physical

activity for 6 months preceding testing, and had no history of lower extremity of low-back surgery. Participants reported to the University of North Carolina at Chapel Hill Sports Medicine Research Laboratory for a single testing session. Each participant wore non-reflective black spandex shorts and shirt and their own athletic shoes. Participants warmed-up on a stationary bike for 5 minutes, at a self-selected pace, prior to completing the jump-landing movement assessment.

Participants were outfitted with 7 cluster sets containing 3 or 4 reflective markers each. The 7 clusters were placed over the: sacrum (1), the thighs (2), the shanks (2), and the feet (2). 21 additional individual reflective markers were placed over the sternal notch (1) and bilaterally over the acromioclavicular joints (2), anterior superior iliac spines (2), greater trochanters (2) medial and lateral epicondyles (4), medial and lateral malleoli (4), the calcanei (2), the first metatarsal-phalangeal joints (2), and the fifth metatarsal-phalangeal joints (2) (**Figure 3.2 – Vicon Marker Placement**). Marker trajectories were tracked via a 10-camera (Vicon Bonita Cameras, version B10) stereophotogrammetry motion capture system (Vicon Motion Systems Ltd., Los Angeles, CA). A right-handed global reference system was defined with the positive x-axis in the anterior direction, the positive y-axis to the left of each participant, and the positive z-axis in the superior direction. Marker trajectory data, sampled at 200Hz, and forceplate data (model #4060-NC;

Figure 3.2 – Vicon Marker Placement



Bertec Co., Columbus, OH), sampled at 1200Hz, were collected and time synchronized with Vicon Nexus software (version 1.8.5; Vicon Motion Systems Ltd., Los Angeles, CA).

Prior to completing the jump-landing biomechanical assessment, a static trial was collected for each participant. The static trial served as the template to calculate trunk and lower extremity joint centers. The location of the hip joint center was approximated using the Bell method.[133] The knee joint center was defined as the midpoint of the femoral epicondyles and the ankle joint center was defined as the midpoint of the malleoli. The greater trochanter, medial and lateral epicondyle, and medial and lateral malleoli markers were removed prior to dynamic trial data collection. Participants completed 5 jump-landing assessments as previously described. Data were simultaneously recorded with the Vicon stereophotogrammetry motion capture system and Microsoft Kinect markerless motion capture system.

3.3c – Baseline Questionnaire (BLQ)

The baseline questionnaire was administered to all study participants to ensure they met study inclusion criteria and to get comprehensive injury and prior physical activity data. The baseline questionnaire is a comprehensive questionnaire that assesses previous and current physical activity levels, previous and current injury history, and overall current physical well-being (**Appendix 3.2**). Baseline questionnaire data were included in our predictive statistical models.

3.3d – Prior Physical Activity

Participants were asked to self-report the frequency (days per week), duration (minutes), and types of physical activity they routinely participated in, immediately preceding Cadet Basic Training. This included completion of the Marx lower extremity physical activity questionnaire.[134] Participants were also asked to self-report if they participated in any physical

activity in the 12 hours preceding the post-Cadet Basic Training blood draw. Participants reported the type and duration of physical activity participated in during the 12 hours preceding the post-Cadet Basic Training blood draw. Prior physical activity, immediately preceding the post-Cadet Basic Training blood draw, was controlled for in statistical analyses.

3.3e – Army Physical Fitness Test (APFT)

The Army Physical Fitness Test (APFT) is administered by the Army prior to and following Cadet Basic Training. The aim of the APFT is to determine the muscular strength, muscular endurance, and cardiorespiratory fitness of each cadet. The APFT includes 2 minutes of push-ups, 2 minutes of sit-ups, and a timed 2-mile run. Raw and standardized (0 – 100 points) event scores and a cumulative score (0 – 300 points) are recorded. Pre-Cadet Basic Training APFT data were included in our predictive statistical models. The study consent form included permission to access APFT scores.

3.3f – Body Mass Index (BMI)

Participant's height and mass were recorded upon entrance into USMA and at the time of the post-Cadet Basic Training Blood Draw. These data were utilized to calculate the body mass index (BMI = mass [kg] / (height $[cm]^2$)[48] for each participant. BMI data were included in our predictive statistical models.

3.3g – Food Consumption Log

Participants were asked to self-report their food and beverage consumption for the 12 hours immediately preceding the post-Cadet Basic Training blood draw. Protein rich food and beverage consumption were controlled for in our statistical analyses.

3.3h – Cadet Basic Training Injury Log

Participants were asked to self-report any musculoskeletal injuries they sustained during

Cadet Basic Training. A musculoskeletal injury was defined as an injury to the musculoskeletal system that resulted in the cadet reporting to the medical staff for evaluation or treatment. The cadet self-reported the body region to which the injury occurred, the injury type (e.g. sprain, strain, fracture), the days the cadet missed or was limited during Cadet Basic Training as the result of the injury, and if the cadet continued to have any signs or symptoms of the injury at the time of the post-Cadet Basic Training blood draw. Musculoskeletal injuries, time missed or limited, and ongoing signs and symptoms were included in our statistical analyses.

3.4 – Data Reduction and Statistical Plan

3.4a – Data Processing and Reduction

3.4a.1 – Biochemical Markers (Biomarkers) of Bone Turnover Analyses

Two (2) commercially available enzyme-linked immunosorbent assays (ELISA) evaluated the serum concentrations of our biomarkers of interest. Specifically, these ELISA kits measured procollagen type I aminoterminal propeptide (PINP; NeoScientific; Cambridge, MA: Product #HP0585) and cross-linked collagen telopeptide (CTx-1; NeoScientific; Cambridge, MA: Product #HC0850) which are biomarkers of bone turnover and represent type I collagen formation and resorption, respectively. PINP and CTx-1 have been shown to increase following military training.[5,17]

All ELISA kits were performed according to the manufacturer's instructions. Serum analyses were conducted by the Biochemistry Laboratory in the Department of Chemistry and Life Science at USMA. All samples were processed by the lab at the same time and biomarker kits were from the same manufacturer and production batch. Specimen samples were assayed in duplicate for each biomarker of interest.

Following data analyses (running of the biomarker assays) ratios of PINP : CTx-1 were calculated (PINP / CTx-1 = PINP : CTx-1 ratio). These ratios are important because they are reflective of the bone remodeling process. The larger the ratio is the more likely the bone is positively remodeling and forming new bone. The smaller the ratio is the more likely the bone is negatively remodeling and is resorbing more bone tissue than it is forming. All biomarker data were log transformed so that the data had a more normal distribution and thus could be modeled using linear regression models.[135]

3.4a.2 – Biomechanical Analyses: Landing Error Scoring System (LESS)

The jump-landing assessments were analyzed in real-time by the PhysiMax™ motion capture system. The PhysiMax™ system provided auto-generated reports for each participant, that included the total LESS score and individual LESS item scores. PhysiMax™ data were congregated into a common Excel spreadsheet (Microsoft Co.; Redmond, WA).

3.4a.3 – Biomechanical Analyses: 3-Dimensional (3D) Joint Angles

Biomechanical data collected with the Vicon Motion Capture system were imported into The MotionMonitor software (Innovative Sports Training, Inc; Chicago, IL). Trunk and lower extremity joint angles of interest were calculated with Euler angels; Euler angles had the following orders of rotation: Y (+ flexion), X (+ varus/adduction), and Z (+ internal rotation). Motion about the hip was defined as the thigh relative to the pelvis, motion about the knee as the shank relative to the thigh, and motion about the ankle as the foot relative to the shank. Trunk motion was calculated relative to the global reference frame. Full extension of the hip, knee, and trunk were defined as 0º, when the individual is standing in an erect, neutral position. All data were filtered (4th-order low-pass Butterworth filter with a cutoff frequency of 12.0Hz) prior to export.

Data were exported from the MotionMonitor software and run through custom Matlab software (version 2013a, The MathWorks; Natick, MA). Frontal and sagittal trunk, hip, knee, and ankle joint angles were calculated at initial ground contact and the peak angle during the "landing phase" of the initial landing. The landing phase was defined as the time from initial ground contact (vertical ground reaction force ≥ 10N) to the point of greatest knee flexion. Joint angle variables were averaged across all jump-landings trials for each time point of interest.

Biomechanical data collected with the Microsoft Kinect markerless motion capture system was analyzed with PhysiMax™ software via secondary data analyses. PhysiMax™ software processed the depth camera data via proprietary kinematic machine learning algorithms. The algorithms extract, track and dynamically refine virtual markers on the individual's body to assess dynamic motion. The algorithms are capable to calculating kinematic parameters including joint angles, ranges, velocities, and accelerations.[16]

Vicon and PhysiMax™ data were averaged across all trials collected with the respective motion capture system. The data were examined for statistical outliers (>3 standard deviations away from the mean); all statistical outliers were removed from the dataset prior to statistical analyses. Data were compared between the trunk and bilateral lower extremity joint angles calculated by each motion capture system. PhysiMax™ data were averaged across lower extremities for the USMA cadets and utilized in our predictive statistical models.

3.4a.4 – Army Physical Fitness Test (APFT)

Pre- and Post-Cadet Basic Training raw and standardized APFT cumulative and individual assessment scores were obtained from the Department of Physical Education at USMA. Differences between the raw and standardized scores were calculated between the pre- and post-Cadet Basic Training APFT tests.

3.4a.5 – Baseline Questionnaire

The key items on the baseline questionnaire for this study were the 11 variables shown in **Table 3.2**.

Table 3.2 – Baseline Questionnaire Variables

Category	Variables	Variables for Analyses
Previous lower extremity musculoskeletal injury	4	• History of lower extremity stress fracture • History of lower extremity acute fracture • History of previous lower extremity musculoskeletal injury (any) • History of previous lower extremity musculoskeletal injury (preceding 6 months)
Previous lower extremity surgery	1	• History of lower extremity musculoskeletal surgery
Previous jump/movement training	1	• History of jump or movement training for injury prevention
Marx Lower Extremity Activity Rating Scale	1	• Marx lower extremity activity rating scale score
Previous athletic/physical activity experiences	4	• Total seasons of physical activity • Total seasons of non-weight bearing physical activity • Total seasons of low impact weight bearing physical activity • Total seasons of high impact weight bearing physical activity

3.4a.6 – Participant Demographics

Body mass index (BMI) was calculated for all participants based on their pre- and post-Cadet Basic Training heights and masses. The absolute difference between the pre- and post-Cadet Basic Training BMI and mass were also calculated.

3.4b – Data Analyses

PASW Statistics for Windows (version 21.0; SPSS Inc.; Chicago, IL) was used to analyze all data. Univariate and multivariate linear regression models determined the extent to which each predictor variable influenced post-Cadet Basic Training biomarker concentrations and turnover ratios. Predictor variables were included in the multivariate models if they

significantly predicted the post-Cadet Basic Training biomarker concentration of either PINP or CTx-1 or significantly predicted the post-Cadet Basic Training PINP : CTx-1 ratio ($p \leq 0.10$). Following linear regression modeling, the antilog of each reported beta-value and corresponding 95% confidence interval (95% CI) were calculated. Trunk and lower extremity joint angles calculated by the Vicon and the markerless motion capture data were compared with mean and 95% CI comparisons, intraclass correlation coefficients (ICC), and Pearson product-moment correlations. 95% CI that overlapped were considered to have significant agreement between the motion capture systems. Statistical significance was set a priori at $\alpha \leq 0.05$ for all analyses. Statistical analyses are summarized in **Table 3.3 – Data Analyses Table**.

3.4b.1 – Specific Aim 1

Univariate and multivariate linear regression models determined how qualitative measures of lower extremity movement patterns predicted each post-Cadet Basic Training biomarker concentration of interest (PINP, CTx-1) and the bone turnover ratio (PINP : CTx-1). Initially, univariate analyses determined how the total LESS score (3 models) predicted each biomarker and the bone turnover ratio. Univariate models then determined how each individual LESS item (63 models) predicted each biomarker and the bone turnover ratio. Individual LESS items that predicted 1 or more of the biomarkers of the bone turnover ratio ($p \leq 0.10$) were then included in 3 multivariate models to predict each biomarker and the bone turnover ratio.

Univariate and multivariate linear regression models then determined how quantitative measures lower extremity kinematics predicted each post-Cadet Basic Training biomarker concentration of interest (PINP, CTx-1) and the bone turnover ratio (PINP : CTx-1). Initially, univariate analyses determined how averaged trunk, hip, knee, and ankle frontal and sagittal plane joint angles at initial ground contact, maximum angle, and displacement values between

initial ground contact and maximum joint angles (63 models) predicted each biomarker and the bone turnover ratio. No kinematic univariate linear regression models were predictive of any biomarker variable of interest. Three (3) multivariate linear regression models including all kinematic variables the determined if any combination of kinematic variables was predictive of the PINP or CTx-1 concentrations or PINP : CTx-1 ratio. Variables that significantly ($p \leq 0.10$) predicted 1 or more biomarker variables were included in multivariate linear regression models (3 models) that determined how the combination of the significant predictors in the overall kinematic multivariate model predicted 1 or more of the biomarkers or the bone turnover ratio.

3.4b.2 – Specific Aim 2

Univariate linear regression models (93 models) determined how previously identified lower extremity stress fracture risk factures predicted each post-Cadet Basic Training biomarker concentration of interest (PINP, CTx-1) and bone turnover ratio (PINP : CTx-1).

3.4b.3 – Specific Aim 3

Multivariate linear regression models (6 models total) determined how each of the significant predictors ($p \leq 0.10$) identified with specific aim 2 modified the effects of lower extremity biomechanics on biomarker profiles at post-Cadet Basic Training. 3 multivariate linear regression models determined how significant individual LESS items and significant stress fracture risk factors predicted each biomarker of interest and the bone turnover ratio. Similarly, 3 multivariate linear regression models determined how significant individual kinematic variables and significant stress fracture risk factors predicted each biomarker of interest and the bone turnover ratio. Food consumption and exercise within the 12 hours preceding the post-Cadet Basic Training blood draw both significantly influenced CTx-1 serum concentrations and were controlled for in the multivariate regression models.

3.4b.4 – Specific Aim 4

The average absolute difference between motion capture systems was calculated for trunk and lower extremity joint angles at initial ground contact, the peak angle for each joint during the landing phase, and the displacement between the 2 time points. Joint angles were compared via comparison of 95% CI surrounding the mean angle reported by each system. Inter-system reliability was assessed with intraclass correlation coefficients (ICC; model 3,1) and Pearson product-moment correlations. Additionally, Bland-Altman plots were calculated to give a visual representation of inter-system agreement.

Table 3.3 – Data Analyses Table

Specific Aim	Variables (Data Source)	Analyses

Aim 1: *Characterize the effects of lower extremity biomechanics on biomarker profiles representing bone turnover. (n=42)*	○ Post-CBT (ln) biomarker concentrations (post-CBT blood draw)
	○ Post-CBT (ln) biomarker turnover ratios (post-CBT blood draw)
	○ Post-CBT jump-landing kinematics (PhysiMax™)
	  ○ Qualitative (LESS)
	  ○ Quantitative (trunk, hip, knee, ankle average angles)
		Linear Regression Models
		• Qualitative Models
		  ○ Univariate (LESS total score) = 3
		  ○ Univariate (LESS items) = 63
		  ○ Multivariate (LESS significant items) = 3
		• Quantitative Models
		  ○ Univariate (individual average angles) = 63
		  ○ Multivariate (all average angles) = 3
		  ○ Multivariate (kinematic significant items) = 3

Aim 2: *Characterize the effects of known stress fracture risk factors on biomarker profiles representing bone turnover. (n=42)*	• Previous physical activity level (post-CBT blood draw questionnaire)
	  ○ Prior exercise volume
	• Previous physical activity type (BLQ)
	  ○ Jump/Movement Training
	  ○ Marx
	  ○ Athletic Seasons (non-weight bearing, low/high-intensity weight bearing)
	• Previous LE injury/surgery history (post-CBT blood draw questionnaire & BLQ)
	  ○ CBT injury
	  ○ CBT injury time loss
	  ○ Previous LE injury (any, within 6 months)
	  ○ Previous stress fracture
	  ○ Previous acute fracture
	  ○ Previous orthopaedic surgery
	• Physical fitness level (pre-CBT APFT)
	  ○ Raw scores (push-ups, sit-ups, run)
	  ○ Standardized scores (push-ups, sit-ups, run)
	  ○ Standardized total score
	• Anthropometrics
	  ○ Pre-CBT (BMI, mass, height)
	  ○ Post-CBT(BMI, mass, height)
	  ○ Pre-to-Post-CBT Difference (BMI, mass)
	• Previous 12 Hours (post-CBT blood draw questionnaire)
	  ○ Food consumption (dinner, snack, breakfast)
	  ○ Exercise
		Linear Regression Models
		• Univariate (individual risk factors) = 93

Aim 3: *Characterize how each predictor variable in Specific Aim 2 modifies the effects of lower extremity biomechanics on biomarker profiles representing bone turnover. (n=42)*	• Same as specific aims 1 and 2; based on what was identified as a significant predictor in each aim.	Linear Regression Models
		• Qualitative Models
		  ○ Multivariate (significant LESS items + significant risk factor variables) = 3
		• Quantitative Models
		  ○ Multivariate (significant kinematic items + significant risk factor variables) = 3

Aim 4: *Validate the trunk and lower extremity angles calculated by the markerless motion capture system (PhysiMax™) against a stereophoto-grammetric system (Vicon). (n=20)*	• Average joint angle for each kinematic variable of interest (Vicon + PhysiMax™)
	  ○ Trunk flexion angle (IC, max, displacement)
	  ○ Trunk lateral flexion (IC)
	  ○ Hip flexion angle (IC, max, displacement)
	  ○ Hip adduction/abduction angle (IC, max, displacement)
	  ○ Knee flexion angle (IC, max, displacement)
	  ○ Knee valgus/varus angle (IC, max, displacement)
	  ○ Ankle plantar flexion angle (IC)
		• 95% CI comparison
		• Intraclass correlation coefficients ($ICC_{3,1}$)
		• Pearson product moment correlations

CHAPTER IV

MANUSCRIPTS

Manuscript 1: Trunk and Lower Extremity Movement Patterns and Stress Fracture Risk Factors Influence Biomarkers of Bone Turnover In Military Training

Introduction

Musculoskeletal injuries affect 63% of non-deployed military personnel[1] and are the most significant medical issue limiting military readiness.[2] Lower extremity stress fractures affect nearly 1 in 3 male service members[5] and result in significant lost duty time, medical costs, and attrition.[4] Given the high prevalence and costs associated with skeletal injuries within the military it is critical to understand the factors that increase the risk of these injuries.[3]

Military training results in high training loads[8] that are associated with high stress fracture rates.[1,4] Musculoskeletal stress occurring during military training may be amplified by aberrant movement patterns which are associated with traumatic and overuse musculoskeletal injuries.[4,9,10]

Clinical movement assessments can identify aberrant movement patterns.[11,12] The Landing Error Scoring System (LESS) is a validated and reliable clinical movement assessment that visually identifies and scores aberrant trunk and lower extremity movement patterns that are associated with musculoskeletal injuries.[4,9,10,12] Furthermore, the LESS can discriminate between individuals at increased lower extremity injury risk from those who are not.[11-13]

Additional modifiable and non-modifiable factors have been identified that increase stress fracture risk during military training. Primarily, these factors include previous physical activity and physical fitness levels,[34,37-40,136] history of musculoskeletal injuries,[37,45] and anthropometric measurements.[17,44,96,97]

Bone is a metabolically active tissue that continuously undergoes remodeling involving bone resorption and formation ("turnover").[63,65,66] Bone turnover occurs throughout life in response to physical load (e.g. ground reaction and muscular forces) and the metabolic environment.[63,65,105] Bone turnover increases in response to military training.[5,17,27] Carboxy-terminal crosslinking telopeptide of type I collagen (CTx-1) is released during bone resorption[18,62,63,66] and procollagen type I aminoterminal propeptide (PINP) is released during bone formation.[66] Some of these particles enter the blood stream where their concentrations can be measured.[62,63] Since all cadets complete similar training, examining bone turnover biomarkers can provide insight into the extent to which known lower extremity risk factors influence bone turnover during military training.[5,17,25,27]

Bone turnover biomarkers can also be acutely influenced by a number of external factors. Protein rich food consumption[63] and acute exercise bouts[63,119] alter circulating levels of bone biomarkers. Bone biomarkers are also influenced by diurnal variations[115-117] and the menstrual cycle.[63,122] Each of these factors should be considered and controlled for during data collection and analyses.

Understanding how the aforementioned stress fracture risk factors influence bone health during military training will allow for the development of targeted intervention strategies to reduce injury risk and optimize performance. Therefore the purpose of this study was to identify how trunk and lower extremity movement patterns and other stress fracture risk factors influence

bone turnover biomarkers. We hypothesized that aberrant movement patterns and other known lower extremity stress fracture risk factors would be predictive of biomarker profiles indicative of high bone turnover.

Materials and Methods

Participants

A total of 45 male military cadets from the United States Military Academy (USMA) were recruited for this study. (**Table 4.1 – USMA Participant Demographics**). The 45 study participants were a convenience sample of participants from a larger prospective study. All male cadets who completed a lower extremity biomechanical assessment for the larger prospective study and completed Cadet Basic Training (n=800) were sent a standardized recruitment email. Cadets who responded to the email and volunteered to participate in this study were consented and further screened to ensure they met the inclusion criteria of this study.

Participants of the larger prospective study were eligible for participation in the present study if they were: 1) 18-26 years old; 2) completed a baseline questionnaire at the beginning of Cadet Basic Training; and 3) completed a jump-landing movement assessment as part of the larger prospective study. Potential study participants were excluded from the present study if they: 1) sustained an injury that precluded them from completing Cadet Basic Training; or 2) had a history of a neurological or metabolic disorder.

Instrumentation

The original 17-item LESS rubric is a validated 2-dimensional (2D) assessment of lower extremity movement patterns with good intra-rater ($ICC_{2,k}$=0.84, SEM=0.42) and inter-rater reliability ($ICC_{2,1}$=0.91, SEM=0.71).[11] The LESS has been expanded to a 22-item scoring rubric that identifies trunk and lower extremity movement patterns during a jump-landing assessment.

LESS items are evaluated at initial ground contact and the time interval between initial ground contact and peak knee flexion.[11,84] A larger LESS score is indicative of more aberrant movement patterns with less neuromuscular control.

Table 4.1 – USMA Participant Demographics Presented as Means ± SD

	Pre-Cadet Basic Training	*Post-Cadet Basic Training*
Age (years)	18.56 ± 1.39	18.71 ± 1.39
Height (cm)	176.95 ± 7.29	181.57 ± 5.70
Mass (kg)	77.20 ± 9.40	76.59 ± 7.31
BMI	24.68 ± 2.87	23.23 ± 1.89
LESS	------	4.86 ± 2.15

We used a markerless motion capture system that has automated LESS scoring. The automated LESS scoring platform allows for accurate real-time scoring of the LESS via a Xbox Kinect camera version 2 (Microsoft Co.; Redmond, WA) and a laptop running proprietary software (PhysiMax^{TM} Technologies Ltd.; Tel-Aviv, Israel). This automated LESS testing platform has been validated against expert LESS raters ($Kappa_{avg}$ = 0.48±0.40; *adjusted* $Kappa_{avg}$ *(PABAK), = 0.71*±0.27; percent agreement = 0.85±0.14), with the majority of LESS items demonstrating near perfect agreement.[16] The Kinect camera collects video depth data at 30Hz. The camera was aligned 3.4m in front of the participant and 0.84cm off of the ground.

Data Collection

Participant Demographics

Participants' ages (years), height (cm), and mass (kg) were recorded at the time of the pre-Cadet Basic Training Army Physical Fitness Test (APFT) and at the post-Cadet Basic Training blood draw. These data were utilized to calculate the body mass index (BMI = mass [kg] / (height [cm]^2) for each participant.[48]

Movement Assessment

Participants completed a jump-landing movement assessment in the second-to-last week

of Cadet Basic Training. They completed 3 trials of a jump-landing movement assessment from a 30cm tall box to a target area located 0.9m in front of the box. Participants were instructed to complete a vertical jump for maximal height immediately following landing in the target area. Participants did not receive feedback or coaching concerning technique, other than what constituted a successful trial. A trial was deemed successful if the participant: 1) jumped off the box with both feet leaving the box at the same time; 2) jumped forward, and not vertically, to reach the target area; 3) landed with both feet in the target area; and 4) completed the task in a fluid motion (**Figure 3.1**).[11] All jump-landing trials were recorded with the Kinetic camera.

Baseline Questionnaire

A self-reported questionnaire assessed previous and current physical activity levels, previous and current musculoskeletal injury history, and overall current physical well-being (**Appendix 3.2**). The Marx lower extremity physical activity questionnaire was included in the baseline questionnaire.[134]

Army Physical Fitness Test (APFT)

The APFT includes 2 minutes of push-ups, 2 minutes of sit-ups, and a timed 2-mile run. Individual event raw and standardized scores (0 – 100 points) and a cumulative standardized score (0 – 300 points) are recorded. The APFT is completed prior to the start of Cadet Basic Training as part of routine military training.

Post-Cadet Basic Training Blood Draw Food, Physical Activity, and Injury Log

Prior to the post-Cadet Basic Training blood draw each participant self-reported food and beverage consumption and physical activity over the preceding 12 hours, and the frequency (days per week), duration (minutes), and types of physical activity they routinely participated in, immediately preceding Cadet Basic Training. Food and beverage consumption and physical

activity within 12 hours of the blood draw were controlled for in our statistical analyses.

Participants also self-reported any musculoskeletal injuries they sustained during Cadet Basic Training. A musculoskeletal injury was defined as an injury to the musculoskeletal system that resulted in the cadet reporting to the medical staff for evaluation or treatment. Injury data included the body region, injury type (e.g. sprain, strain, fracture), number of days the cadet missed or was limited during Cadet Basic Training as a result of the injury, and if the cadet continued to have any signs or symptoms of the injury at the time of the post-Cadet Basic Training blood draw.

Post-Cadet Basic Training Blood Draw

Post-Cadet Basic Training blood draws were completed on site at USMA within 2 weeks of the end of Cadet Basic Training. All blood draws were completed between 0600 and 0800. Blood was collected in 1 (5ml) red top tube without additives. Upon collection, the serum tube was inverted 3-5 times and allowed to clot at room temperature for at least 30 minutes but no longer than 60 minutes. Immediately after clotting, each sample was centrifuged at room temperature at 1300g for 10 minutes to separate the serum from the clot. The serum was extracted from the collection tubes and aliquoted into cryotubes. Cryotubes were stored at -80ºC until analyses were completed.

Data Reduction

Movement Assessment: Landing Error Scoring System (LESS)

Jump-landing assessments were analyzed in real-time by the PhysiMax^{TM} motion capture system. If a movement error was observed during a minimum of 2 of the 3 trials the error was recorded and counted towards the total LESS score.[11] The PhysiMax^{TM} system provided auto-generated reports (total LESS scores and individual LESS item scores) for each participant. The

data were congregated into a common Excel spreadsheet (Microsoft Co.; Redmond, WA).

Biomarkers of Bone Turnover

Serum samples were batched and bioassayed at the end of all data collections to minimize inter-assay variability. Two commercially available enzyme-linked immunosorbent assays (ELISA) evaluated PINP (NeoScientific; Cambridge, MA: Product #HP0585) and CTx-1 (NeoScientific; Cambridge, MA: Product #HC0850) serum concentrations. All ELISA kits were from the same manufacturer and production batch. Serum samples were processed at the same time and assayed in duplicate for each biomarker of interest.

Bone formation (PINP) to bone resorption (CTx-1) ratios were calculated (PINP / CTx-1 = PINP : CTx-1 ratio). These ratios are indicative of the amount of bone remodeling activity. The larger the ratio is the more likely the bone is positively remodeling and forming sufficient new bone. The smaller the ratio is the more likely the bone is negatively remodeling and is resorbing more bone tissue than it is forming.[65] Biomarker data were log transformed so that the data had a more normal distribution.[135]

Data Analyses

PASW Statistics for Windows (version 21.0; SPSS Inc.; Chicago, IL) was used to analyze all data. Univariate and multivariate linear regression models determined how qualitative measures of lower extremity movement patterns and other stress fracture risk factors predicted each post-Cadet Basic Training biomarker concentration (PINP, CTx-1) and bone turnover ratio (PINP : CTx-1). Initially, univariate analyses determined how the total LESS score predicted each biomarker and the bone turnover ratio. Univariate models then determined how each individual LESS item and stress fracture risk factor predicted each biomarker and the bone turnover ratio. Stress fracture risk factors included: previous physical activity quantity and type,

history or lower extremity injury and surgery, pre-Cadet Basic Training fitness, anthropometric measures (height, mass, BMI, and the change in each), and food consumption and physical activity in the 12 hours preceding the post-Cadet Basic Training blood draw. Individual LESS items and stress fracture risk factors that predicted 1 or more of the biomarkers or the bone turnover ratio ($p \leq 0.10$) were then included in multivariate models to predict each biomarker and the bone turnover ratio. Means are reported in the original (unstransformed) score. Statistical significance for the multivariate models was set a priori at $\alpha \leq 0.05$ for all analyses.

Movement data were unavailable for 3 cadets. Therefore, our final sample size was 42 cadets for statistical analyses.

Results

Trunk and Lower Extremity Movement Patterns – Landing Error Scoring System (LESS)

Univariate linear regression revealed a number of significant predictors for PINP and CTx-1 concentrations and the PINP : CTx-1 ratio. The presence of foot internal rotation was associated with increased PINP concentrations and an increased PINP : CTx-1 ratio. Similarly, excessive trunk flexion displacement increased PINP concentrations and the PINP : CTx-1 ratio. Lower extremity sagittal plane displacement increased the PINP : CTx-1 ratio. The only significant predictor of CTx-1 concentrations was the presence of heel-to-toe landing. The total LESS score was not a significant predictor of any biomarker variable.

Multivariate regression analyses incorporating only movement data did not significantly predict changes in PINP, CTx-1, or PINP : CTx-1 ratios. In the multivariate models, foot internal rotation increased PINP concentrations and PINP : CTx-1 ratios. Lower extremity sagittal plane displacement also increased PINP : CTx-1 and excessive trunk flexion displacement increased PINP. Heel-to-toe landings increased CTx-1 concentrations. No other variables were significant

predictors within the multivariate models. The results of the univariate and multivariate analyses and the overall multivariate models are reported in **Table 4.4 – Predictability of the Landing Error Scoring System on Biomarkers of Bone Turnover**.

Lower Extremity Stress Fracture Risk Factors

Univariate linear regression revealed a number of significant predictors for PINP and CTx-1 concentrations and the PINP : CTx-1 ratio. An injury during Cadet Basic Training increased PINP concentrations and PINP : CTx-1 ratios. The raw sit-up score also increased PINP and PINP : CTx-1. As post-Cadet Basic Training mass increased, so did CTx-1 concentrations, and the difference in pre-to-post-Cadet Basic Training cadet mass increased PINP concentrations and PINP : CTx-1 ratios.

Lower Extremity Stress Fracture Risk Factors and Movement Quality

Multivariate linear regression models incorporating both movement quality and other stress fracture risk factors significantly predicted PINP concentrations and PINP : CTx-1. Foot internal rotation continued to increased PINP concentrations and PINP : CTx-1 ratios. Excessive trunk flexion displacement also increased PINP and PINP : CTx-1. Heel-to-toe landings increased CTx-1. Injury during Cadet Basic Training increased PINP and PINP : CTx-1. The changes in mass from pre-to-post-Cadet Basic Training increased PINP and CTx-1. The results of the univariate and multivariate analyses and the overall multivariate models are reported in **Table 4.5 – Predictability of Stress Fracture Risk Factors and Movement Quality on Biomarkers of Bone Turnover**.

Regression Model Covariates

Breakfast prior to the post-Cadet Basic Training blood draw significantly increased PINP : CTx-1 ratios by0.81 (95% CI: 0.66, 0.99; p=0.04). Exercise within 12 hours of the post-Cadet

Basic Training blood draw significantly increased CTx-1 concentrations by 1.34µg/L (95% CI: 1.11, 1.62; $p<0.01$) and PINP : CTx-1 by 0.62 (95% CI: 0.45, 0.87; $p<0.01$). Thus, both variables were entered into the movement quality and other stress fracture risk factor multivariate regression models as covariates.

Discussion

Lower extremity stress fracture risk factors predicted post-Cadet Basic Training bone turnover biomarker concentrations. Qualitative analysis of movement quality is capable of identifying movement patterns that predict bone turnover biomarkers. Similarly, other known stress fracture risk factors (e.g. previous injury, mass) are also predictive of bone turnover biomarkers. These findings provide important insight into how previously identified lower extremity stress fracture risk factors influence bone health at the molecular level and thus influence stress fracture risks.

Trunk and Lower Extremity Movement Patterns – Landing Error Scoring System (LESS)

Trunk and lower extremity movement patterns observed during a validated clinical movement assessment predict post-Cadet Basic Training bone turnover concentrations.[11,84] Surprisingly, overall movement quality was not predictive of PINP or CTx-1 concentrations or PINP : CTx-1 ratios. Overall movement quality was examined in 2 ways: 1) the total cumulative LESS score; and 2) the "overall impression" as scored by the LESS. These findings were surprising as a higher LESS score is indicative of overall poor movement quality,[11] which would result in more skeletal stress and thus more bone turnover,[63,65,105] and total LESS score has been associated with stress fracture risk.[13]

The LESS was developed to identify anterior cruciate ligament (ACL) risk factors.[11] The LESS is capable of identifying these risk factors,[11] as well stress fracture risk factors.[13] Thus, the

"overall impression" item scored on the LESS may be identifying factors that are irrelevant to or even protective against stress fracture risk (e.g. excessive trunk flexion displacement). Similarly, when the total LESS score is calculated, the presence of some LESS items that increase ACL injury risk may actually reduce stress fracture risks; when these items are included in the total LESS score, the score is higher, but the net stresses on the skeletal system may actually be less than an individual who displays fewer movement errors (smaller LESS score). However, stress fracture risk was not directly examined in this study. Bone turnover biomarkers were examined that are not a direct proxy for stress fracture risk.

Multivariate analyses including only significant LESS items did not predict any biomarker variables. These findings highlight another important aspect of the LESS: individuals can have the same cumulative LESS score, but may have scored differently on individual LESS items. For example, 1 individual may display medial knee displacement at initial ground contact, a narrow stance, and no knee flexion displacement; a second individual could display asymmetrical foot contact (timing) at initial ground contact, foot internal rotation, and excessive trunk flexion displacement. Both of these individuals' LESS scores would be 3. Thus there is substantial variability in how individuals can obtain the same cumulative LESS score. For these reasons, individual LESS items are better predictors of bone turnover biomarker concentrations and ratios than overall movement profiles.

A number of individual LESS items predicted post-Cadet Basic Training bone turnover concentrations and ratios. These LESS items include: heel-to-toe landings, lower extremity sagittal plane displacement, foot internal rotation, and excessive trunk flexion displacement. However, some LESS items, including lower extremity sagittal plane displacement and foot internal rotation, predicted post-Cadet Basic Training biomarker concentrations in the opposite

direction than was hypothesized.

If a heel-to-toe landing was present it increased the post-Cadet Basic Training CTx-1 concentration by 0.73µg/L. Greater CTx-1 concentrations may be indicative of excessive bone resorption, accelerated bone remodeling, and compromised bone strength.[111] The mean post-Cadet Basic Training CTx-1 concentration was 3.68µg/L (±1.53µg/L), thus the presence of a heel-to-toe landing accounted for 20% of the post-Cadet Basic Training CTx-1 concentration, but a minimum change in CTx-1 concentrations of 54% has been suggested to be needed in order to be considered clinically meaningful.[66] However, this was observed in an older, osteoporotic population, so the smaller percent changes observed in our study should be further examined to determine their clinical meaningfulness.

Our findings agree with previous studies that examined LESS items and lower extremity stress fracture risk.[13] Cameron et al.[13] found a relationship between ankle plantar flexion angle and stress fracture risk in military cadets. Furthermore, relationships exist between ankle dorsiflexion angles and vertical ground reaction forces during landings.[137,138] Minimal plantar flexion, as is the case with heel-to-toe landings, results in higher peak vertical ground reaction forces as compared to toe-to-heel landings.[137,138] Heel-to-toe landings also increase the vertical ground reaction loading rate, which is a known stress fracture risk factor.[139]

Foot internal rotation increased PINP concentrations at post-Cadet Basic Training. This indicates the bone is positively remodeling and increasing in strength. This was surprising as torsion and bending stresses concentrate in the bone cortex and stimulate osteoclasts to begin the bone remodeling process.[27,97] Furthermore, previous work with military cadets found that cadets who displayed knee internal rotation greater than 5° during a jump-landing assessment were 2-4 times more likely to sustain a stress fracture than individuals who had a neutral or externally

rotated knee.[9] The potential exist that what is visually observed as foot internal rotation during a jump-lading assessment occurs at the time of initial ground contact when individuals commonly have a plantar flexed foot and ankle. Foot and ankle plantar flexion causes the tibia to externally rotate.[140] Thus, when the ground reaction forces are greatest, at initial ground contact, the tibia is in a safer externally rotated position while the feet appear to be internally rotated. Furthermore, foot and ankle plantar flexion at initial ground contact mitigate ground reaction forces and loading rates which may be protective against stress fractures.[139]

Bone turnover is initiated by osteoclastic activity that outpaces osteoblast activity, resulting in greater bone resorption than formation.[44,61] Bone resorption takes 7-10 days while formation takes 2-3 months.[39,41] Thus, the post-Cadet Basic Training blood samples were likely collected after the cadets had passed the initial bone breakdown period and occurred bone formation was outpacing resorption. This is also 1 potential reason that we did not observe many variables that predicted post-Cadet Basic Training CTx-1 concentrations.

A lack of trunk and lower extremity sagittal plane displacement resulted in larger PINP : CTx-1 ratios. A larger PINP : CTx-1 ratio is indicative of more bone formation than resorption. Overall trunk and lower limb displacement can be scored as a 0 (no error, sufficient sagittal plane displacement), 1 (some sagittal plane displacement), or 2 (no/minimal sagittal plane displacement). This indicates that individuals who scored a 2 had the largest increases in their PINP : CTx-1 ratio. This was surprising as previous research has shown that stiffer landings (less sagittal plane displacement) increases ground reaction forces and ground reaction force loading rates that can increase stress fracture risk.[139,141]

Excessive trunk flexion displacement mitigates ground reaction forces during jump-landings[142] and therefore may be protective against lower extremity stress fractures. Our findings

support this. Excessive trunk flexion displacement increased post-Cadet Basic Training PINP concentrations and PINP : CTx-1, indicating more bone formation was occurring than bone resorption.

It was surprising that medial knee displacement did not predict any biomarker variable. Medial knee displacement is a clinical proxy for knee valgus alignment[11,82] which increases lower extremity stress fracture risk.[9] In the authors' experiences, individuals commonly display foot external rotation in conjunction with medial knee displacement. This is supported by the "position of no return" as described by Ireland et al.[143]; the foot and tibia are externally rotated, the knee is abducted (valgus alignment), and the hip is adducted and internally rotated. It is also possible that visual observation of medial knee displacement may not be sensitive enough to identify the multiplanar factors that contribute to 3-dimensional (3D) knee valgus.[82]

Lower Extremity Stress Fracture Risk Factors

Previously identified lower extremity stress fracture risk factors are predictive of post-Cadet Basic Training biomarker concentrations. Significant predictors include injury during Cadet Basic Training, performance on the APFT sit-up assessment, and post-Cadet Basic Training mass and the change in pre-to-post-Cadet Basic Training mass. Some previously identified stress fracture risk factors predicted bone biomarkers as we hypothesized (e.g. sit-ups and post-Cadet Basic Training mass) while others did not (e.g. injury during Cadet Basic Training and the change in pre-to-post-Cadet Basic Training mass). Furthermore, some risk factors that we hypothesized would strongly influence post-Cadet Basic Training bone biomarker concentrations (e.g. APFT run times and previous physical activity) were not predictive at all.

Pre-Cadet Basic Training physical fitness influenced post-Cadet Basic Training bone biomarker concentrations. Each additional sit-up a cadet completed during the pre-Cadet Basic

Training APFT increased PINP concentrations and PINP : CTx-1 ratios. Increases in PINP concentrations and PINP : CTx-1 ratios are indicative of bone formation, which is protective against stress fractures. Our findings agree with previous work that showed better performance on the sit-up component of standardized military physical fitness assessments reduced injury risk.[34,37,45,97]

We anticipated that pre-Cadet Basic Training APFT run times would strongly influence post-Cadet Basic Training biomarker concentrations, this was not observed in our study. Poor aerobic fitness increases musculoskeletal stress and injury risks.[35,41] The post-Cadet Basic Training blood sample collection may have occurred late enough in the training regimen that any initial negative changes in bone biomarkers (i.e. increased CTx-1 concentrations) had passed and the bones were beginning to rebuild.[39,41] Thus, no relationship was observed between pre-Cadet Basic Training APFT run times and post-Cadet Basic Training biomarker concentrations. We also excluded individuals who sustained an injury during Cadet Basic Training that precluded them from finishing the training. Any individuals who may have been severely out of shape at the beginning of the study may have become injured during Cadet Basic Training and were excluded from our study.

Sustaining an injury during Cadet Basic Training increased PINP and PINP : CTx-1. This finding opposed what we hypothesized because previous injury increases future injury risk.[37,45] This relationship has been observed for stress fractures among military cadets.[99] Again, the potential exist that the acute response to injury had passed and the bones and other tissues containing type I collagen (e.g. tendons) were rebuilding and an increase in PINP and PINP : CTx-1 were observed. Previous stress fracture history was hypothesized to be a strong predictor of post-Cadet Basic Training biomarker concentrations; however, no participants in the study

had a history of a stress fracture or had sustained an acute lower extremity fracture in the 6 months preceding Cadet Basic Training. It is possible that no study participants had a history of stress fracture because individuals with a previous history of stress fracture may have sustained a new stress fracture during Cadet Basic Training, and thus they were excluded from our study.

Overweight individuals have increased stress fracture risk.[33] We observed similar findings in our study. Post-Cadet Basic Training mass predicted CTx-1 concentrations. Larger mass resulted in greater post-Cadet Basic Training CTx-1 concentrations. Conversely, previous research has also shown that individuals with low body weight are also at increased stress fracture risk.[17,40,44,97]

We hypothesized that large changes in cadet pre-to-post-Cadet Basic Training mass would predict bone biomarker turnover rates. We observed greater changes in pre-to-post-Cadet Basic Training mass resulted in greater PINP and PINP : CTx-1, but not CTx-1 concentrations. This may indicate that these individuals lost a sufficient amount of weight and their bones were able to begin to rebuild bone because the extra stress had been removed. Our findings in combination with previous research suggest military personnel should aim to maintain a healthy weight, within "normal" body mass index (BMI) to minimize stress fracture risk.[17,33,40,44,97]

Previous physical activity level and type are both strong lower extremity stress fracture risk factors.[96] However, we did not observe any relationships between previous physical activity and bone biomarkers. The potential exist that all cadets entered Cadet Basic Training with similar experiences with sports and activities, however this does not appear to be the case. There were wide ranges of the number of activity seasons that cadets participated in (16.58±9.72), including non-weight bearing (1.36±2.49), low-intensity weight bearing (4.96±4.83), and high-intensity weight bearing (9.24±5.60) activities.

Regression Model Covariates

Eating breakfast and exercising prior to the post-Cadet Basic Training blood draw increased CTx-1 concentrations. Protein rich food (e.g. meat, eggs, milk) consumption can alter the concentrations of collagen byproducts in the serum, which may be incorrectly identified as bone resorption byproducts.[63]

Exercise can also lead to artificially elevated levels of bone biomarker serum concentrations.[63,119] Bone formation and resorption biomarkers are both reported to increase[22,23,120] and decrease[12359,60] following endurance exercise. One proposed explanation for the changes in bone biomarker concentrations following acute exercise bouts is the presence or absence of plasma volume expansion that may occur after some exercise events.[23,124]

Lower Extremity Stress Fracture Risk Factors and Movement Quality

Trunk and lower extremity movement patterns and other stress fracture risk factors combine to significantly predict PINP concentrations and PINP : CTx-1 ratios following Cadet Basic Training. This strongly supports research that shows stress fracture risks are multifactorial and all aspects of health and wellness should be considered and monitored to identify folks at increased stress fracture risk.[108]

In our combined multivariate models foot internal rotation increased PINP and PINP : CTx-1 to a similar extent as to what was observed in the multivariate movement model. Heel-to-toe landings also similarly increased CTx-1 concentrations. Excessive trunk flexion displacement was a significant predictor in the combined multivariate model but not in the movement multivariate model, indicating that excessive trunk flexion displacement may interact with other stress fracture risk factors and significantly increase PINP concentrations.

Limitations

Our study is not without its limitations and these limitations should be considered when interpreting the results of this study. First, serum samples were only collected post-Cadet Basic Training. Understanding how bone biomarkers change throughout military training may also be of interest, but has been previously studied.[5,17,27] Yanovich et al.[5] also demonstrated that bone turnover biomarkers did not differ between males who went on to sustain a stress fracture and those who did not.[5] Second, we were unable to obtain resting or fasting blood samples as we could not interfere with normal military training. However, military personnel eat a standardized diet so the risk of sample contamination from food consumption was equally likely for all study participants.[109] We also controlled for food and exercise contamination in our statistical models. Third, only male cadets were examined in this study as we aimed to limit the potential of confounding variables, such as sex. It is known that males and females display different movement patterns,[11,85] and bone biomarkers can be influenced by the female menstrual cycle.[63,122] Furthermore, bone biomarkers respond differently to military training in male and female populations.[5,17] Future studies should look at females and other vulnerable populations (e.g. distance runners).

Conclusions

Lower extremity stress fracture risk factors predict post-Cadet Basic Training bone turnover biomarkers. Our study expands on previous research as it provides insight into how known stress fracture risk factors alter bone health at the molecular level. This information is useful because it lays the basis for future research that can track bone turnover biomarkers throughout military training; these studies will help to identify when bones are most susceptible to stress fracture. Once these vulnerable periods are identified, military administrators can alter

training so that external stresses are reduced during these vulnerable time periods. A reduction of external forces will in turn lower stress fracture risks See **Appendix 4.1** and **Appendix 4.2** for a summary of how trunk and lower biomechanical and other stress fracture risk factors influence bone turnover biomarkers.

Table 4.3 – Summary of Landing Error Scoring System (LESS) Items

LESS Item	Number of Participants Displaying The LESS Error (%)
Knee Flexion Angle – IC	5 (11.63%)
Hip Flexion Angle – IC	3 (6.98%)
Trunk Flexion Angle – IC	8 (18.60%)
Heel-to-Toe Landing	5 (11.63%)
Asymmetrical Foot Contact	4 (9.30%)
Asymmetrical Foot Contact Timing	1 (2.33%)
Asymmetrical Heel-Toe/Toe-Heel Landing	1 (2.33%)
Lateral Trunk Flexion Angle – IC	7 (16.28%)
Medial Knee Position – IC	5 (11.63%)
Stance Width - Narrow	0 (0.00%)
Stance Width - Wide	17 (39.53%)
Foot Internal Rotation	2 (4.65%)
Foot External Rotation	8 (18.60%)
Knee Flexion – DSP	1 (2.33%)
Hip Flexion – DSP	4 (9.30%)
Trunk Flexion – DSP	15 (32.56%)
Excessive Trunk Flexion – DSP	7 (16.28%)
Maximum Medial Knee Position	14 (32.56%)
Asymmetrical Loading	13 (30.23%)
Knee "Wobble"	2 (4.65%)
Sagittal Plane Joint – DSP	1 = 34 (79.07%); 2 = 0 (0.00%)
Overall Impression	1 = 33 (76.74%); 2 = 8 (18.60%)

[1]IC = Initial Ground Contact
[2]Max = Maximum joint angle during the descent phase of the jump-landing
[3]DSP = Joint angle displacement from initial ground contact the maximum joint angle during the descent phase of the jump-landing

Table 4.4 – Predictability of the Landing Error Scoring System on Biomarkers of Bone Turnover

Biomarker	Overall Model	Predictors	Univariate Models Mean Change (95% CI)	*p*	Multivariate Model Mean Change (95% CI)	*p*
PINP	R-square = 0.16 *p* = 0.15	LESS Total	1.03 (0.93, 1.14)	0.58	------	------
		Heel-to-Toe	0.98 (0.49, 1.95)	0.96	0.86 (0.44, 1.69)	0.67
		Foot IR	**0.40 (0.15, 1.11)**	**0.09**	**0.40 (0.15, 1.11)**	**0.09**
		Excessive TFD	**1.72 (0.97, 3.05)**	**0.07**	1.54 (0.85, 2.76)	0.16
		Sagittal Joint DSP	1.36 (0.78, 2.37)	0.29	1.34 (0.76, 2.36)	0.32
CTx-1	R-square = 0.14 *p* = 0.23	LESS Total	0.97 (0.92, 1.02)	0.27	------	------
		Heel-to-Toe	**0.73 (0.52, 1.00)**	**0.06**	**0.74 (0.53, 1.04)**	**0.09**
		Foot IR	0.90 (0.53, 1.50)	0.68	0.91 (0.55, 1.52)	0.73
		Excessive TFD	1.13 (0.84, 1.51)	0.42	1.17 (0.87, 1.58)	0.29
		Sagittal Joint DSP	0.84 (0.64, 1.11)	0.23	0.86 (0.64, 1.14)	0.29
PINP : CTx-1	R-square = 0.22 *p* = 0.06	LESS Total	1.06 (0.97, 1.16)	0.20	------	------
		Heel-to-Toe	1.35 (0.75, 2.43)	0.32	1.16 (0.67, 2.03)	0.60
		Foot IR	**0.45 (0.19, 1.08)**	**0.08**	**0.44 (0.19, 1.03)**	**0.07**
		Excessive TFD	1.53 (0.93, 2.51)	0.10	1.31 (0.80, 2.14)	0.29
		Sagittal Joint DSP	**1.61 (1.01, 2.56)**	**0.05**	**1.56 (0.97, 2.51)**	**0.07**

[1]IR = Internal rotation
[2]TFD = Trunk flexion displacement
[3]DSP = Joint angle displacement from initial ground contact the maximum joint angle during the descent phase of the jump-landing

Table 4.5 – Predictability of Stress Fracture Risk Factors and Movement Quality on Biomarkers of Bone Turnover

Biomarker	Overall Model	Predictors	Univariate Models Mean Change (95% CI)	*p*	Multivariate Model Mean Change (95% CI)	*p*
PINP	**R-square = 0.47 *p* = 0.02**	Heel-to-Toe	0.98 (0.49, 1.95)	0.96	0.79 (0.43, 1.43)	0.44
		Foot IR	**0.40 (0.15, 1.11)**	**0.09**	**0.45 (0.18, 1.19)**	**0.10**
		Excessive TFD	**1.72 (0.97, 3,05)**	**0.07**	**1.68 (0.96, 2.96)**	**0.08**
		Sagittal Joint DSP	1.36 (0.78, 2.37)	0.29	1.08 (0.64, 1.79)	0.78
		CBT Injury	**0.47 (0.23, 0.94)**	**0.04**	**0.40 (0.21, 0.79)**	**0.01**
		Sit-ups Raw Score	**0.99 (0.97, 1.00)**	**0.08**	0.99 (0.97, 1.01)	0.49
		Mass – Post-CBT	0.99 (0.97, 1.02)	0.68	0.99 (0.96, 1.03)	0.75
		Mass – Difference	**0.94 (0.89, 0.98)**	**0.01**	**0.95 (0.90, 1.00)**	**0.05**
CTx-1	R-square = 0.39 *p* = 0.08	**Heel-to-Toe**	**0.73 (0.52, 1.00)**	**0.06**	**0.74 (0.53, 1.03)**	**0.09**
		Foot IR	0.90 (0.53, 1.50)	0.68	0.97 (0.58,1.61)	0.91
		Excessive TFD	1.13 (0.84, 1.51)	0.42	1.16 (0.85, 1.59)	0.36
		Sagittal Joint DSP	0.84 (0.64, 1.11)	0.23	0.83 (0.63, 1.11)	0.22
		CBT Injury	0.89 (0.62, 1.27)	0.52	0.87 (0.60, 1.26)	0.48
		Sit-ups Raw Score	1.00 (0.99, 1.01)	0.51	1.00 (0.99, 1.01)	0.82
		Mass – Post-CBT	**1.01 (1.00, 1.03)**	**0.07**	1.01 (0.99, 1.03)	0.38
		Mass – Difference	0.99 (0.96, 1.02)	0.49	**0.97 (0.95, 1.00)**	**0.07**
PINP : CTx-1	**R-square = 0.66 *p* < 0.01**	Heel-to-Toe	1.35 (0.75, 2.43)	0.32	1.06 (0.70, 1.61)	0.77
		Foot IR	**0.45 (0.19, 1.08)**	**0.08**	**0.46 (0.24, 0.87)**	**0.02**
		Excessive TFD	**1.53 (0.93, 2.51)**	**0.10**	**1.45 (0.98, 2.14)**	**0.07**
		Sagittal Joint DSP	**1.61 (1.01, 2.56)**	**0.05**	1.29 (0.91, 1.83)	0.17
		CBT Injury	**0.53 (0.29, 0.97)**	**0.05**	**0.46 (0.29, 0.73)**	**<0.01**
		Sit-ups Raw Score	**0.98 (0.97, 1.00)**	**0.01**	1.00 (0.98, 1.01)	0.41
		Mass – Post-CBT	0.98 (0.96, 1.00)	0.12	0.99 (0.96, 1.01)	0.24
		Mass – Difference	**0.95 (0.91, 0.99)**	**0.01**	0.97 (0.93, 1.01)	0.14

[1]IR = Internal rotation
[2]TFD = Trunk flexion displacement
[3]DSP = Joint angle displacement from initial ground contact the maximum joint angle during the descent phase of the jump-landing
[4]BMI = Body mass index
[5]CBT = Cadet Basic Training

Manuscript 2: Trunk and Lower Extremity Kinematics and Stress Fracture Risk Factors Influence Biomarkers of Bone Turnover In Military Training

Introduction

Lower extremity stress fractures affect nearly 1 in 3 male military service members.[5] Lower extremity fractures result in the greatest cumulative lost duty time of any non-battle related injury,[34] stress fractures also result in significant medical costs and attrition from service.[4] These overuse lower extremity fractures are preventable.[1,4,59] Therefore it is essential to identify factors that increase lower extremity stress fractures risks.

Musculoskeletal injuries are associated with both military training[1,4] and aberrant biomechanical patterns.[9,10,12,14,35,36] Military training[8] and aberrant biomechanics[61] result in high stresses acting on lower extremity skeletal segments. When military training and aberrant biomechanics occur simultaneously they result in abnormal forces acting on the skeletal system that increase injury risk.[61]

Laboratory based jump-landing assessments can identify individuals at increased musculoskeletal injury risk.[9-12] Cameron et al.[9] utilized an electromagnetic motion tracking system to identify biomechanical factors during a jump-landing assessment that increased stress fracture risk. Markerless motion capture systems have the potential to also identify biomechanical patterns associated with injury risk.[126-130]

In addition to military training and biomechanical patterns a number of additional factors increase stress fracture risk during military training. These factors include physical fitness levels,[34,37-40,136] previous musculoskeletal injuries,[37,45] and sex.[44,46] Each of these factors may also influence biomarkers of bone formation and resorption ("turnover"). Bone turnover occurs

throughout life in response to physical load (e.g. ground reaction and muscular forces) and the metabolic environment in order to maintain a healthy bone density.[63,65,105] Bone turnover biomarkers are also influenced by diurnal variations,[115-117] protein rich food consumption,[63] acute exercise bouts,[63,119] and the female menstrual cycle.[63,122] Thus, all of these factors should be controlled during data collection and analyses.

As bone remodels carboxy-terminal crosslinking telopeptide of type I collagen (CTx-1)[18,62,63,66] and procollagen type I aminoterminal propeptide (PINP)[66] are released, respectively. Some PINP and CTx-1 particles are released into the blood where their concentrations can be measured.[5,17,25,27] Thus, biomarkers are useful in determining how stress fracture risk factors influence bone turnover during military training.[5,17,25,27]

It is essential to not only identify the risk factors associated with stress fractures but also how these risk factors influence bone tissue itself. Understanding these relationships is essential so that efficacious injury prevention strategies may be developed and implemented. Therefore, the purpose of this study was to identify how trunk and lower extremity kinematics and other stress fracture risk factors influence biomarkers of bone turnover. We hypothesized that aberrant biomechanical patterns and known lower extremity stress fracture risk factors would result in biomarker profiles indicative of high bone turnover rates.

Materials and Methods

Participants

A convenience sample of 45 males was recruited from a larger study sample of cadets at USMA. All participants were first year cadets completing a 6-week Cadet Basic Training course. (**Table 4.1**). All male cadets who completed a lower extremity biomechanical assessment for the larger prospective study and completed Cadet Basic Training (n=800) were sent a standardized

recruitment email. Cadets who responded to the email and volunteered to participate in this study were consented and further screened to ensure they met the inclusion criteria of this study.

Cadets were eligible for participation in this study if they were: 1) 18-26 years old; 2) completed a baseline questionnaire at the beginning of Cadet Basic Training; and 3) completed a jump-landing movement assessment as part of a larger prospective study. Cadets were excluded if they: 1) sustained an injury that precluded them from completing Cadet Basic Training; or 2) had a history of a neurological or metabolic disorder.

Instrumentation

A markerless motion capture system utilizing a Xbox Kinect camera version 2 (Microsoft Co.; Redmond, WA) and a laptop running proprietary software (PhysiMax^{TM} Technologies Ltd.; Tel-Aviv, Israel) recorded all jump-landing movement assessments. The Kinect camera collected video depth data at 30Hz. The Kinetic camera was aligned 3.4m in front of the participant on a tripod with the camera 0.84cm off of the ground. The markerless motion capture system is capable of automatically capturing and calculating full-body kinematics. Similar markerless motion capture systems can reliably calculate sagittal and frontal plane hip and knee angles during dynamic movement assessments.[126-130]

Data Collection

Participant Demographics

Participants' ages (years), heights (cm), and masses (kg) were recorded at the time of the pre-Cadet Basic Training Army Physical Fitness Test (APFT) and at the post-Cadet Basic Training blood draw. Body mass index (BMI = mass [kg] / (height [cm]^2)[48] was calculated for each participant.

Biomechanical Assessment

Participants completed 3 trials of a jump-landing movement assessment during the second-to-last week of Cadet Basic Training. Cadets jumped from a 30cm tall box to a target area located 0.9m in front of the box and completed a vertical jump for maximal height immediately following landing in the target area. Participants did not receive feedback or coaching concerning technique, other than what constituted a successful trial. A trial was deemed successful if the participant: 1) jumped off the box with both feet leaving the box at the same time; 2) jumped forward, and not vertically, to reach the target area; 3) landed with both feet in the target area; and 4) completed the task in a fluid motion (**Figure 3.1**).[11]

Baseline Questionnaire

The baseline questionnaire assessed previous and current physical activity levels and injury history (**Appendix 3.2**). The Marx lower extremity physical activity questionnaire[134] was included in the Baseline Questionnaire.

Army Physical Fitness Test (APFT)

Cadets complete the APFT prior to the start of Cadet Basic Training as part of their standard military training. The APFT includes 2 minutes of push-ups, 2 minutes of sit-ups, and a timed 2-mile run. Raw and standardized scores (0 – 100 points) were calculated for each individual assessment. The individual standardized scores were summed together for a cumulative standardized score (0 – 300 points).

Post-Cadet Basic Training Blood Draw Questionnaire

At the time of the post-Cadet Basic Training blood draw, participants self-reported the frequency (days per week), duration (minutes), and types of physical activity they routinely participated in immediately preceding Cadet Basic Training. They also self-reported their food

and beverage consumption and physical activity (type and duration) during the preceding 12 hours. Food and beverage consumption and physical activity were controlled for in our statistical analyses.

Self-reported musculoskeletal injuries during Cadet Basic training were recorded. A musculoskeletal injury was defined as an injury to the musculoskeletal system that resulted in the cadet reporting to the medical staff for evaluation or treatment. The following information was recorded for each injury: 1) body region; 2) type of injury (e.g. sprain, strain, fracture); 3) number of days the cadet missed or was limited during Cadet Basic Training as a result of the injury; and 4) if the cadet continued to have any signs or symptoms of the injury at the time of the post-Cadet Basic Training blood draw.

Post-Cadet Basic Training Blood Draw

Post-Cadet Basic Training blood draws were completed on site at USMA. Blood draws occurred within 2 weeks of the end of Cadet Basic Training and were completed between 0600 and 0800. Blood was collected in a 5ml red top tube without additives. The tube was inverted 3-5 times and allowed to clot at room temperature for at least 30 minutes but no longer than 60 minutes. Samples were centrifuged at room temperature at 1300g for 10 minutes. Serum was extracted from the collection tubes and aliquoted into cryotubes and stored at -80ºC until analyses were completed.

Data Reduction

Biomechanical Analyses

Biomechanical data collected with the markerless motion capture system was analyzed with PhysiMax^{TM} software via secondary data analyses with machine learning algorithms. The algorithms extract, track and dynamically refine virtual markers on the individual's body to

assess dynamic motion. The algorithms are capable of calculating kinematic parameters including joint angles, ranges, velocities, and accelerations.[16]

Trunk and lower extremity frontal and sagittal joint angles were calculated at initial ground contact, maximum angles during the descent phase (initial ground contact to peak knee flexion), and the displacement during the descent phase. Kinematic data were averaged across the lower extremities and all trials. The data were examined for statistical outliers (>3 standard deviations away from the mean); all statistical outliers were removed from the dataset prior to statistical analyses.

Biomarkers of Bone Turnover

Serum samples were batched and bioassayed at the end of all data collections to minimize inter-assay variability. Commercially available enzyme-linked immunosorbent assays (ELISA) evaluated PINP (NeoScientific; Cambridge, MA: Product #HP0585) and CTx-1 (NeoScientific; Cambridge, MA: Product #HC0850) serum concentrations. ELISA kits were from the same manufacturer and production batch. All serum samples were processed simultaneously and assayed in duplicate for PINP and CTx-1.

Bone turnover ratios (PINP / CTx-1 = PINP : CTx-1 ratio) were calculated. These ratios are indicative of the amount of the bone remodeling activity. The larger the ratio is the more likely the bone is positively remodeling and forming new bone. The smaller the ratio is the more likely the bone is negatively remodeling and is resorbing more bone tissue than it is forming.[65] Biomarker data were log transformed so that the data had a more normal distribution and could be analyzed via linear regression models.[135]

Data Analyses

PASW Statistics for Windows (version 21.0; SPSS Inc.; Chicago, IL) was used to

analyze all data. Linear regression models determined how each predictor variable influenced post-Cadet Basic Training biomarker concentrations and turnover ratios. Statistical significance was set a priori at $\alpha \leq 0.05$ for all multivariate analyses.

Univariate regression analyses determined how averaged trunk, hip, knee, and ankle frontal and sagittal plane joint angles at initial ground contact, maximum angle, and displacement values predicted PINP and CTx-1 concentrations and PINP : CTx-1 bone turnover ratios. No univariate linear regression models were predictive of any biomarker variable ($p \geq 0.10$). Multivariate linear regression models including all kinematic variables then determined if any combination of kinematic variables was predictive of PINP, CTx-1, or PINP : CTx-1.

Univariate linear regression models also determined how previously identified lower extremity stress fracture risk factors predicted each post-Cadet Basic Training biomarker concentration (PINP, CTx-1) and the bone turnover ratio (PINP : CTx-1). Stress fracture risk factors included: previous physical activity experience and type, history or lower extremity injury and surgery, pre-Cadet Basic Training fitness, anthropometric measures (height, mass, BMI, and the change in each), and food consumption and physical activity in the 12 hours preceding the post-Cadet Basic Training blood draw. Kinematic and other stress fracture risk factor variables that significantly predicted ($p \leq 0.10$) 1 or more biomarker variables were included in multivariate linear regression models to predict each biomarker and the bone turnover ratio.

Food consumption and exercise within the 12 hours preceding the post-Cadet Basic Training blood draw significantly influenced CTx-1 serum concentrations and were controlled for in all multivariate regression models.

Mean changes are reported in the original (untransformed) score. Kinematic data were

unavailable for 3 cadets. Therefore, our final sample size was 42 cadets for all statistical models.

Results

Trunk and Lower Extremity Kinematics

Univariate linear regression analyses did not identify any kinematic variables that were significant predictors of PINP or CTx-1 concentrations or PINP : CTx-1 ratios. The multivariate linear regression models incorporating all kinematic variables identified a number of significant predictors for post-Cadet Basic Training CTx-1. These variables include hip flexion angle at initial ground contact and maximum hip flexion angle. Knee flexion angle at initial ground contact and knee flexion displacement increased post-Cadet Basic Training CTx-1 concentrations. CTx-1 concentrations were increased by maximum knee valgus angle and knee varus angle displacement. Finally CTx-1 was significantly increased by ankle plantar flexion angle at initial ground contact. No other kinematic variables predicted any biomarker variable.

The parsimonious multivariate linear regression analyses incorporating only kinematic data did not significantly predict PINP, CTx-1, or PINP : CTx-1 ratios. Hip flexion angle at initial ground contact, maximum hip flexion angle, knee flexion displacement, and knee varus displacement continued to significantly predict post-Cadet Basic Training CTx-1 concentrations. No other kinematic variables significantly predicted any post-Cadet Basic Training biomarker variable. The results of the univariate and multivariate analyses and the overall multivariate models are reported in **Table 4.7 – Predictability of Trunk and Lower Extremity Kinematics on Biomarkers of Bone Turnover**.

Lower Extremity Stress Fracture Risk Factors

Univariate linear regression identified significant predictors for PINP and CTx-1 concentrations and the PINP : CTx-1 ratios. An injury during Cadet Basic Training increased

PINP concentrations and PINP : CTx-1 ratios. The raw sit-up score also increased PINP and PINP : CTx-1. Post-Cadet Basic Training mass increased CTx-1 concentrations and the difference in pre-to-post-Cadet Basic Training cadet mass increased PINP concentrations and PINP : CTx-1 ratios.

Lower Extremity Stress Fracture Risk Factors and Trunk and Lower Extremity Kinematics

Multivariate linear regression models incorporating trunk and lower extremity kinematic variables and other stress fracture risk factors significantly predicted PINP : CTx-1. Injury during Cadet Basic Training increased PINP and PINP: CTx-1. Changes in mass from pre-to-post-Cadet Basic Training increased PINP and PINP : CTx-1 . Maximum hip flexion angle, knee flexion displacement, maximum knee valgus angle, and knee varus displacement predicted CTx-1 concentrations in the combined multivariate models. No other stress fracture risk factors or kinematic variables were predictive of any biomarker variable of interest. The results of the univariate and multivariate analyses and the overall multivariate models are reported in **Table 4.8 – Predictability of Stress Fracture Risk Factors and Trunk and Lower Extremity Kinematics on Biomarkers of Bone Turnover**.

Regression Model Covariates

Breakfast prior to the post-Cadet Basic Training blood draw increased PINP : CTx-1 ratios by 0.81 (95% CI: 0.66, 0.99; p=0.04). Exercise within 12 hours of the post-Cadet Basic Training blood draw increased CTx-1 concentrations by 1.34µg/L (95% CI: 1.11, 1.62; p<0.01) and PINP : CTx-1 by 0.62 (95% CI: 0.45, 0.87; p<0.01). Thus, both variables were entered into the multivariate regression models as covariates.

Discussion

Previously identified lower extremity stress fracture risk factors are predictive of post-

Cadet Basic Training bone turnover biomarker concentrations. Quantitative analyses of trunk and lower extremity kinematic patterns had minimal ability to predict post-Cadet Basic Training bone turnover biomarkers. This was surprising as the jump-landing assessment has previously been used to identify lower extremity stress fracture risk factors.[9,13] Biomechanical and non-biomechanical lower extremity stress fracture risk factors interact and alter their influence on post-Cadet Basic Training biomarker concentrations. Our findings provide important insight into how previously identified lower extremity stress fracture risk factors influence bone health and stress fracture risks during military training.

Trunk and Lower Extremity Kinematics

We hypothesized individual measures of trunk and lower extremity biomechanical patterns measured during a functional movement assessment would predict PINP or CTx-1 concentrations and PINP : CTx-1 ratios, this was not observed. Overall movement quality as assessed by multivariate linear regressions incorporating all trunk and lower extremity kinematic variables was also did not predict post-Cadet Basic Training bone turnover concentrations. This was unexpected as poor movement quality results in more musculoskeletal stress and thus greater bone turnover.[63,65,105]

The jump-landing movement assessment can identify lower extremity stress fracture risk factors.[9,13] However, laboratory based movement analysis equipment was required to identify differences between military cadets who went on to sustain a stress fracture and those who did not.[9] While the motion capture system used in this study is capable of qualitatively analyzing movement quality during a jump-landing assessment it may not be sensitive enough to detect minute differences in trunk and lower extremity kinematics that could be predictive of lower extremity stress fracture risk and bone turnover biomarkers.[16]

Multivariate linear regression models incorporating all kinematic variables identified a number of variables that were predictive of post-Cadet Basic Training CTx-1 concentrations. Post-Cadet Basic Training CTx-1 concentrations were influenced by hip and knee flexion angles, knee frontal plane angles, and ankle plantar flexion angles. These findings agree with previous research that has identified kinematic risk factors for lower extremity stress fractures.[9,13]

Lower extremity sagittal plane joint angles predicted post-Cadet Basic Training CTx-1 concentrations. Smaller hip flexion and larger knee flexion angles increased CTx-1 concentrations. Previous research has shown that stiffer landings (less sagittal plane displacement) increases ground reaction forces.[141] Larger ground reaction forces result in more bending and torsional forces on the lower extremity bones. Bending and torsional forces stimulate osteoclasts which initiate bone resorption and the bone remodeling process.[27] Furthermore, stiff landings would increase the vertical ground reaction loading rate, which is a known stress fracture risk factor.[139] Therefore we hypothesized more sagittal plane motion would reduce CTx-1 concentrations.

Post-Cadet Basic Training CTx-1 concentrations were also influenced by knee frontal plane joint angles. As frontal plane knee angle increased so did post-Cadet Basic Training CTx-1 concentrations. This indicates that knee frontal plane position increases the amount of bone resorption that is occurring. If bone resorption outpaces bone formation bone tissue is lost, bone mineral density drops, and there is a loss in trabecular integrity, and increased fracture risk.[63,65] This may explain why knee valgus angle during jump-landing assessments is predictive of lower extremity stress fracture.[9]

Markerless motion capture systems, similar to the 1 utilized in this study, have been validated against stereophotogrammetric systems that calculate 3-dimensional (3D) kinematic

angles.[126-130] Sagittal plane kinematics calculated by markerless motion capture systems are most reliable,[126-130] while frontal plane kinematics are less so,[130] and transverse kinematics have not been validated. However, the markerless motion capture system utilized in this study has yet to be validated, thus the potential exist it may not have been capable of correctly identifying jump-landing biomechanical patterns. If the markerless motion captures system utilized in this study does not accurately calculate trunk and lower extremity kinematics, this could explain why there was a lack of relationships observed between jump-landing kinematics and post-Cadet Basic Training bone turnover biomarkers.

Lower Extremity Stress Fracture Risk Factors

Previously identified lower extremity stress fracture risk factors predict post-Cadet Basic Training bone turnover biomarker concentrations. Sustaining an injury during Cadet Basic Training, APFT sit-up assessment performance, and post-Cadet Basic Training mass and the change in pre-to-post-Cadet Basic Training mass predict 1 or more post-Cadet Basic Training biomarker variables. Some previously identified stress fracture risk factors predicted post-Cadet Basic Training bone biomarkers as we anticipated (e.g. sit-ups and post-Cadet Basic Training mass) while others did not (e.g. injury during Cadet Basic Training and the change in pre-to-post-Cadet Basic Training mass). Additionally, risk factors that we hypothesized would be strong predictors of post-Cadet Basic Training bone biomarkers (e.g. APFT run times and previous physical activity) did not predict any bone biomarker.

Our findings and the work of others demonstrate that better pre-Cadet Basic Training physical fitness is protective against stress fractures.[34,37,45,97] Previous work found United States infantrymen in the lowest quartile for the number of sit-ups are 1.9 times more likely to be injured than those in the highest quartile.[34,37] In our study, for each additional sit-up a cadet

completed during the pre-Cadet Basic Training APFT increased PINP concentrations and PINP : CTx-1 ratios. Increases in PINP concentrations and PINP : CTx-1 ratios are indicative of bone formation that is protective against stress fractures.

We anticipated that pre-Cadet Basic Training APFT run times would be strong predictors of post-Cadet Basic Training biomarker concentrations, this was not observed. Previous work showed non-deployed United States Army infantrymen in the slowest 2-mile run time quartile are 1.6 times more likely to be injured than those in the fastest quartile.[34,37] Poor aerobic fitness increases the work the body has to do and in-turn increase musculoskeletal stress and injury risk.[35,41]

One potential explanation for the lack of a relationship between APFT run times and post-Cadet Basic Training bone turnover biomarker concentrations is our blood samples were collected at the end of the training period. At this point in training individuals may have improved their aerobic fitness and their bodies may have adapted to the training regimen, thus any initial changes in bone biomarkers related to pre-Cadet Basic Training aerobic fitness had passed. The bone remodeling process is initiated by a period of bone resorption followed by a prolonged period of bone formation,[39,41] we may have observed all cadets during the bone formation phase of the remodeling process.

Sustaining an injury during Cadet Basic Training increased PINP and PINP : CTx-1. This finding opposed what was anticipated as a history of previous musculoskeletal injury increases future injury risk.[37,45,99] It is important to note that none of the cadets who sustained an injury during Cadet Basic Training were symptomatic at the time of the post-Cadet Basic Training blood draw or missed more than 2 days of Cadet Basic Training. Therefore, the potential exist that the acute response to injury had passed and the bones and other tissues containing type I

collagen (e.g. tendons) were rebuilding and thus we observed an increase in PINP and PINP : CTx-1.

Greater post-Cadet Basic Training mass was predictive of greater post-Cadet Basic Training CTx-1 concentrations. This aligns with previous work that showed heavier individuals are at increased stress fracture risk.[33] We hypothesized that large changes in cadet pre-to-post-Cadet Basic Training mass would be predictive of bone biomarker profiles representative of high turnover rates. Armstrong et al.[96] found military cadets who went on to sustain a stress fracture lost weight throughout the training period, up until the time of their injury. The opposite was observed in our study; greater changes in pre-to-post-Cadet Basic Training mass resulted in greater PINP and PINP : CTx-1, and not CTx-1 concentrations. Heavier individuals at the start of Cadet Basic Training may have lost a sufficient amount of weight and their bones were able to begin to rebuild because the extra stress of excess weight was removed. Military personnel should aim to maintain a healthy weight prior to and during military training to reduce stress fracture risk.[17,33,40,44,97]

Previous physical activity level and type are both strong predictors of lower extremity stress fracture during military training,[33,40,43,96] but did not predict any biomarker variable in the present study. The potential exists that all cadets entered Cadet Basic Training with similar experiences with sports and activities and thus no relationships were observed between previous physical activity and bone turnover biomarkers. However, we observed wide ranges in the number of activity seasons that cadets participated in (16.58±9.72), including non-weight bearing (1.36±2.49), low-intensity weight bearing (4.96±4.83), and high-intensity weight bearing (9.24±5.60) activities. All physical activity experiences were self-reported and individuals entering the military may overestimate the amount of physical activity they previously

participated or may be unaware of how much physical activity they actually participate.[37,39]

Regression Model Covariates

Eating breakfast and exercising prior to the post-Cadet Basic Training blood draw increased CTx-1 concentrations. Protein rich food (e.g. meat, eggs, milk) consumption can alter collagen byproduct concentrations in the serum, which may be incorrectly identified as bone resorption byproducts (CTx-1).[63] Exercise may also lead to artificially elevated levels of bone biomarker serum concentrations.[63,119] Long-distance running in well trained individuals may temporarily inhibit bone formation and stimulate bone resorption,[22] but the opposite has also been observed.[20,21] One proposed explanation for the differences in bone biomarker concentrations following acute exercise bouts is the presence or absence of plasma volume expansion that may occur after some exercise events but not others.[23,124]

Lower Extremity Stress Fracture Risk Factors and Trunk and Lower Extremity Kinematics

Trunk and lower extremity movement patterns and other stress fracture risk factors combine to significantly predict PINP : CTx-1 ratios following Cadet Basic Training. In our combined multivariate models only non-kinematic risk factors predicted changes in PINP and PINP : CTx-1. It appears that kinematic and non-kinematic risk factors interact and alter the extent to which they influence post-Cadet Basic Training bone biomarker concentrations is altered. The effect of injury during Cadet Basic Training on PINP and PINP : CTx-1 is tempered by kinematic variables. However, the addition of non-kinematic risk factors did not alter the effects of any of the kinematic variables. Our findings support research that shows stress fracture risks are multifactorial and all aspects of past and present physical health and physical activity should be considered when identifying individuals at increased stress fracture risk.[108]

Limitations

The following limitations should be considered when interpreting the results of our study. Serum samples were only collected at the end of Cadet Basic Training. However, examining how bone biomarkers change throughout military training has been previously studied.[5,17,27] We were unable to obtain resting and fasting blood samples as we could not interfere with normal military training. Previous work with similar study populations reports that military personnel eat a "standard diet" so the risk of sample contamination from food consumption was equally likely for all study participants.[109] Also food consumption and physical activity were controlled for in our statistical models. Finally, only male cadets were examined in this study as we aimed to limit the potential of confounding variables, such as sex. Future studies should examine females and other vulnerable populations (e.g. distance runners).

Conclusions

Lower extremity stress fracture risk factors significantly predict post-Cadet Basic Training bone turnover biomarkers. Overall, previously identified biomechanical risk factors[9] were not observed in the current study, which may indicate that our biomechanical analyses were not sensitive enough to accurately detect trunk and lower extremity kinematics during a jump-landing assessment. However, our study expands on previous research as it provides insight into how other known stress fracture risk factors alter bone health at the molecular level. This information can help guide future work to develop injury mitigation strategies and reduce stress fracture risk. See **Appendix 4.1** and **Appendix 4.2** for a summary of how trunk and lower biomechanical and other stress fracture risk factors influence bone turnover biomarkers.

Table 4.6 – Summary of Trunk and Lower Extremity Kinematic Variables

Kinematic Variable	Mean (Standard Deviation)
Trunk Flexion – IC	33.88° (9.74)
Trunk Flexion – Max	52.55° (7.86)
Trunk Flexion – DSP	18.67° (3.89)
Lateral Trunk Flexion – IC	-0.36° (1.82)
Hip Flexion – IC	-14.99° (2.15)
Hip Flexion – Max	-45.99° (3.13)
Hip Flexion – DSP	30.99° (3.46)
Hip Frontal Plane – IC	5.56° (3.55)
Hip Adduction – Max	4.61° (3.68)
Hip Abduction – Max	9.25° (21.08)
Hip Adduction – DSP	1.68° (3.61)
Hip Abduction – DSP	3.69° (19.35)
Knee Flexion – IC	4.59° (8.42)
Knee Flexion – Max	94.78° (11.06)
Knee Flexion – DSP	86.63° (9.37)
Knee Front Plane – IC	-2.36° (2.09)
Knee Varus – Max	11.94° (17.70)
Knee Valgus – Max	6.41° (6.41)
Knee Varus – DSP	13.72° (15.89)
Knee Valgus – DSP	8.22° (8.88)
Ankle Plantar Flexion – IC	19.01° (16.13)

[1]IC = Initial Ground Contact
[2]Max = Maximum joint angle during the descent phase of the jump-landing
[3]DSP = Joint angle displacement from initial ground contact the maximum joint angle during the descent phase of the jump-landing

Table 4.7 – Predictability of Trunk and Lower Extremity Kinematics on Biomarkers of Bone Turnover

Biomarker	Overall Model	Predictors	Univariate Models		Multivariate Model	
			Mean Change (95% CI)	*p*	Mean Change (95% CI)	*p*
PINP	R-square = 0.10 *p* = 0.81	Hip Flexion – IC	0.95 (0.85, 1.06)	0.35	0.99 (0.61, 1.63)	1.00
		Hip Flexion – Max	1.00 (0.93, 1.07)	0.99	1.06 (0.79, 1.41)	0.69
		Knee Flexion – IC	1.02 (0.99, 1.05)	0.17	1.04 (0.89, 1.22)	0.64
		Knee Flexion – DSP	0.99 (1.00, 1.02)	0.59	1.01 (0.91, 1.12)	0.81
		Knee Valgus – Max	1.01 (0.98, 1.05)	0.58	1.03 (0.97, 1.10)	0.32
		Knee Varus – DSP	1.00 (0.98, 1.01)	0.85	1.01 (0.99, 1.04)	0.35
		Ankle Plantar flexion – IC	0.99 (0.98, 1.01)	0.22	1.00 (0.96, 1.03)	0.90
CTx-1	R-square = 0.27 *p* = 0.15	**Hip Flexion – IC**	**0.96 (0.91, 1.01)**	**0.13**	**0.80 (0.64, 0.99)**	**0.05**
		Hip Flexion – Max	**1.00 (0.97, 1.04)**	**0.80**	**1.17 (1.03, 1.33)**	**0.02**
		Knee Flexion – IC	1.01 (0.99, 1.02)	0.36	0.99 (0.92, 1.06)	0.67
		Knee Flexion – DSP	**1.00 (0.99, 1.01)**	**0.65**	**1.05 (1.00, 1.10)**	**0.05**
		Knee Valgus – Max	1.00 (0.98, 1.02)	0.94	1.02 (1.00, 1.05)	0.11
		Knee Varus – DSP	**1.00 (1.00, 1.01)**	**0.52**	**1.02 (1.01, 1.03)**	**0.01**
		Ankle Plantar flexion – IC	1.00 (0.99, 1.00)	0.43	0.99 (0.97, 1.00)	0.15
PINP : CTx-1	R-square = 0.09 *p* = 0.89	Hip Flexion – IC	0.99 (0.90, 1.08)	0.81	1.26 (0.82, 1.93)	0.31
		Hip Flexion – Max	0.99 (0.93, 1.06)	0.87	0.91 (0.70, 1.17)	0.45
		Knee Flexion – IC	1.01 (1.00, 1.04)	0.29	1.05 (0.92, 1.21)	0.46
		Knee Flexion – DSP	1.00 (0.98, 1.02)	0.72	0.97 (0.99, 1.06)	0.75
		Knee Valgus – Max	1.01 (0.98, 1.04)	0.49	1.01 (0.96, 1.06)	0.75
		Knee Varus – DSP	1.00 (0.98, 1.01)	0.56	1.00 (0.97, 1.02)	0.74
		Ankle Plantar flexion –IC	0.99 (0.98, 1.01)	0.34	1.01 (0.98, 1.04)	0.58

[1]IC = Initial ground contact of jump-landing
[2]Max = Maximum joint angle during the descent phase of the jump-landing
[3]DSP = Joint angle displacement from initial ground contact the maximum joint angle during the descent phase of the jump-landing

Table 4.8 – Predictability of Stress Fracture Risk Factors and Trunk and Lower Extremity Kinematics on Biomarkers of Bone Turnover

Biomarker	Overall Model	Predictors	Univariate Models		Multivariate Model	
			Mean Change (95% CI)	*p*	Mean Change (95% CI)	*p*
PINP	R-square = 0.44 *p* = 0.19	Hip Flexion – IC	0.95 (0.85, 1.06)	0.35	0.75 (0.45, 1.25)	0.28
		Hip Flexion – Max	1.00 (0.93, 1.07)	0.99	1.07 (0.78, 1.46)	0.69
		Knee Flexion – IC	1.02 (0.99, 1.05)	0.17	0.94 (0.80, 1.10)	0.45
		Knee Flexion – DSP	0.99 (1.00, 1.02)	0.59	1.03 (0.93, 1.14)	0.58
		Knee Valgus – Max	1.01 (0.98, 1.05)	0.58	1.04 (0.99, 1.10)	0.16
		Knee Varus – DSP	1.00 (0.98, 1.01)	0.85	1.01 (0.99, 1.04)	0.34
		Ankle Plantar flexion – IC	0.99 (0.98, 1.01)	0.22	0.98 (0.94, 1.01)	0.20
		CBT Injury	**0.47 (0.23, 0.94)**	**0.04**	**0.37 (0.17, 0.82)**	**0.02**
		Sit-ups Raw Score	**0.99 (0.97, 1.00)**	**0.08**	1.00 (0.98, 1.02)	0.95
		Mass – Post-CBT	0.99 (0.97, 1.02)	0.68	1.00 (0.96, 1.04)	0.89
		Mass – Difference	**0.94 (0.89, 0.98)**	**0.01**	**0.93 (0.87, 0.98)**	**0.02**
CTx-1	R-square = 0.48 *p* = 0.11	Hip Flexion – IC	0.96 (0.91, 1.01)	0.13	0.88 (0.68, 1.13)	0.33
		Hip Flexion – Max	1.00 (0.97, 1.04)	0.80	**1.17 (1.00, 1.37)**	**0.06**
		Knee Flexion – IC	1.01 (0.99, 1.02)	0.36	1.02 (0.94, 1.11)	0.63
		Knee Flexion – DSP	1.00 (0.99, 1.01)	0.65	**1.05 (1.00, 1.10)**	**0.08**
		Knee Valgus – Max	1.00 (0.98, 1.02)	0.94	**1.03 (1.00, 1.05)**	**0.07**
		Knee Varus – DSP	1.00 (1.00, 1.01)	0.52	**1.02 (1.01, 1.03)**	**0.01**
		Ankle Plantar flexion – IC	1.00 (0.99, 1.00)	0.43	0.99 (0.98, 1.01)	0.43
		CBT Injury	0.89 (0.62, 1.27)	0.52	0.99 (0.57, 1.41)	0.65
		Sit-ups Raw Score	1.00 (0.99, 1.01)	0.51	1.00 (0.99, 1.01)	0.79
		Mass – Post-CBT	**1.01 (1.00, 1.03)**	**0.07**	1.01 (0.98, 1.03)	0.57
		Mass – Difference	0.99 (0.96, 1.02)	0.49	0.97 (0.94, 1.01)	0.12
PINP : CTx-1	**R-square = 0.56 *p* = 0.03**	Hip Flexion – IC	1.00 (0.99, 1.00)	0.43	0.85 (0.58, 1.26)	0.43
		Hip Flexion – Max	0.99 (0.90, 1.08)	0.81	0.91 (0.72, 1.16)	0.45
		Knee Flexion – IC	0.99 (0.93, 1.06)	0.87	0.92 (0.81, 1.04)	0.20
		Knee Flexion – DSP	1.00 (0.98, 1.02)	0.79	0.98 (0.91, 1.06)	0.66

	Knee Valgus – Max	0.95 (0.87, 1.05)	0.34	1.01 (0.97, 1.06)	0.51
	Knee Varus – DSP	1.01 (0.98, 1.04)	0.49	0.99 (0.97, 1.02)	0.60
	Ankle Plantar flexion – IC	1.00 (0.98, 1.01)	0.56	0.98 (0.96, 1.01)	0.25
	CBT Injury	0.99 (0.98, 1.01)	0.34	**0.38 (0.21, 0.69)**	**<0.01**
	Sit-ups Raw Score	**0.98 (0.97, 1.00)**	**0.01**	1.00 (0.99, 1.02)	0.86
	Mass – Post-CBT	0.98 (0.96, 1.00)	0.12	1.01 (0.97, 1.04)	0.75
	Mass – Difference	**0.95 (0.91, 0.99)**	**0.01**	**0.95 (0.90, 0.99)**	**0.02**

[1]IC = Initial ground contact of jump-landing
[2]Max = Maximum joint angle during the descent phase of the jump-landing
[3]DSP = Joint angle displacement from initial ground contact the maximum joint angle during the descent phase of the jump-landing
[4]BMI = Body mass index
[5]CBT = Cadet Basic Training

Manuscript 3: Validation of a Markerless Motion Capture System Trunk and Lower Extremity Joint Angles During a Jump-Landing Assessment

Introduction

Laboratory[9-12] and field[11-14] based jump-landing movement assessments can identify individuals at increased musculoskeletal injury risk. Laboratory based movement assessments require expensive and cumbersome equipment to measure biomechanical patterns during movement assessments.[11] Thus, there is a need for highly portable motion capture systems that accurately calculate trunk and lower extremity kinematics so that movement assessments can be employed in field based settings.

Markerless motion capture systems utilizing Microsoft Kinect depth cameras to track trunk and lower extremity movement patterns have been developed.[125-131] Overall, these systems provide valid measures of sagittal[125,126,130] and frontal[125-127] plane joint angles, but are unable to provide valid measures of transverse plane angles. These systems demonstrate moderate-to-good validity and reliability during squatting[126,130] landing tasks.[127,130] Hip and knee sagittal plane kinematics consistently have the best validity, while hip and knee frontal plane joint angles only display poor-to-fair validity.[130]

A new commercially available markerless motion capture system reliably qualitatively analyzes movement patterns during jump-landing movement assessments.[16] The findings of this study are promising, as this system automates a valid and reliable clinical movement assessment that is capable of identifying individuals at increased musculoskeletal injury risk.[11,12] However, the joint angles reported by this markerless motion capture system have yet to be validated against the current gold standard of three-dimensional (3D) motion assessment, marker based

stereophotogrammetry. Validation of this markerless motion capture system is needed before wide-spread implementation can occur and aid clinicians in identifying lower extremity injury risks.

Therefore, the aim of this study was to validate the sagittal and frontal plane trunk and lower extremity joint angles reported by a commercially available markerless motion capture system during a jump-landing assessment. We hypothesized the markerless motion capture system would validly calculate trunk and lower extremity sagittal and frontal plane joint angles.

Materials and Methods

Participants

A convenience sample of 20 participants (male = 10, female = 10) were recruited from the general student body population at the University of North Carolina at Chapel Hill (**Table 4.9 – Markerless Motion Capture System Reliability Participant Demographics**). The primary investigator recruited participants in-person from Exercise and Sport Science classes using a standardized recruitment flyer and script. Participants were physically active a minimum of 30 minutes 3 times a week, free of lower extremity injury that required 3 consecutive days of missed physical activity for 6 months preceding testing, and had no history of lower extremity or low-back surgery.

Table 4.9 – Markerless Motion Capture System Reliability Participant Demographics

	All	*Males*	*Females*
Age (years)	20.50 ± 2.78	20.60 ± 3.72	20.40 ± 1.58
Height (cm)	170.36 ± 9.82	176.65 ± 6.66	164.07 ± 8.44
Mass (kg)	68.38 ± 10.07	71.53 ± 9.34	65.21 ± 10.21
BMI	23.50 ± 2.40	22.87 ± 2.31	24.13 ± 2.43

Participants reported to the University of North Carolina at Chapel Hill Sports Medicine Research Laboratory for a single testing session. Each participant wore non-reflective black

spandex shorts and shirt and their own athletic shoes.

Instrumentation

Markerless Motion Capture System

A markerless motion capture system utilizing a Xbox Kinect camera version 2 (Microsoft Co.; Redmond, WA) and a laptop running proprietary software (PhysiMax^{TM} Technologies Ltd.; Tel-Aviv, Israel) recorded all jump-landing movement assessments. The Kinect camera collected video depth data at 30Hz. The Kinetic camera was aligned 3.4m in front of the participant on a tripod so that the camera was 0.84cm off of the ground. The markerless motion capture system is capable of automatically capturing and calculating full-body kinematics without the use of reflective markers or electromagnetic sensors. Similar markerless motion capture systems can reliably calculate sagittal and frontal plane hip and knee joint angles during dynamic movement assessments.[126-130]

Stereophotogrammetry Motion Capture System

Participants were outfitted with 7 cluster sets containing 3 or 4 reflective markers each. The 7 clusters were placed over the: sacrum (1), the thighs (2), the shanks (2), and the feet (2). 21 additional individual reflective markers were placed over the sternal notch (1) and bilaterally over the acromioclavicular joints (2), anterior superior iliac spines (2), greater trochanters (2) medial and lateral epicondyles (4), medial and lateral malleoli (4), the calcanei (2), the first metatarsal-phalangeal joints (2), and the fifth metatarsal-phalangeal joints (2) (**Figure 3.2**). Prior to the biomechanical assessment the greater trochanter, medial and lateral epicondyle, and medial and lateral malleoli markers were removed from the participants.

Marker trajectories were tracked via a 10-camera (Vicon Bonita Cameras, version B10) stereophotogrammetry motion capture system (Vicon Motion Systems Ltd., Los Angeles, CA).

A right-handed global reference system was defined with the positive x-axis in the anterior direction, the positive y-axis to the left of each participant, and the positive z-axis in the superior direction. Marker trajectory data, sampled at 200Hz, and force platform data (model #4060-NC; Bertec Co., Columbus, OH), sampled at 1200Hz, were collected and time synchronized with Vicon Nexus software (version 1.8.5; Vicon Motion Systems Ltd., Los Angeles, CA).

Data Collection

Demographic data (sex, age, mass, height) were collected for each participant. Participants then warmed-up on a stationary bike for 5 minutes, at a self-selected pace. A static trial was then collected for each participant. The static trial served as the template for the stereophotogrammetric system to calculate trunk and lower extremity joint centers.

Participants completed 5 jump-landing assessments. Participants jumped from a 30cm tall box to the force platforms located 0.9m in front of the box. Participants were instructed to complete a vertical jump for maximal height immediately following landing on the force platforms. Participants did not receive feedback or coaching concerning technique, other than what constituted a successful trial. A trial was deemed successful if the participant: 1) jumped off the box with both feet leaving the box at the same time; 2) jumped forward, and not vertically, to reach the force platforms; 3) landed with each foot on its respective force platform; and 4) completed the task in a fluid motion (**Figure 3.1**).[11] Data were simultaneously recorded with the markerless motion capture system and the Vicon stereophotogrammetric motion capture system.

Data Reduction

Markerless Motion Capture System

Biomechanical data collected with the markerless motion capture system were analyzed with PhysiMax^{TM} software via secondary data analyses. PhysiMax^{TM} software processes the

depth camera data via proprietary kinematic machine learning algorithms. The algorithms extract, track and dynamically refine virtual markers on the individual's body to assess dynamic motion. The algorithms are capable of calculating kinematic parameters including joint angles, ranges, velocities, and accelerations.[16] Sagittal and frontal plane trunk, hip, knee, and ankle joint angles were reported at initial ground contact, the maximum angle during the "landing phase" of the initial landing, and the displacement between initial ground contact and the maximum angle during the landing phase. The landing phase was defined as the time from initial ground contact (the frame before the entire foot was in contact with the ground) to the point of greatest knee flexion.

Stereophotogrammetry Motion Capture System

Kinematic and kinetic data collected with the Vicon Motion Capture system were imported into The MotionMonitor software (Innovative Sports Training, Inc; Chicago, IL). The location of the hip joint center was approximated using the Bell method.[133] The knee joint centers were defined as the midpoints of the femoral epicondyles and the ankle joint centers were defined as the midpoints of the malleoli. Trunk and lower extremity joint angles were calculated with Euler angels; Euler angles had the following orders of rotation: Y (+ flexion), X (+ varus/adduction), and Z (+ internal rotation). Motion about the hip was defined as the thigh relative to the pelvis, motion about the knee as the shank relative to the thigh, and motion about the ankle as the foot relative to the shank. Trunk motion was calculated relative to the global reference frame. Full extension of the trunk, hip, knee was defined as 0º, when the individual is standing in an erect, neutral position. All kinematic and kinetic data were filtered within The MotionMonitor software (4th-order low-pass Butterworth filter with a cutoff frequency of 12.0Hz).

Data were exported from the MotionMonitor software and run through custom Matlab software (version 2013a, The MathWorks; Natick, MA). Sagittal and frontal plane trunk, hip, knee, and ankle joint angles were reported at initial ground contact, the maximum angle during the "landing phase" of the initial landing, and the displacement between initial ground contact and the maximum angle during the landing phase. The landing phase was defined as the time from initial ground contact (vertical ground reaction force ≥ 10N) to the point of greatest knee flexion.

General

PhysiMax™ and Vicon data were averaged for each time point of interest across all trials collected with the respective motion capture system. The data were examined for statistical outliers (>3 standard deviations away from the mean); all statistical outliers were removed from the dataset prior to statistical analyses.

Data Analyses

The percent difference between motion capture systems was calculated for trunk and lower extremity joint angles at initial ground contact, the peak angle for each joint during the landing phase, and the displacement between initial ground contact and the peak angle. The joint angle reported by each system was compared via comparison of 95% confidence intervals (CI) surrounding the mean of that angle. 95% CIs that overlapped were considered to significantly agree.

PASW Statistics for Windows (version 21.0; SPSS Inc.; Chicago, IL) was used to assess inter-system reliability via intraclass correlation coefficients (ICC; model 3,1) and Pearson product moment-correlations. Statistical significance was set a priori at $\alpha \leq 0.05$. Bland-Altman plots were calculated to give a visual representation of inter-system agreement (**Figure 4.1 –**

Bland-Altman Plots of Agreement for the Stereophotogrammetric and Markerless Motion Capture Systems).

Results

Trunk and Ankle

Sagittal plane trunk motion displayed fair to good agreement between the markerless and stereophotogrammetric motion capture systems. The 95% CIs for trunk flexion at initial ground contact, maximum joint angle, and joint angle displacement overlapped and there was significant agreement between the systems for maximum trunk flexion and trunk flexion displacement. Lateral trunk flexion at initial ground contact had poor agreement between the systems.

The agreement between motion capture systems for ankle plantar flexion angles at initial ground contact differed between the right and left limbs. The right limb had good agreement between systems while the left limb had fair agreement between systems. Trunk and ankle joint angles and statistics of agreement are presented in **Table 4.10 – Trunk and Ankle Joint Angle Means, 95% Confidence Intervals, Intraclass Correlation Coefficients, and Pearson Product-Moment Correlation Coefficients**.

Hip

Sagittal plane hip angle agreement between systems ranged from fair to excellent. The 95% CI overlapped for hip flexion angles at initial ground contact, maximum hip flexion angles, and hip flexion angle displacements. There was significant agreement between the systems for all sagittal plane joint angles, with the exception of the right hip flexion angle at initial ground contact.

Poor to fair agreement was observed for all frontal plane hip joint angles. Overlap between the 95% CIs was only present for right hip adduction angle displacement and left hip

abduction angle displacement. Significant correlations were observed for maximum left hip adduction angle, maximum left hip abduction angle and maximum right hip abduction angle. No other significant findings were observed for frontal plane hip angles. Hip joint angles and statistics of agreement are presented in **Table 4.11 – Hip Joint Angle Means, 95% Confidence Intervals, Intraclass Correlation Coefficients, and Pearson Product-Moment Correlation Coefficients**.

Knee

Sagittal plane knee joint angle agreement ranged from poor to excellent. Right and left knee flexion angles at initial ground contact had poor agreement. Significant agreement was observed for maximum knee flexion angles and joint displacements . The 95% CIs overlapped for all knee flexion maximum angles and joint displacements with the exception of left knee flexion displacement.

Overall, there was fair agreement for frontal plane knee joint angles. Right and left maximum knee varus angles had excellent agreement between systems. Left knee frontal plane initial ground contact angle displayed good agreement between systems. All other knee frontal plane joint angles had poor to fair agreement between systems. Knee joint angles and statistics of agreement are presented in **Table 4.12 – Knee Joint Angle Means, 95% Confidence Intervals, Intraclass Correlation Coefficients, and Pearson Product-Moment Correlation Coefficients**.

Discussion

Moderate agreement was observed between the markerless motion capture system and the gold-standard stereophotogrammetric systems. In general, there was better agreement between sagittal plane kinematic measures than frontal plane measures and maximum and

displacement values had better agreement than joint angles at initial ground contact. Our findings are in agreement with previous work that compared markerless and stereophotogrammetric motion capture systems.[126,127,130]

Overall, poor agreement was observed for 16 variables, fair agreement for 8 variables, good agreement for 3 variables, and excellent agreement for 11 variables. Better agreement existed between motion capture systems for sagittal plane variables (poor=2, fair=4, good=2, excellent=9) than frontal plane variables (poor=14, fair=4, good=1, excellent=2). There was also better agreement between systems for maximum (poor=6, fair=0, good=1, excellent=6) and displacement (poor=4, fair=5, good=0, excellent=4) joint angles than initial ground contact angles (poor=6, fair=3, good=2, excellent=1).

Differences in sagittal and frontal plane levels of agreement observed in our study are similar to those previously reported.[125-127] These findings were not surprising but counterintuitive as to what would be expected. The Microsoft Kinect camera is aligned perpendicular to the frontal plane so you would except the camera would be better able to detect frontal rather than sagittal plane joint angles. However, sagittal plane joint angles are typically larger than frontal plane angles, especially for maximum angles, so any limitations in the markerless motion capture systems ability to detect minute changes in joint angles may be minimized because of the larger overall joint angles. Similar findings are observed between validated three-dimensional (3D) motion capture systems.[144]

The Bland-Altman plots visually comparing the markerless and stereophotogrammetric motion capture system showed trends for the sagittal and frontal plane joint angles. In general, no trends or relationships were observed for sagittal plane joint angles. There were data points equally distributed above and below the mean difference line. Frontal plane angles did show

common trends. Overall, the markerless motion capture system underestimated smaller frontal plane joint angles and overestimated larger frontal plane joint angles. The Bland-Altman plots also showed that in general the mean difference between the two motion capture systems was more closely centered on zero for sagittal plane variables than frontal plane variables.

Significant correlations were observed for frontal plane hip joint angles. However, with the exception of left hip abduction displacement all correlations were negative, indicating that the motion capture systems were potentially reporting hip abduction and adduction in the opposite directions. The markerless motion capture system may have been limited in its ability to calculate hip frontal plane angles because individuals landing from a jump go into deep knee flexion and the knees can block the Kinect camera from visualizing the hip joints. Thus, the markerless motion capture system may be unable to track the hip joint markers. This may also explain why the markerless motion capture system did slightly better at identifying smaller frontal plane hip angles (those occurring at or near initial ground contact) than larger frontal plane hip angles occurring at or near peak knee flexion. Overall, the markerless motion capture system is unable to accurately calculate frontal plane hip angles.

Our findings are also comparable to those reported by Mauntel et al.[16] who compared a markerless motion capture system to the gold-standard (expert raters) for qualitative analysis of trunk and lower extremity movement patterns during a jump-landing assessment. Mauntel et al.[16] reported better agreement between the markerless motion capture system and expert raters for maximum joint angle and displacements movement errors than movement errors identified at initial ground contact.

Mauntel et al.[16] validated a markerless motion capture system's ability to accurately assess the Landing Error Scoring System (LESS).[11,84] The markerless motion capture system in

that study reliably identified trunk and lower extremity movement errors during a jump-landing movement assessment ($Kappa_{avg}$ = 0.48±0.40; *adjusted* $Kappa_{avg}$ *(PABAK), = 0.71*±0.27; percent agreement = 0.85±0.14), with the majority of LESS items demonstrating almost perfect agreement.[16] Gross movement quality is visually scored by the LESS and thus minute changes in joint angles are less important. The markerless motion capture system was also able to identify these gross differences in movement patterns. Collectively, these findings suggest markerless motion capture systems are limited in their abilities to identify small differences in trunk and lower extremity kinematics. However markerless motion capture systems can effectively identify larger movement patterns and may be useful in automating clinical movement screenings that have previously involved visual identification of gross movement patterns.

Inherit limitations of markerless motion capture systems inhibit their abilities to identify trunk and lower extremity kinematics at initial ground contact. Microsoft Kinect depth cameras collect video data at 30Hz while the force platform data in this study were sampled at 1200Hz and standard 2-dimensional (2D) video cameras collect data at 60Hz. Fewer data points (frames) inhibit the Microsoft Kinect's ability to accurately identity initial ground contact, and the actual frame where ground contact occurs may be missed by the Kinect camera. The PhysiMax™ software attempts to correct for this limitation by identifying initial ground contact and the frames immediately preceding and following that frame. The software then averages the trunk and hip joint angles across those 3 frames.

The markerless motion capture and stereophotogrammetric systems also defined initial ground contact differently. The markerless motion capture system defined initial ground contact as the frame prior to the entire foot being in contact with the ground. The stereophotogrammetric system identified initial ground contact as when the vertical ground reaction forces exceeded

10N. This difference in definitions could have led to some of the discrepancies observed between the systems for trunk and lower extremity kinematics at initial ground contact.

Limitations

The following limitations should be considered when interpreting the findings of our study. Only 1 movement assessment was examined; the examination of addition movement assessments is needed to develop this markerless motion capture system into a more robust system. Our study sample only included healthy individuals. Thus, the system must be validated in individuals with previous lower extremity injuries as they are at the greatest risk of future injury. Transverse plane joint angles were not assessed in this study. However, previous studies that examined the ability of Microsoft Kinect markerless motion capture systems to accurately calculate transverse plane joint demonstrated poor agreement against stereophotogrammetric systems.[125] Similar findings are observed between validated 3D motion capture systems.[144]

Conclusions

Moderate agreement exist between markerless and stereophotogrammetric motion capture systems for trunk and lower extremity kinematics during a jump-landing assessment. The markerless motion capture system is better at calculating sagittal plane joint angles than frontal plane joint angles. Furthermore, the markerless motion capture system is limited in its abilities to accurately calculate joint angles at initial ground contact and transverse plane joint angles, which may have important implications for injury risk. For these reasons markerless motion capture systems should be used with caution for identifying small differences in joint kinematics during high velocity functional tasks until further refinement occurs. However, Microsoft Kinect based markerless motion capture systems can correctly identify differences in gross movement patterns and thus can aid clinicians in identifying individuals at increased risk of injury.

Table 4.10 – Trunk and Ankle Joint Angle Means, 95% Confidence Intervals, Intraclass Correlation Coefficients, and Pearson Product-Moment Correlation Coefficients

Variable	Mean (95% CI)	$ICC_{3,1}$		Pearson-r Correlation	
		ICC	*p*	r-value	*p*
Trunk Flexion – IC*	Vicon: 31.86 (26.35, 37.37) PMax: 36.81 (33.59, 40.03) *Difference (%): 14.42%*	0.17	0.24	0.19	0.43
Trunk Flexion – Max*†	Vicon: 43.99 (35.60, 52.38) PMax: 52.46 (43.95, 60.94) *Difference (%): 17.56%*	0.41	0.03	0.41	0.07
Trunk Flexion – DSP*†‡	Vicon: 12.69 (7.82, 17.56) PMax: 13.40 (6.95, 19.85) *Difference (%): 5.44%*	0.58	<0.01	0.60	<0.01
Lateral Trunk Flexion – IC*	Vicon: 0.61 (-0.76, 1.98) PMax: 0.40 (-0.48, 1.28) *Difference (%): 41.58%*	-0.15	0.75	-0.17	0.48
Ankle Plantar flexion (Right) – IC*†‡	Vicon: 35.28 (27.58, 42.98) PMax: 26.08 (16.30, 35.86) *Difference (%): 29.99%*	0.51	<0.01	0.53	0.02
Ankle Plantar flexion (Left) – IC‡	Vicon: 34.32 (26.37, 42.47) PMax: 9.74 (7.62, 11.86) *Difference (%): 111.58%*	0.32	0.08	0.65	<0.01

[1]PMax = PhysiMax motion capture system
[2]IC = Initial ground contact of jump-landing
[3]Max = Maximum joint angle during the descent phase of the jump-landing
[4]DSP = Joint angle displacement from initial ground contact the maximum joint angle during the descent phase of the jump-landing
*Indicates 95% CI overlap
†Indicates significant ICC value ($p \leq 0.05$)
‡Indicates significant correlation ($p \leq 0.05$)

Table 4.11 – Hip Joint Angle Means, 95% Confidence Intervals, Intraclass Correlation Coefficients, and Pearson Product-Moment Correlation Coefficients

Variable	Mean (95% CI)	$ICC_{3,1}$		Pearson-r Correlation	
		ICC	*p*	r-value	*p*
Hip Flexion (Right) – IC*	Vicon: -21.43 (-26.82, -16.04) PMax: -17.98 (-19.33, -16.52) *Difference (%): 17.51%*	0.10	0.33	0.22	0.35
Hip Flexion (Left) – IC*†‡	Vicon: -20.38 (-23.82, -16.94) PMax: -18.05 (-19.4, -16.7) *Difference (%): 12.13%*	0.46	0.02	0.67	<0.01
Hip Flexion (Right) – Max*†‡	Vicon: -50.59 (-60.02, -41.16) PMax: -49.22 (-57.77, -40.67) *Difference (%): 2.75%*	0.66	<0.01	0.67	<0.01
Hip Flexion (Left) – Max*†‡	Vicon: -53.91 (-62.74, -45.08) PMax: -49.66 (-57.97, -41.35) *Difference (%): 8.21%*	0.77	<0.01	0.77	<0.01
Hip Flexion (Right) – DSP*†‡	Vicon: -29.16 (-38.13, -20.19) PMax: -31.24 (-39.02, -23.46) *Difference (%): 6.89%*	0.68	<0.01	0.69	<0.01
Hip Flexion (Left) – DSP*†‡	Vicon: -31.71 (-40.91, -22.51) PMax: -31.71 (-39.3, -24.12) *Difference (%): 0.00%*	0.70	<0.01	0.71	<0.01
Hip Frontal (Right) – IC	Vicon: -6.68 (-8.52, -4.84) PMax: 10.18 (8.96, 11.40) *Difference (%): 963.43%*	-0.21	0.81	-0.23	0.35
Hip Frontal (Left) – IC	Vicon: -8.69 (-10.26, -7.12) PMax: 10.58 (8.49, 12.67) *Difference (%): 2039.15%*	-0.25	0.84	-0.26	0.31
Hip Adduction (Right) – Max	Vicon: -3.19 (5.52, -0.84) PMax: 12.78 (9.02, 16.54) *Difference (%): 333.06%*	-0.23	0.84	-0.26	0.29
Hip Adduction (Left) – Max‡	Vicon: -6.85 (-9.91, -3.79) PMax: 28.51 (21.85, 35.17) *Difference (%): 326.50%*	-0.47	0.98	-0.62	<0.01
Hip Adduction (Right) – DSP*	Vicon: 3.49 (2.50, 4.48) PMax: 2.60 (-0.44, 5.64) *Difference (%): 29.23%*	-0.03	0.55	-0.05	0.83
Hip Adduction (Left) – DSP	Vicon: 2.15 (0.85, 3.45) PMax: 18.15 (11.51, 24.79) *Difference (%): 157.64%*	-0.13	0.69	-0.35	0.19
Hip Abduction (Right) – Max‡	Vicon: -9.98 (-11.83, -8.13) PMax: 23.44 (18.07, 28.81) *Difference (%): 496.58%*	-0.37	0.92	-0.61	0.02
Hip Abduction (Left) – Max‡	Vicon: -14.03 (-17.04, -11.02) PMax: 15.05 (11.69, 18.41) *Difference (%): 5701.96%*	-0.54	0.99	-0.55	0.02

Hip Abduction (Right) – DSP	Vicon: 2.31 (1.11, 3.51) PMax: 12.89 (8.13, 17.65) *Difference (%): 139.21%*	-0.01	0.51	-0.01	0.97
Hip Abduction (Left) – DSP*	Vicon: 4.68 (2.61, 6.75) PMax: 4.49 (1.40, 7.58) *Difference (%): 4.14%*	0.33	0.08	0.35	0.14

[1]PMax = PhysiMax motion capture system
[2]IC = Initial ground contact of jump-landing
[3]Max = Maximum joint angle during the descent phase of the jump-landing
[4]DSP = Joint angle displacement from initial ground contact the maximum joint angle during the descent phase of the jump-landing
*Indicates 95% CI overlap
[†]Indicates significant ICC value ($p \leq 0.05$)
[‡]Indicates significant correlation ($p \leq 0.05$)

Table 4.12 – Knee Joint Angle Means, 95% Confidence Intervals, Intraclass Correlation Coefficients, and Pearson Product-Moment Correlation Coefficients

Variable	Mean (95% CI)	$ICC_{3,1}$		Pearson-r Correlation	
		ICC	*p*	r-value	*p*
Knee Flexion (Right) – IC	Vicon: 29.52 (20.44, 38.60) PMax: 7.83 (3.07, 12.59) *Difference (%): 116.14%*	0.12	0.30	0.14	0.54
Knee Flexion (Left) – IC	Vicon: 30.56 (21.62, 39.50) PMax: 6.87 (2.49, 11.25) *Difference (%): 126.58%*	0.16	0.25	0.20	0.41
Knee Flexion (Right) – Max*†‡	Vicon: 88.84 (76.38, 101.3) PMax: 91.63 (80.01, 103.25) *Difference (%): 3.09%*	0.78	<0.01	0.78	<0.01
Knee Flexion (Left) – Max*†‡	Vicon: 89.69 (78.85, 100.53) PMax: 89.97 (78.31, 101.63) *Difference (%): 0.31%*	0.95	<0.01	0.95	<0.01
Knee Flexion (Right) – DSP*†‡	Vicon: 59.32 (44.24, 74.40) PMax: 76.82 (68.15, 85.49) *Difference (%): 25.71%*	0.59	<0.01	0.68	<0.01
Knee Flexion (Left) – DSP†‡	Vicon: 59.13 (45.01, 73.25) PMax: 83.10 (73.74, 92.46) *Difference (%): 33.71%*	0.64	<0.01	0.69	<0.01
Knee Frontal (Right) – IC*‡	Vicon: 2.85 (-0.44, 6.14) PMax: -1.61 (-4.1, 0.88) *Difference (%): 719.35%*	-0.52	0.99	-0.54	0.01
Knee Frontal (Left) – IC*†‡	Vicon: 4.36 (1.24, 7.48) PMax: 7.24 (2.41, 12.07) *Difference (%):49.66%*	0.59	<0.01	0.65	<0.01
Knee Varus (Right) – Max*†‡	Vicon: 6.90 (3.66, 10.14) PMax: 9.75 (6.96, 12.54) *Difference (%): 34.23%*	0.60	<0.01	0.61	<0.01
Knee Varus (Left) – Max*‡	Vicon: 9.75 (5.67, 13.83) PMax: 11.25 (7.84, 14.66) *Difference (%): 14.29%*	0.69	<0.01	0.71	<0.01
Knee Varus (Right) – DSP	Vicon: 4.05 (2.92, 5.18) PMax: 11.36 (6.43, 16.29) *Difference (%): 94.87%*	0.17	0.23	0.40	0.08
Knee Varus (Left) – DSP*	Vicon: 4.42 (2.59, 6.25) PMax: 3.40 (0.95, 5.85) *Difference (%): 26.09%*	-0.42	0.97	-0.44	0.06
Knee Valgus (Right) – Max*	Vicon: -2.93 (-8.08, 2.22) PMax: 3.32 (0.79, 5.85) *Difference (%): 3205.45%*	0.28	0.11	0.35	0.13
Knee Valgus (Left) – Max*	Vicon: -0.36 (-4.07, 3.35) PMax: 3.46 (0.85, 6.07) *Difference (%): 246.45%*	0.24	0.14	0.26	0.27

Knee Valgus (Right) – DSP*	Vicon: -5.77 (-8.54, -3.00) PMax: 4.93 (0.3, 9.56) *Difference (%): 2547.62%*	0.17	0.19	0.23	0.33
Knee Valgus (Left) – DSP*	Vicon: -4.50 (-6.12, -2.88) PMax: -4.68 (-7.83, -1.53) *Difference (%): 3.92%*	-0.17	0.77	-0.21	0.38

[1]DSP = Joint angle displacement from initial ground contact the maximum joint angle during the descent phase of the jump-landing
2Indicates 95% CI overlap
[3]Indicates significant ICC value ($p \leq 0.05$)
[4]Indicates significant correlation ($p \leq 0.05$)

Table 4.13 – Overall Trunk and Lower Extremity Joint Angle Agreement

Variable	Qualitative Ranking
Trunk Flexion – IC	Fair
Trunk Flexion – Max	Good
Trunk Flexion – DSP	Excellent
Lateral Trunk Flexion – IC	Poor
Hip Flexion (Right) – IC	Fair
Hip Flexion (Left) – IC	Excellent
Hip Flexion (Right) – Max	Excellent
Hip Flexion (Left) – Max	Excellent
Hip Flexion (Right) – DSP	Excellent
Hip Flexion (Left) – DSP	Excellent
Hip Frontal (Right) – IC	Poor
Hip Frontal (Left) – IC	Poor
Hip Adduction (Right) – Max	Poor
Hip Adduction (Left) – Max	Poor
Hip Adduction (Right) – DSP	Fair
Hip Adduction (Left) – DSP	Poor
Hip Abduction (Right) – Max	Poor
Hip Abduction (Left) – Max	Poor
Hip Abduction (Right) – DSP	Poor
Hip Abduction (Left) – DSP	Fair
Knee Flexion (Right) – IC	Poor
Knee Flexion (Left) – IC	Poor
Knee Flexion (Right) – Max	Excellent
Knee Flexion (Left) – Max	Excellent
Knee Flexion (Right) – DSP	Excellent
Knee Flexion (Left) – DSP	Good
Knee Frontal (Right) – IC	Poor
Knee Frontal (Left) – IC	Good
Knee Varus (Right) – Max	Excellent
Knee Varus (Left) – Max	Excellent
Knee Varus (Right) – DSP	Poor
Knee Varus (Left) – DSP	Fair
Knee Valgus (Right) – Max	Poor
Knee Valgus (Left) – Max	Poor
Knee Valgus (Right) – DSP	Poor
Knee Valgus (Left) – DSP	Fair
Ankle Plantar Flexion (Right) – IC	Good
Ankle Plantar Flexion (Left) – IC	Fair

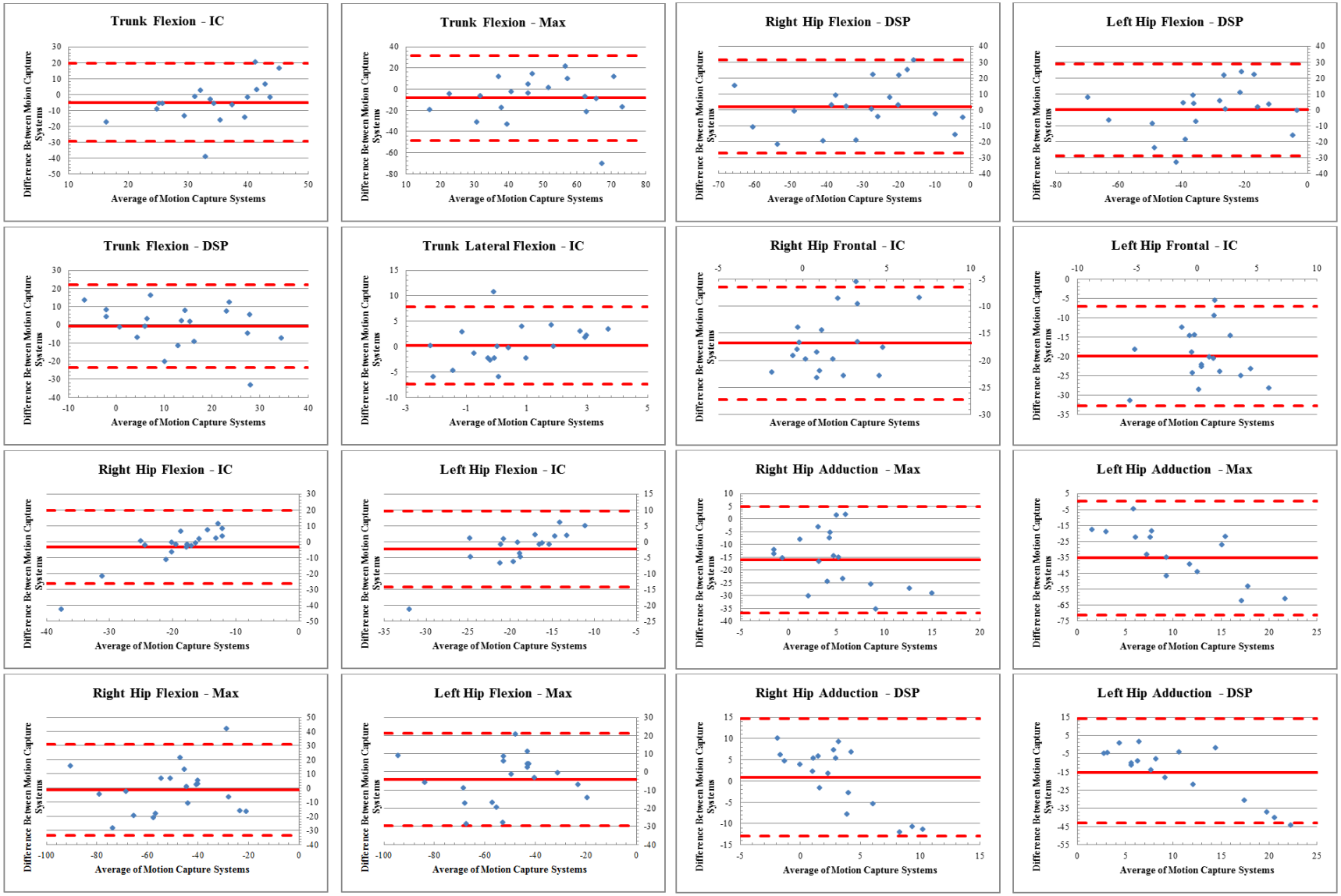


Figure 4.1 – Bland-Altman Plots of Agreement for the Stereophotogrammetric and Markerless Motion Capture Systems

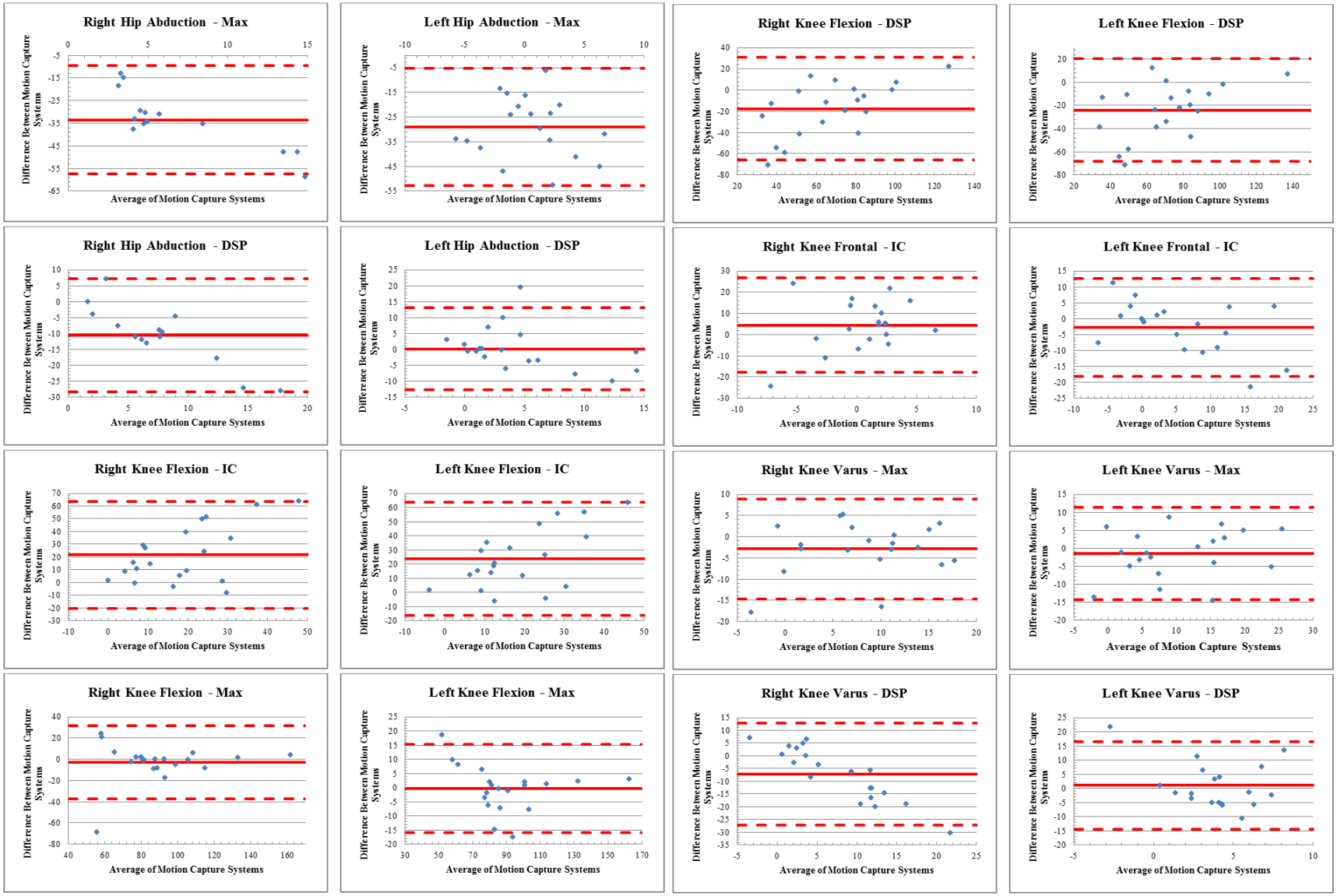


Figure 4.1 – Bland-Altman Plots of Agreement for the Stereophotogrammetric and Markerless Motion Capture Systems

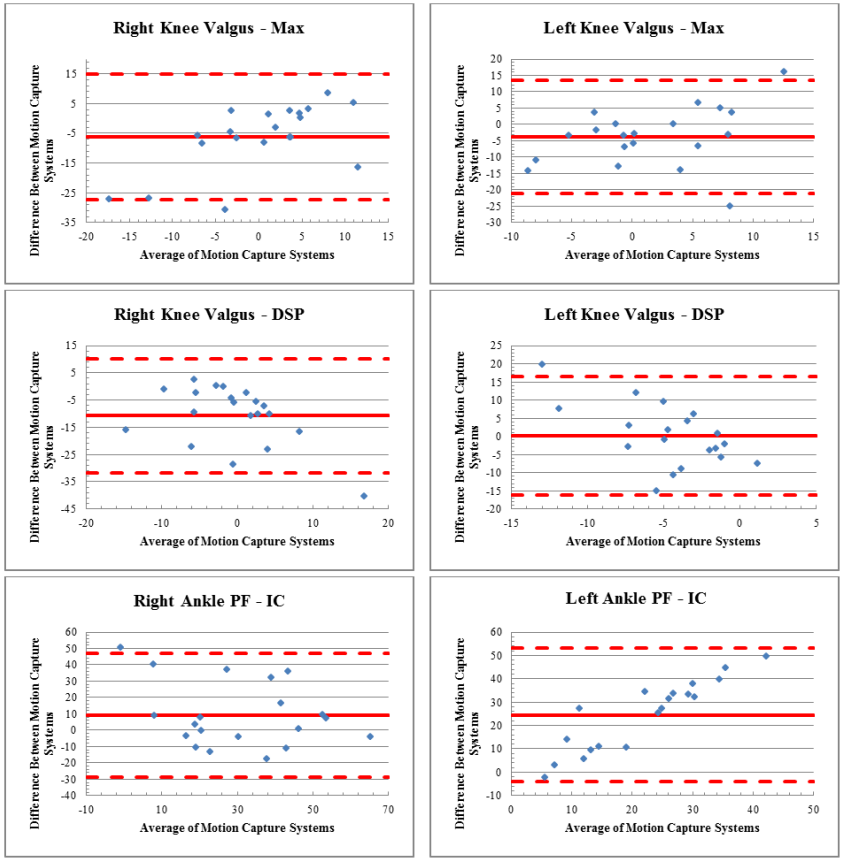


Figure 4.1 – Bland-Altman Plots of Agreement for the Stereophotogrammetric and Markerless Motion Capture Systems

CHAPTER V

SUMMARY

5.1 – Introduction

Lower extremity stress fractures affect 1 in 3 male service members and result in significant medical costs and attrition from military service.[4,5] Biomechanical patterns, physical fitness, and previous physical activity and musculoskeletal injuries affect stress fracture risk.[9,34,37-40,45,136] It is essential to not only identify the risk factors associated with stress fractures but also understand how these risk factors influence bone tissue itself. Biochemical markers (biomarkers) indicative of bone "turnover" (formation: procollagen type I aminoterminal propeptide [PINP]; resorption: cross-linked collagen telopeptide [CTx-1]) can be used to track acute bone health changes.[5,17,25,27] The purpose of this study was to identify how trunk and lower extremity biomechanical patterns and other stress fracture risk factors influence bone turnover biomarkers. Understanding these relationships is essential so that efficacious injury prevention strategies may be developed and implemented to reduce stress fracture risk.

5.2 – Methods

We assessed the influence of stress fracture risk factors (independent variables), with: 1) a lower extremity movement assessment; 2) self-reported injury and physical activity history questionnaires; and 3) military physical fitness tests. Bone turnover biomarkers (dependent variables) were assessed via a serum sample. Male military cadets (n=45) completing Cadet Basic Training participated in this study. Cadets who sustained an injury that precluded them

from completing Cadet Basic Training or had a neurological or metabolic disorder were excluded. A markerless motion capture system recorded and analyzed trunk and lower extremity biomechanical patterns during 3 jump-landing trials.[11] Serum samples were collected following Cadet Basic Training (post-Cadet Basic Training). ELISAs determined PINP and CTx-1 serum concentrations. Bone turnover biomarker ratios (PINP : CTx-1) were calculated. Univariate and multivariate linear regression models determined how each independent variable influenced PINP, CTx-1, and PINP : CTx-1. Food consumption and exercise prior to the post-Cadet Basic Training blood draw were controlled for in our statistical analyses.

5.3 – Results

Quantitative and qualitative trunk and lower extremity biomechanical analyses identified significant predictors of PINP and CTx-1 concentrations and the PINP : CTx-1 ratio. Qualitatively, PINP and PINP : CTx-1 were predicted by: foot internal rotation; excessive trunk flexion displacement; and limited lower extremity sagittal plane displacement. CTx-1 was predicted by: heel-to-toe landings. The total LESS score was not a significant predictor of any biomarker variable. Quantitatively, CTx-1 was predicted by: hip flexion angle at initial ground contact; maximum hip flexion angle; knee flexion angle at initial ground contact; knee flexion displacement; maximum knee valgus angle; knee varus angle displacement; and ankle plantar flexion angle at initial ground contact.

A number of non-biomechanical stress fracture risk factors predicted post-Cadet Basic Training biomarker concentrations. PINP and PINP : CTx-1 were predicted by: an injury during Cadet Basic Training; the raw-sit up score; and the difference in pre-to-post-Cadet Basic Training cadet mass. CTx-1 was predicted by: post-Cadet Basic Training mass.

5.4 – Interpretation of Results

Known lower extremity stress fracture risk factors predicted post-Cadet Basic Training bone turnover biomarker concentrations. Qualitative and quantitative analyses of trunk and lower extremity biomechanical patterns are capable of identifying movement patterns that predict bone turnover biomarkers. Additionally, other known stress fracture risk factors predicted bone turnover biomarkers. These findings provide important insight into how previously identified lower extremity stress fracture risk factors influence bone health at the molecular level and influence stress fracture risks.

Overall trunk and lower extremity movement quality did not predict post-Cadet Basic Training bone turnover concentrations. This was unexpected as poor movement quality results in more musculoskeletal stress and thus greater bone turnover.[63,65,105] However, the qualitative biomechanical analysis (Landing Error Scoring System [LESS]) used in our study was originally developed to identify anterior cruciate ligament (ACL) risk factors.[11] Some of the biomechanical patterns identified with the LESS increase ACL injury but actually reduce stress fracture risks. Furthermore, individuals can have the same cumulative LESS score, but may have scored differently on individual LESS items. Thus, individual biomechanical variables are better predictors of post-Cadet Basic Training bone turnover biomarker concentrations and ratios.

Lower extremity sagittal plane joint angles predicted post-Cadet Basic Training CTx-1 concentrations. As small hip flexion and larger knee flexion angles increased CTx-1 concentrations and limited trunk and lower extremity sagittal plane displacement resulted in larger PINP : CTx-1 ratios. These findings were somewhat surprising as stiffer landings (less sagittal plane displacement) increase ground reaction forces and force loading rates which increase stress fracture risk.[139,141] Conversely, excessive trunk flexion also increased PINP and

PINP : CTx-1. Sagittal plane trunk displacement mitigates ground reaction forces during jump-landings and therefore can be protective against lower extremity stress fractures.[142]

Heel-to-toe landings increased the post-Cadet Basic Training CTx-1 concentrations. Minimal plantar flexion at initial ground contact increases vertical ground reaction forces and loading rates, which increases bone stress[137-139] and stress fracture risk.[13] Paradoxically, our quantitative biomechanical analyses showed that greater ankle plantar flexion angle at initial ground contact increased CTx-1 concentrations. The markerless motion capture system used in our study had limited ability to accurately detect foot placement at initial ground contact, so it may not have accurately calculated ankle plantar flexion angle at initial ground contact and thus the discrepancies in the relationships between ankle plantar flexion and bone turnover biomarkers were observed. Further research is needed to fully understand the relationships between ankle plantar flexion and post-Cadet Basic Training bone turnover biomarkers.

Knee valgus and varus alignment increased bone resorption. This may explain why knee valgus angle during jump-landing assessments is predictive of lower extremity stress fracture.[9] Medial knee displacement is a clinical proxy for knee valgus alignment[11,82] but did not predict any biomarker variable. The potential exists that visual observation of medial knee displacement may not be sensitive enough to identify the multiplanar factors that contribute to 3-dimensional (3D) knee valgus.[82]

Knee varus angle displacement resulted in greater CTx-1 concentrations. This was surprising as knee varus alignment during jump-landings reduces stress fracture risks.[9] However, a negative correlation was observed between knee frontal plane angle at initial ground contact between the markerless motion capture system and the stereophotogrammetric systems. Thus, it is likely that what the markerless systems reported as a varus value was actually valgus

alignment at initial ground contact and the relationship between knee varus angle displacement and CTx-1 concentrations was observed.

Visual observation of foot internal rotation increased PINP concentrations. This contradicts research that shows knee internal rotation during a jump-landing assessment is a stress fracture risk factor.[9] Foot internal rotation during jump-landings generally occurs at the time of initial ground contact when individuals commonly have a plantar flexed foot and ankle. Foot and ankle plantar flexion results in tibial external rotation.[140] Thus, when the ground reaction forces are greatest the tibia is in a safer, externally rotated, position even though the feet appear internally rotated. Plantar flexion at initial ground contact also helps to mitigate ground reaction forces which could result in greater PINP concentrations.

Better pre-Cadet Basic Training physical fitness is protective against stress fractures.[34,37,45,97] In our study, each additional sit-up increased PINP concentrations and PINP : CTx-1 ratios, indicating bone formation was occurring. We hypothesized that pre-Cadet Basic Training run times would be strong predictors of post-Cadet Basic Training biomarker concentrations as poor aerobic fitness increases the work the body has to do and in-turn increase musculoskeletal stress and injury risk.[35,41] This was not observed in our study.

Previous physical activity level and type are both strong predictors of lower extremity stress fractures during military training,[33,40,43,96] but did not predict any biomarker variable in the present study. It is likely that cadets did not accurately report their previous physical activity experiences, and thus no relationships were observed.[37,39]

Sustaining an injury during Cadet Basic Training increased PINP and PINP : CTx-1. This finding opposed what was hypothesized as a history of previous musculoskeletal injury increases future injury risk.[37,45,99] It is important to note that none of the cadets who sustained an injury

during Cadet Basic Training were symptomatic at the time of the post-Cadet Basic Training blood draw or missed more than 2 days of Cadet Basic Training. Thus, the potential exist that the acute response to injury had passed and tissues containing type I collagen (eg. tendons) were rebuilding and we observed an increase in PINP and PINP : CTx-1.

Greater post-Cadet Basic Training mass increased post-Cadet Basic Training CTx-1 concentrations. This aligns with previous work that showed heavier individuals are at increased stress fracture risk.[33] Greater changes in pre-to-post-Cadet Basic Training mass resulted in greater PINP and PINP : CTx-1 concentrations. Our findings, and those of others indicate that military personnel should aim to maintain a healthy weight prior to and during military training to reduce stress fracture risk.[17,33,40,44,97]

Our findings demonstrate the multifactorial nature of stress fracture risk factors. All aspects of and individual's health and wellness should be considered and monitored to identify individuals at increased stress fracture risk. See **Appendix 4.1** and **Appendix 4.2** for a summary of how known stress fracture risk factors influence bone turnover biomarkers.

5.5 – Strengths and Limitations

The biggest strength of our study is the controlled nature in which it was conducted. Our population was restricted to male military cadets who were new to military training. This helped to limit the variability in our sample and potential factors that could confound the study results. However this well controlled design also limited the generalizability of our study. It is known that sex[11,63,85,122] and prior physical activity influence stress fracture risks and bone biomarker responses to physical activity.[5,17,19,27] Thus, future research must examine how known stress fracture risk factors affect bone biomarker concentrations in females, distance runners, and

individuals with metabolic disorders that may affect bone health as these populations are most vulnerable for stress fractures.

The motion capture system utilized in this study is equipped with fixed algorithms that automatically qualitatively and quantitatively evaluate trunk and lower extremity biomechanics. This allowed for consistent analysis of all cadets. This motion capture system can validly identify gross movement patterns and is affordable and quick and easy to use.[16] However, this system is limited in its ability to accurately calculate joint angles at initial ground contact and transverse plane joint angles, which may have important implications for injury risk. For these reasons further refinement of the markerless motion capture system should occur so that more accurate measurements of trunk and lower extremity joint angles can be calculated during functional movement assessments. More sensitive kinematic measures (3-dimensional biomechanical analyses) should be utilized in future studies examining how trunk and lower extremity biomechanics influence bone turnover biomarkers.

Kinetic measurements were not recorded in this study. This is a major limitation as kinetic measurements can identify stress fracture risk factors and thus could greatly influence bone biomarker concentrations.[139,141] Future research should examine how kinetic measurements, including ground reaction forces and internal joint moments, alter bone turnover biomarkers.

Blood samples were only collected at the end of Cadet Basic Training. Thus, we were unable to determine how stress fracture risk factors alter bone turnover biomarkers throughout military training. Understanding how stress fracture risk factors alter bone turnover biomarkers throughout military training is important so that critical periods can be identified when bones are most vulnerable to fracture. Once these critical periods and the factors that influence bone health during these periods are identified targeted stress fracture risk mitigation strategies can be

implemented.

Finally, resting and fasting serum samples were unable to be collected for this study. However, military personnel eat a standardized diet so the risk of food consumption sample contamination was equally likely for all study participants.[109] Also, food and exercise contamination were controlled for in our statistical models.

5.6 – Conclusions

Lower extremity stress fracture risk factors significantly predict post-Cadet Basic Training bone turnover biomarkers. Overall, previously identified biomechanical risk factors[9] were significant predictors of post-Cadet Basic Training bone turnover biomarkers. However, our biomechanical analyses may not have been sensitive enough to accurately calculate all trunk and lower extremity kinematics during a jump-landing assessment. Our study expands on previous research as it provides insight into how stress fracture risk factors alter bone health at the molecular level. This information is useful as it can help guide the development of targeted stress fracture risk mitigation strategies. Reducing stress fracture risk will mitigate the effects of stress fractures on our nation's military and improve force health and readiness.

APPENDIX 3.1 – LESS OPERATIONAL DEFINITIONS

Item #	LESS item	Operational Definition
1	Knee flexion angle at initial contact	At the time point of initial contact, if a knee is flexed less than 30°, score ERROR. If both knees are flexed more than 30°, score NO ERROR.
2	Hip flexion angle at initial contact	At the time point of initial contact, if a thigh is in line with the trunk, score ERROR. If both thighs are flexed on the trunk, score NO ERROR.
3	Trunk flexion angle at initial contact	At the time point of initial contact, if the trunk is vertical or extended on the hips, score ERROR. If the trunk is flexed on the hips, score NO ERROR.
4	Ankle plantar flexion angle at initial contact	At the time point of initial contact, if 1 foot lands heel-to-toe or flat foot, score ERROR. If both feet land toe-to-heel, score NO ERROR.
5	Asymmetrical foot contact	If 1 foot lands before the other or if 1 foot lands heel-to-toe or foot flat and the other lands differently (i.e. toe-to-heel), score ERROR. If the feet land symmetrically, score NO ERROR.
6	Asymmetrical Timing	If 1 foot lands before the other, score ERROR. If the feet land at the same time, score NO ERROR.
7	Asymmetrical Heel-Toe/ Toe-Heel	If 1 foot lands heel-to-toe or foot flat and the other lands toe-to-heel, score ERROR. If the feet land symmetrically, score NO ERROR.
8	Lateral trunk flexion angle at initial contact	At the time point of initial contact, if the midline of the trunk is flexed to the left or the right side of the body, score ERROR. If the trunk is not laterally flexed, score NO ERROR.

9	Medial knee position at initial contact	At the time point of initial contact, imagine a line straight down from the center of the patella. If the line is medial to the midfoot, score ERROR. If the line goes through the midfoot, score NO ERROR.
10-11	Stance width	Once the entire foot is in contact with the ground, imagine a line down from the tip of each shoulder. If a line falls inside a foot, score ERROR for greater than shoulder width. If a line falls outside of a foot, score ERROR for less than shoulder width. If both lines fall on the feet, score NO ERROR. ***If a foot is internally or externally rotated, grade the stance width based on heel placement.
12-13	Foot position	At the point of maximum rotation between initial contact and maximum knee flexion, if a foot is externally or internally rotated more than 30°, then score ERROR. If the feet are not internally or externally rotated more than 30° between the time period of initial contact to max knee flexion, score NO ERROR.
14	Knee flexion displacement	If a knee does not flex more than 45° from initial contact to maximum knee flexion, score ERROR. If the knees flex more than 45°, score NO ERROR.
15	Hip flexion displacement	If a thigh does not flex more on the trunk from initial contact to maximum knee flexion angle, score ERROR. If a thigh flexes more on the trunk from initial contact to maximum knee flexion, score NO ERROR.
16	Trunk flexion displacement	If the trunk does not flex more from the point of initial contact to maximum knee flexion, score ERROR. If the trunk does flex more from the point of initial contact to maximum knee flexion, score NO ERROR.
17	EXCESSIVE Trunk flexion displacement	If the trunk flexes past parallel with the lower leg, score ERROR. If the trunk appears parallel with the lower leg or less, score NO ERROR.

18	Maximum medial knee position	At the point of maximal medial knee position, imagine lines straight down from the center of each patella. If a line runs through the great toe or is medial to the great toe, score ERROR. If both lines are lateral to the great toe, score NO ERROR.
19	Asymmetrical Loading	If the participant appears to have a weight-shift, or loading 1 side more than the other, score ERROR. If weight seems to be loaded evenly across both limbs, score NO ERROR.
20	Wobble	Watch landing REAL-TIME. If 1 or both of participant's knees appears to "wobble", or demonstrate quick varus/valgus motion, score ERROR. If no wobble is present, score NO ERROR.
21	Joint displacement	Watch the sagittal plane motion at the trunk, hips, and knees from initial contact to maximum knee flexion angle. If the participant goes through large displacement of the trunk, hips, and knees then score SOFT. If the participant goes through some trunk, hip, and knee displacement but not a large amount, then score AVERAGE. If the participant goes through very little, if any trunk, hip, and knee displacement, then score STIFF.
22	Overall impression	Score EXCELLENT if the participant displays a soft landing and no frontal/transverse plane motion. Score POOR if the participant displays a stiff landing and at least some frontal or transverse plane lower extremity motion OR large frontal or transverse plane lower extremity motion. All other landings score AVERAGE.

APPENDIX 3.2 – BASELINE QUESTIONNAIRE

ID: _____

Developing Lower Extremity Injury Prediction Models and
Evaluating Targeted Injury Prevention Intervention Strategies

BASELINE QUESTIONNAIRE

STUDY ID NUMBER: _____

NAME: _____

COMPANY NAME: _____

PLATOON NAME: _____

SSN: _____

This questionnaire is part of a research project about identifying people who are at higher risk for lower extremity injury and preventing these injuries.

Your participation in this study is voluntary. Neither your military career nor your medical treatment will be affected should you choose not to fill out any or all of this questionnaire. No participants will be identified in any report or publication. All research records will be kept confidential.

The study is being led by Dr. Kenneth Cameron, Department of Orthopedic Research, at Keller Army Community Hospital, in collaboration with Dr. Stephen W. Marshall, epidemiologist at the University of North Carolina at Chapel Hill, Dr. Darin Padua, sports medicine specialist at the University of North Carolina at Chapel Hill, and Dr. Lindsay Distefano, biomechanist at the University of Connecticut.

PART 1 – Injury History

1. **Today's date and current time:**
   a. **30 June 2015; morning (AM)**
   b. **30 June 2015; afternoon (PM)**
   c. **1 July 2015; morning (AM)**
   d. **1 July 2015; afternoon (PM)**

The remaining questions refer to injuries or conditions that you have **ever** experienced.

2. **Have you ever had an injury to a ligament in either (or both) knee(s)?**
   a. **No**
   b. **Yes**

3. **Have you ever had an injury to the Anterior Cruciate Ligament (ACL)?**
   a. **No**
   b. **Yes**

4. **If you answered YES to QUESTION 3, which knee(s) was/were injured?**
   a. **Not applicable (no history of ACL injury)**
   b. **Left**
   c. **Right**
   d. **Both**

5. **If you answered YES to QUESTION 3, when (what year) did the injury occur?**
   a. **Not applicable (no history of ACL injury)**
   b. **2015**
   c. **2014**
   d. **2013**
   e. **2012**
   f. **2011**
   g. **2010**
   h. **2009**
   i. **2008**
   j. **2007 or before**

6. **If you answered YES to QUESTION 3, did this ACL injury (or injuries) require surgery?**
   a. **Not applicable (no history of ACL injury)**
   b. **No**
   c. **Yes**

---

7. **Have you ever had an injury to the Medial Collateral Ligament (MCL)?**
   a. **No**
   b. **Yes**

8. **If you answered YES to QUESTION 7, which knee(s) was/were injured?**
   a. **Not applicable (no history of MCL injury)**
   b. **Left**
   c. **Right**
   d. **Both**

9. **If you answered YES to QUESTION 7, did this MCL injury (injuries) require surgery?**
   a. **Not applicable (no history of MCL injury)**
   b. **No**
   c. **Yes**

---

10. **Have you ever had an injury to the Lateral Collateral Ligament (LCL)?**
    a. **No**
    b. **Yes**

11. **If you answered YES to QUESTION 10, which knee(s) was/were injured?**
    a. **Not applicable (no history of LCL injury)**
    b. **Left**
    c. **Right**
    d. **Both**

12. **If you answered YES to QUESTION 10, did this LCL injury (or injuries) require surgery?**
    a. **Not applicable (no history of LCL injury)**
    b. **No**
    c. **Yes**

---

13. **Have you ever had an injury to the Posterior Cruciate Ligament (PCL)?**
    a. **No**
    b. **Yes**

14. **If you answered YES to Question 13, which knee(s) was/were injured?**
    a. **Not applicable (no history of PCL injury)**
    b. **Left**
    c. **Right**
    d. **Both**

15. **If you answered YES to QUESTION 13, did this PCL injury (or injuries) require surgery?**
    a. **Not applicable (no history of PCL injury)**
    b. **No**
    c. **Yes**

---

16. Have you *ever* had an injury to the meniscus of the knee(s)?
    a. No
    b. Yes

17. If you answered YES to QUESTION 16, which knee(s) was/were injured?
    a. Not applicable (no history of meniscus injury)
    b. Left
    c. Right
    d. Both

18. If you answered YES to QUESTION 16, did this injury (or injuries) require surgery?
    a. Not applicable (no history of meniscus injury)
    b. No
    c. Yes

---

19. Have you had knee surgery, ***within the past 10 years***, other than that listed in the previous questions?
    a. No
    b. Yes

20. If you answered YES to Question 19, which knee(s) was/were operated on?
    a. Not applicable (no history other knee injury in the past 10 years)
    b. Left
    c. Right
    d. Both

---

21. Within the past ***six months***, have you had episodes of ***severe*** pain in your knee(s)?
    Severe *means pain that would make you stop what you were doing, or limit or interfere with your activities.*
    a. No
    b. Yes

22. If you answered YES to QUESTION 21, which knee(s) was/were involved?
    a. Not applicable (no history of severe knee pain in the past six months)
    b. Left
    c. Right
    d. Both

23. If you answered YES to QUESTION 21, was/is it worse when you exercise?
    a. Not applicable (no history of severe knee pain in the past six months)
    b. No
    c. Yes

24. If you answered YES to QUESTION 21, do you currently have this problem, or has it resolved?
    a. Not applicable (no history of severe knee pain in the past six months)
    b. Still a problem
    c. Resolved

---

25. Have you had a lower limb stress fracture within the past six months?
    a. No
    b. Yes

26. If you answered YES to QUESTION 25, which leg(s) was/were injured?
    a. Not applicable (no history of lower limb stress fracture in the past six months)
    b. Left
    c. Right
    d. Both

---

27. Have you had some other lower limb bone fracture within the past six months?
    a. No
    b. Yes

28. If you answered YES to QUESTION 27, which leg(s) was/were injured?
    a. Not applicable (no history of other lower limb fracture in the past six months)
    b. Left
    c. Right
    d. Both

---

29. Have you had patellofemoral pain (severe knee pain or runner's knee) within the past six months?
    a. No
    b. Yes

30. If you answered YES to QUESTION 29, which knee(s) was/were involved?
    a. Not applicable (no history of patellofemoral pain in the past six months)
    b. Left
    c. Right
    d. Both

31. If you answered YES to QUESTION 29, does it currently interfere with physical activity?
    a. Not applicable (no history of patellofemoral pain in the past six months)
    b. No
    c. Yes

---

32. Have you had swelling, clicking or popping, or a feeling of the knee giving way within the past six months?
    a. No
    b. Yes

33. If you answered YES to QUESTION 32, which knee(s) was/were involved?
    a. Not applicable (no history swelling, clicking, popping, or giving way in the past six months)
    b. Left
    c. Right
    d. Both

34. If you answered YES to QUESTION 32, does it currently interfere with physical activity?
    a. Not applicable (no history swelling, clicking, popping, or giving way in the past six months)
    b. No
    c. Yes

---

35. In the past six months, have you had an injury to your hip?
    a. No
    b. Yes

36. If you answered YES to QUESTION 35, which hip(s) was/were injured?
    a. Not applicable (no history of hip injury in the past six months)
    b. Left
    c. Right
    d. Both

37. If you answered YES to QUESTION 35, does it currently interfere with physical activity?
    a. Not applicable (no history of hip injury in the past six months)
    b. No
    c. Yes

---

38. In the past six months, have you had an injury to your ankle?
    a. No
    b. Yes

39. If you answered YES to QUESTION 38, which ankle(s) was/were injured?
    a. Not applicable (no history of ankle injury in the past six months)
    b. Left
    c. Right
    d. Both

40. If you answered YES to QUESTION 38, does it currently interfere with physical activity?
    a. Not applicable (no history of ankle injury in the past six months)
    b. No
    c. Yes

---

41. In the past six months, have you had an injury to your foot?
    a. No
    b. Yes

42. If you answered YES to QUESTION 41, which foot(s) was/were involved?
    a. Not applicable (no history of foot injury in the past six months)
    b. Left
    c. Right
    d. Both

43. If you answered YES to QUESTION 41, does it currently interfere with physical activity?
    a. Not applicable (no history of foot injury in the past six months)
    b. No
    c. Yes

---

44. In the past six months, have you had any other lower leg injury other than that listed in the previous questions?
    a. No
    b. Yes

45. If you answered YES to QUESTION 44, which lower leg(s) was/were injured?
    a. Not applicable (no history of other lower leg injury in the past six months)
    b. Left
    c. Right
    d. Both

46. If you answered YES to QUESTION 44, does it currently interfere with physical activity?
    a. Not applicable (no history of other lower leg injury in the past six months)
    b. No
    c. Yes

---

47. In the *past six months*, have you been using a training program that involves repeated jumping? (Such programs are sometimes referred to as plyometric exercises)
    a. No
    b. Yes

48. If you answered YES to QUESTION 47, how many months, *out of the past six*, have you been doing this program?
    a. Not applicable (have not completed a training program with repeated jumping in the past six months)
    b. 1 month
    c. 2 months
    d. 3 months
    e. 4 months
    f. 5 months
    g. 6 months

49. In the *past six months*, have you been doing a training program designed to reduce the risk of ACL injury?
    a. No
    b. Yes

50. If you answered YES to QUESTION 49, how many months, *out of the past six*, have you been doing this program?
    a. Not applicable (have not completed a training program designed to reduce the risk of ACL injury in the past six months)
    b. 1 month
    c. 2 months
    d. 3 months
    e. 4 months
    f. 5 months
    g. 6 months

51. If you answered YES to QUESTION 49, please name the program, or its developer:
    a. Not applicable (have not completed a training program designed to reduce the risk of ACL injury in the past six months)
    b. USMA or prep school
    c. My coach
    d. My athletic trainer
    e. Other

52. If you answered YES to QUESTION 49, how often (days per week) did you do the program?
    a. Not applicable(have not completed a training program designed to reduce the risk of ACL injury in the past six months)
    b. 1 day
    c. 2 days
    d. 3 days
    e. 4 days
    f. 5 days
    g. 6 days
    h. 7 days

PART 2 – Activity History

Please indicate how often you performed each activity in your healthiest and most active state, **in the past year (prior to R-Day).**

53. Running: running while playing a sport or jogging
    a. Less than one time *in a month*
    b. One time *in a month*
    c. One time *in a week*
    d. 2 or 3 times *in a week*
    e. 4 or more times *in a week*

54. Cutting: changing directions while running
    a. Less than one time *in a month*
    b. One time *in a month*
    c. One time *in a week*
    d. 2 or 3 times *in a week*
    e. 4 or more times *in a week*

55. Decelerating: coming to a quick stop while running
    a. Less than one time *in a month*
    b. One time *in a month*
    c. One time *in a week*
    d. 2 or 3 times *in a week*
    e. 4 or more times *in a week*

56. Pivoting: turning your body with your foot planted while playing a sport. For example: skiing, skating, kicking, throwing, hitting a ball (golf, tennis, squash), etc.
    a. Less than one time *in a month*
    b. One time *in a month*
    c. One time *in a week*
    d. 2 or 3 times *in a week*
    e. 4 or more times *in a week*

PART 3 – Sports Participation History

Please fill in the appropriate answer for all of the sports listed below. A "**SEASON**" is defined as participating at least 3 times a week for at least 3 months. Please fill in the appropriate bubble on your Scantron.

Question	Sport or Activity	Never, or Less than 1 complete season (<3/wk for 3 months)	1-2 Seasons over the past 10 years	3-5 Seasons over the past 10 years	6 or more Seasons Over the past 10 years
57.	Baseball/Softball	A	B	C	D
58.	Basketball	A	B	C	D
59.	Boxing	A	B	C	D
60.	CrossFit	A	B	C	D
61.	Canoeing/ Kayaking	A	B	C	D
62.	Crew	A	B	C	D
63.	Cheerleading	A	B	C	D
64.	Cycling/Mountain Biking	A	B	C	D
65.	Dance	A	B	C	D
66.	Fencing	A	B	C	D
67.	Football	A	B	C	D
68.	Field Hockey	A	B	C	D
69.	Golf	A	B	C	D
70.	Gymnastics	A	B	C	D
71.	Ice Hockey/In-line Hockey	A	B	C	D
72.	Ice Skating	A	B	C	D
73.	Kickboxing	A	B	C	D
74.	Lacrosse	A	B	C	D
75.	Martial Arts	A	B	C	D
76.	Rock Climbing	A	B	C	D
77.	Rugby	A	B	C	D
78.	Running/Jogging	A	B	C	D
79.	Skateboarding	A	B	C	D
80.	Skiing/ Snowboarding	A	B	C	D
81.	Soccer	A	B	C	D
82.	Squash/ Racquetball	A	B	C	D
83.	Surfing/Windsurfing	A	B	C	D
84.	Swimming	A	B	C	D
85.	Team Handball	A	B	C	D
86.	Tennis	A	B	C	D
87.	Cross Country/Track and Field	A	B	C	D
88.	Triathlon	A	B	C	D
89.	Ultimate Frisbee	A	B	C	D
90.	Volleyball	A	B	C	D
91.	Water Skiing/Wakeboarding	A	B	C	D
92.	Weight Training	A	B	C	D
93.	Wrestling	A	B	C	D
94.	Yoga/Tai Chi	A	B	C	D
95.	Other Physical Activities	A	B	C	D

THANK YOU FOR YOUR PARTICIPATION!

GO ARMY!

APPENDIX 4.1 – MOVEMENT QUALITY AND BIOMARKERS OF BONE TURNOVER

Kinematic Variable	Effect on Bone Turnover Biomarkers	Discussion
Overall Movement Quality	• Total LESS score did not predict any biomarker variable. • The "overall impression" item on the LESS did not predict any biomarker variable. • Multivariate regression analyses incorporating only LESS movement data or kinematic data did not predict any biomarker variable.	• The LESS was developed to identify anterior cruciate ligament (ACL) risk factors but LESS scores have been associated with lower extremity stress fracture risk factors. o The "overall impression" item may identify factors that are irrelevant to or protective against stress fracture risk. o The total LESS score includes items that increase ACL injury risk but may reduce stress fracture risks. • The kinematic variables reported by the markerless motion capture system were highly variable and may have limited their ability to predict post-Cadet Basic Training biomarker concentrations.
Sagittal Plane Joint Displacement	• Lower extremity sagittal plane displacement (LESS) increased PINP : CTx-1 but not PINP. • Excessive trunk flexion displacement (LESS) increased PINP concentrations and PINP : CTx-1 ratios. • Multivariate linear regression models incorporating all kinematic variables identified predictors of post-Cadet Basic Training CTx-1. o Hip flexion angle at initial ground contact and maximum hip flexion angle o Knee flexion angle at initial ground contact and knee flexion displacement	• A lack of trunk and lower extremity sagittal plane displacement increased PINP : CTx-1 ratios indicating with the smallest amount of sagittal plane displacement had the largest increases in PINP : CTx-1 ratio. o Surprising as stiffer landings increase ground reaction forces and loading rates; both increase stress fracture risk. • Trunk flexion displacement during jump-landings mitigates ground reaction forces. o May be protective against lower extremity stress fractures. Supported by the observed increases in PINP and PINP : CTx-1 ratios. • The relationship between greater hip flexion and increased CTx-1 concentrations is reasonable as smaller negative hip flexion values are actually greater hip flexion angles. • The relationship between knee flexion angle and CTx-1 is opposite of what was anticipated as greater knee flexion angles should mitigate ground reaction forces and reduce the forces acting on the bones. o The markerless motion capture system had poor agreement with the stereophotogrammetric system for initial contact angles and may not have reported accurate joint angles.
Frontal Plane Hip	• Medial knee displacement	• Medial knee displacement was hypothesized to be predictive of CTx-1

and Knee Position	(LESS) was not predictive of any biomarker variable.
	• Multivariate linear regression models incorporating all kinematic variables identified predictors of post-Cadet Basic Training CTx-1.
	  ○ Maximum knee valgus angle
	  ○ Knee varus angle displacement
		as medial knee displacement is a proxy for knee valgus alignment which increases lower extremity stress fracture risk.
		  ○ Individuals commonly display foot external rotation in conjunction with medial knee displacement which results in tibial external rotation and may reduce stress fracture risk.
		  ○ Visual observation of medial knee displacement may not be sensitive enough to identify all of the multiplanar factors that contribute to three-dimensional (3D) knee valgus angle.
		• Maximum knee valgus angle reported by the markerless motion capture system was predictive of post-Cadet Basic Training CTx-1 concentrations, this supports previous research that found knee valgus alignment during a jump-landing increases stress fracture risk.
		• The relationship between greater knee varus angle displacement and increased CTx-1 concentrations was surprising as knee varus alignment during jump-landings reduces stress fracture risks.
		  ○ A negative correlation was observed between knee frontal plane angle at initial ground contact between the markerless motion capture system and the stereophotogrammetric systems. It is likely that what the markerless systems reported as a varus value was actually valgus alignment at initial ground contact and thus the relationship between knee varus angle displacement and CTx-1 concentrations was observed.

Foot Position at Initial Ground Contact	• Heel-to-toe landings (LESS) increased CTx-1 concentrations.
	• Foot internal rotation (LESS) increased PINP concentrations and PINP : CTx-1.
	• Multivariate linear regression models incorporating all kinematic variables identified predictors of post-Cadet Basic Training CTx-1.
	  ○ Ankle plantar flexion angle at
		• Heel-to-toe landings increased CTx-1 concentrations. Heel-to-toe landings result in higher peak vertical ground reaction forces and loading rates, compared to toe-to-heel landings. Greater vertical ground reaction forces and loading rates are stress fracture risk factors.
		• Foot internal rotation increased PINP concentrations. This was surprising as previous research found knee internal rotation during a jump-landing assessment increases stress fracture risk.
		  ○ The potential exist that visually observed foot internal rotation occurs at initial ground contact when individuals commonly have a plantar flexed foot and ankle. Foot and ankle plantar flexion causes the tibia to externally rotate. Thus, when the ground reaction forces are

	initial ground contact	greatest the tibia is in a safer externally rotated position. The plantar flexed position of the foot would also help to mitigate ground reaction forces and reduce ground reaction force loading rates. ○ Torsion and bending forces stimulate the bone remodeling process. Initially bone resorption outpaces formation. Bone resorption takes 7-10 days while formation takes 2-3 months. Thus, the post-Cadet Basic Training blood samples were likely collected after the cadets had passed the initial bone breakdown period and occurred when bone formation was outpacing resorption and the relationship with PINP was observed. • The relationship between greater ankle plantar flexion angle at initial ground contact and increased CTx-1 concentrations was an unexpected finding and difficult to explain as there was good validity between the markerless and stereophotogrammetric motion capture systems. ○ Further study is needed to understand this relationship.

APPENDIX 4.2 – STRESS FRACTURE RISK FACTORS AND BIOMARKERS OF BONE TURNOVER

Stress Fracture Risk Factor	Effect on Bone Turnover Biomarkers	Discussion
Musculoskeletal Injury History	• Injury during Cadet Basic Training increased PINP and PINP : CTx-1. • Previous injury history did not predict any biomarker variable.	• Sustaining an injury during Cadet Basic Training increased PINP and PINP : CTx-1. This finding opposed what we hypothesized because previous injury increases future injury risk. ○ The potential exist that the acute response to injury had passed and the bones and other tissues containing type I collagen were rebuilding and an increase in PINP and PINP : CTx-1 were observed. • Previous stress fracture history was hypothesized to be a strong predictor of post-Cadet Basic Training biomarker concentrations; however, no participants in the study had a history of a stress fracture or had sustained a lower extremity fracture in the 6 months preceding Cadet Basic Training.
Physical Fitness Test Performance	• The raw sit-up score predicted PINP and PINP : CTx-1. • No other measures of physical fitness predicted any biomarker variables.	• Pre-Cadet Basic Training physical fitness influenced post-Cadet Basic Training bone biomarker concentrations. Each additional sit-up a cadet completed during the pre-Cadet Basic Training APFT increased PINP concentrations and PINP : CTx-1 ratios. Our findings support previous work that showed better performance on the sit-up component of standardized military physical fitness assessments decreased injury risk. • We anticipated that pre-Cadet Basic Training APFT run times would strongly influence post-Cadet Basic Training biomarkers, this was not observed. ○ The post-Cadet Basic Training blood sample collection may have occurred late enough in the training regimen that any initial increases in CTx-1 had passed and the bones were beginning to rebuild. ○ Individuals who sustained an injury during Cadet Basic Training that precluded them from finishing the training were excluded from our study. Individuals who may have been severely out of shape at the beginning may have become injured during Cadet Basic Training and were excluded from our study.
Previous Physical Activity	• No previous physical activity variable predicted any biomarker	• Previous physical activity level and type have both been identified as strong risk factors for lower extremity stress fracture risk factors.

	variable.	○ The potential exist that all cadets entered Cadet Basic Training with similar experiences with sports and activities; this does not appear to be the case with our sample. ○ Participants may not have accurately recalled their previous physical activity experiences and thus no relationships were observed.
Anthropometric Measurements	• Post-Cadet Basic Training mass increased CTx-1. • The difference in pre-to-post-Cadet Basic Training increased PINP and PINP : CTx-1 ratios.	• Overweight individuals have increased stress fracture risk. We observed similar findings in our study. Larger mass resulted in greater post-Cadet Basic Training CTx-1 concentrations. ○ Conversely, individuals with low body weight are also at increased stress fracture risk. • We hypothesized that large changes in pre-to-post-Cadet Basic Training mass would predict high rates of bone resorption. We observed the opposite; greater changes in pre-to-post-Cadet Basic Training mass resulted in greater PINP and PINP : CTx-1, and not CTx-1 concentrations. ○ May indicate that these individuals lost weight, reduced stresses on their bones, and the bones began to rebuild. • Our findings in combination with previous research suggest military personnel should aim to maintain a healthy weight throughout military training to minimize stress fracture risk.
Food Consumption Preceding the post-Cadet Basic Training Blood Draw	• Breakfast prior to the post-Cadet Basic Training blood draw increased PINP : CTx-1 ratios	• Eating breakfast increased CTx-1 concentrations. Protein rich foods can alter the concentrations of collagen byproducts in the serum, which may be incorrectly identified as bone resorption byproducts (CTx-1).
Exercise Preceding the post-Cadet Basic Training Blood Draw	• Exercise prior to the post-Cadet Basic Training blood draw increased CTx-1 concentrations and PINP : CTx-1	• Exercise can artificially elevate or reduce bone biomarker concentrations in the blood. The presence or absence of plasma volume expansion may occur after exercise, which can influence biomarker concentrations measured in the serum.

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