The Prevalence of Low-Level Exertional Rhabdomyolysis and Muscle Damage During Military Field Training Exercises

by

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Abstract

Exertional rhabdomyolysis (ER) is a condition characterized by skeletal muscle breakdown due to strenuous physical exertion. ER has a higher prevalence in populations undergoing rigorous training, such as military personnel. ER incidence rates in military settings are up to 20 times that of civilian populations. Despite this prevalence, many ER cases remain undiagnosed or underreported particularly among Reserve Officer Training Corps (ROTC) cadets. This research aims to explore physiological and psychological changes occurring during a two-to-three-day ROTC field training exercise (FTX), focusing on biomarkers such as creatine kinase and myoglobin, energy availability, hydration status, and training adaptations. The findings contribute to a deeper understanding of ER risk factors and recovery mechanisms in ROTC members.

The first portion of this project examined the effects of an ROTC FTX on cadet nutrition and energy balance. The results demonstrated that cadets often fail to consume sufficient nutrition to maintain energy balance, leading to short-term low energy availability. This suggests that strategic fueling strategies, improved ration compositions, and enhanced nutrition education are necessary to sustain both training performance and long-term health.

The second portion of this project explored the effects of an ROTC FTX on physical and psychological strain, measured through pain pressure threshold, mood, energy, and restfulness. The results demonstrated that cadets experienced a significant decline in mood, energy, and restfulness early in the FTX, particularly among Marines. Pain Pressure Thresholds (PPT) values correlated with fatigue and muscular strain, with novice cadets experiencing greater physiological stress. This suggests that recovery strategies should be tailored to branch-specific needs, experience level, and conditioning.

The third portion of this research evaluated the effects of a ROTC FTX on hydration markers, electrolyte balance, and metabolic biomarkers. The results demonstrated significant changes in hydration

status and metabolic markers, though blood markers of renal function remained stable. This suggests that monitoring hydration and metabolic responses is crucial in training environments.

The final portion of this project brought together the three prior sections to evaluate the effects of an ROTC FTX on systemic stress, metabolic shifts, and muscular adaptations. The results demonstrated that cadets exhibited physiological changes in hydration status, metabolic markers, and pain tolerance. Higher body fat percentages and older age were correlated with greater metabolic fluctuations, and fitness levels appeared to buffer metabolic perturbations. These findings suggest that physiological responses to military training are transient, with adaptations primarily reflecting immediate physical demands rather than long-term alterations.

Collectively, this body of research provides insight into the physiological and psychological adaptations experienced by ROTC cadets during a FTX. The findings suggest that cadets generally adapt well to training demands, and that nutrition, hydration, recovery strategies, and individual variability play key roles in optimizing performance and minimizing risks.

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List of Abbreviations

µg/L – microgram / liter

ACE – angiotensin-converting enzyme

AKI- Acute Kidney Injury

ALP – alkaline phosphate

ALT – alanine aminotransferase

AMR – Active Metabolic Rate

ASIS – Anterior Superior Iliac Spine

AST – Aspartate Transaminase

ATP – Adenosine triphosphate

B- Brain

BCT – Basic Combat Training

bFGF – basic fibroblast growth factor

BMI – Body Mass Index

BUN – Blood Urea Nitrogen

Ca- Calcium

CK – Creatine Kinase

cm – centimeters

CMP- Complete Metabolic Panel

ComRAD- U.S. Army Combat Rations Database

CRP – C-reactive protein

cTnI – Cardiac troponin I

DHPRs – Dihydropyridine Receptors

DOMS – Delayed Onset Muscle Soreness

EB- Energy Balance

ECC – Excitation-contraction Coupling

EE – Energy Expenditure

ER – Exertional Rhabdomyolysis

EST – Electronic Simulation Training

FFM – Fat Free Mass

FTX – Field Training Exercise

g – grams

G6PD- glucose-6-phosphate dehydrogenase

HBAS – heterozygous HBB gene

HBSS – homozygous HBB gene

HOT-A – Hot meal (from the post dining facility)

IOC – International Olympic Committee

K- Potassium

kcal – kilocalories

kcal- kilocalories

kcal/day – kilocalories per day

kcal/kg/day – kilocalories per kilogram per day

kDa- kilodalton

kg – kilograms

lbs. – pounds

LDH- lactate dehydrogenase

LEA- Low Energy Availability

M- Muscle

Mb – Myoglobin

MDRI – Military Dietary Reference Intake

mg/dL – milligrams per deciliter

MH – Malignant Hyperthermia

ml- milliliter

MRE- Meal Ready-to-eat

MS- Military Science

MSKI – Musculoskeletal Injury

MTFs- Military Treatment Facilities

Na – Sodium

ng/ml – nanograms per milliliter

NI- Nutritional Intake

nmol/L – nanomole per liter

oz- ounces

PPT – Pain Pressure Threshold

RAS – renin-angiotensin system

RED-S – Relative Energy Deficiency in Sport

ROM – Range of Motion

ROTC – Reserve Officer Training Corps

RYR1 – Ryanodine Receptors

SCA – Sickle Cell Anemia

SCT- Sickle Cell Trait

SD – Standard Deviation

SE – Standard Error

sTnI – skeletal troponin I

TCCC- Trauma combat casualty care

TnC- calcium-binding subunit

TnI – actomyosin-adenosine triphosphate-inhibiting subunit

TnT – tropomyosin-binding subunit

U.S. – United States

u/L – Units per Liter

USAF – United States Air Force

USG – Urine Specific Gravity

yrs – years

Introduction

Exertional rhabdomyolysis (ER) is a condition characterized by skeletal muscle breakdown due to strenuous physical exertion.[1-3] ER is prevalent in populations undergoing rigorous training, including military personnel.[4-6] High levels of physical activity release intracellular muscle contents into the bloodstream, triggering a cascade with detrimental effects on bodily systems.[7,8] Biological sex, race, dehydration, and unaccustomed exercise exacerbate susceptibility.[9] The total incidence rates of ER are difficult to determine, as many ER cases go unreported or are underreported in medical settings.[10] There are approximately 26,000 cases of ER annually in the United States.[11]

The majority of reported cases are in tactical athlete populations (military: 0.3%- 3.0%, law enforcement: 0.2%, and firefighters: 0.2%).[10,11] Service members experience an ER rate 20 times that found in civilians.[12] Military ER incidents peaked at 43.1 per 100,000 person-years between 2017-2021.[13] Recovery is prolonged and impacts unit stress, mission completion, and military medical costs. ER incidents occur most often in training regiments (e.g., basic training), as military training exacerbates the risk of ER prevalence.[14,15]These incidents often occur at critical times in the individual's development and military career, delaying training progression or resulting in dismissal from military service.[16,17]

Though the origins of rhabdomyolysis are diverse, development follows a common mechanistic pathophysiology (Figure 1).[7] Rhabdomyolysis can be trigged by a variety of muscular insults categorized as hypoxic, chemical, biological or physical. When rhabdomyolysis is caused specifically by damage to the muscle by physical training or exercise it is termed ER.[1,2,18] The

term ER will be used in the cases of rhabdomyolysis caused by exertional means while the term rhabdomyolysis will be used when referring to the overall condition.

ER arises from the leakage of intracellular muscle contents into the bloodstream, including creatine kinase and myoglobin. This initiates a chemical and hormonal cascade that disrupts diverse bodily systems.[2,18-20] Myoglobinuria is the occurrence of myoglobin (Mb) in the urine. This generally occurs after Mb has been released from the muscle following muscular damage. Myoglobinuria is a hallmark of exertional rhabdomyolysis and can result in renal impairment as myoglobin precipitates in renal tubules.[21,22] The ensuing cascade involves the release of cellular contents into the bloodstream, leading to a variety of metabolic disturbances, including possible renal failure and even leading to death.[23,24] The precise etiology of ER remains multifactorial. Factors such as poor physical fitness, biological sex, race, dehydration, warm or hot weather, especially with high humidity, unaccustomed exercise, and underlying genetic factors have been recognized to exacerbate susceptibility.[1,14,15,25] Timely recognition and management are crucial to mitigate the risk of complications.[17] It is critical to understand the sequela of exertional rhabdomyolysis in diverse physically demanding contexts, including military training environments.[14]

The Reserve Officer Training Corps (ROTC) is the largest commissioning body for the U.S. Armed Forces.[26] ROTC programs are designed to develop civilians into future military leaders.[27] Nearly 60% of U.S. Army second lieutenants and over 40% of active duty general officers have been commissioned from a ROTC program.[28-30] ROTC members are a unique group, with cadets completing military training while attending a university full-time. In addition to a full

class schedule and maintaining the requirements of a traditional college student, ROTC members are required to complete physical fitness sessions, military skills laboratories, and military-specific courses. Additional cognitive, mental, and physical demands that ROTC members must withstand, along with the normal stressors as college students, contribute to the increased occurrence of training-related injuries.[27]

ROTC members are generally less accustomed to military-specific events and tasks than active-duty members despite regular physical and military training. ROTC programs complete a biannual three-day field training exercise (FTX). Members stay in a field training environment while completing military training such as obstacle courses, range weapon training, land navigation, and ruck marching. The escalation in rigorous training increases the concern for ER in ROTC members. Research on ER in ROTC populations has relied on retrospective military reports. [31,32] However, these studies may not accurately represent ROTC components, as many cadets and midshipmen opt for external medical care from personal primary care providers who do not report these instances to military agencies.[32]

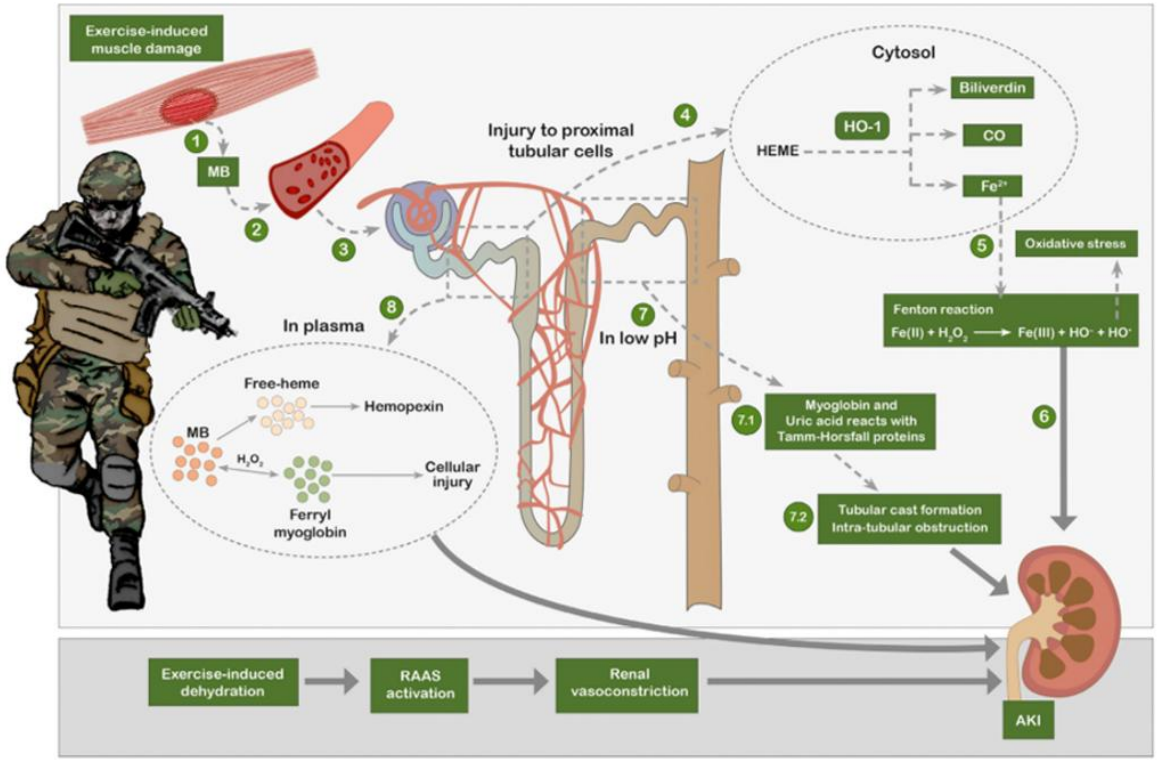


Figure 2. Pathophysiology and exercise-induced factors leading to exertional rhabdomyolysis and acute kidney injury. Image taken from Carneiro et al [1]

Understanding additional moderators (e.g., biological sex, race, dehydration, and unaccustomed exercise) may lead to decreased undiagnosed incidents and the development of preventative measures. This improved comprehension of ER may contribute to the prevention of musculoskeletal injury, the effectiveness of recognizing and preventing ER, and reducing instances of acute kidney injury or even death caused by ER.[33,34] This research addresses a critical gap in understanding this condition within a specific demographic, with the potential to enhance preventive measures and medical interventions. Findings may reduce the impact of ER on individual cadets and the broader military community. Our goal is to determine the unrecognized occurrence and contributing moderators of exertional rhabdomyolysis in ROTC members during a 2-3 day extended military field training exercise. Creating a more in-depth understanding of

specific moderators will lead to a better understanding of the development and necessary medical treatment and possibly decreased incidence of exertional rhabdomyolysis occurrences.

The main objective of this research is to conduct an observational study assessing both creatine kinase and myoglobin levels along with subjective measures prior to and after completing a two-to-three-day military field training exercise. Our central hypothesis is that military field training exercises will result in low-level or otherwise undiagnosed rhabdomyolysis in some ROTC cadets, as measured by exceedingly heightened creatine kinase and myoglobin levels.

The primary aim of this study is to determine if above normal physical intensity, short-term military training produces low-level rhabdomyolysis, putting military members at risk for serious injury.

Aim 1A: Determine how a two-to-three-day ROTC field training exercise (FTX) alters creatine kinase and myoglobin to levels associated with exertional rhabdomyolysis.
Hypothesis: Creatine kinase and myoglobin levels in ROTC Cadets will increase to three times baseline levels after a two-to-three-day field training exercise.

Aim 1B: Determine if fitness level, body composition, biological sex, race, or age correspond with changes in creatine kinase and myoglobin levels after a two-to-three day ROTC FTX.
Hypothesis: Cadets with lower fitness level, higher body fat percentage, and black biological males ages 20 years or younger will be more likely to experience creatine kinase and myoglobin levels elevated to sub-clinical levels, above that typically found due to exercise.

Aim 2: Examine how recorded physical activity during a two-to-three-day ROTC FTX is related to increased creatine kinase and myoglobin after the FTX.
Hypothesis: Physical activity above a 300% individual daily norm during a two-to-three-day

ROTC FTX will correspond with increased values of creatine kinase and myoglobin biomarkers.

Aim 3: Investigate if clinical signs and symptoms of rhabdomyolysis (dark urine color, prolonged muscular soreness, or inordinate fatigue) are present when creatine kinase and myoglobin reach levels that are considered low or mild rhabdomyolysis.

Hypothesis: Clinical signs and symptoms of exertional rhabdomyolysis will be present at low levels (e.g., three to four times base-level values) of creatine kinase and raised myoglobin.

Literature Review

Injuries impact readiness and deployability more than any other medical issue in the U.S. military.[13,35] Musculoskeletal injuries (MSKI) are the leading cause of disability among service members,[13,36] resulting in over 8 million limited duty days in a single year.[37] Along with the common ailments of sprains, strains, and fractures, exertional rhabdomyolysis (ER) has attracted significant interest in the U.S. military.[13,14] ER is a pathological condition involving the breakdown of skeletal muscle tissue following unaccustomed (novel) or intense physical exertion.

[1] The military experiences an ER incidence rate 20 times higher than the civilian population.[12] ER incidents peaked at 43.1 per 100,000 person-years within active-duty military branches Between 2017 and 2021.[1,13] The combination of a large amount of training and a population entering military training that is overweight and unfit increases the prevalence of ER incidence in this population.[13,14]

The etiology of rhabdomyolysis is broad and often requires multiple muscular insults to trigger the condition. The muscular insult is typically categorized into one of four mechanisms: hypoxic, chemical, biological, or physical (Table 1).[17,18] The most common causes of rhabdomyolysis in pediatric patients are trauma, connective tissue disorders, viral myositis, exercise, and drug overdose.[2,17,18,24,38] The most frequent causes in adults include illicit drug use, alcohol abuse, certain medications, muscular diseases, trauma, neuroleptic malignant syndrome (NMS), seizures, and prolonged immobility.[2,17,18,24,38] In the military population, rhabdomyolysis most often occurs with excessive training, lack of acclimatization before heavy training, and novel training. When the cause of rhabdomyolysis is exercise or physical training, it is termed Exertional Rhabdomyolysis.[1,2,17,18,24] Military personnel are a unique population and are at a heightened risk for developing ER due to the rigorous and often novel physical training they undergo.[1] Military training frequently involves high-intensity exercises, prolonged physical activity, and exposure to diverse environmental conditions such as heat, humidity, and cold. These factors can overwhelm the body, leading to ER.[5] Throughout the remainder of this review, ER will be used in the cases of rhabdomyolysis caused by exertional means. In contrast, the term rhabdomyolysis will be utilized when referring to the condition in totality.

Table 1. Causes of Rhabdomyolysis

Hypoxic	Physical	Chemical	Biologic
External	External	External	External

Carbon monoxide exposure	Crush injury	Alcohol	Bacterial, viral, and parasitic myositis
Cyanide exposure	Trauma	Prescription medication	Organic toxins
Internal	Burns	Over-the-counter medications	Snake venom
Compartment syndrome	Electrocution	Illicit drugs	Spider bites
Vascular compression	Hypothermia	Internal	Insect stings (ants, bees, wasps)
Immobilization	Hyperthermia	Hypokalemia	Internal
Bariatric surgery	Internal	Hypophosphatemia	Dermatomyositis, polymyositis
Prolonged surgery	Prolonged and extreme exertion	Hypocalcemia	Endocrinopathies
Sickle cell trait	Seizures	Hypo-/hypernatremia	Adrenal insufficiency
Vascular thrombosis	Status asthmaticus		Hypothyroidism
Vasculitis	Severe agitation (delirium tremens, psychosis)		Hyperaldosteronism
	Neuroleptic malignant syndrome		Diabetic ketoacidosis
	Malignant hyperthermia		Hyperosmolar state

Adapted from Zimmerman et al.[17]

All rhabdomyolysis causes follow the same mechanistic pathophysiological pathway despite the wide variety of causes leading to the development of the condition.[7] Factors such as poor physical fitness, biological sex, race, lack of acclimatization, dehydration, unaccustomed exercises, and psychological factors may increase susceptibility to the development of ER.[1,14,15,25] Although the precise etiology of rhabdomyolysis or ER development is diverse, the development follows the same ending pathophysiological pathway.[2,39] Many genetic deficiencies or mutations may also increase the susceptibility to rhabdomyolysis and ER.[1,40-42] A thorough evaluation of subjective and objective measures, including physical assessment and laboratory diagnostics, is required in evaluating a potential rhabdomyolysis case.[1,2,39] It is important to comprehend contributing factors and diagnostic measures, particularly in at-risk populations. Timely recognition and management of rhabdomyolysis is crucial to mitigating the risk of complications or death.[17] It is important that we gain a better understanding of ER, especially in diverse, physically demanding contexts such as military environments.[1,14,16] A thorough understanding of

the causation, pathophysiology and diagnostic assessments is important for accurately evaluating a rhabdomyolysis incident. Increased awareness of causes and the associated signs and symptoms of rhabdomyolysis may lead to expedited diagnosis and medical care for the individual.

Rhabdomyolysis results from the leakage of intracellular muscle contents, including creatine kinase (CK) and myoglobin (Mb), into the bloodstream which initiates a chemical and hormonal cascade that disrupts many bodily systems.[2,18,19,27,43] This may lead to a myriad of complications, quickly affecting the respiratory, muscular, renal, cardiac contraction, and/or cardiovascular systems.[2,19,20] A single incidence of rhabdomyolysis can also lead to prolonged recovery taking days to months, additional concern for recurrent incidences, and other prolonged bodily interruptions.[1,2,18,20,39]

Researchers have extensively studied rhabdomyolysis through case studies and epidemiological reports, but no research to date has prospectively examined rhabdomyolysis in observation of a training event.[13,44-48] Studies also aim to qualify and quantify rhabdomyolysis and ER incidents by intentionally inducing delayed muscle soreness and examining factors contributing to ER diagnosis through eccentric exercises.[49-51] A knowledge gap remains in understanding the causation, incidence occurrence, changes in biomarkers leading to ER and proper diagnosis. Our goal is to explore this gap through an observational study during a multi-day military field training exercise (FTX). This dissertation seeks to determine if the ROTC FTX increases muscle injury biomarkers to levels associated with ER. As secondary aims, this dissertation investigates whether additional factors such as fitness level, body composition, biological sex, race, or age correspond with increases in the analyzed muscular injury biomarkers. Furthermore, it compares measured physical activity during the FTX to regular daily physical activity and examines whether increased physical activity correlates with the rise in muscle injury

biomarkers. Answering these questions is an important first step in proactively examining ER incidence in ROTC cadets and applying our findings to the greater military population.

This literature review is organized into two main sections. The first section briefly explains human anatomy and physiology and the currently accepted etiology of ER. The second section delves into additional factors that may protect against or predispose individuals to developing an ER incident.

Physiology and Affected Anatomy

A brief introduction and explanation of the associated human anatomy and physiology are essential to enhance comprehension of the causes, etiology, and ramifications of an ER incident.

Muscle tissue and cellular characteristics

The human body contains approximately 600 skeletal muscles, comprising 40-45% of body weight.[52] Each skeletal muscle consists of numerous muscle fibers ranging from 10-80 μm in diameter. These muscle fibers are made up of cells, including the cell membrane called the sarcolemma. Hundreds to thousands of myofibrils within each muscle fiber contain the muscular contraction machinery known as sarcomeres. The sarcomeres contain myosin and actin filaments, which are polymerized protein molecules that facilitate muscular contraction. The sarcomeres reside within a cellular matrix called the sarcoplasm, which contains phosphate, magnesium, potassium, proteins, and enzymes. Adenosine triphosphate (ATP) provides the energy for muscular contraction and relaxation by transporting calcium into the sarcoplasm during the excitation-contraction coupling (ECC) phase.[7,53,54]

Muscle Fiber Types and Contents

Skeletal muscles perform task-specific functions based on their heterogeneous makeup. The construction of different muscle fiber types creates a variety of slow to fast fibers, with each muscle containing different percentages of each type.[55] Human skeletal muscle generally divides into three types: slow oxidative fibers (Type I), fast oxidative glycolytic fibers (Type IIa), and fast glycolytic fibers (Type IIx). Fiber type classifications are based on the metapbolic characteristics and function of the fiber, as well as the type of the myosin-heavy chain prominently expressed within the fiber.[56] These classifications simplify the properties and capabilities of muscle fibers and they help distinguish the effects of diseases, ailments, and exercise training changes within the musculature.

Type I fibers, or "slow-twitch fibers," primarily function in endurance activities and lower-intensity exercises.[55,57,58] These fibers contract slowly and rely on aerobic respiration. Due to their higher capillary density, blood perfusion, and myoglobin content, they are also referred to as "red" fibers. Type I fibers are relatively fatigue-resistant and produce a large amount of Adenosine Triphosphate (ATP) from aerobic metabolism. With a small diameter, they do not generate significant tension and can be easily damaged with prolonged high-level exertion. These fibers are abundant in muscles involved in multiple slow contractions, such as postural muscles and the diaphragm.

Type IIa fibers, known as "intermediate fast-twitch fibers," contract quickly and use aerobic and glycogen metabolism for ATP production.[55,58] These fibers have a high number of mitochondria and a significant number of capillaries that supply oxygen, enabling aerobic metabolism. Additionally, Type IIa fibers have a high glycolytic capacity, allowing for anaerobic

metabolism. They contract faster and are less fatigue-resistant than Type I fibers but more fatigue-resistant than Type IIx fibers. Muscles with a high composition of Type IIa fibers include the gastrocnemius, quadriceps, gluteal muscles, deltoids, and pectoralis major. These adaptable fibers are critical for activities requiring strength and endurance, such as many athletic events.

Type IIx fibers, or "fast-twitch glycolytic fibers," have the highest contraction rate and are the most powerful of the three muscle fiber types.[55,58] Type IIx fibers contain fewer mitochondria and a lower blood supply than the other two fiber types. Low oxidative capacity makes these fibers highly fatigable and rely heavily on anaerobic metabolism. They generate significant power in a short amount of time, making them essential for movements requiring high power and explosiveness. Activities that utilize Type IIx fibers include sprints, jumping, and heavy weightlifting.

Muscle Contraction

Muscular contraction is a complex event that requires a cohesive effort between neurological, mechanical, and chemical processes.[2,17,52] Disruption of these systems can alter the ability for proper muscular contraction.[1,39] During the initial phase of muscular contraction, presynaptic acetylcholine release induces depolarization, activating the L-type calcium channels (dihydropyridine receptors or DHPRs). Skeletal muscle contraction relies intracellular calcium release and the interaction between DHPRs and type I ryanodine receptors (RYR1). This interaction releases Ca^{2+} from the intracellular sarcoplasmic reticulum stores during excitation-contraction coupling (ECC). The additional Ca^{2+} enables the reaction between actin and myosin in the sarcomere by binding to troponin C, resulting in muscular contraction. Ca^{2+} must be restored via active transport by Ca^{2+}-ATPase to end the contraction. ATP is critical for restoring Ca^{2+}

stores. At this stage of ECC, the initial skeletal muscle breakdown associated with ER begins. If ATP stores are severely depleted, ATP-dependent transporters, Ca^{2+}- ATPase and Na/K^{+}-ATPase, may be affected. Under normal circumstances, low intracellular Na^{+} and Ca^{2+} concentrations and high intracellular K+ concentrations are maintained.[7,52,59]

Sarcolemmal Damage

Excessive or novel exercise may directly injure the sarcolemma. Researchers assess muscular damage in two primary ways: 1) direct observation through muscle biopsy, or 2) indirect observation through subjective measures and laboratory tests for muscle injury biomarkers in blood or urine.[60,61] Previous research indicates smaller muscle fibers are more likely to sustain exercise-induced damage than larger muscle fibers.[62] The amount and severity of muscular damage vary with the type and amount of exercise, specific muscle activity, and the muscle's makeup, including fiber type and protein content.[62] When muscular damage occurs, observations have recorded a decrease in muscular strength and force within minutes.[50] This decrease is attributed to a developed deficit in excitation-contraction coupling (ECC), disruption or loss of contractile components (actin and myosin), and loss of force-transmitting structures (desmin) of the muscle.[50,59] Research suggests that these damaged fibers may become permanently depolarized and unable to regain normal function.[62,63]

Two main pathways lead to sarcolemmal damage. The first is overuse and physical damage from excessive physical activity. The second pathway is when depleted ATP from repetitive contraction leads to an imbalance within the cellular structure, resulting in Ca^{2+}-ATPase and $Na^{-/+}$ / K^{+}-ATPase pump dysfunction. Increased intracellular concentrations of Ca2+ due to higher cell permeability to sodium ions with continued muscle contraction exacerbate the ATP

deficit. Disruption of ATP-dependent transporters creates a cyclical effect, increasing intracellular Ca2+ and enhancing calcium-dependent phospholipids and proteases. This increase leads to excessive myofibrillar, membrane protein, and cytoskeletal damage. As the damage progresses, large amounts of metabolites (phosphate, urate, and potassium), intracellular electrolytes, and intracellular proteins (myoglobin (Mb), creatine kinase (CK), aldolase, lactate dehydrogenase (LDH), aspartate transaminase) leak into circulation.

A small number of muscle fibers swell and exhibit edema immediately after exercise. This minimal damage does not cause subjective discomfort and objective muscle force deficit observed. Increased muscular damage becomes visible with increased inflammatory cell infiltrations and a disrupted sarcoplasm, as numerous swollen fibers appear two-to-three days post-exercise. When viewed longitudinally via electron microscopy, fibers can show damage via Z-line streaming, or an appreciable disruption of the Z-lines and associated sarcomeres. Peak Z-line streaming occurs three days after exercise and decreases as the tissue repair process ensues.[59]

Prolonged and novel exercise disrupts other cytoskeletal elements, including desmin and dystrophin. These elements maintain cell membrane integrity and Z-disc structure. Without proper desmin and dystrophin maintenance, skeletal muscle cells can collapse leading to a leakage of intracellular components.[59] Desmin forms part of the structural scaffolding by maintaining Z-disc structure and proper sarcomeric alignment within and between myofibrils. Dystrophin is a large cytoskeletal protein associated with cell membrane integrity. Dystrophin maintains the cellular integrity during repeated mechanical loading experienced daily through movement. Loss of dystrophin leads to Duchenne muscular dystrophy, causing severe muscle degeneration by losing the skeletal membrane stabilizing function. Research has linked dystrophin loss with beta-spectrin loss.[59] Beta-spectrin is another membrane protein associated with stabilization. Additional studies have shown alpha-sarcoglycan loss following eccentric contractions. Alpha-sarcoglycan is a

member of the dystrophin-associated protein complex. A genetic mutation of this complex results in Limb-Girdle muscular dystrophy. Rapid loss of these proteins makes muscle cells more susceptible to damage from additional contractions.

Large-scale loss of membrane integrity poses a danger to individuals for severe or extensive muscular damage. Widespread degradation compromises muscle fiber homeostasis by disrupting the barrier that maintains the balance of intracellular and extracellular molecules. Small amounts of transient membrane disruptions provide a pathway for molecule release and uptake in tissues exposed to repeated mechanical stress, leading to adaptation and hypertrophy. Transient membrane disruptions also regulate basic fibroblast growth factor (bFGF).[59] This stimulates the proliferation of cells that contribute to muscle repair and act as one of likely several chemotactic agents for muscular repair. The contribution of bFGF and corresponding cellular changes play a critical role in muscle repair and adaptation, particularly following exercise. Transient sarcolemmal disruption acts as a signaling event in skeletal muscle's response to exercise, releasing growth factors and other molecules important for repair and adaptation. Depending on the injury's magnitude and additional contributing factors, muscle cells will either repair and adapt or undergo cellular death. Mild-to-moderate muscle damage in healthy adults generally does not cause significant issues. These individuals can usually clear the leaked intracellular contents from the blood in seven-to-nine days after the muscle damage occurs.[64]

Table 2. Serum biomarker levels usually found after exercise

Biomarkers	Myoglobin	CK	LDH	AST	Troponin
Exercise	Up to 4-fold	Up to 3 to 4- fold	Up to 2-fold	Up to 2-fold	Slight increase

Adapted from Brancaccio et al.[65]

Studies on sarcolemmal disruption reveal a significant deficit in force production immediately after exercise despite minimal structural damage within the muscle. The small amount of tissue affected by structural damage does not account for the force deficit. Immediate disruption and damage to the excitation-contraction coupling (ECC) from exercise better explains the immediate drop in force production. Impairment of the ECC can occur at any point in the chain of events between the depolarization of the muscle cell membrane and the release of calcium from the sarcoplasmic reticulum. These impairments reduce calcium activation of myofibrils and subsequent force production. Research has shown that calcium release during electrical activation is impaired in both single muscle fibers and whole muscles when the ECC is disrupted. Eccentric contractions alter the structure of T-tubules, contributing to ECC impairment. The disruption occurs at the junctions between the T-tubules and the sarcoplasmic reticulum. This gap is susceptible to damage due to the progressive development of length discrepancies between neighboring sarcomeres and increases the likelihood of ECC failure.

Creatine Kinase

Cytosolic creatine kinase (CK) is a dimeric globular protein consisting of two subunits whereas mitochondrial CK exists as a larger octomeric protein complex.[66] Cellular CK levels are highly expressed in high-energy-demand tissues. Each subunit possesses a molecular mass of 43-45 kDa, totaling ~85- 90 kDa for cytosolic complexes and ~340 kD for mitochondrial complexes. The two CK polypeptide subunits include muscle (M) and brain (B). Tissues containing CK comprise three different types of tissue-specific isoenzymes, with varying proportions of the two polypeptides. The commonly accepted distribution of CK isoenzymes includes the brain

predominantly composed of CK-BB, cardiac muscle consisting of 70-80% CK-MM and 20-30% CK-MB, and skeletal muscle composed of 98% CK-MM and 2% CK-MB.[64]

CK levels in the blood and urine rise corresponding with muscular damage. This increase illustrates a close association between muscular damage and serum CK.[64] Elevated CK levels are also linked with other forms of muscular disruption or disease such as muscular dystrophy as well as muscular damage from exercise.[1,2,39] Evaluation of CK levels is the most widely used method for diagnosing and monitoring muscle injuries.[67] Increased CK presence in the blood and urine indicates muscular damage, but it is not a reliable gauge of the extent of the damage.[68] The amount of serum CK increase has not been shown to reflect the amount of muscular damage directly.[1,17,69] Previous research suggests that CK activity may relate to muscle fiber type, the cross-sectional area of the muscle involved in the exercise, and muscular protein content.[68] Damage to the muscle occurs at differing aspects of the muscular sarcomere. Myofibrillar CK-MM binds to the M-line of the sarcoplasmic reticulum and is found in the I-band space of the sarcomere, providing support for muscle energy.[59,67] This sarcomeric location is particularly susceptible to disruption and will release CK from the damaged muscle cell. CK is released by the damaged muscle cells into the lymphatic system, transported into the thoracic duct, and then into the bloodstream.[67] This pattern of CK release after muscular cell damage forms the basis for using CK as a biomarker to indicate muscular destruction. Elevated CK levels have been shown to indicate underlying myositis or cardiac concern in addition to muscular damage.[20,43,64] The cause of myositis increasing serum CK is often unknown (Baird 2012). In the absence of myocardial infarction, physical trauma, or disease, serum CK levels rising above 5,000 u/L indicate severe muscle disruption.[64] Base levels of CK are generally accepted to be 35-175 u/L. In cases of subclinical disorders, minor injuries, genetic factors, physical activity status, and medications, CK ranges have been observed between 20-16,000 u/L.[64]

Research has shown that individual CK responses to exercise vary significantly. The same exercise protocol can produce highly variable levels of serum CK among different individuals.[64,68] Some individuals may be "high responders," exhibiting higher CK levels after exercise, or "low responders," who show lower CK levels.[64,68] This variability may relate to the predominance of specific muscle fiber types, or the type and intensity of the exercise completed.[68,70] It has been found that individuals diagnosed with heat stroke and ER had a predominance of type II muscle fibers.[68,71] Further studies suggest that high responders develop increased muscular tension and have a greater number of type II muscle fibers. This may lead to a higher energy substrate requirement to maintain exercise intensity, resulting in faster muscular fatigue.[68] Additionally, different genetic polymorphisms may explain the differing CK reactions in high or low responders.[68,72]

The medical community has not wholly agreed upon an absolute quantitative measure of CK in the diagnosis of ER. Nevertheless, it is generally accepted that CK values above five times the upper limit of normal or a CK level of 10,000 u/L indicate rhabdomyolysis.[64,67,73] This level of circulating CK signals a significant disturbance or disruption of striated muscle tissue, with associated leakage of intracellular muscle contents. Although this significant rise in CK levels is unique to muscular cell damage, it is important to recognize that CK levels will rise in all individuals engaging in higher-intensity activities. Mildly elevated serum CK levels and other biomarkers or a small CK flux are expected and are a natural response to exercise (Table 2).[67]

CK levels alone may not fully reflect structural damage to cells, as factors such as hydration status can influence CK measures. Although CK measures are typically at the forefront of laboratory diagnostics for ER, they should not be solely relied upon. Additional subjective and objective assessments, such as other muscular damage biomarkers, delayed onset muscle soreness

(DOMS), decreased range of motion (ROM), and decreased muscular strength, should also be utilized in the diagnosis of ER.[64]

Myoglobin

Myoglobin (Mb) is a dark red oxygen-carrying hemoprotein found in striated muscles, such as cardiac and skeletal muscle.[67] It belongs to the super globin family of proteins and comprises a single polypeptide chain of 154 amino acids and a porphyrin ring with a central ferrous iron molecule. Mb is similar to hemoglobin in its oxygen-binding capability but differs in having only one oxygen-binding site compared to hemoglobin's four. Despite having fewer binding sites, Mb has a much higher oxygen-binding affinity. The highest amounts of Mb are found in the sarcoplasm of oxidative skeletal muscle fibers, with lower amounts present in smooth muscle tissues.

Mb has a lower molecular weight of 18 kDa and quickly enters the bloodstream within hours following muscular injury.[67] Mb serves as one of the earliest biomarkers to detect myocardial infarction and diffuse muscular damage. The released Mb is easily filtered through the kidneys and excreted in the urine in healthy individuals with mild to moderate muscular damage. The large amount of Mb released into the bloodstream overwhelms the filtering capabilities of the glomerulus, leading to the formation of Mb casts and creating renal tubular obstruction in cases of extreme muscle damage or rhabdomyolysis. These precipitants, known as casts, more commonly form when the urine is more acidic. Urine is more acidic when the urine specific gravity is higher and indicates dehydration. The nephrotoxic properties of Mb increase the risk of developing acute kidney injury (AKI). The release of Mb is often accompanied by the release of CK, lactate dehydrogenase (LDH), and alanine aminotransferase (ALT). The release of these substances

during a rhabdomyolysis incident also leads to the development of hyperkalemia, hypocalcemia, and AKI, which are of additional concern causing further homeostatic disruption and bodily damage.

Mb comprises approximately 1-2% of dry weight muscle in moderately trained individuals, with lower amounts present in less trained individuals and up to 3.5% in highly trained personnel.[67] Low levels of Mb are commonly found in the circulating blood. Serum blood levels of Mb of 0-85 ng/ml are accepted as normal and Mb levels of 0 to 0.003 mg/dL are considered normal while Mb are bound to plasma globulins.[74] Serum Mb levels increase in the circulating blood when muscular damage occurs. When serum Mb levels reach 0.5 to 1.5 mg/dL, the binding capacity is overwhelmed, and Mb is rapidly excreted into the urine in a condition known as myoglobinuria. [74,75]

Myoglobinuria

Myoglobinuria is the presence of excessive myoglobin (Mb) from damaged muscle excreted in the urine. Mb exists in muscle cells, and myoglobinuria invariably indicates damage to muscle cell membranes and subsequent leakage of Mb into the bloodstream. Myoglobinuria is not exclusive to rhabdomyolysis; many injuries and illnesses can lead to its development.[76] Common causes include direct trauma to muscle (e.g., crush injuries, burns, shock, physical beatings), Haff's disease (consumption of cellulose-contaminated fish), regional enteritis, arterial thrombosis, alcohol ingestion (with or without sedatives), primary muscle diseases (e.g., polymyositis, muscular dystrophy), idiopathic paroxysmal myoglobinuria (occurring spontaneously or following mild-to-moderate exertion without primary muscle disease), and exertional rhabdomyolysis after severe exertion.

The discoloration of urine caused by myoglobinuria is often considered a hallmark of rhabdomyolysis. The discoloration results from the pigment of excreted Mb in the urine and is commonly described as tea-like or cola-like color.[22,77,78] Significant damage to as little as 100g of skeletal muscle can produce a laboratory-detectable result of myoglobinuria. The brown discoloration of urine occurs when 200g or more of skeletal muscle is damaged.[79] It is pertinent to note that not all rhabdomyolysis cases present with visible, dark-colored urine. Approximately 17% of rhabdomyolysis cases present with detectable myoglobinuria.[2,73] Therefore, myoglobinuria is not necessary for diagnosing rhabdomyolysis.[80] Urine Mb levels must exceed 5700 nmol (100 mg/dL) to change the urine to the known prominent dark colors. The urine may also contain pigmented brown granular casts, epithelial cells, or other brown debris.[80] It is important to distinguish between myoglobinuria and hematuria, especially after possible kidney injury or an endurance event. Bladder bleeding can occur during long-distance events such as long-distance running. The likelihood of hematuria from exercise increases when an individual voids before the activity.[81]

Troponin

Troponin is a regulatory protein found in striated muscle. It is a contractile protein complex located on the thin filament of a sarcomere. Three isoforms of troponin exist in both skeletal and cardiac muscle: tropomyosin-binding subunit (TnT), calcium-binding subunit (TnC), and actomyosin-adenosine triphosphate-inhibiting subunit (TnI). Measuring TnI allows for a fast differential diagnosis during complex clinical scenarios of skeletal muscle injury with possible cardiac involvement. Cardiac troponin I (cTnI) is specific to myocardial injuries such as myocardial infarction, cardiac muscle damage, or necrosis.[82] While elevated CK levels indicate overall musculature damage, additional analysis of cTnI provides an immediate way to

differentiate between skeletal and cardiac muscle damage.[63] The use of cTnI can detect cardiac injury after any trauma leading to cardiac damage.[63]

Researchers have previously recommended the use of plasma levels of skeletal troponin I (sTnI) as a marker of contraction-induced muscle damage. Previous research has shown that sTnI rises and peaks parallel to CK and remains elevated for up to two days after exercise-induced muscle injury. These features make sTnI an ideal biomarker for ER cases. The molecular weight of sTnI is approximately 18.5 kDa, smaller than CK but larger than Mb.[83,84] The differences in molecular weight affect the capability of analysis and the expediency of the test after the incident occurs. Ultimately, developing and using an adequate assay for sTnI has been costly and only as effective as the commonly used CK assay. Currently, it is most efficient to continue using the accepted measurement combination of CK, Mb, and physical signs and symptoms in diagnosing ER.[83]

Electrolyte Disturbances

Additional electrolyte disturbances, such as hyponatremia or hypokalemia, may occur during exercise, which increases healthcare complications.[52,59,85] Potassium released from cells mediates appropriate muscular blood flow and vasodilation. When potassium decreases due to cellular membrane imbalance, blood flow to muscles during exercise reduces and limits energy supply, increasing the risk of developing ER. Continuing hyponatremia leads to further Na+/Ca2-ATPase dysfunction. This progressive dysfunction causes additional cell lysis due to the activation of proteases and lipases. Another complication of hyponatremia is the failure of volume regulation in the cell. This failure will lead to further external cell lysis and potentially stimulate vasopressin secretion. These factors, along with other cellular responses during exercise, such as increased

oxidative stress and the release of proinflammatory cytokines, result in cell death and the spilling of intracellular components into the surrounding tissue.

Electrolyte disorders, including hypokalemia, hypophosphatemia, hyponatremia, and hypernatremia, can lead to rhabdomyolysis by disrupting the function and permeability of the sarcolemma.[52] Hypokalemia is the most common electrolyte disturbance associated with rhabdomyolysis and exertional rhabdomyolysis (ER). The muscle membrane depolarizes and inhibits glycogen production and storage in myocytes. Potassium depletion is also linked to inadequate muscle blood flow during exercise, ischemia, and rhabdomyolysis.

Hyponatremia exacerbates rhabdomyolysis through extracellular hypoosmolality and subsequent cell swelling. This often acts in conjunction with hypokalemia.[11,75,79] Hypophosphatemia is another significant concern in rhabdomyolysis, as phosphate is crucial for ATP production and oxygen affinity in hemoglobin. Potassium and phosphate serum levels may appear normal or elevated due to the release of these intracellular ions from damaged myocytes in hypokalemic and hypophosphatemia cases. Hyperphosphatemia can be managed with phosphate binders when levels exceed 7 mg/dL. Hypokalemia is particularly critical due to its potentially lethal effects on cardiac rhythm and function, necessitating early identification and prompt treatment tailored to the severity of hyperkalemia. Hypocalcemia may develop early but should only be treated if severe symptoms or hyperkalemia occur to avoid calcium phosphate deposition in tissues. Hypercalcemia typically arises later in patients with acute renal failure during the diuretic phase and can usually be managed with volume expansion and diuretics. Hypermagnesemia may occur in rhabdomyolysis patients with renal insufficiency and requires appropriate treatment. In ER severe cases of myonecrosis can lead to metabolic acidosis and hyperuricemia. While xanthine oxidase inhibitors may be ineffective, uricosuric agents can be beneficial if renal function is intact. Hypoalbuminemia may indicate extensive vascular

permeability or a dilution effect due to declining renal function and expanded intravascular volume. In extreme cases, emergent hemodialysis may be necessary to address severe metabolic acidosis, electrolyte imbalances, and acute renal failure.

Definition and Diagnosis of Exertional Rhabdomyolysis (ER)

The definition and diagnosis of ER continually evolve with medical findings. Rhabdomyolysis and ER may be challenging to diagnose due to the unique presentation. Individuals may not seek medical care in some mild or low-level cases and will self-treat the condition. Currently, four key diagnostic factors of ER are accepted.[7] The first is a CK elevation 12-36 hours after exercise, with a maximum increase at three-to-four days and normalization within several weeks. The second diagnostic factor is the CK increase follows exercise that surpasses an individual's fatigue levels and is unaccustomed. The third key factor is the CK increase is symptomatic, with additional signs and symptoms including muscular swelling, weakness, and/or muscle soreness. The fourth key factor is the presence of myoglobinaemia and/or myoglobinuria, assessed by laboratory testing or visual inspection. It is uncommon for individuals to present with the accepted rhabdomyolysis trilogy of elevated CK levels, myoglobinuria, and extreme muscular fatigue or soreness.[7] The extent of muscular and bodily systemic damage is not dictated by the plasma CK levels. Although CK levels may stay elevated for a limited amount of time, they do not indicate total healing or a lack of additional complications from rhabdomyolysis.

Genetics

Specific genetic polymorphisms have been identified as risk factors for ER development, particularly with highly demanding physical training.[1,7,40,41,86] It is medically recommended for

individuals who sustain recurrent episodes of ER to undergo genetic screening to rule out possible causative genetic concerns,[2,25] Concerns such as miRNA, histone alterations, and DNA methylation contribute to increased ER risk. DNA methylation is a heritable epigenetic mark involving a methyl group transfer to the C-5 position of the cytosine ring.[87,88] Recent research has shown the effects of these epigenetic modifications, particularly in regulating diseases and injury response.[1,89-91] Different genes involved in energy metabolism can be methylated after exercise. In particular, the PGC-1α, GLUD1, PDK-4, PPAR-δ, TFAM, ADIPOR1, ADIPOR2, and BDKRB2 genes can alter ER risk through metabolism.[1,92] Additionally, genes related to MEF2A (muscular metabolism), RUNX1 (blood cell production), and ASC (inflammation) have also been linked to increased ER risk. Previous research has shown dynamically altered DNA methylation in skeletal muscle during and after exercise.[1,92-94] Genetic testing may be pertinent for the knowledge and safety of individuals with ER or recurrent ER, but it is not always practical due to cost and efficiency.[1,7,86]

Previous studies have concluded that mutations in certain genes known to lead to rhabdomyolysis were present in 43% of ER cases with unknown etiology, suggesting a wide heterogeneity of rhabdomyolysis and ER.[1,86] Genetic screening can be an important preventative tool, particularly for those who sustain recurrent episodes of rhabdomyolysis or ER.[7,40,41,86] Genes affecting fatty acid metabolism include PCCB, ACADVL, CPT2, EFTA, EFTB, ETFDH, and MTP. Genes related to glycogen metabolism disorders include GYG1, GYS1, GBE1, PYGM, PFKM, ENO3, PGAMZ, PGK1, PGM1, PHKA1, and PHKB. Additional genetic disorders dealing with intramuscular calcium release and excitation-contraction coupling (ECC) include RYR1, CACNA1S, and CAV3. Mitochondrial genetic disorders posing increased risk for ER include CO1 (MTCo1), COII (MCO2), COIII (MTCO3), FDX1L, HADHA, HADHB, ISCU, MTCYB, and mtDNA. More specific mitochondrial genetic disorders related to the inner

membrane and matrix enzymes of the mitochondria are TIMM50 and OAT. Mitochondrial respiratory chain complex disorders include ELAC2, NDUFA6, NDUFS8, NDUFA10, and NUBPL. Genetic disorders of muscular dystrophies include FKTN, ANO5, DYS, DYSF, FKRP, ATP2A1, GMPPB, and SGCA genes. These inherited neuromuscular disorders are often associated with an increased incidence of developing ER. Most of these muscular diseases are abnormalities in muscle fibers and are seen either at birth or in early childhood, often presenting as skeletal deformities, muscular weakness, or muscular hypotonia. While these genetic disorders increase ER concern, they often develop in ways that prevent affected individuals from completing typical or high-level physical activity.[1,65,95,96] Genes related to phosphatidic acid and phosphate deficiency, such as LPIN1, LPIN2, and LPIN3, also raise ER concern. Finally, one of the most well-known genetic disorders involves the HBB gene.

Sickle Cell Anemia & Sickle Cell Trait

Sickle cell trait (SCT) is a well-known contributor to both malignant hyperthermia (MH) and ER development.[1,97] Sickle cell disease is a condition where an individual carries two copies of the altered HBB gene. This disease affects approximately 100,000 Americans.[98] SCT occurs when an individual carries only one copy of the altered HBB gene. SCT is much more prevalent than sickle cell anemia. An estimated 3 million Americans and 300 million people are diagnosed with SCT worldwide.[98,99] SCT has been well explored in the general, athletic, and military populations.[1,7,40,41,61] SCT has garnered significant media attention due to numerous events occurring during athletic activities.[99,100]

Individuals with sickle cell concerns can be homozygous (HBSS) or heterozygous (HBAS), depending on heredity.[99,101,102] A mutation of the HBB gene causes SCT, where the

replacement of glutamic acid by valine alters the stability and solubility of hemoglobin. The sickling shape of erythrocytes occurs under low oxygen tension, causing the cells to stretch and bend into the characteristic sickle shape.[99,103] This variation in hemoglobin causes sickle-shaped blood cells to adhere to endothelial adhesions, triggering an inflammatory response and potentially causing occlusion of the blood vessel where the sickling event occurs.[97,104]

Military studies have focused on individuals with SCT and the increased risk of ER. Research has indicated an increased risk for ER events in individuals with SCT, comparable to soldiers with obesity or tobacco use.[101] Previous research has shown that SCT-positive trainees in the U.S. Air Force (USAF) had a 16.7% increased risk of rhabdomyolysis and a 7.1% increased risk of hospitalization due to rhabdomyolysis.[97] Understanding the increased complications that can occur with SCT-positive individuals is necessary when comprehending the implications of ER.

Angiotensin-converting Enzyme (ACE) Genotypes

Extensive research has connected angiotensin-converting enzyme (ACE) polymorphism with physical activity performance and exercise heat tolerance.[72] These genes also predict high versus low CK responders.[51,72] Located on chromosome 17 at 17q23.3 in humans, the ACE gene is critical in regulating the renin-angiotensin system (RAS). The RAS regulates fluid balance, blood pressure, and thermoregulation.[51,72,105] The regulation of these systems in the context of ER development is important and influenced by both ACE polymorphism and hydration levels. The ACE gene contains a polymorphism termed an insertion or deletion ("I" or "D") in three genotypes: insertion (II), insertion/deletion (ID), and deletion (DD). Research suggests that genotypes with the I allele are associated with better aerobic performance and exercise heat

tolerance, providing a physiological advantage over those with the D allele.[72,106] The DD allele has been associated with lower physiological tolerance to exercise heat stress compared to the II and ID genotypes.[72] Additionally, the DD allele is linked with clinical severity among individuals with McArdle's myopathy.[72,107] In contrast, other studies indicate that individuals with ID and DD alleles have a lower CK response to exercise and better overall physical performance.[51] A study conducted in 2007 reported that the ID and DD alleles were common among Israeli elite endurance athletes.[108] This research found that the highest CK responders had the II allele, intermediate CK response in those with the ID allele, and the lowest CK responders had the DD allele.[51,108]

ACE genotypes may also affect the metabolic efficiency of muscle during repeated contractions. During exercise metabolic efficiency may protect the muscle from prolonged mechanical tension and metabolic stress.[72] Previous research has postulated a possible association between the ACE gene and the CK-MM gene at chromosome 19q13.2-13.3. This location is also within the same region where the genetic coding for myotonic dystrophy disease (DMPK) is located (19q13.3).[72,109] There may also be a gene linkage with RYR1.[72,110] A mutation of the RYR1 gene has been associated with increased susceptibility to malignant hyperthermia (MH) and ER development,[72,111]as well as a possible influence on muscle function during both mechanical work and metabolic stress.[72]

Malignant Hyperthermia (MH)

Malignant hyperthermia (MH) is a pharmacogenetic disorder where factors can trigger life-threatening conditions affecting skeletal muscle.[74,112] Anesthetics and depolarizing muscle relaxants can cause muscle rigidity or contracture, fever, metabolic acidosis, and possibly death

due to dysregulation of Ca2+ levels.[112,113] MH can lead to the development of rhabdomyolysis, and conversely, rhabdomyolysis can also be an expression of MH.[7,112,113] Individuals with a ryanodine receptor type 1 (RYR1) variant may remain clinically asymptomatic until a case of MH or rhabdomyolysis occurs.[7,113] Previous research suggests that individuals presenting with ER or rhabdomyolysis, especially recurrent episodes, should be immediately assessed for MH.[113,114] MH is a disqualifying condition for military employment, and if diagnosed during service, the individual may be dismissed. Most genetic disorders related to MH stem from RYR1 mutations.[1,25,41,115] The RYR1 gene regulates calcium channels during the excitation-contraction coupling (ECC) phase. Improper moderation of this channel by the RYR1 protein allows for additional calcium leakage, creating abnormal muscular contraction.

CPT1 & CPT2 Deficiencies

Deficiencies in carnitine palmitoyl transferase (CPT1 and CPT2) are genetic disorders that increase the risk of ER or recurrent ER episodes.[41] The CPT2 protein is located in the inner membrane of the mitochondria, with CPT1 on the outer membrane.[1] CPT1 and CPT2 facilitate the transfer of long-chain fatty acids from the cytosol to the mitochondria for β-oxidation. There may be a connection between CPT2 deficiency and MH due to calcium channels remaining in an open state with poorly functioning CPT2.[115,116]

G6PD Deficiency

Glucose-6-phosphate dehydrogenase (G6PD) deficiency in the military setting has gained significant attention over the last 20 years.[117] Properly functioning G6PD protects erythrocytes from oxidative injury by reducing NADP+ through the pentose phosphate pathway. Inadequate

amounts of NADPH prevent the reduction of oxidized glutathione which causes its levels to build up and leads to erythrocyte hemolysis due to the additional oxidative stress.[118] G6PD deficiency is the most common erythrocyte enzymatic disorder in humans With over 300 variants affecting more than 400 million people worldwide.[117] The U.S. military has recognized the prevalence and importance of this disorder by mandating genetic testing for G6PD deficiency before deploying service members to malaria-prone locations.[119] The U.S. Army now tests trainees at Basic Combat Training reception before they join their training units due to the pervasiveness of G6PD deficiency and its association with both heat illness and ER. This deficiency results from an X-linked recessive inherited genetic disorder. A mutation of the G6PD gene leads to absent or inefficient expression, causing the deficiency.[119] Research has linked unexplained ER, especially recurrent ER cases, to G6PD deficiency.[117,119,120] Both conditions present similarly, with fever, dark urine, muscular pain, and fatigue.[119] Given these similarities, it is essential to distinguish between G6PD deficiency and other more characteristic causes of ER.

Additional Causes of ER

Several diseases, drugs, and genetic polymorphisms contribute to ER. A full discussion of these numerous causative factors is outside the scope of this review; however, a few key components will be highlighted. Specific supplements, statins, and a history of recent viral illness increase the risk of developing rhabdomyolysis, but do not necessarily predispose individuals to ER. Genetic disorders such as fatty acid and glycogen metabolism disorders, intramuscular calcium release and excitation-contraction coupling disorders, mitochondrial disorders, muscular dystrophies, phosphatidic acid or phosphate deficiency, and sickle cell trait have all been linked to high ER risk rates. Additionally, genetic polymorphisms affecting the ACTN3 R577X,[121,122] ACE

I/D,[51,123] MYLK C37885A,[41,122,124] MYLK C49T,[41,122,124] and CKM[72] genes also increase the risk of ER by lowering CK tolerance, reducing heat tolerance, and increasing CK and Mb release after exercise. Many of these conditions are rare and generally not evaluated unless there is a known inherited trait or multiple ER events.

Another causative factor for developing rhabdomyolysis is the contraction of COVID-19 or receiving a COVID-19 vaccination. Specific case studies have documented rhabdomyolysis incidence in combination with the COVID-19 virus.[125-127] Hospital cases of rhabdomyolysis secondary to COVID-19 have not been exertional in nature but rather due to overall damage to striated and cardiac musculature from the illness.[125-127] Both the virus and the COVID-19 vaccination have been associated with increased rhabdomyolysis incidents.[128,129] In most reported cases rhabdomyolysis occurred shortly after the COVID-19 vaccination.[128] However, this does not limit the concern for a decreased ability to mitigate susceptibility to rhabdomyolysis or ER after receiving the vaccine. These increased incidents of rhabdomyolysis are often attributed to the COVID-19 viral illness and the overall decrease in physical activity during societal lockdowns, followed by individuals returning to normal or more advanced physical activity.[128]

Laboratory Testing Techniques

Laboratory testing is an important part of rhabdomyolysis and ER diagnosis. Assessing serum levels of CK and Mb and urinalysis of Mb are the most common laboratory tests used in healthcare settings. Several other serum blood tests and testing methodologies are also available depending on the testing location and the time elapsed since the exertional event leading to ER and rhabdomyolysis.

Use of Urine Testing and Urine Dipstick Testing

A urine dipstick quickly and effectively assesses myoglobinuria.[79] However, a urine dipstick test does not differentiate between Mb, hemoglobin, and erythrocytes.[80] When a urine dipstick detects "blood," especially with diagnostically elevated CK levels, it likely indicates myoglobinuria in the case of ER or rhabdomyolysis. This can be further confirmed by microscopic urine analysis showing relatively few erythrocytes.[19,75,80,130] Urine dipsticks can reveal other factors in ER In addition to assessing myoglobinuria. Protein presence has been noted in 45% of rhabdomyolysis cases.[2] Proteinuria occurs from releasing Mb and other proteins from damaged muscle.[2,61,75] A urine dipstick examination in a patient with potential ER may show a positive (3+ or 4+) level of heme/blood, an acidic pH (5-6), protein positivity, reddish-brown urine color, possible positive bilirubin, and, upon visual inspection, Mb casts or dead epithelial cells.[75] However, exercising caution with ER diagnoses using urine dipstick diagnostics is important, as they may produce false positives and negatives.

Creatine Kinase as ER Diagnosis

The evaluation of creatine kinase (CK) levels is the most common laboratory test used for diagnosing ER.[25,75,79,131] CK levels are significantly elevated during rhabdomyolysis incidents.[2] The CK test is one of the most sensitive diagnostic tests for ER. No other condition is known to cause such significant increases in CK levels.[79] Serum CK levels generally rise 2-12 hours after the onset of muscular damage and peak 24-72 hours later.[25] Population norms accept CK levels to be 55-170 units/L in males and 30-135 units/L in females.[132] ER diagnosis occurs when CK levels reach five times the individual's norm or the upper level of the population norm (~1,000 units/L). This can be problematic as rigorous endurance or resistance exercise can promote these levels

without other classical ER markers being affected.[64] A differential diagnosis of hyperCKemia is made When CK levels exceed ten times the accepted upper limit.[25]During ER incidence CK levels may reach 100,000 units/L or higher.[133] Testing CK levels after exercise exposure is more beneficial in a healthcare setting than testing for Mb. The one and a half day (1.5 day) half-life for CK is longer than the two-to-three-hour half-life of Mb. The longer half-life of CK allows for laboratory blood serum testing for a comparatively prolonged time compared to Mb testing.[25]

Myoglobin as ER Diagnosis

A primary concern of rhabdomyolysis is the immense release of myoglobin (Mb) from myofibers into the bloodstream following extensive myocellular damage.[76,134] Mb is the skeletal oxygen carrier and is usually bound to plasma globulins. Mb levels peak after leakage from the myocyte potentially exceeding the plasma-protein binding capacity during rhabdomyolysis. When an excess of Mb occurs in the bloodstream it is filtered across the glomerulus of the kidney. This Mb excess has the potential of obstructing renal tubules and causing significant kidney damage. Another concern of increased Mb is its ability to exert a direct cytotoxic effect by enhancing local oxidative stress in tubular cells. This creates a high urinary excretion rate of uric acid, compounding tubular obstruction by uric acid casts. The intratubular degradation releases free iron from Mb, increasing free radical production and leading to further ischemic damage.

Although Mb has a very short half-life it is still commonly assessed in the differential diagnosis as a biomarker for ER. Mb is only released into circulation due to muscle injury and rises before CK levels.[2,135] The release of excessive Mb into the bloodstream is one of the primary concerns in ER and can lead to acute kidney injury (AKI).[39,136] A major limiting factor is that Mb's half-life is only two-to-four hours and tends to normalize within six-to-eight hours after muscular damage.[19] A well-known characteristic of ER is tea or cola-colored urine.[76,77] The

darkened urine color is from the large quantity of Mb released into the bloodstream after muscular injury. The Mb is then filtered through the kidneys and excreted through the urinary system.[2,19,135] This dark-colored urine, known as myoglobinuria, is produced when over 100 g of muscle is damaged and Mb levels exceed 0.5-1.5 mg/dL, surpassing the binding capacity.[74,131] Normal blood serum myoglobin concentration is below 5.7 nmol/L (100 µg/L), and in urine, it is below 0.57 nmol/L (10 µg/L).[2] In cases of ER, Mb levels usually increase three-to-four times the upper reference limit, with some cases reaching up to 50-100 times the upper reference limit after muscular damage.[2,59,131]

LDH, AST, ALT with ER Diagnosis

Examining abnormal liver function in any rhabdomyolysis case is pertinent. Less traditional ER and rhabdomyolysis related laboratory diagnostic testing assesses levels of lactate dehydrogenase (LDH), aspartate aminotransferase (AST), and alanine aminotransferase (ALT).[137,138] Liver and kidney damage may occur when LDH, AST, and ALT are elevated.[138] Small differences exist between the kinetics of AST and LDH in response to physical exercise. After low to moderate-intensity exercise, LDH is released into the bloodstream, whereas AST levels do not respond similarly.[131] Both LDH and AST levels increase with heavy muscular activity, especially in the case of an ER incident.[131] Previous research has shown very high blood serum concentrations of LDH in rhabdomyolysis patients, raising additional concern for acute kidney injury (AKI) development.[137,139,140] Research in 2021 indicates that evaluating LDH levels is a cost-effective and appropriate indicator for assessing rhabdomyolysis, including ER, and the risk of AKI.[137]

Part I – Physiology and Affected Anatomy Summary

In summary, the comprehensive understanding of ER involves examining numerous physiological, genetic, and diagnostic factors. The intricate physiology and anatomy of muscle fibers, the mechanisms of muscle contraction, and the types of muscle fibers play crucial roles in ER development. Genetic predispositions, such as those related to sickle cell trait, ACE genotypes, and multiple metabolic disorders, significantly influence ER risk. Laboratory diagnostics, including the assessment of CK, Mb, LDH, AST, and ALT levels, alongside noninvasive methods like girth measurements can aid in an accurate rhabdomyolysis diagnosis. It is important to understand the genetic and biochemical aspects of ER particularly for groups such as the military that regularly complete physically demanding training. Comprehensive knowledge of ER is required for effective diagnosis, management and prevention. This holistic approach ensures a thorough understanding of ER, aiding in the development of targeted interventions and improved outcomes for affected individuals.

Part II

ER Additional Signs and Symptoms

Diagnosing ER requires a comprehensive patient assessment, beyond just laboratory testing of specific biomarkers. Conducting a thorough physical examination that includes both subjective and objective signs and symptoms is required. Patients with ER typically exhibit a combination of increased body temperature, muscular weakness, localized myalgia, edema, and dark brown urine upon physical examination.[34,61]

At-Risk Populations

Physical, environmental, and psychological characteristics can either protect against or increase the risk of developing ER. The most investigated moderators in relation to ER development are age, biological sex, race, and physical fitness levels. Previous epidemiological research has indicated that those most at risk are young (<23 years old) Black males in the first 90 days of military service.[13] despite personality metrics and mentality often being studied in relation to injury rates and recovery There has been minimal investigation into psychological factors related to ER incidence. Exploring these moderators and how each influences ER incidence is beneficial for a comprehensive understanding of the condition.

Psychological and Emotional Attributes for Injury Incidence and Response

Specific research on psychological and emotional factors related to ER has not been directly explored. However, assessing psychological and emotional factors pertaining to injury incidence and recovery from injuries has been well researched.[47,141-143] It is pertinent to examine the psychological and emotional aspects as they relate to ER incidence. ER is classified as a musculoskeletal injury (MSKI) often linked with heat illness and may follow similar psychological aspects as other injuries and illnesses. Psychological factors and evaluation methods have been used as predictive tools to gauge recovery success and return to duty particularly after illness or injury.

Illness and injury are inevitable in military training. Approximately 42% of males and 62% of females sustain some form of MSKI during Army Combat Basic Training (BCT).[144-146] These high injury rates correlate with a significant incidence of voluntary military separation

rather than separation due to medical necessity. One study found a 57% separation rate in BCT following an injury that affected the trainee's training schedule and graduation timeline.[47] Illness and injury are common during a military career, with a higher incidence of MSKI occurring within the first 90 days of military training.[13] An individual's attitude and mentality may significantly influence recovery despite being unable to prevent the occurrence of an injury, illness, or ER incident. Research has demonstrated that motivation for job or military success enhances graduation rates, overall success, favorable MSKI healing, and return to full duty.[147]

Psychological and Emotional Attributes for Injury Incidence and Response

Researchers have acknowledged the significant impact of psychological factors predicting success rates through injury recovery.[141,147,148] Two well-known psychological assessments, the five-factor model of personality and the Rorschach inkblot test, have shown promise in determining personality traits and stress management capabilities.[141,149-151] The five-factor model of personality indicates that individuals scoring higher on Extroversion, Conscientiousness, and Emotional Stability, but lower on Agreeableness, are more likely to succeed in high-risk occupation training programs.[141,142,150,152] Successful training completion has been particularly correlated with Emotional Stability, and to some extent, Extroversion and Openness.[141,143,153]

The Rorschach inkblot test is another personality assessment used to predict military success rates.[141,154] A significant difference between these two tests is that while individuals may alter their responses to appear "right" or "correct" on the five-factor model, they are less capable of doing so with the Rorschach assessment.[141,155] This difference allows for a more comprehensive and less biased examination of the individual. The Rorschach method may better represent ongoing mental processes and personality characteristics, serving as a predictor of training performance.[141,156] This method not only indicates the overall cognition of the individual

due to its balance of freedom and reality support, but also may better predict how the individual copes with stress, difficult situations, or critical challenges.[141,156,157]

Psychological and Emotional Attributes for Injury Incidence and Response

Assessments of stress coping abilities of individual grit and hardiness have been shown to predict success rates.[141,150,158] Grit and hardiness are frequently used interchangeably even though they have distinct definitions. Grit is defined as perseverance and passion for long-term goals,[159] and has been shown to predict retention, but not performance, in U.S. military academy cadets.[160,161] In contrast, hardiness focuses on the ability to withstand challenges and is defined as the capacity to endure, manage, and respond to challenging events using positive coping strategies.[158,160,162] Hardiness has been identified as a predictive factor for successful training outcomes in military settings.[158,162,163]

Both grit and hardiness are crucial factors to consider when estimating how an individual will cope with stress and stressful situations. It has been suggested that there could be a relationship between levels of hardiness and grit and injury risk, which may be influenced by injury occurrence in both positive and negative ways.[158] One study followed 104 athletes over two years and found that those with higher levels of hardiness had a lower injury risk and were better able to recover from sustained injuries.[164] A similar effect was observed in BCT indicating a correlation between high self-reported hardiness and grit with lower MSKI rates, higher return to duty, and higher graduation rates.[158] Another study found that positive expectations of completing BCT (grit) significantly impacted success rates and reduced overall attrition rates.[147] Understanding and properly applying these psychological and emotional factors assist in comprehending risk and recovery from an ER incident or sustaining an MSKI in initial military

training. It is important not to overlook the clinical implications of psychological and emotional influences, particularly how grit and hardiness potentially play a role in the risk and recovery of injury and illness.[165]

Biological Sex

Biological sex has been linked to the development of ER.[1,39,166] Previous literature indicates a male predominance in ER development.[39,166] This increased incidence is thought to be due to the larger muscle mass and tissue in males compared to females. Additionally, studies have shown a higher increase in CK levels in males during exercise than in females.[166] A larger number of males typically participate in high-intensity exercises, increasing the likelihood of developing ER.[1] Research also suggests that higher levels of estrogen in females provide a protective effect.[1,167] A decrease in CK and LDH secretion in menopausal women taking estrogen hormone supplements further supports the protective nature of estrogen.[166,168] Biological sex may also influence the excretion rates of CK and Mb from the bloodstream and body. Females exhibited lower CK levels 24 hours after completing a marathon.[77,135] This finding suggests that females secrete less CK into the bloodstream after muscular damage and have a faster removal of CK from the body compared to males, indicating a potential protection against ER incidence. Additionally, research has shown the structural similarity between estrogen and Vitamin E which may allow estrogen to suppress excessive oxidative stress from exercise and reduce neutrophil leukocyte and macrophage inflammatory cells.[169,170]

Nutrition

Nutritional intake and dietary composition have been linked to ER incidence. Carbohydrate-heavy diets or "carbo-loading" prior to physical events have been associated with increased CK levels, brown urine (indicating myoglobinuria), ER development, and acute renal failure.[61,77] This incidence appears to result from the reduction of the typical muscular energy stores due to acidification and increased lactic acid from the elevated glycogen levels in carbohydrates.[171]

Increased ER rates have also shown a direct negative correlation with protein intake.[172] Vegan and vegetarian individuals have a higher ER incidence rate compared to those consuming an omnivorous diet.[172,173] Researchers have ascertained that the increase for ER in vegans and vegetarians are due to the protein deficiency inherent in such diets.[172,173] Previous literature has documented one case-study where a vegetarian athlete exhibited high CK levels and ER, along with muscle pain, general malaise, and temporary tachycardia.[174] Nutritional protein deficits, combined with excessive carbohydrate consumption, appear to predispose individuals to ER events.

This concern is particularly relevant in military training, where individuals often consume one to two meal-ready-to-eat (MRE) meals daily. With a 20-minute time constraint for eating[175] individuals tend to consume the carbohydrate-heavy sections of the MRE due to ease of eating, chewing, and flavor preference.[176,177] Nutritionists cannot account for personal meal or taste preferences despite the careful design of MREs to optimize military performance. Increased nutritional education may help address concerns about protein deficiency and carbohydrate overconsumption, as nutritional intake is one of the few controllable factors in developing ER.[177-

Girth Measurement

Muscle swelling often occurs due to muscular trauma and enzyme leakage from sarcolemma damage. Measuring the muscle is an alternative, noninvasive method to detect this muscular swelling. Girth measurements are taken at consistent, specific anatomical landmarks on the body to compare the affected area.[59,60] These measurements are often used in cases of infection, effusion or fluid retention, joint swelling after injury, and other healthcare concerns. A hallmark symptom of ER is muscular swelling from the accumulation of intracellular contents in interstitial space. Girth measurements, even if pre-incident measurements are unknown, can monitor the progression of muscular swelling over time. A continued increase in the swelling of the affected muscles can indicate that the condition is worsening.[180] If the swelling is not adequately managed and quickly reduced, blood flow can be occluded, leading to muscular necrosis. It is recommended to use girth measurements in conjunction with other clinical and laboratory assessments to form a more comprehensive examination of an individual with ER.[17]

Pressure Algometry

Pressure algometry is a tool for measuring pain sensitivity in specific tissues to quantify the pain pressure threshold (PPT).[181,182] Pre- and post-event measurements can be taken and compared to assess the change in PPT and values can also be compared to normative data.[183,184] A digital algometer can assess PPT after an ER or DOMS incident as an objective measure of muscular discomfort.[181,185] Severe muscular soreness or tenderness is a common symptom in ER from muscular breakdown, associated swelling, and edema.[2,25,39] Use of pressure algometry

provides a non-invasive technique to quantify and monitor the muscular soreness of the individual.[184,186-188]

Physical Activity and Fitness Levels

Physical activity and overall fitness levels are significant factors in ER development.[2,17,39] There is a consensus among researchers that fitter individuals are generally better adapted to physical demands that reduces ER incidents.[31,69,189,190] Fit individuals seem to have a protective factor against ER even when the physical demands are novel or individuals are not well adapted to the specific physical rigors. Current literature broadly states that individuals that are fit are more protected against developing ER.[1,5,12,191]

Previous literature has emphasized the importance of proper acclimatization (hot weather, humidity, cold weather) to decrease ER occurrence.[53,100] There is likely a link between poor acclimatization and ER development, especially during the first 90 days of military training when individuals are adapting to increased physical activity levels, psychological stressors, and often a new geographical environment.[189] ER is often associated with heat illness even though it is not a heat injury. Increased heat and humidity amplify dehydration and hypovolemia, accelerating the pathophysiological process of ER development at the cellular level.[53,100] Individuals who are better adapted to their operating climate are generally less susceptible to developing ER and are more protected from the effects of environmental illnesses both heat concerns and cold concerns.[25,192]

Types of Fitness Regime (Excessive and Novel Training Events)

Novel training with inadequate rest periods and highly physically demanding tasks are a primary contributor to ER occurrence.[17,25,39,69] Previous research indicates that extreme or novel exercises affect the three muscle fiber types in different ways.[55,56] The Z-line structure of the sarcomeres in fast-twitch muscle fibers (type II) is thinner compared to type I fibers. Type II fibers also have fewer M-lines (three compared to five in type I fibers). These anatomical differences may lead to the higher susceptibility of type II fibers to damage, especially during eccentric exercise-induced muscular damage.[55,56,83]

Epidemiology and Reporting for ER Incidents

The first laboratory factor to consider is the leakage of CK and Mb into the bloodstream from damaged muscle when diagnosing and reporting ER incidents. Recent research suggests that CK levels alone, especially without the ability to compare individual norms or rises, should not be the sole diagnostic factor for ER. CK levels vary drastically between individuals and have been shown to differ between biological sexes and races.[193] Healthcare providers should be cautious in assuming baseline CK levels from population norms, as these can vary greatly. Typical baseline levels are reported to be 55-170 units/L for males and 30-135 units/L for females.[132] However, recent literature reveals that baseline levels vary based on muscle mass, daily activity levels, and race.[132,194-196]

Many reports and articles on ER occurrence rely on retrospective epidemiological studies. The data evaluated is typically derived from reports using ICD-9 or ICD-10 codes from hospital

medical records or military healthcare databases (AHLTA or now, GENESIS).[13,37] Therefore, it is important to question the validity of these diagnoses, as many reports do not specify how the individual was diagnosed or by what means. This raises concerns about improper reporting and incidence rates. Active-duty personnel and military academy settings have the most accurate representation in these systems due to access to Military Treatment Facilities (MTFs). However, service members may seek healthcare in other locations that may not be reported in their military records.

A more pressing concern is the underreporting of injury and illness in the ROTC setting.[26] In 2020, there were 1,000 colleges and universities with ROTC programs in the United States.[32,197] Most of these programs do not have a designated military healthcare provider in-house. Therefore, when ROTC members become injured or ill, they often seek care through university medical clinics or local medical facilities. Due to the "gray area" of an ROTC member's military standing, these illnesses and injuries often go unreported. The lack of reporting to military databases can easily confound the occurrence rates of illnesses and injuries throughout the ROTC component nationwide.

Exertional Rhabdomyolysis Concerns in ROTC

The majority of ER incidence occurs in active-duty service members completing either BCT or bootcamp, or when initiating high level training, or immediate training in a new environment.[6,13,31] Given these primary risk factors, it is intuitive that ER incidence numbers would be lower in military academies and even lower for ROTC components due to overall training time and training-to-rest ratios. However, ROTC components are particularly vulnerable during their annual or bi-annual field training exercises (FTX). The percentage of the ROTC

population must be taken into consideration when monitoring incident rates of ER. The actual number of ER incidents reported to be higher in the active-duty military compared to the ROTC component is due to the number of individuals within ROTC (n=30,000) compared to active duty, national guard, and reserves (n=2,079,142). ROTC cadets and midshipmen face primary risk factors for ER, including novel training, inadequate rest periods, high physical demand tasks, and the stress of being full-time university students.[32,48,197,198] This places ROTC members at the highest risk for developing ER during FTX events.[197,199]

Most ROTC components do not have a specified military medical provider. Previous literature has indicated that up to two-thirds of sustained injuries in ROTC cadets go unreported, a higher rate than in active-duty components.[199,200] It is unclear whether these injuries go unreported due to intentional concealment or a lack of knowledge about the healthcare system. Previous reports showed that over 50% of ROTC members surveyed were unfamiliar with available healthcare providers or how to access them.[26] Another study found that 39% of ROTC members did not report injuries due to "inconvenient access to medical care".[199] This lack of familiarity with healthcare options leads to decreased reporting of ROTC illnesses and injuries, especially in reports used for retrospective epidemiological data.

Military training is physically demanding. Due to their unique niche, military members experience higher levels of ER than many other special populations. A variety of innate and developed physical and psychological aspects, environmental influences, and fitness status affect the likelihood of ER. The high incidence rate of ER has brought significant attention to its causes and management. ER incidents and MSKIs significantly affect the readiness and deployability of military forces. Little research has been conducted on ROTC components regarding injury and illness tracking. Most research has focused on active-duty components. Conducting additional

research with a focus on ROTC components and the development of ER incidents is a critical step in ensuring the safety of future officers in our armed services.

Literature Review Summary / Conclusion

This literature review highlights several critical factors influencing the incidence and management of ER. Comprehensive patient assessment, including subjective and objective signs and symptoms, is essential for accurate diagnosis. Psychological attributes such as grit and hardiness significantly impact recovery and success rates, particularly in military training environments.

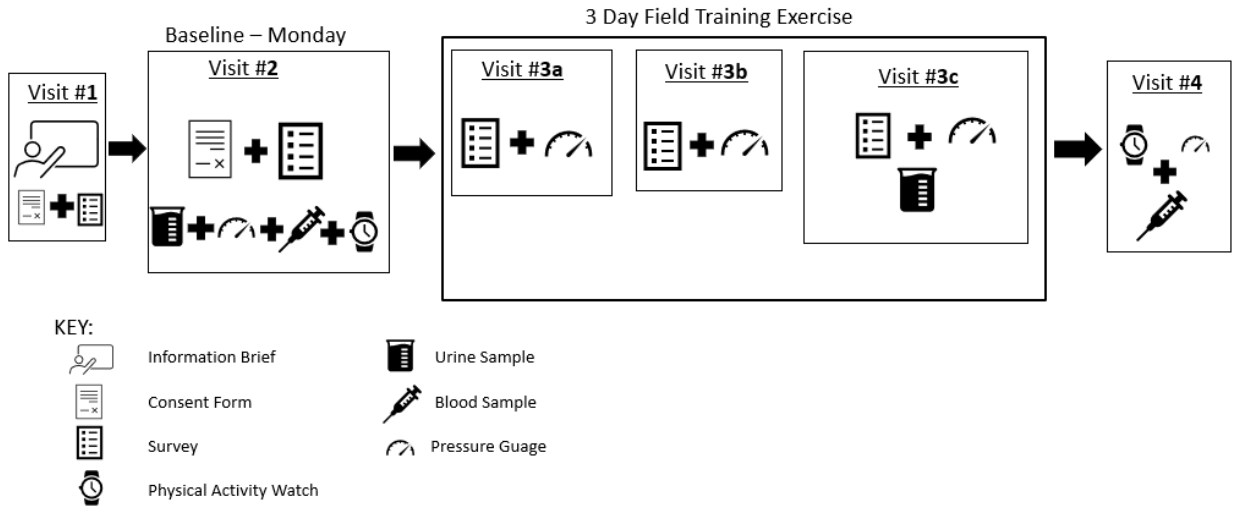
Biological sex plays a crucial role, with males being more prone to ER due to larger muscle mass and higher CK levels during exercise, while estrogen in females offers a protective effect. Nutritional education in military settings with balanced diets and adequate protein intake are important to reduce ER risk. Physical fitness and proper acclimatization are protective factors against ER, with fitter individuals better adapted to physical demands. Novel training regimes with inadequate rest periods and high physical demands are primary contributors to ER, particularly affecting fast-twitch muscle fibers. Epidemiological data on ER often relies on retrospective studies, raising concerns about diagnosis validity and underreporting, especially in ROTC settings. Young Black males in the initial stages of military service are identified as the most at-risk population, highlighting the need for further research into additional factors influencing ER incidence. ROTC members face unique challenges during field training exercises, with inadequate injury reporting and access to medical care exacerbating the risk of ER. Addressing these issues through targeted research and improved healthcare access is crucial for the safety and readiness of future military officers. In summary, a multifaceted approach

considering physical, psychological, and environmental factors is essential for understanding and managing ER, particularly in military contexts. Further research is needed to refine diagnostic criteria, improve reporting accuracy, and develop effective prevention and management strategies.

Methods

Study Design:

This observational cohort study investigates levels of creatine kinase, myoglobin, and associated moderators during an ROTC Military Field Training Exercise. The Auburn University Institutional Review Board has approved this study #24-843 FB.



Participants:

We aimed to recruit 50 ROTC members.

Participants were required to meet the following inclusion criteria to participate in the study:

1. Current member of Auburn University Marine or Army ROTC, 17-45 years of age
   *All participants under the age of 18 must have written parental/guardian consent.
2. No current physical restrictions limiting the individual from full participation in military training (not on “profile/ chit”).
3. Free of any known medical conditions that would be disqualifying for Department of Defense service.
4. Free of any medical condition that would contradict providing a blood sample, such as diagnosis of a blood clotting disorder or taking blood thinner medications.

Participants were eligible for a $50 compensation upon study completion.

Overview: Data Collection Components and Procedures

Demographics and Health History Form: Participants completed one initial survey detailing personal information on demographics (e.g., biological sex, academic year, ROTC level), typical physical activity (e.g., frequency, intensity, time, and type of physical activity), and basic pertinent health history (e.g., history of rhabdomyolysis, history of diagnosed and/or hospitalized heat illness, current medications, and supplements, basic disease overview for self and family).

Muscle Soreness and Mood Survey: Participants completed a short survey each day inquiring about muscle soreness and fatigue in major muscle groups. The survey also included questions regarding overall mood during military training tasks throughout the day, sleep quality and length, and nutritional intake (food and liquid estimates). These specific measurements were taken to subjectively assess the developing muscle soreness in major muscle groups, mood, perceived sleep quality, and reported macronutrient intake from meal-ready-to-eat (MREs). The survey utilized in this study was designed and developed by the research team after careful consideration of inclusion and exclusion factors after a comprehensive literature review. The survey was tailored to focus on the specific population in this study, as well as to directly address the research questions.

Muscle Soreness Measurements: Pain pressure threshold (PPT) was measured in N/cm^2 using a digital force gauge (Force Ten FDX Compact, Wagner Instruments, Greenwich CT, USA) as objective measurements of major muscle group soreness. In the evening of each training day, participants completed a muscle soreness assessment, with the researcher using a digital force

pressure gauge over major muscle groups (calves, quadriceps, hamstrings, biceps, triceps, latissimus dorsi, rhomboids).

Muscle Girth Measurements: Muscle girth measurements of major muscle groups were assessed using a flexible girth measurement tape as an objective measure of muscular swelling. During the initial screening and in the evening of each training day, participants completed a muscle girth measurement assessment at specified locations of the upper and lower leg using a flexible girth measurement tape (Gulick tape).

Blood Sample: Blood samples were used to assess creatine kinase and myoglobin levels before and after the FTX. Participants provided a blood sample (20 ml- two red top blood collection tubes). Research team members who have completed and been awarded phlebotomy certification through the Auburn University Department of Clinical Laboratory Science collected the blood sample following standard blood collection practice and procedure.

Blood collection and transport: Blood samples were collected in two plasma blood collection tubes (red top with hemogard). The samples will be set for 30 minutes at an ambient temperature (40-75°F) and then placed on ice in an appropriately labeled biohazard cooler. One sample tube was transported to a local Hospital Laboratory for analysis of CK, Mb, and CMP. The secondary sample was transported back to Auburn University School of Kinesiology, where they were properly centrifuged, aliquoted, and stored in the -80°C freezer as additional samples.

Urine Sample: Urine samples were used to assess creatine kinase, myoglobin, and urine dipstick levels before and after the FTX. Participants provided a urine sample for analysis (3-4 oz) in the specified container. Participants were given an appropriately labeled urine collection specimen cup and utilized the restroom to provide the sample. Urine samples were immediately assessed for

the specific gravity of the sample using a portable refractometer (Vollrath Company, Sheboygan, WI, USA), then stored for later analysis.

Urine collection and transport: Urine samples were collected in a single specimen cup and immediately tested for urine specific gravity. The samples were then placed on ice in an appropriately labeled biohazard cooler. They were then transported back to Auburn University School of Kinesiology, where they were properly aliquoted and stored in the -80°C freezer as additional samples.

Body Composition Measures: Body composition was used to assess body fat percentage, lean muscle mass weight, and body water content. Participants were required to be fasted for eight hours prior to the body composition measures. After ensuring proper hydration levels, the body composition measures were assessed using a TANITA BC-568 InnerScan Segmental Body Composition Monitor (TANITA Corporation, Preston, WA, USA). Body Impedance Assessment (BIA) measures were only taken once during each participant's baseline assessment.

STEP 1: Information Brief – Recruitment and Informed Consent

Participants were invited to join an informational brief detailing the purpose and methodology of the study. Cadre (military instructors) were not present to reduce coercion. The research team reassured all individuals that participation in the study was completely voluntary and individuals could withdraw from the study at any time. Additionally, they were assured that if they choose not to participate or drop out, it would not impact the individual's relationship with Auburn University, Auburn ROTC, or the Warrior Research Center.

After completing the informational brief and ensuring no Cadre are present, individuals volunteering to participate met with a research team member to read over the informed consent and were allowed to ask any questions regarding the study protocol.

In the case that an individual preferred to have time to consider participation, or if an individual was unable to attend the initial informational brief, they were allowed to complete a one-on-one meeting with a member of the research team to read over and complete the informed consent form and ask any questions they had regarding the study protocol. After completion of the informed consent, the participant took part in the baseline screening. All participants under the age of 18 completed a parental/guardian consent form prior to completing any additional paperwork or sample collection.

STEP 2: Baseline/ Sample Collection

Participants were instructed beforehand to refrain from any physical exercise or "workouts" for 24 hours before baseline data collection. Baseline measures were completed Monday morning with participants in a fasted state for at least eight hours to help avoid compromising sample collection of creatine kinase and myoglobin from Saturday training or activities and reduce interference with ROTC training schedules.

Volunteers met at the ROTC building on the Auburn University campus. Individuals who had not been consented met one-on-one with a research team member and discussed the protocol and consent form and answered any questions they had regarding the protocol. After completing the consent form, the participant completed the demographics and health history form before completing the remaining baseline examinations.

Participants completed the following baseline examinations:

1. Provided a urine sample for analysis (3-4 oz).
    a. Specific gravity of urine samples were immediately tested using a portable refractometer (Vollrath Company, Sheboygan, WI, USA)
    b. A spot urinalysis using a urine dipstick (Rapid response Urinalysis Reagent Test Strips, BTNX Inc, Pickering, Ontario, Canada) was immediately used to assess urine urobilinogen, glucose, bilirubin, ketone, specific gravity, blood, pH level, protein, nitrites, and leukocytes.
    c. The urine sample was kept after completion of the urine specific gravity and urine dipstick assessments for myoglobin analysis.

2. Completed Body Impedance Analysis (BIA).
    a. TANITA BC-568 InnerScan Segmental Body Composition Monitor (TANITA Corporation, Preston, WA, USA).

3. Completed muscle soreness and mood survey.

4. Completed muscle soreness measurements with the researcher.
    a. Digital Force Pressure Gauge (Force Ten FDX Compact, Wagner Instruments, Greenwich CT, USA)

5. Completed muscle girth measurements with the researcher.

6. Provided a blood sample for analysis (20 ml – 2 blood collection tubes).

a. Blood samples were utilized for later creatine kinase, CMP, and myoglobin analysis.

7. Received an accelerometer to wear on a non-dominant wrist for ten days.
    a. Accelerometer; ActiGraph xGT3X-BT (ActiGraph Technologies, Pensacola FL, USA)

8. Received instructions for data collection steps/visits during FTX.

STEP 3: Field Training Exercise – Data Collection

Field Data Collection Procedures: (FTX days)

Day 1: Participants met with the researcher at approximately 5:30 pm and completed the following:

1) Muscle Soreness and Mood Survey

2) Muscle Soreness and Girth Measurements

Day 2: Participants met with the researcher at approximately 5:30 pm and completed the following:

1) Muscle Soreness and Mood Survey

2) Muscle Soreness and Girth Measurements

Day 3: Participants met with the researcher after the conclusion of the final ruck march and completed the following:

1) Muscle Soreness and Mood Survey

2) Muscle Soreness and Girth Measurements

4) Urine Sample

STEP 4: Return Physical Accelerometers and Follow-Up Assessment

Participants were instructed to continue wearing the physical activity trackers for another four days (Monday to Wednesday following the event) to assess daily activity after returning to a regular weekly schedule. This information was used for comparison (regular daily activity compared to FTX activity). Participants met with a research team member at the Auburn University ROTC building on Wednesday, the week following the completion of the FTX, to return the physical activity trackers and completed the following:

1) Muscle Soreness and Mood Survey

2) Muscle Soreness and Girth Measurements

4) Blood Sample

After completing the baseline assessment, FTX data collection, and physical activity tracker return, participants were provided with an Auburn University payment form for compensation.

STEP 5: Analysis of Biological Samples

Biochemical and Histological Analysis

All blood and urine sample processing was completed through East Alabama Medical Center (EAMC) Laboratory Services (Opelika, Alabama, USA). Gathered samples were prepared per EAMC laboratory instructions at the collection site. Blood samples were collected in the requested blood collection tubes for CK, Mb, and CMP testing. They were packaged in a biohazard-compliant container for transport. Urine samples were tested on-site for specific gravity and urine dipstick analysis. The urine samples were then aliquoted and frozen according to EAMC laboratory instructions and placed in a biohazard-compliant container for transport. Both the blood and urine samples were placed in spill preventative containers, placed on ice per the EAMC laboratory instructions and transported via personal vehicle to the EAMC laboratory the day of collection.

Step 6: Statistical Analysis

Data was analyzed in a multi-step fashion. Statistical analyses will be completed in the R statistical Program Software Version (4.2.2(RStudio; Boston, MA, USA) using the psych, lattice, ggplot2, dplyr, tidyr, and lme4 packages. All data was imported and removal of missing data, or data that constituted as outliers due to improbable recording (e.g. extremely low nutritional intake records) using the statistical software. A variety of statistical analyses were conducted to address each aim of the study. The significance level of $\alpha = 0.05$ will be set for each analysis *a priori* to determine statistical significance.

To address Aim 1A in determining whether a 2-3 day FTX significantly alters CK and Mb levels in ROTC cadets, a paired t-test was conducted to evaluate these changes as the t-test compares the means of the pre-and post-levels of CK and Mb to determine if there is a statistically significant difference. An assumption check was completed to assess the normality of the data distribution, including the difference between the paired observations. The Central Limit theorem

was considered to mitigate normality concerns due to the predicted sample size. A pair-wise t-test was conducted if the data was normally distributed.

$$t = \frac{\bar{d}}{s_d/\sqrt{n}}$$

If the data was found not to be normal, a Wilcoxon signed rank test was used.

$$W = \sum_{i=1}^{n} R_i^+$$

These statistical assessments were appropriate to address Aim 1A as the data consisted of paired observations comparing the pre-FTX blood and urine biomarkers to the post-FTX levels. The dependent variable in these tests was the biomarker level measured at the pre and post time points. The null hypothesis will state no difference in the biomarker levels at the two time points. The alternative hypothesis is that the CK or Mb levels will increase three times the baseline levels after FTX completion.

A multiple regression analysis was conducted to address Aim 1B in determining the relationship between fitness level (measured by the military fitness test), body composition, biological sex, race and age, and changes in CK and Mb after the FTX. This method allows for assessing the impact of multiple independent variables on a single dependent variable. Assumptions of all data will be checked for linearity, independence, homoscedasticity, and normality of residuals. The dependent variables of this assessment was used to calculate differences of the CK and Mb between the post-FTX and the pre-FTX levels. The independent variables for this assessment consisted of the moderators above. A linear regression was utilized rather than a logistic regression because the CK and Mb levels will be taken as continuous numerical values instead of a binary assessment of an estimated or proposed rise in biomarker

value. Two separate models were conducted to address Aim 1B. One model was specific to the changes in the CK levels, and the other was specific to the Mb changes. The models were constructed using the following equations:

1. Change in CK levels = β0 + β1(Fitness Level) + β2(Body Composition) + β3(Biological Sex) + β4(Race) + β5(Age) + ε

2. Change in Mb levels = β0 + β1(Fitness Level) + β2(Body Composition) + β3(Biological Sex) + β4(Race) + β5(Age) + ε

The results were determined by assessing the coefficients, standard errors, t-values, and p-values to determine the statistical significance and strength of the relationships between the moderators and the changes in CK and Mb levels.

A correlation analysis and a regression analysis were conducted to examine the direct effect of an increased PA level (measured through step count and estimated kilocalories expended) during the FTX and how it was related to the increase in CK and Mb levels. A Pearson correlation or Spearman rank correlation was conducted based on the normality of the data. A Pearson correlation helped assess the strength and direction of the linear relationship between PA and the CK or Mb levels if the data were continuous and normally distributed. If the data was not normally distributed, then a Spearman rank correlation was utilized as a non-parametric alternative to a Pearson correlation.

$$r = \frac{\sum(X_i - \bar{X})(Y_i - \bar{Y})}{\sqrt{\sum(X_i - \bar{X})^2 \sum(Y_i - \bar{Y})^2}}$$

Pearson Correlation

$$\rho = 1 - \frac{6\sum d_i^2}{n(n^2 - 1)}$$

Spearman Rank Correlation

After completing the correlation calculations, a simple linear regression analysis was performed. In this analysis, the CK and Mb levels were dependent variables, while the PA were the independent variables. This analysis illustrated how the changes in the PA levels impacted or predicted changes in the CK and Mb levels.

To address Aim 3, multiple statistical analyses was used to investigate whether ER clinical signs and symptoms were present when CK and Mb levels are 3-4x baseline levels. These CK and Mb levels are considered low-level or mild ER. Descriptive and summary statistics were calculated to evaluate the mean, median, standard deviation, and range for CK and Mb levels and the frequency and percentage of existing clinical signs and symptoms. Additional correlations were evaluated similarly to the previous aims of either Pearson or Spearman correlations based on the distribution and normality of the gathered data. These correlations assessed the relationship between the CK and Mb levels and the presence of clinical signs and symptoms. Furthermore, two separate linear regressions were used to analyze the probability of CK and Mb levels based on the presence or absence of ER's clinical signs and symptoms. The dependent variable was the CK and Mb levels, while the independent variable was a binary coding for the presence of the clinical signs and symptoms. The models were constructed as follows:

1. $CK\ Levels=\beta0+\beta1(Dark\ Urine)+\beta2(Muscular\ Soreness)+\beta3(Fatigue)+\epsilon CK\ Levels=\beta0+\beta1(Dark\ Urine)+\beta2(Muscular\ Soreness)+\beta3(Fatigue)+\epsilon$

2. $Mb\ Levels=\beta0+\beta1(Dark\ Urine)+\beta2(Muscular\ Soreness)+\beta3(Fatigue)+\epsilon Mb\ Levels=\beta0+\beta1(Dark\ Urine)+\beta2(Muscular\ Soreness)+\beta3(Fatigue)+\epsilon$

These results provided insights into how the presence or absence of ER's clinical signs and symptoms may predict CK and Mb levels. The results also illustrated if there were significant

differences in CK and Mb levels between groups with and without the clinical signs and symptoms.

Nutritional Intake and Energy Expenditure of ROTC Members During an FTX

Introduction

Service members often engage in physically and mentally demanding tasks. Proper nutrition is essential for sustaining peak performance. The Military Dietary Reference Intake

(MDRI) provides nutritional guidelines for military personnel based on training settings.[201] An important training event for Reserve Officers' Training Corps (ROTC) Cadets is the required twice yearly Field Training Exercise (FTX). MDRI recommends normal daily caloric intake ranging from 1,785 to 3,417 kcal for males and 1,432 to 2,756 kcal for females. Macronutrient distribution should consist of 50-55% carbohydrates, 10-35% protein, and no more than 30% fat.[201] Micronutrient requirements are expressed as percentages of daily intake. During FTXs, Cadets primarily rely on Meal-Ready-to-Eat (MRE) rations provided by their training unit. MREs are designed to meet the nutritional demands of field training, offering high-calorie, nutrient-dense meals. However, food preferences, hunger levels, and meal timing often cause Cadets to self-limit their intake.[202]

A negative energy balance of 30-45 kcal/kg/day increases the risk of decreased physical performance and impaired physiological function.[203,204] Insufficient nutritional intake combined with high activity levels has been linked to increased risk of bone stress injuries,[205] menstrual and fertility disorders in women,[203] and reduced testosterone levels in men.[202,206,207] Inadequate energy availability also affects cellular growth, maintenance, and thermoregulation.[208] Research has further demonstrated that nutritional deficits during military training can impair physical and cognitive performance, endocrine and metabolic function, gastrointestinal health, iron status, mood, and immune function.[209]

Key differences exist although military nutritional needs are often compared to those of athletes. Variations in training intensity, duration, and personnel demographics influence nutritional demands. Military personnel often require higher fat intake to support nutrient absorption and overall caloric needs.[204,210] Adequate fat in the military members' diet also supports prolonged tasks, such as an FTX. Athletic nutrition recommendations typically base intake on body composition and focus on the desired performance need of the athlete based upon

sport, position, and activity requirements.[37,202] In contrast, military nutritional recommendations are percentage based for macronutrients and recommended in terms of grams of macronutrient per kilograms of body weight during the day.[201] These macronutrient per kilogram body weight recommendations are altered during operational and restricted rations.[201]

The United States ROTC units conduct biannual FTXs lasting two to three days. These exercises involve military training activities such as marksmanship, obstacle courses, land navigation, and ruck marches.[211] Cadets must complete training while living in field conditions requiring them to adapt their nutrition to meet physical demands.[212] Proper nutritional intake reduces fatigue, injury risk, and illness.[213] However, studies indicate that military members often experience a nutritional deficit compared to their energy expenditure during multi-day exercises.[202,204,214,215] This study aims to: (a) assess self-reported nutritional intake (NI) among ROTC Cadets during a short-term FTX compared to military nutrition guidelines; and (b) compare self-reported NI with calculated energy expenditure (EE) during the FTX .

Methods

This study was part of a larger observational cohort study conducted with a university ROTC program. The university's Institutional Review Board, Army and Marine ROTC Commands and Army HPRO approved the study (protocol #24-843 FB). Participants wore accelerometers to monitor activity levels and self-reported their estimated nutritional intake during a two-to-three-day FTX. The training program, duration, meal availability, and physical demands varied by ROTC branch (Army or Marine) (Figure 1).

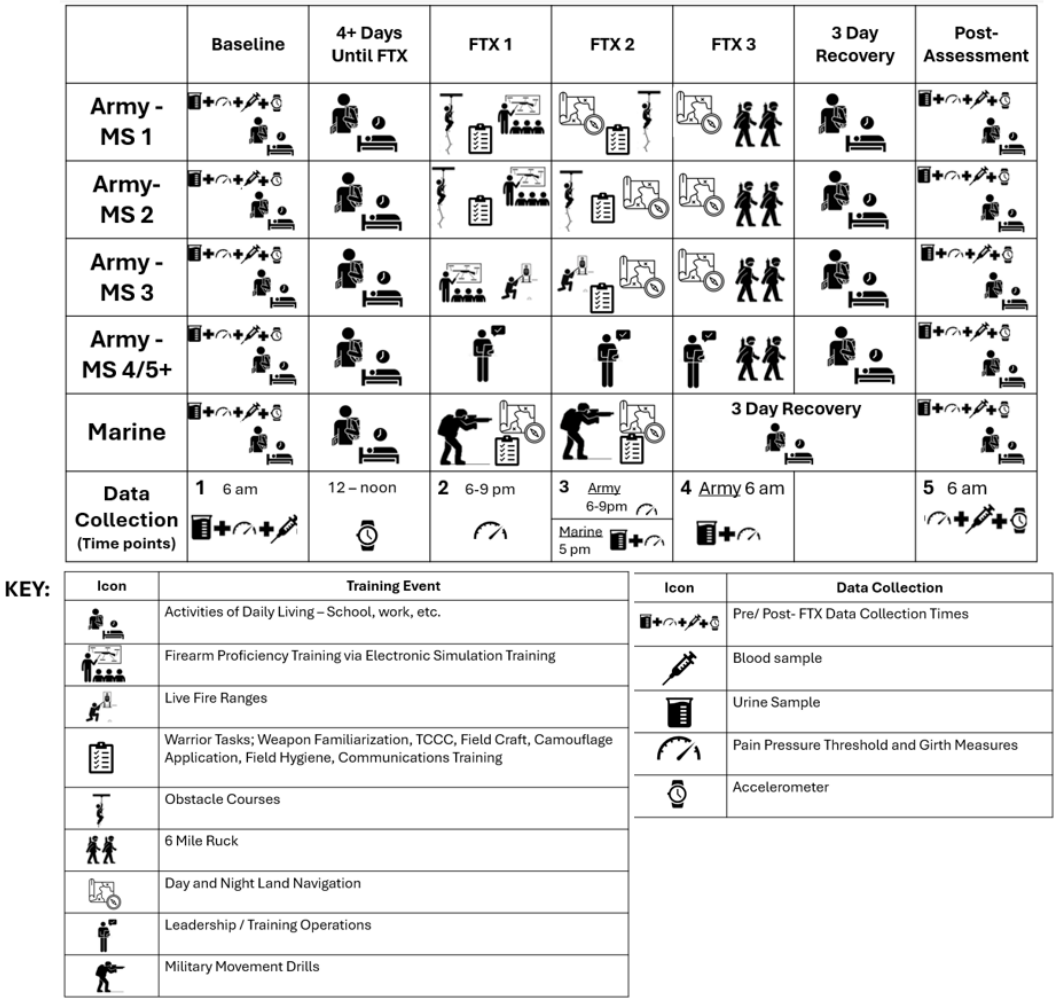


Figure 1. Field Exercise Training (FTX) and Data Collection Time Point Schedule

Marine ROTC Training Protocol:

- Duration: Two days, one night of continuous training.
- Activities: Squad movement drills and patrol, bivouac infiltration and exfiltration, camouflage application, weapon system familiarization, communication system use, trauma combat casualty care (TCCC), day and night land navigation, logistics operations, leadership exercises, and ruck marches between activities.

Army ROTC Training Protocol:

- Duration: Three days, two nights with designated six-hour sleep periods per night.
- Activities varied by Military Science (MS) year:
    - **MS1 and MS2:** Firearm proficiency training via Electronic Simulation Training (EST), obstacle course, conditioning course, team development course, and a six-mile ruck march.
    - **MS3:** Firearm proficiency training via EST, live firearm training, and qualification at 25-meter and modified record fire ranges.
    - **MS1-MS3:** Day and night land navigation, team-building events, warrior tasks (weapon familiarization, TCCC, field craft, camouflage application, field hygiene, and communications training).
    - **MS4:** Operational planning and support for MS1-MS3 events.

Participants could supplement MREs with self-provided food and beverages. All additional intake was recorded alongside military-provided meals.

Participants

All participants voluntarily joined this study and signed a written informed consent after receiving a detailed verbal and written explanation of the requirements. To be eligible, individuals had to be active members of the university ROTC program, between 17 and 45 years old, free of physical restrictions, not on a medical profile or chit, and without known medical conditions that would disqualify them from Department of Defense service.

Fifty-seven individuals initially enrolled in the study. Fourteen participants were later excluded from the analysis due to incomplete data or other complications (Figure 2). The final analysis included 43 participants (33 males: 20.39 ± 3.37 years, 175.96 ± 5.72 cm, 79.65 ± 8.61

kg; 10 females: 23.30 ± 6.5 years, 166.68 ± 5.17 cm, 67.75 ± 7.92 kg). Additional demographic details are presented in Table 1.

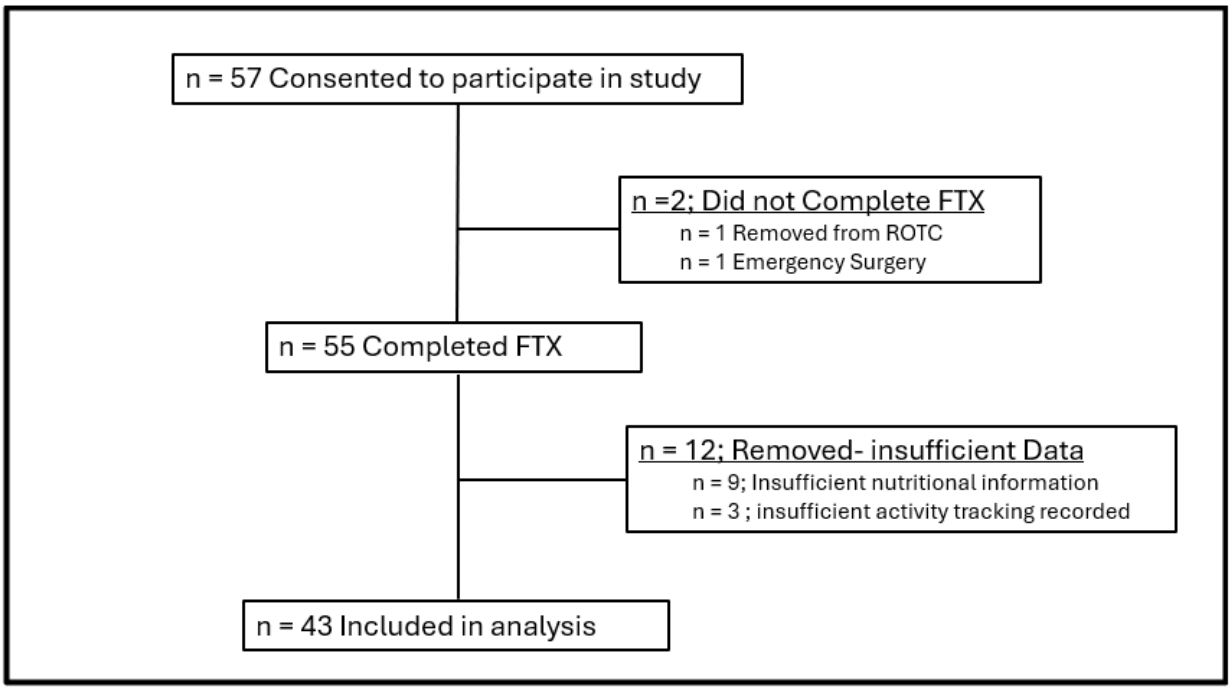


Figure 2. Participant flow and sample size

Table 1. Participant Demographics

Participants	43
MS1	11 (10 male/ 1 female)
MS2	11 (11 male /0 female)
MS3	8 (5 male / 3 female)
MS4	11 (6 male/ 5 female)
MS5+	2 (1 male / 1 female)
Male/ Female	33/10
Age (yrs)	21 ± 4 years
Height (cm)	174 ± 7cm

Body mass (kg)	76.9 ± 9.8 kg
BMI	25.4 ± 2.6
Body Fat Percentage (group)	20.2 ± 7.5 %
Male Body Fat Percentage	17.0 ± 5 %
Female Body Fat Percentage	31.3 ± 3.4 %

Note: MS = Military Science Year

Participants recorded all nutritional intake from meals and snacks using a written food log. The log included pre-printed Meal-Ready-To-Eat (MRE) check sheet with additional space for documenting extra food items and portion sizes. The Army ROTC unit received two MREs per day during the FTX and one hot meal (HOT-A) in the field from the Army Post dining facility. The Marine ROTC unit received two or three MREs in advance and had the option to fieldstrip the MREs by discarding any unwanted included items before the training event to minimize packing in/out load.

Caloric values for recorded food items were calculated using nutritional data from the Combat Rations Database (ComRAD), a resource developed by Human Performance Resources (Consortium for Health and Military Performance, CHAMP), the U.S. Army Combat Capabilities Development Command (DEVCOM) Soldier Center, and the U.S. Army Research Institute of Environmental Medicine.[216] Additional food items were analyzed using the U.S. Department of Agriculture's Food Data Central.[217] Because the Marine FTX lasted two continuous days while the Army FTX spanned three days, reported nutritional values were adjusted to reflect daily intake for direct comparison.

Energy expenditure was estimated using a triaxial accelerometer (ActiGraph xGT3X-BT, ActiGraph Technologies, Pensacola, FL, USA). Accelerometers were set to a 60 Hz sampling frequency and programmed for each participant with sex, age, height, body mass, and dominant hand. Participants wore the devices continuously throughout the FTX. Raw acceleration data were analyzed using ActiLife Software v6.13.4 (ActiGraph, Pensacola, FL, USA). Energy expenditure

was determined using built-in algorithms, including the Freedson VM3 Combination (2011) and Swartz Adult Overground & Lifestyle (2000) for metabolic equivalents (METs). Moderate-to-vigorous physical activity (MVPA) was assessed using Montoye cut points.[218] Data were excluded from analysis if participants wore the device for less than 10 hours per day.

Estimated energy balance (EB) was calculated by comparing energy expenditure (EE) with recorded nutritional intake (NI). A surplus of intake was recorded as a positive value, while a deficit was recorded as a negative value, following the equation:

$$E(\text{nergy})B(\text{alance}) = N(\text{utritional})I(\text{ntake}) - E(\text{nergy})E(\text{xpenditure}) \text{ or } EB = NI - EE$$

This calculation was applied to each ROTC participant to assess daily energy balance.

Data Analysis

Statistical analyses were conducted using R (R Core Team, 2020) and RStudio (RStudio Team, 2023) version 4.2.2, utilizing the psych, lattice, car, dplyr, tidyr, emmeans, and effsize packages. Statistical significance was set *a priori* at $\alpha = 0.05$. Normality of dependent variables was assessed using Shapiro-Wilk tests and visualized with Q-Q plots. One sample t-tests with Cohen's d effect sizes and 95% confidence intervals compared participants' nutritional intake to MDRI recommendations for total caloric intake and macronutrient distribution. Analyses were conducted based on MDRI macronutrient guidelines for males and females compared carbohydrate, protein, and fat intake setting μ as the recommended macronutrient intake in grams or percentage of total diet. Levene's test confirmed homogeneity of variances. Paired t-tests or Wilcoxon signed-rank tests assessed energy balance (EB) relative to energy expenditure (EE) and nutritional intake for each FTX day and the entire event.

Results

Comparison of recorded nutritional intake to MDRI guidelines revealed substantial nutritional deficits and poor macronutrient distribution among ROTC cadets participating in the Fall FTX. MDRI guidelines provide a recommended macronutrient range both in grams per day, and in percentage of daily nutritional intake. For example, carbohydrates should compose 50-55% daily nutritional intake, male recommendations spread from 340 g to 680 g per day for males, 276 g to 552 g for females. These recommendations will be referred to the lower and upper recommendations based on sex (Table 2).

Table 2. General MDRI Guidelines for Daily Nutritional Intake

Macronutrient		Male	Female
Carbohydrates	Daily % NI	50-55 %	50-55%
	Grams	340-680g	275 – 552g
Protein	Daily % NI	10-35%	10-35%
	Grams	68-136g	55-110g
Fats	Daily % NI	30%	30%
	Grams	17g	12g

Note: % = percentage of daily nutritional intake, g = grams, NI = nutritional intake

Many male cadets failed to meet carbohydrate percentage intake recommendations. Only 60.6% ($t= 0.90$, $df= 32$, $p = 0.38$) met the recommended 50% of daily intake from carbohydrates, while all male cadets ($t= -3.01$, $df= 32$, $p= 0.005$) consumed less than the upper 55% recommendation. Female cadets also failed to meet the upper daily carbohydrate gram ($t=-22.37$, $df=9$, $p<0.001$) and lower daily carbohydrate gram ($t=-7.63$, $df=9$, $p <0.001$) MDRI guidelines. However, 80% of female cadets met the lower carbohydrate percentage recommendation (50% of daily nutritional intake) ($t= -7.63$, $df=9$, $p <0.001$), and 70% met the upper percentage recommendation ($t= 0.07$, $df=9$, $p =0.95$).

Protein intake analyses showed that 94% of male cadets did not meet the lower daily protein recommendation in grams (t= -10.03, df= 32, p <0.001) and all male cadets consumed less than the upper MDRI protein recommendation (t= -34.70, df= 32, p <0.001). However, 97% exceeded the lower 10% daily protein intake recommendation (t=8.65, df = 32, p <0.001) while none surpassed the upper limit (t=-38.15, df=32, p<0.001). Only 10% of female cadets met the lower MDRI protein recommendation (t= -4.62, df=9, p = 0.001) and none reached the upper limit (t= -16.56, df = 9, p <0.001). Like the males, all female cadets met the lower protein percentage recommendation (t=4.87, df=9, p <0.001) but none exceeded the upper MDRI percentage (t=-25.99, df= 9, p <0.001).

The MDRI provides a single fat intake recommendation based on biological sex. Most male cadets (94%) met or exceeded the daily fat gram recommendation (t=6.50, df=32, p <0.001). Additionally, 72% met or exceeded the daily fat percentage recommendation (t=3.37, df=32, p = 0.002). Among female cadets, 90% met or exceeded the daily fat gram recommendation (t=4.19, df=9, p= 0.002) while 60% met or exceeded the daily fat percentage recommendation (t=0.43, df=9, p= 0.680).

Active metabolic rate (AMR) was calculated for each cadet based on body weight and reported activity levels. Male cadets had an AMR of 2,879 ± 176 kcal/day, while accelerometer data estimated their energy expenditure at 2,471 ± 533 kcal/day during the FTX. Female cadets had an AMR of 2307 ± 1478 kcal/day, with accelerometer-based energy expenditure averaging 1,946 ± 348 kcal/day.

Cadets consumed significantly fewer calories than they expended during the FTX (t= -12.07, df=42, p <0.001) with a large Cohen's effect size. Low Energy Availability (LEA) caloric thresholds were calculated using guidelines from the International Olympic Committee[208] and

compared to reported intake. Cadets consumed significantly fewer calories than their LEA-calculated requirements ($t=6.47$, $df=57.54$, $p<0.001$). This trend remained significant when analyzed separately for male ($t=5.23$, $df=40.88$, $p<0.001$) and female cadets ($t=4.54$, $df=10.02$, $p = 0.001$).

Discussion

This study examined the self-reported nutritional intake and estimated energy expenditure of ROTC cadets during a Fall FTX, providing insights into the balance between these factors under demanding field training conditions. The findings highlight the risk of energy deficits among cadets which can significantly impact physical performance, cognitive function, and overall health.[202]

Energy balance depends on multiple factors, including assigned duties, task duration, environment, and food availability.[219] Poor energy balance is associated with negative health outcomes, including impaired immune and endocrine function, decreased bone mass, reduced strength and power, and unfavorable changes in body composition that can lower operational performance.[209] Previous research found that soldiers expended approximately 595 more kcal per day than they consumed during the initial two weeks of basic training.[215] While a 500 kcal daily deficit may not pose immediate health risks, prolonged energy imbalances can negatively affect body composition, performance, as well as mental and physical recovery. Severe energy depletion (e.g., 5% body fat loss or over 10% total weight loss) has been shown to impair health and job performance in military personnel.[220-222]

Energy balance plays a crucial role in sustaining cognitive function, endurance, reaction time, and overall physical performance, all essential for military effectiveness. A negative energy balance like the one observed in the current study can increase fatigue, impair decision making, and reduce muscular endurance. These deficits may limit a cadet's ability to perform effectively

during training and in real-world operations. Research has shown that prolonged energy deficits hinder mission success, especially in environments where soldiers operated for extended periods with limited nutritional intake.[223,224]

Cadets in the present study exhibited a statistically significant negative energy balance throughout the FTX. Energy deficits did not vary between ROTC branches despite differences in food provisions. Army ROTC cadets received scheduled mealtimes and were provided with two MREs and one hot meal per day, while Marine ROTC cadets received two to three MREs before the event and were instructed to field strip non-essential items before training allowing the Marines to carry less weight and space of food in their rucksacks into and out of the FTX. Research suggests energy deficits in training environments can contribute to low energy availability (LEA) even in short-term settings such as a two-to-three-day FTX.

LEA presents a significant challenge for military personnel during FTX where prolonged physical demands often coincide with insufficient caloric intake. Sustained LEA can impair performance, delay recovery, and hinder cognitive function, all of which are critical for mission success.[215,222,225-227] Chronic LEA can also lead to relative energy deficiency in sport (RED-S), a condition characterized by compromised health and performance.[208,227] Cadets in the present study not only failed to meet recommended caloric intake but also fell below the IOC's LEA threshold defined as <30 kcal/kg fat-free mass (FFM) per day for females and <25 kcal/kg FFM/day for males (Figure 2).[208] These findings emphasize the need for proper energy balance to support health and performance during training.

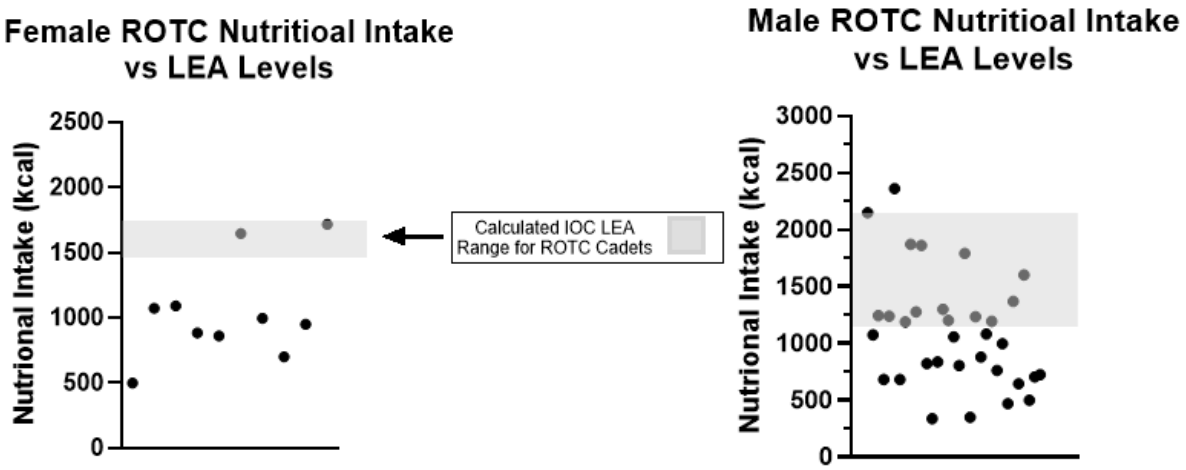


Figure 3. Low Energy Availability Amount vs ROTC Nutritional Intake
Note: LEA = Low Energy Availability, IOC = International Olympic Committee

Energy deficits during training may also have long-term consequences for the health and career longevity of cadets. Chronic LEA increases the risk of stress fracture, muscle loss, and endocrine dysfunction which can all lead to limited military performance and retention. In this study most cadets failed to meet recommended caloric intake and fell below the IOC LEA threshold. These findings suggest that early-stage training environments may already predispose individuals to physiological adaptations that negatively impact performance. The purpose of the ROTC is to create future military leaders. It is imperative for the ROTC cadets to learn and establish proper nutrition practices in these short-term controlled environments to utilize the best practices after commissioning where these training real-world events will span a much longer time frame. As military officers and leaders, the knowledge and practices that cadets' gain in an ROTC program will shape the future of our military force. Optimizing nutritional intakes early in their military career will enhance resilience and improve long-term operational readiness, and this knowledge can be transitioned to other military personnel. Integrating nutrition education into ROTC programs could help cadets develop effective fueling strategies before they enter active-duty service.

Proper macronutrient distribution is essential for maintaining health, optimizing energy levels, and supporting physiological functions. Military doctrine provides specific dietary guidelines based on estimated energy needs for male (85 kg, 175 cm) and female (69 kg, 163 cm) service members.[201] Self-reported dietary intake remains a common method for estimating military nutritional intake despite inherent limitations.[176,224,228] This study implemented a self-reported nutritional intake questionnaire throughout the FTX. One of the primary challenges in military nutrition is balancing caloric intake with the logistical constraints of field operations. In this study Army ROTC cadets received their scheduled meals, but marines parred down their MREs to what they wanted and were willing to carry. This tradeoff between food availability and load carriage is well documented in military research.[222,229] Continuing to improve and optimize ration composition to provide nutrient-dense, palatable, and lightweight food options could help reduce energy deficits while minimizing physical burden.

Carbohydrates play a crucial role in exercise performance,[230] power output,[231] and endurance.[232] Recommended carbohydrate intake for athletes ranges from 3-12 g/kg/day,[233,234] while military guidelines suggest 4-8 g/kg/day. IET soldiers have been reported to consume 5 g/kg/day,[215] while other studies found an intake of 240 g/day.[214] In this study, cadets consumed approximately 142 ± 63 g (53 ± 8% of daily intake) with none meeting the recommended carbohydrate intake levels (Figure 3). Although 61% of males and 80% of females met the recommended 50% carbohydrate intake, the overall caloric deficit remains a concern. Preloading carbohydrates before field training may be an effective strategy to improve energy availability. Endurance research emphasizes glycogen storage as a key factor in sustaining prolonged physical performance. Applying similar principles to military personnel may enhance energy reserves before field operations. Structured fueling strategies before and during FTX may help sustain

energy levels and mitigate performance declines as none of the cadets in this study met recommended daily carbohydrate intake levels.

Protein intake is vital for muscle maintenance, recovery, and athletic performance.[235-238] Recommended protein intake ranges from 1.0-2.2 g/kg/day for athletes[233,239] and 0.8-1.6 g/kg/day per military guidelines.[201] Nearly 94% of male cadets and 90% of female cadets failed to meet the lower recommended protein intake with none reaching the upper recommendation (Figure 3). Previous studies have reported higher protein intakes among IET soldiers, averaging 114 g/day[215] and 78 g/day.[214]

Dietary fat is essential for hormone production, cell membrane integrity, and nutrient absorption.[233,240,241] Essential fatty acids support inflammation reduction, brain health, and cardiovascular function.[233,241,242] Fat serves as a dense energy source, particularly valuable for endurance activities like FTX.[243,244] Military guidelines recommend limiting fat intake to 30% of total daily calories, estimating 17 g/day for males and 12 g/day for females.[201] Previous studies have reported military fat intake levels well above these guidelines, with IET soldiers consuming 89 g/day[215] and 77 g/day.[214] In the current study, 94% of male and 90% of female cadets exceeded the recommended fat intake (Figure 3). Many cadets relied on high-fat snack options such as meat jerky, which likely contributed to satiety and reduced overall caloric intake.

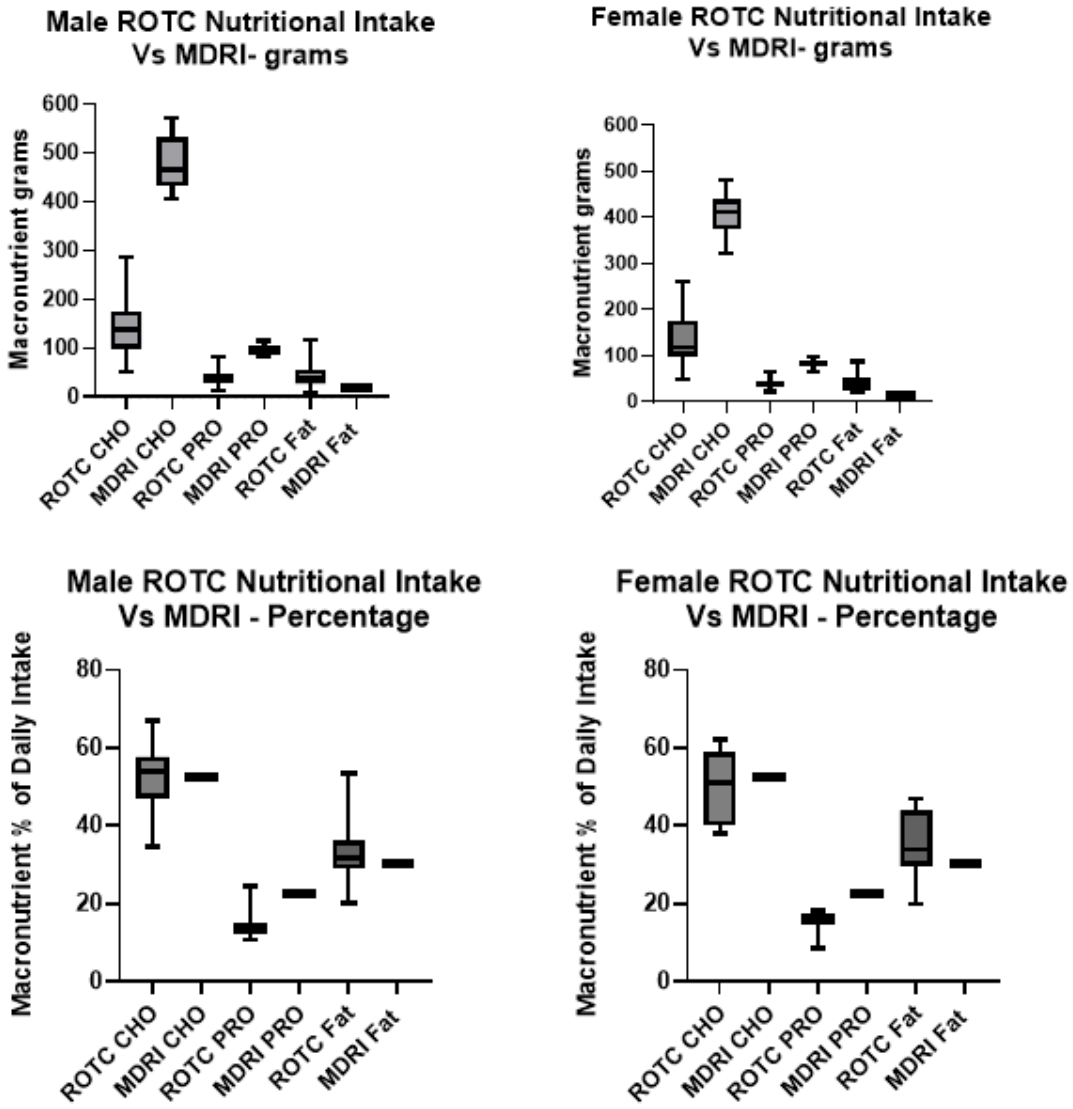


Figure 4. ROTC Participant Nutritional Intake Compared to MDRI Recommendations
Note: CHO = Carbohydrate, PRO = Protein

Previous research has reported military personnel expending 3,700-6,300 kcal/day in training environments[245] and 3,441.6 kcal/day in garrison.[246] Energy expenditure varies based on occupational tasks, with infantry soldiers often exhibiting the highest demands. Reported energy requirements range from 2,342 kcal/day for female administrative personnel[223] to 7,122 kcal/day for male Marines in mountain warfare training.[222,247] In this study, male cadets expended 2,540.8

± 638.9 kcal/day, while female cadets expended 1,942.67 ± 368.58 kcal/day, both lower than previous literature (Figure 4).

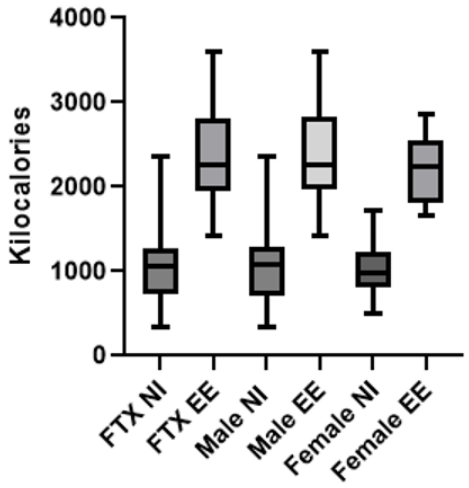


Figure 5. Energy Deficit; Self-Reported Nutritional Intake vs Energy Expenditure
Note: FTX = Field Exercise Training, NI = Nutritional Intake, EE = Energy Expenditure

The energy expenditure values in this study were lower than those reported in previous military research. This may be due to several factors including variations in activity level, training intensity, the shorter duration of this FTX compared to longer military exercises, or the methodology of estimating energy expenditure. Doubly labeled water remains the gold standard for measuring energy expenditure, however its high cost and time-intensive analysis limit feasibility in larger studies.[248-250] Prior research supports accelerometers as a valid tool for assessing physical activity and energy expenditure, particularly in military settings.[204,215,229,251-253] Additionally, accelerometers offer a non-invasive alternative that improves participant adherence without adding unnecessary burden.[226,254-256]Accelerometers measure movement and the rate of movement. They do not, however, measure the effort required to conduct the recorded movement. Heart rate variability over the course of an FTX would provide a more insightful measure to the

true effort of the cadets. A difference in energy expenditure and effort is likely to occur when comparing a six mile walk to a six-mile ruck with an additional 11.5 kgs (25 lbs.) of gear and equipment. Measuring heart rate variability would provide a more insightful assessment of the effort that cadets are withstanding rather than just the distance.

Military personnel and elite athletes share similar physiological demands that require precise nutrition strategies to sustain performance and recovery. Both groups rely on adequate carbohydrates, proteins, and fats to fuel prolonged exertion, maintain muscle mass, and support cognitive function. However, the cadets in this study fell short of recommended macronutrient intake levels. Their inadequate carbohydrate and protein consumption likely contributed to suboptimal recovery. Sport science research shows that targeted nutrition strategies such as increasing protein intake after exercise and consuming high-glycemic carbohydrates during sustained activity enhance endurance and reduce fatigue. Incorporating these targeted nutrition strategies can also increase the overall caloric intake for the cadets. Previous research has shown that providing protein shakes can increase caloric intake and enhance performance in Army Basic Combat Training.[257] Applying these principles to ROTC military training could improve cadets' ability to maintain high performance throughout the training event.

A variety of practical recommendations can be made to address the energy deficits observed in this study. Several strategies could help improve nutritional intake during FTX and other high-demand training environments. Encouraging cadets to carry nutrient-dense lightweight snacks such as energy gels or carbohydrate-dense meal replacements may help maintain caloric intake without increasing ruck sack weight or space. Implementing structured meal planning before field exercises including carbohydrate-loading strategies may enhance energy availability. Teaching cadet's military-specific nutrition strategies can impower them to make better fueling decisions especially when in rationed environments. Encouraging optimized post-event nutrition

including protein and carbohydrate replenishment may help in accelerating recovery and improving subsequent training performance.

Limitations

This study has several limitations that should be considered when interpreting the findings. First, dietary intake was assessed using self-reported methods, which are prone to recall bias and underreporting, particularly in field training environments where accurate tracking is challenging. Additionally, energy expenditure was estimated using accelerometry, which, while practical and validated in military settings, does not account for load carriage or effort variations, potentially leading to underestimations. The study was also limited by its short duration, capturing only a snapshot of energy balance during a single FTX, which may not fully represent long-term patterns or cumulative effects of energy deficits. Furthermore, while differences in ROTC branch provisions were noted, individual variations in food consumption and adherence to nutritional recommendations were not controlled.

Conclusion

This study highlights the critical role of nutrition in supporting cadet performance and long-term health during military training. The observed energy deficits during FTX underscore the need for strategic fueling strategies, improved ration compositions, and enhanced nutrition education to sustain both training and operational performance. ROTC cadets may fail to consume sufficient nutrition to maintain energy balance, with dietary choices leading to excessive fat intake while falling short on carbohydrates and protein. The energy demands of FTX exceed caloric intake, resulting in short-term low energy availability (LEA) which may impact performance and increase injury risk. Integrating sports nutrition principles and tactical meal planning into military training programs can help optimize energy balance, enhance mission readiness, and improve long-term force sustainability. Future research should further investigate the effects of LEA on

military readiness and develop targeted nutritional strategies to sustain performance and resilience.

Acknowledgements

We would like to thank the entire Warrior Research Center Laboratory, Auburn University, graduate and undergraduate members with their assistance in the data collection for this project. We would also like to thank Auburn University's Army and Marine ROTC programs for working with us for the data collection for this project during FTX training.

Quantifying Muscle Soreness, Mood Changes, and Activity Level in ROTC Members During a Fall FTX

Introduction

Field training exercises (FTX) are important components for Reserve Officers' Training Corps (ROTC) program cadets. The FTX provides cadets with hands-on opportunities to develop and test their leadership, resilience, and operational skills in realistic military scenarios.[211] These FTX events create physical and mental demands and present unique challenges, particularly in muscle soreness, mood changes, and increased physical activity levels.[199,226,228] Understanding these variables allows for optimizing training protocols and minimizing injury and illness risks for ROTC members.

Muscle soreness is a common outcome of increased physical workloads, especially when the body is subjected to unfamiliar or strenuous movements. The FTX requires cadets to engage in activities such as carrying heavy loads, prolonged marching, and performing tactical maneuvers, activities significantly different from their daily routines. The increase in workload can result in delayed onset muscle soreness (DOMS), characterized by stiffness, tenderness, and reduced muscle function.[49,258,259] This soreness can affect performance during training and can alter biomechanical patterns leading to additional soreness, fatigue, and altered gait and increasing joint stress and the risk of injury.[260-262]

The psychological demands of FTX also play a pivotal role in shaping cadets' experiences and outcomes[263-267] Mood and mentality is a key factor influencing task performance, particularly in challenging environments.[147] Positive mood states are associated with enhanced focus, motivation, and problem-solving abilities necessary for navigating military scenarios. Conversely, decreased mood, often exacerbated by physical fatigue and soreness, can impair decision-making,

reduce perseverance, and heighten the perception of task difficulty.[149,151,158] When combined, these factors create a cycle where decreased mood and elevated soreness amplify fatigue and further increasing vulnerability to injury.[158-160]

Increased activity levels during FTX represent a significant departure from the typical daily energy expenditure of ROTC cadets. The heightened physical demands include extended periods of marching, climbing, and other endurance-based tasks requiring cadets to sustain elevated energy outputs over prolonged durations.[215,226,228] These activities are integral to military training, however, the abrupt increase in intensity and duration can strain the musculoskeletal and cardiovascular systems. Measures of external and internal load may improve our understanding of the (mal)adaptations that occur during an FTX. External load is the work performed, speed, and distance covered. Internal load is the strain on biological systems in attempts to maintain homeostasis from the external load placed upon the individual.[226] Both internal and external load are needed to illicit overall physical and physiological adaptations.[226,268] Too much external and/or internal load can lead to overuse musculoskeletal injuries, overreaching, and overtraining, ultimately causing an overall decrement rather than continued positive adaptations.[269-271]

Previous research has investigated muscle soreness in military training,[49,258] how mood affects military training and success,[147,165] and changes in activity levels during military training exercises.[215,222,228] To date, no study has examined the interaction of these factors during a ROTC FTX event. The aim of this study is to evaluate the interplay between the physical and psychological factors in a military training environment by quantifying muscle soreness, mood changes, and activity levels during a fall FTX.

Methods

This study was part of a larger study conducted with a University ROTC program. The Institutional Review Board of the host University, ROTC Marine Commander and the Army

HPRO approved the study (protocol #24-843 FB). The general approach to this study was for participants to complete a pre-FTX screening, recorded measures during the FTX, and post-FTX measures. The program of instruction (POI), length and requirements of the FTX were based upon the ROTC Branch (Army or Marine) directives (Figure 1).

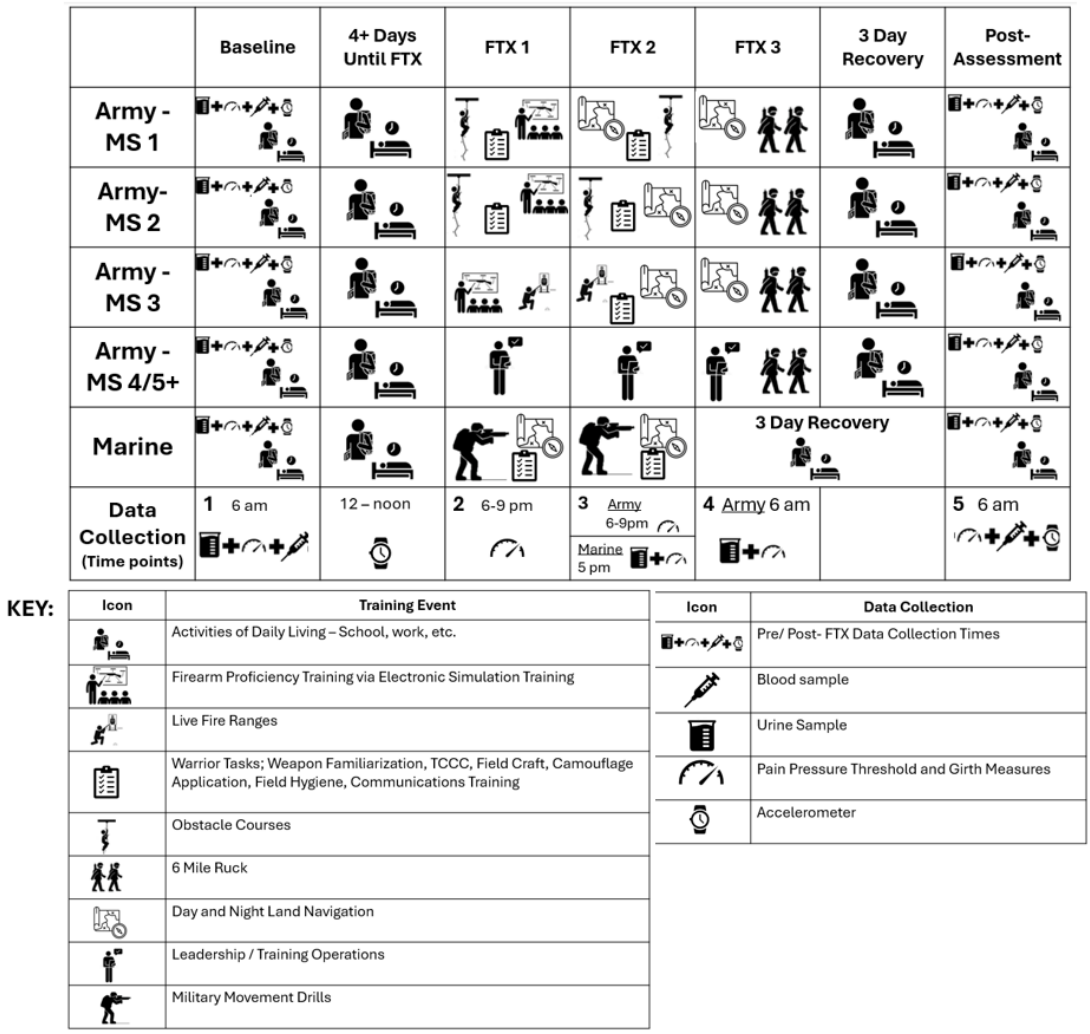


Figure 1. Field Exercise Training (FTX) and Data Collection Time Point Schedule

Marine Corps Training: A two-day, one-night continuous training event, featuring military squad movement drills and patrols. The training included fieldcraft skills such as bivouac infiltration and exfiltration, applying camouflage paint and gear, weapon system familiarization, and communication system operations. Participants also trained in trauma combat casualty care (TCCC), day and night land navigation, leadership exercises, and ruck marches between activities.

Army Training: A three-day, two-night training regimen with scheduled sleep periods (six hours per night), with training activities tailored to the Military Science (MS) year level.

MS1 and MS2: Training focused on firearm proficiency using Electronic Simulation Training (EST), obstacle courses, conditioning courses, team development exercises, and a six-mile ruck march.

MS3: Included advanced firearm proficiency through EST, live-fire training and qualifications at the 25-meter range, and modified record fire ranges.

MS1-MS3: Day and night land navigation, inter-platoon team-building events, and warrior tasks such as weapon familiarization, TCCC, fieldcraft (camouflage application, field hygiene, and communications training/setup).

MS4: Responsible for operational planning and support for MS1-MS3 events.

Members of the Army and Marine ROTC components were provided with a detailed verbal and written explanation of the requirements of the study via an educational brief and informed consent packet. Individuals that met the inclusion criteria of being a member of the University ROTC program, 17-45 years old, not having any current physical restrictions or being on a profile or chit and being free of any known medical conditions that would disqualify them from Department of Defense service were allowed to volunteer to participate in the observational cohort study in conjunction with their mandatory FTX. Fifty-seven individuals signed written informed consents and volunteered to participate in this study. Upon completion, two participants were excluded from the analysis due to lack of study completion or insufficient recorded data (consort diagram below, Figure 2). The 46 participants who successfully completed the study were included in the final data analysis. Additional demographics of the included participants are displayed in Table 1.

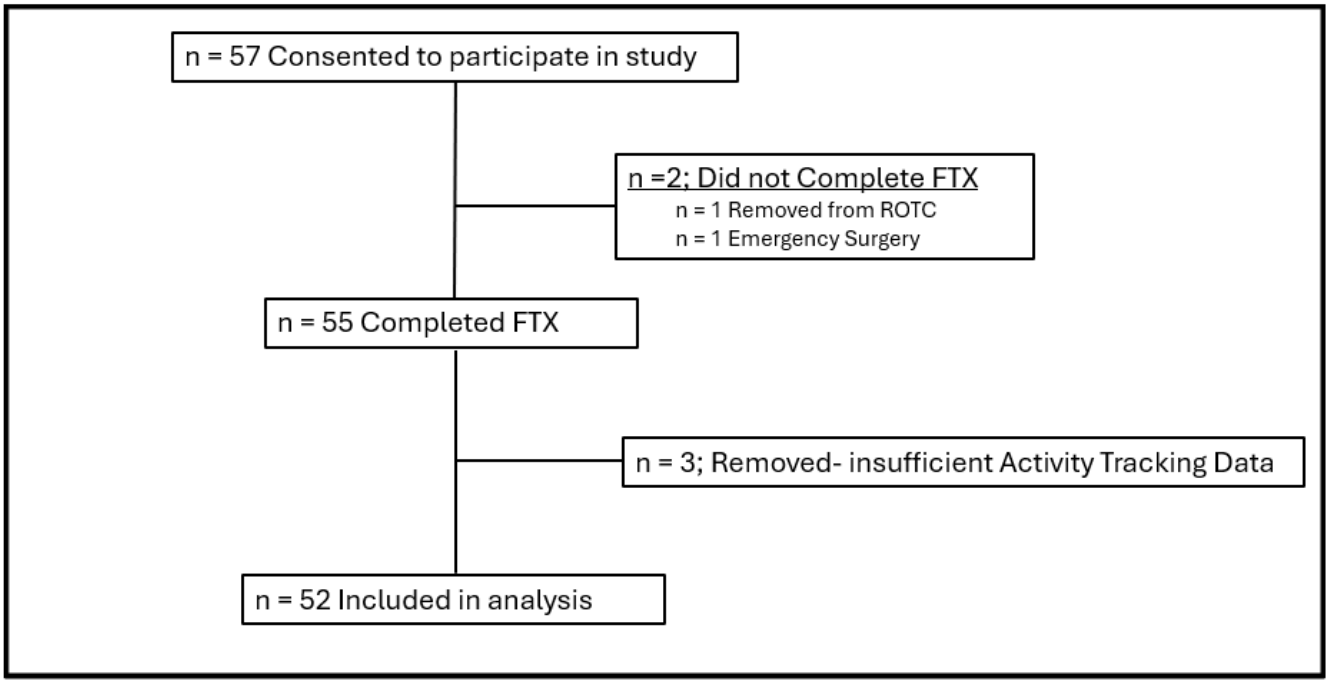


Figure 2. Participant flow and sample size

Table 1. Participant Demographics

Participants	52
MS1	11 (10 male / 1 female)
MS2	12 (12 male / 0 female)
MS3	12 (8 male / 4 female)
MS4	15 (10 male / 4 female)
MS5+	2)1 male / 1 female)
Male/ Female	41/11
Age (years)	21 ± 4 years
Height (cm)	174 ± 7 cm
Height (cm) Male	176 ± 7 cm
Height (cm) Female	166 ± 6 cm
Body mass (kg)	77 ± 10 kg
Body mass (kg) Male	81 ± 10 kg
Body mass (kg) Female	68 ± 9 kg
BMI	25.4 ± 2.6
Body Fat Percentage (group)	20.2 ± 7.5%
Body Fat Percentage Male	17.0 ± 4.5%
Body Fat Percentage Female	31.3 ± 3.4%

Note: cm= centimeters; kg = kilograms

Pressure algometry (Digital Force Pressure Guage (Force Ten FDX Compact, Wagner Instruments, Greenwich, CT, USA) was utilized to assess quantitative measures of muscle soreness over 14 locations comprising major muscle groups (Figure 3). The pressure algometer was outfitted with the provided rubber tip for all measurements to decrease discomfort of the test sites. Each measurement was completed with a research team member applying the pressure algometer perpendicular to the muscle belly, increasing the pressure until a verbal cue from the participant when the pressure becomes uncomfortable and slightly painful. Measures were taken during the pre-FTX screening assessment, throughout the FTX, and at the post-FTX assessment. Additionally, daily PPT data

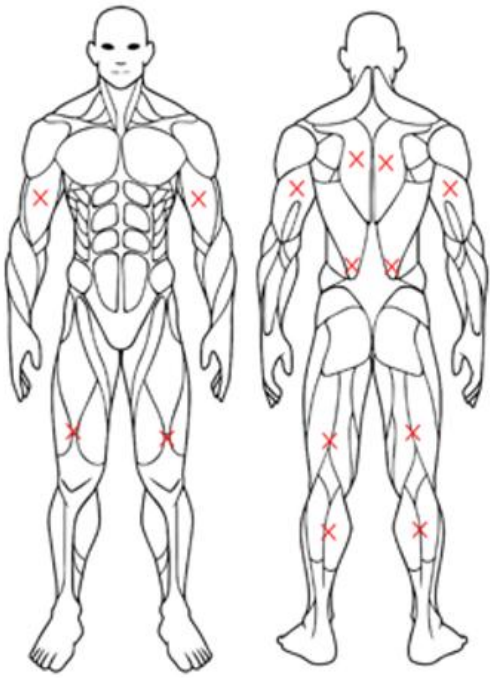


Figure 3. Pain Pressure Threshold (PPT) Sites

was averaged for each participant to provide a single, overall soreness measure. All measurements were recorded in newtons (N).

Lower limb length was measured during the baseline assessment utilizing a flexible Gulick measuring tape. To determine the location of girth measurements for consistency and rapidity length of the participants upper leg measuring from the Anterior Superior Iliac Spine (ASIS) of the ox coxae to the suprapatellar pole, and length of the low leg measuring from the infrapatellar pole to the talar dome/window were utilized. Limb section lengths were divided into thirds and rounded to the nearest tenth of a centimeter for girth measurement locations. Thigh measurements were taken at one-third the distance of the upper leg from the suprapatellar pole, and calf measurements were taken at one-third the distance of the lower leg from the infrapatellar pole (Figure 4). Lower limb girth measurements were evaluated at the participant's baseline assessment, each day of the FTX, and during the post-FTX assessment.

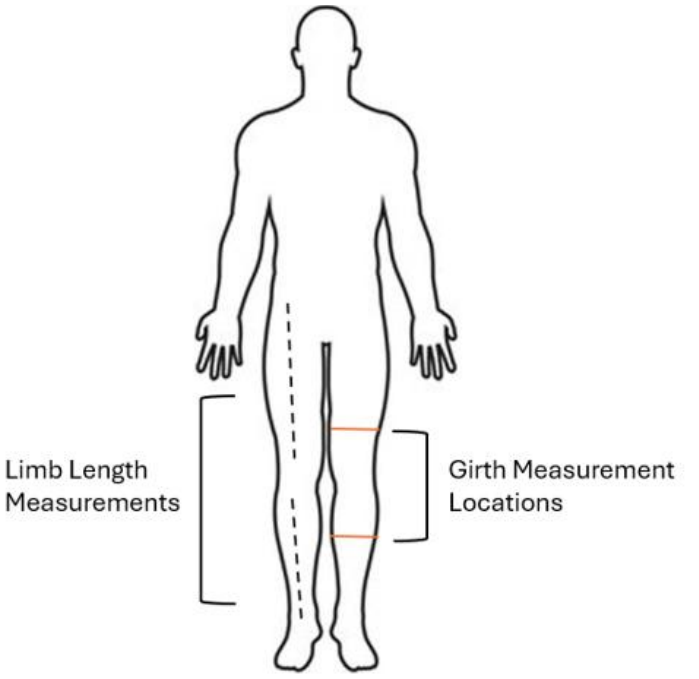


Figure 4. Limb Length and Girth Measurement Locations

Participants completed a brief, three-question disposition survey during the pre-FTX screening assessment, the end of each FTX day, and the post-FTX assessment. The goal was to ensure minimal training disruption and brevity. Each question was scaled from 1-5 with 1 being the lowest feeling, and 5 being the highest feeling. The three questions included in the survey:

1. Compared to “your normal,” how do you feel today?

2. Compared to “your normal,” how is your energy level today?

3. Compared to “your normal,” how rested do you feel today?

Physical activity was monitored using a triaxial accelerometer (ActiGraph xGT3X-BT, ActiGraph Technologies, Pensacola FL, USA). While doubly labeled water remains the gold standard for estimating energy expenditure, it is cost-prohibitive for larger studies (over 10-15 participants) and the extensive analysis time can compromise measurement accuracy.[248,249] Previous research supports the use of accelerometers as valid tools for assessing physical activity and energy expenditure, especially in military contexts.[204,215,229,251-253,272,273] The non-invasive nature of accelerometers enhances participant adherence without adding extra burden.[226,254-256] Accelerometers were configured with a sampling frequency of 60 Hz and programmed with participant-specific data including sex, age, height, body mass, and dominant hand. Participants wore the devices continuously except during showers. Raw acceleration data were processed with ActiLife Software v6.13.4 (ActiGraph, Pensacola FL, USA) using built-in algorithms for estimating energy expenditure (Freedson VM3 Combination, 2011), metabolic equivalents (METs, Swartz Adult Overground & Lifestyle 2000), and Montoye cutpoints for moderate-to-vigorous physical activity (MVPA). Data from participants wearing the device for less than 10 hours a day were excluded from the analysis.

Data Analysis

Statistical analyses were conducted using R (R Core Team, 2020) and R Studio (RStudio Team, 2023) version 4.2.2, utilizing the psych, lattice, dplyr, effsize, tidyr, ordinal, lme4 packages. The significance level was established a priori at $\alpha = 0.05$. Data was fitted into linear models to assess the change over time for mood, energy, and rest subjective measures, and also girth and

PPT objective measures. A mixed effect model was used to ascertain the interplay between the recorded measurements and groupings of age, sex, military educational years, and military branch. Assumptions for the models were appropriately checked for homoscedasticity and normality using a Shapiro Wilke test and visualizing with a Q-Q plot, then utilizing a Levene's test before conducting either Tukey or Bonferroni post-hoc testing.

Results

Linear mixed-effects models were used to examine changes in PPT measures across five time points for multiple bilateral body sites. Each model included time as a fixed effect and participant as a random effect to account for within-subject variability (Table 2).

The right PPT measures for biceps, triceps, rhomboids, latissimus dorsi, hamstrings, quadriceps and calf muscles all had significant increases over time from baselines measures through post-FTX measures. Each location sustained a significant increase throughout each time point compared to baseline measures. The left PPT measures for biceps, and triceps all had significant increases over time compared to baseline. A model assessing the overall average PPT changes revealed significant increases across all time points compared to the baseline measures. The increases of PPT measures over the course of the study at each time point extrapolates that muscle soreness in these locations decreased over time, or participants became less sensitive to the PPT testing.

Table 2. Pain Pressure Thresholds Over Time

Body Part	Baseline	Time 2 (FTX)	Time 3 (FTX)	Time 4 (FTX)	Time 5 (Post)
Right Bicep	38.52 (2.89, 13.34)	28.45 (3.61, 13.34)	22.48 (3.61, 7.86)	23.69 (3.83, 6.18)	12.64 (3.61, 3.50)
Right Triceps	39.23 (3.09, 12.69)	32.14 (3.84, 8.37)	26.55 (3.84, 6.92)	28.31 (4.07, 6.95)	13.38 (3.84, 3.49)
Right Rhomboid	53.38 (3.49, 15.31)	37.17 (4.21, 8.82)	29.80 (4.21, 7.07)	35.67 (4.47, 7.96)	20.51 (4.21, 4.87)
Right Lat	52.06 (2.82, 18.49)	30.75 (3.11, 9.87)	27.11 (3.11, 8.71)	34.24 (3.31, 10.34)	20.92 (3.11, 6.72)
Right Hamstring	60.04 (3.26, 18.43)	43.97 (3.65, 12.05)	42.35 (3.65, 11.60)	42.65 (3.88, 10.99)	26.26 (3.65, 7.19)
Right Quadriceps	64.97 (3.12, 20.79)	44.52 (3.83, 11.63)	44.61 (3.83, 11.66)	42.59 (4.06, 10.48)	30.61 (3.83, 7.99)
Right Calf	63.58 (3.21, 19.83)	44.26 (3.73, 11.88)	39.59 (3.73, 10.62)	37.94 (3.96, 9.58)	25.72 (3.73, 6.90)
Left Bicep	33.93 (3.10, 10.94)	32.27 (3.63, 8.90)	25.40 (3.63, 7.00)	28.47 (3.85, 7.39)	13.78 (3.63, 3.80)
Left Triceps	36.60 (3.28, 11.17)	37.36 (3.99, 9.36)	32.09 (3.99, 8.04)	33.76 (4.24, 7.97)	14.91 (3.99, 3.73)
Left Rhomboid	54.46 (3.07, 17.75)	33.90 (3.52, 9.63)	32.14 (3.52, 9.13)	34.68 (3.74, 9.26)	19.37 (3.52, 5.50)
Left Lat	50.97 (3.14, 16.24)	33.27 (3.65, 9.12)	28.00 (3.65, 7.63)	29.92 (3.88, 7.71)	18.59 (3.65, 5.10)
Left Hamstring	62.88 (3.41, 18.46)	44.34 (3.92, 11.32)	40.85 (3.92. 10.43)	43.98 (4.16, 10.56)	25.13 (3.92, 6.41)
Left Quadriceps	64.49 (3.08, 20.97)	43.18 (3.41, 12.67)	41.53 (3.41, 12.18)	40.15 (3.63, 11.07)	29.58 (3.41, 8.68)
Left Calf	63.23 (3.25, 19.46)	46.24 (3.58, 12.91)	43.11 (3.58, 12.04)	39.64 (3.81, 10.41)	24.81 (3.58, 6.93)
Average PPT	52.74 (2.69, 19.60)	37.99 (3.03, 12.55)	33.93 (3.03, 11.21)	35.34 (3.22, 10.98)	21.38 (3.03, 7.07)

Note: All results are structured as: Baseline = Intercept; Value (Standard Error, t-value). Lat = Latissimus Dorsi. FTX = Field Training Exercise

Linear mixed-effects models were used to assess the effect of time on four dependent variables: right thigh, left thigh girth, right calf girth, left calf girth. Each model included time as a fixed effect and a random intercept for each participant (Table 3).

Table 3. Low Limb Girth Measurements Over Time

Body Part	Baseline	Time 2 (FTX)	Time 3 (FTX)	Time 4 (FTX)	Time 5 (Post)
Right Thigh	53.36 (0.53, 100.71)	1.37 (0.31, 4.48)	0.91 (0.31, 2.95)	1.06 (0.33, 3.23)	-0.26 (0.31, -0.84)
Right Calf	37.23 (0.43, 86.02)	1.70 (0.44, 3.88)	0.05 (0.44, 0.120	1.45 (0.47, 3.11)	-0.71 (0.44, -1.61)
Left Thigh	51.93 (0.58, 89.39)	1.82 (0.49, 3.74)	1.51 (0.52, 4.34)	2.25 (0.52, 4.34)	1.08 (0.49, 2.21)
Left Calf	36.45 (0.35, 105.42)	2.14 (0.25, 8.54)	0.95 (0.25, 3.800	2.32 (0.27, 8.69)	-0.11 (0.25, -0.45)

Note: All results are structured as: Baseline = Intercept; Value (Standard Error, t-value). FTX = Field Training Exercise

The model for right thigh girth revealed a significant effect of time on right thigh girth scores with increases at time 2, time 3, and time 4. However, there was no significant difference in the post-FTX assessment. The random intercept variance was 12.83, and residual variance was 2.61, indicating moderate between-subject variability. For right calf girth, a significant effect of

time was observed at time 2 and time 4. No significant differences were observed at time 3 or post- FTX assessment (time 5). The random intercept variance was 5.02, with a residual variance of 5.28. The model for left thigh girth also demonstrated a significant effect of time. Compared to baseline, left thigh girth significantly increased at time 2, time 4, and time 5. The variance of the random intercept was 12.02, and the residual variance was 6.55, indicating higher variability in left thigh girth scores across participants. The analysis of left calf girth revealed significant increases in left calf girth at time throughout the FTX from time 2 to time 4. No significant difference was found at the post-FTX assessment (time 5). The random intercept variance was 4.85, and residual variance was 1.73.

Linear mixed models were employed to examine changes in girth of lower limb girth sections over the time points of the FTX. The analyses were conducted separately based on years in ROTC (MS year 1-5) (Table 4).

First year ROTC members had a significant right thigh girth increase over time at time 2, time 3, and time 4. The random effects showed a variance of 7.88 for MS1 participants, indicating variability in baseline right thigh girth measurements across participants. MS2 (second year ROTC members) experienced a significant increase in right thigh girth at time 2, but no significant changes at subsequent time points. The variance for baseline measures for MS2's was 10.95, indicating notable variability in initial right thigh girth measures. Third year ROTC members (MS3) had minimal right thigh girth changes over time that were not significant. The participant-level variance was the largest across groups (22.23), suggesting considerable individual differences in right thigh girth. Fourth year ROTC students (MS4) were also not significantly different across time points. All time points showed non-significant results, and the variance for participants' intercepts was 10.22. For participants in five or more ROTC years

(MS5), no significant changes in right thigh girth were observed at any time point. The model was based on a small sample size and the variance for both random effects was 5.52, indicating some between-participant variation.

MS1 participants had significant increases in the girth of the left thigh at time 2 , time 3, time 4, and post-FTX assessment (time 5). The variance in baseline left thigh girth measurements was 9.37. MS2 participants had significant increases during the FTX at times 2 through time 4. There was no significant change found at time 5. The variance for baseline measurements was 11.01. In the MS3 group, significant increases in left thigh girth were observed across all time measures compared to baseline. The variance for baseline left thigh girth was 18.48. For MS4 participants, changes in left thigh girth were minimal and not statistically significant at any time point. The participant=level variance was 10.19, and residual variance was 7.54. For the MS5 participants, the model indicated no significant changes over time. The small sample size and high variance in residuals (3.80) may have limited the power to detect changes.

The MS1 participants right calf girth did not significantly change over time and no time points reached statistical significance. MS2 participants had a significant increase in right calf girth observed at time 4, while a significant decrease was noted at time 5. Other time points were not significantly different from baseline. MS3 participants showed no statistical significance in right calf girth over time. For MS4 participants, right calf girth significantly increased at time 2, but no other time points showed significant changes. For MS5 participants, a significant increase in right calf girth was observed at time 2 and time 4.

The model revealed a significant effect of time on left calf girth for all participants. MS1, MS2, and MS3 participants had significant observed increases at time 2 and time 4. Changes at time 3 and time 5 were not statistically significant for these participants. For MS2 participants, a

significant effect of time on left calf girth was found at time 2 and time 4. MS4 and MS5 participants had significant increases in left calf girth at time 2, time 3, and time 4. No significant difference was detected at time 5 for either the MS4 or MS5 participants.

Table 4. Military Science (MS) Year Low Limb Girth Measurement Comparisons

Right Thigh					
MS year	Baseline	Time 2 (FTX)	Time 3 (FTX)	Time 4 (FTX)	Time 5 (Post)
1	50.73 (1.01, 47.56)	3.61 (0.92, 3.93)	3.35 (0.92, 3.93)	3.39 (0.98, 3.47)	1.19 (0.92, 1.30)
2	55.31 (1.00, 55.24)	1.35 (0.57, 2.38)	0.78 (0.57, 1.34)	0.67 (0.60, 1.12)	-0.70 (0.57, -1.24)
3	54.08 (1.36, 39.77)	0.33 (0.53, 0.63)	-0.06 (0.53, -0.12)	0.40 (0.59, 0.68)	-0.49 (0.53, -0.94)
4	53.16 (0.86, 61.82)	0.48 (0.45, 1.06)	0.04 (0.45, 0.10)	0.33 (0.47, 0.70)	-0.71 (0.45, -1.57)
5+	52.25 (2.35, 22.24)	3.15 (2.35, 1.34)	1.55 (2.35, 0.66)	1.10 (2.35, 0.47)	-0.25 (2.35, -0.110
Right Calf					
MS year	Baseline	Time 2 (FTX)	Time 3 (FTX)	Time 4 (FTX)	Time 5 (Post)
1	37.95 (0.88, 42.94)	0.76 (1.08, 0.71)	-0.45 (1.08, -0.41)	1.06 (1.14, 0.93)	-0.18 (1.08, -0.17)
2	38.73 (0.56, 69.09)	0.81 (0.47, 1.72)	-4.17 (0.47, -0.36)	1.58 (0.50, 3.20)	-1.20 (0.47, -2.56)
3	37.34 (1.08, 34.45)	0.36 (1.13, 0.32)	-0.42 (1.13, -0.37)	1.32 (1.27, 1.04)	-1.78 (1.13, -1.57)
4	35.72 (0.88, 40.75)	3.70 (0.82, 4.52)	0.63 (0.82, 0.77)	1.47 (0.85, 1.72)	-0.89 (0.82, -0.11)
5+	34.75 (1.05, 32.96)	5.30 (1.36, 3.90)	2.60 (1.36, 1.91)	3.65 (1.36, 2.69)	1.65 (1.36, 1.21)
Left Thigh					
MS year	Baseline	Time 2 (FTX)	Time 3 (FTX)	Time 4 (FTX)	Time 5 (Post)
1	49.36 (1.29, 38.19)	4.70 (1.28, 3.67)	3.95 (1.28, 3.08)	5.30 (1.36, 3.89)	2.97 (1.28, 2.32)
2	53.88 (0.99, 54.45)	2.44 (0.51, 4.74)	1.40 (0.51, 2.72)	1.84 (0.54, 3.39)	-0.08 (0.51, -0.15)
3	51.19 (1.38, 36.97)	2.42 (0.10, 2.43)	1.72 (0.10, 1.72)	2.55 (1.12, 2.27)	2.59 (0.10, 2.60)
4	52.58 (1.05, 49.95)	-1.09 (0.97, -1.13)	-0.01 (0.97, -0.01)	0.58 (1.01, 0.57)	-1.09 (0.97, -0.09)
5+	53.00 (2.09, 25.34)	1.50 (1.95, 0.77)	-0.45 (1.95, -0.23)	-0.10 (1.95, -0.05)	-2.30 (1.95, -1.18)
Left Calf					
MS year	Baseline	Time 2 (FTX)	Time 3 (FTX)	Time 4 (FTX)	Time 5 (Post)
1	36.50 (0.79, 46.24)	2.25 (0.76, 0.95)	1.44 (0.76, 1.88)	2.61 (0.81, 3.21)	0.48 (0.76, 0.63)
2	38.08 (0.56, 68.16)	1.32 (0.48, 2.75)	0.58 (0.48, 1.22)	2.10 (0.50, 4.17)	-0.88 (0.48, -1.85)
3	36.38 (0.71, 51.27)	1.54 (0.37, 4.11)	0.44 (0.37, 1.17)	2.24 (0.42, 5.29)	-0.23 (0.37, -0.62)
4	35.22 (0.68, 51.43)	2.88 (0.45, 6.35)	1.14 (0.45, 2.53)	2.18 (0.47, 4.61)	0.08 (0.45, 0.17)
5+	36.00 (1.00, 35.90)	4.95 (0.73, 6.78)	2.45 (0.73, 3.35)	3.95 (0.73, 5.41)	0.90 (0.73, 1.23)

Note: All results are structured as: Baseline = Intercept; Value (+ /- Compared to baseline) (Standard Error, t-value)

We assessed four or five total time points; base line, each of the two or three days of the FTX, and post-FTX. Disposition (mood) scores significantly decreased from baseline (time 1) to time 2 (M= 3.13 vs M = 1.84, $p = 0.009$). While Mood showed a trend toward recovery at later time points (Time 5: M = 2.88), no other pairwise comparisons reached statistical significance after Bonferroni adjustment (all $p > 0.05$).

Significant decreases in mood were observed for Army ROTC members at several time points compared to baseline measures (time 1). Specifically, mood was significantly lower at time 2 (estimate = -0.97, SE = 0.43, z= -2.27, p = 0.02) and showed a trend toward decreased mood at time 3 (estimate = -0.70, SE = 0.42, z= -1.87, p = 0.06). There was no significant difference between baseline (time 1) and post-FTX assessments (time 5) (estimate = -0.33, SE = 0.42, z= -0.78, p = 0.44), indicating a partial recovery in mood by post-FTX assessment.

Marine ROTC members' moods significantly decreased (time 2) (estimate = -2.74, SE = 0.98, z= -2.77, p= 0.006) and time 3 (estimate = -1.82, SE = 0.93, z= -1.96, p = 0.049) compared to baseline (time 1). However, no significant difference was found between baseline measures and post-FTX assessment (Estimate = 1.197×10^{-7}, SE=0.89, z= 0.000, p=1) suggesting that mood stabilized by post-FTX assessment.

Cumulative link mixed models were used to examine mood fluctuations over time by military education year. Random intercepts accounted for individual variability. For first year ROTC members mood declined over time with a decrease at time 4 (estimate = -1.68, SE = 0.98, z= -1.71, p= 0.09). However, none of the time points showed statistically significant change compared to baseline, indicating variability in mood but no consistent trend. ROTC members in their second year had significant mood decreases at time 2 (estimate = -1.96, SE = 0.79 z= -2.45, p = 0.014), suggesting an early drop in mood. However later time points (time 3-post-FTX assessment) did not show significant differences, indicating partial stabilization or recovery. ROTC members in their third year, mood significantly declines at time 2 (estimate = -2.18, SE= 0.83, z=-2.63, p=0.009) and time 3 (estimate = -1.76, SE = 0.81, z= -2.182, p=0.029). These findings indicate that mood deterioration occurred earlier but did not persist at later time points (time 4 and post-FTX assessment). For ROTC members in their fourth year no significant mood changes were observed at any time point (p>0.05), suggesting relative mood stability over time.

The model for individuals with five or more ROTC years did not converge, likely due to an insufficient number of observations or limited variability in mood responses.

Feelings of energy level scores significantly fluctuated over time ($p < 0.001$). Compared to baseline (time 1), Energy significantly decreased at time 2 (-1.76, $p < 0.001$), time 3 (-2.26, $p < 0.001$), and time 4 (-1.77, $p < 0.001$). However, by the post-assessment (time 5), energy levels were not significantly different from baseline (-0.14, p=0.69), suggesting complete recovery of this domain. Post hoc pairwise comparisons revealed that energy levels at time 3 were significantly lower than the post-FTX assessment (time 5) ($p = 0.0002$), confirming a temporary decline. No significant differences were observed between time 2, time 3, and time 4, suggesting a sustained period of low energy before recovery.

Army ROTC members showed significant declines in energy at time 2 (Estimate = -1.57, SE = 0.43, z= -37, p = 0.0002), time 3 (estimate = -1.62, SE = 0.44, z= -3.65, p= 0.0003), and time 4 (estimate = -1.64, SE= 0.44, z= -3.78, p = 0.0001), compared to baseline. However, no significant change was observed at the post-FTX assessment (estimate = -0.18, SE = 0.41, z= -0.44, p=0.66), indicating a lack of recovery in energy levels by the post-FTX assessment. Marine ROTC members had significantly reduced energy measures at time 2 (estimate = -4.07, SE = 1.33, z=-3.07, p = 0.002) and time 3 (estimate = -8.098, SE = 2.00, z = -4.05, p <0.001) compared to baseline levels (time 1). No significant changes in energy scores were observed at the post-FTX assessment (estimate = -5.964 x10-7, SE = 0.9177, z= 0.00, p = 1), suggesting no noted recovery by the final time point. A series of cumulative link mixed models were conducted to examine changes in self-reported energy levels over time, stratified by military education year. Each model included time as a fixed effect and participant as a random effect. Results are present through MS1 (first years) to MS4 (fourth years) as the model for members with 5 or more ROTC years did not converge due to insufficient variability in the random effects.

First year ROTC members there was a significant decrease in energy levels at time 2 (β = -2.07, p = 0.021), time 3 (β = -2.71, p = 0.006), and time 4 (β= -3.37, p = 0.001) relative to baseline. No significant difference was observed at time 5 (post-FTX assessment) (β= 0.03, p =1.00). The random intercept variance was 0.3425, indicating moderate between-participant variability in baseline energy levels. A significant decrease in energy was observed for second year TOC members at time 2 (β= -1.90, p= 0.01), while time 3 showed a trend toward significance (β= -1.37, p = 0.096). No significant differences were found at time 4 (β = 0.76, p = 0.32) or at the post-FTX assessment (time 5) (β = 0, p=1.00). The random intercept variance was 0.70, suggesting moderate individual variability. Third year ROTC members showed a significant decrease in energy levels at time 2 (β= -1.84, p=0.038), time 3(β= -3.34, p=0.001), and time 4(β= -3.00, p= 0.004). No significant change was detected at the post-FTX assessment (time 5) (B= 0, p= 1). The random intercept variance was 1.61, indicating relatively high between-subject variability. The model for ROTC members that had five or more years in ROTC failed to converge, likely due to insufficient variability in the random effects structure.

Self-reported restfulness (sleep) scores indicated a significant effect (p <0.001). Compared to baseline (time 1), sleep scores significantly decreased at time 2 (Estimate = -3.15, p <0.001), time 3 (estimate = -2.75, p < 0.001), and time 4 (estimate = -2.14, p <0.001). However, by the post-FTX assessment (time 5) sleep rating was not significantly different from baseline (estimate = -0.27, p= 0.47) suggesting a recovery in this domain. Post hoc analyses confirmed that sleep at time 2 was significantly lower than all the other time points (p<0.001), and significant improvements were observed from time 3 to the post-FTX assessment (p<0.001). These findings suggest that sleep ratings were most disrupted at time 2, with gradual recovery by the post-FTX assessment (time 5).

Army ROTC members had significant declines in restfulness measures at time 2 (estimate = -3.27, SE = 0.47, z= -6.95, p <0.001), time 3 (estimate = -2.28, SE = 0.45, z= -5.11, p<0.001), and time 4 (estimate = -2.16, SE = 0.44, z= -4.94, p< 0.001), compared to baseline. However, no significant difference was found at the post-FTX assessment (time 5) (estimate = -0.34, SE= 0.41, z= -0.83, p = 0.41), suggesting that restfulness showed signs of recovery by the post-FTX assessment. Marine ROTC members sustained noted significant reductions in restfulness at time 2 (estimate = -3.89, SE = 1.29, z=-3.02, p=0.003) and time 3 (estimate = -6.15, SE = 1.54, z= -3.998, p<0.001) compared to baseline. No significant change in restfulness was observed at the post-FTX assessment (estimation = 2.086×10^{-7}, SE= 0.86, z=0.00, p=1.0) indicating that restfulness may have returned to baseline by the final time point of the post-FTX assessment.

Both Army and Marine ROTC members exhibited significant declines in restfulness overtime, with Marines showing a larger drop at time 3 (Estimate = -6.15) compared to the Army (Estimate -2.28). However, while the Army demonstrated a partial recovery by the post-FTX assessment, restfulness among Marines appeared to fully return to baseline by the post-FTX assessment. The Marines also exhibited a greater variability in restfulness decline, as indicated by the larger standard errors.

A series of Cumulative Linked Mixed Models (CLMM) were conducted to examine the effect of time on self-reported restfulness measures across military education year groups. Time was treated as a categorical predictor and participant ID was included as a random effect to account for repeated measures. First year ROTC members revealed a significant effect of time on restfulness, with notable decrease in restfulness at multiple time points. Compared to baseline, restfulness was significantly lower at time 2 (estimate = -1.86, SE = 0.84, p = 0.027), time 3 (estimate = -2.43, SE = 0.88, p=0.006), and time 4 (estimate = -2.50, SE = 0.90, p = 0.006). No significant difference was observed at the post-FTX assessment (time 5) (p=0.88). The variance of

the random intercept for participants was 0.063 (SD= 0.25), suggesting minimal variability in individual differences. For the second year ROTC members (MS2), the analysis indicated a significant reduction in restfulness at time 2 (estimate = -3.52, SE = 0.86, p <0.001), Time 3(estimate = -1.91, SE = 0.81, p = 0.018), and time 4 (estimate = -1.63, SE = 0.80, p = 0.04). No significant change was observed at the post-FTX assessment (time 5) (p=0.44). The random intercept variance for participants was 1.935 (SD = 1.39), indicating greater variability in individual responses. Third year ROTC members (MS3) restfulness showed significant declines at time 2 (estimate = -3.65, SE= 0.96, p<0.001), time 3 (estimate = -3.72, SE 0.99, p<0.001), and time 4 (estimate = -2.98, SE = 0.93, p=0.011). No significant change was found at the post-FTX assessment (time 5) (p=1.00). The random intercept variance was 1.156 (SD = 1.08), reflecting moderate variability among participants. Similarly, fourth year ROTC members (MS4) demonstrated a significant decrease in restfulness at time 2 (estimate = -3.87, SE= 0.87, p<0.001), time 3 (estimate = -3.19, SE = 0.85, p<0.001), and time 4 (estimate = -2.07, SE = 0.86, p = 0.02). No significant change was detected at time 5(p=0.58). The random intercept variance for participants was the highest among the groups at 8.359 (SD = 2.89), indicating substantial individual differences. The model for ROTC members with five or more years failed to converge due to insufficient variation in the dataset. This suggests that there were too few participants or insufficient variability in reported restfulness to estimate the model parameters reliably.

A linear mixed- effects model was conducted to examine the effect of time on average activity levels while accounting for individual differences using a random intercept for participants. The model demonstrated a significant main effect of time, indicating that average activity levels fluctuated across the measured time points. The fixed effects estimates indicated that the mean activity level prior to the FTX was 1344.03 ± 82.42 kcal, during the FTX was 2460.39 ± 82.42 kcal, and after FTX was 1178.01 ± 82.94 kcal. Post hoc pairwise comparisons,

using Tuckey's adjustment, revealed significant differences between specific time points. Activity levels when comparing pre-FTX and during FTX were significantly higher during the FTX than before (estimate = -1116 kcal, SE = 77.2, t= -14.46, $p<0.001$). No significant difference was found between the pre-FTX and post-FTX activity levels (estimate = 166 kcal, SE = 77.7, t= 2.14, p= 0.09). Activity levels during the FTX were significantly higher than the three days measured post-FTX (estimate = 1282 kcal, SE = 77.7, t= 16.50, $p<0.001$). The random effects indicated considerable inter-individual variability in activity levels.

A linear mixed-effects model was used to analyze the effects of time and military branch (Army vs Marines) on activity level. Military branch did not significantly affect overall activity level ($\beta = 137.99$, SE = 229.39, t= 0.60), nor did the interaction between branch and time reach statistical significance. However, the negative interaction coefficient for Marine post-FTX activity levels ($\beta = -320.54$, SE = 222.97, t = -1.44) suggests that Marines exhibited a steeper decline in activity expenditure during the post-FTX time point compared to the Army. Estimated marginal means indicated that at pre-FTX, Army personnel had an estimated activity level of 1323 kcal (SE= 211). During the FTX both groups exhibited a marked increase in activity levels (Army = 2452 kcal, SE = 90; Marines = 2504 kcal, SE = 211). At the post-FTX time point activity levels declined in both groups, but the decrease was more pronounced for marines (Army 1202 kcal, SE = 90; Marines = 1019 kcal, SE = 220). Pairwise comparisons revealed significant differences in activity levels across time. Notably, activity levels during the FTX was significantly higher than pre-FTX for both the Army ($p<0.001$), and the Marines ($p < 0.001$). A significant decline in activity levels was observed between the FTX and post-FTX measures for both groups (Army: $p < 0.001$; Marines: $p<0.001$), with the Marines experiencing a larger reduction. No significant differences were found between pre-FTX and post-FTX activity levels within either group.

Between-group comparisons showed no significant difference between the Army and Marines at any single time point ($P > 0.05$).

A linear mixed-effects model was used to analyze the effects of time and Military Science year (MS) on average activity level through caloric energy expenditure, with participant included as a random effect. The model demonstrated significant individual variability. MS year did not have a significant main effect on activity level ($\beta = -57.99$, SE = 69.03, $t = -0.84$), suggesting that MS year alone does not strongly predict activity level. The interaction effects between MS year and time were also not significant, though there was a trend toward an increasing effect time at the post-FTX time point ($\beta = 110.74$, SE = 64.18, $t = 1.73$), indicating that higher MS year participants may be associated with relatively higher activity levels after the FTX.

Discussion

This study examined activity levels, PPT, low limb girth measurements, and disposition (mood, energy, and sleep) before, during and after an ROTC FTX. Previous research has demonstrated that military training significantly increases muscle soreness, induces muscular inflammation, and alters psychological well-being. Our findings contribute to this growing body of evidence by providing insight into the physiological and psychological responses to short-term military training in two ROTC components with important implications for continuing to optimize training protocols and recovery strategies.

Activity levels fluctuated significantly throughout the study, with a marked increase during the FTX, followed by a sharp decline in the post-FTX recovery phase. The return to pre-FTX activity levels suggests a transient but intense physiological demand associated with the training. This aligns with previous research highlighting elevated energy expenditure during military training, with male personnel demonstrating a 38% increase and female personnel a 17% rise over energy expenditure while in garrison.[222,250]

Energy expenditure increased by approximately 1,116 kcal during the FTX due to sustained physical tasks such as rucking, running, and land navigation exercises (Figure 4). Energy expenditure decreased by 1,282 kcal following the FTX, reinforcing the expected recovery trajectory after rigorous exertion. These are substantial increases in activity levels, however, they are well below reported activity levels of active-duty servicemembers.[215,222,247] No significant differences emerged between Army and Marine ROTC participants, however, Marines exhibited a steeper post-FTX decline in activity. This may be attributed to differences in recovery strategies. Marine ROTC members trained continuously overnight whereas Army ROTC participants adhered to a structured six-hour sleep routine. Sleep deprivation has been linked to hormonal imbalances, decreased resilience, and impaired cognitive function, likely contributing to these observed disparities.[258,274]

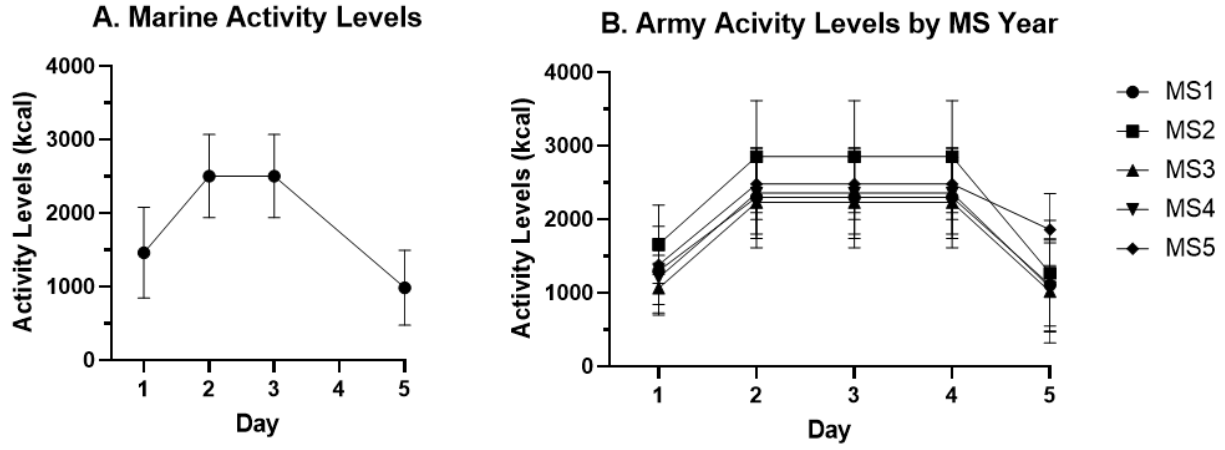


Figure 4. Energy Expenditure (Average Kilocalories Expended)
Note: Day 1= Baseline, Day 5 = post-assessment; kcal = kilocalorie

No significant differences in activity levels were found across Military Science (MS) year groups, suggesting that academic progression does not strongly influence physical activity patterns during an FTX despite conducting differing training events (Figure 1). However, a trend

indicated that higher MS-year cadets maintained slightly elevated post-FTX activity levels, potentially due to superior conditioning, experience with recovery strategies, or additional post-training responsibilities. Conversely, MS1 cadets (first-year ROTC members) exhibited lower post-FTX activity levels possibly reflecting self-selected recovery measures such as increased sleep and reduced physical exertion. Unlike active-duty military personnel, ROTC cadets must transition immediately back to their academic roles, potentially influencing their post-FTX recovery patterns.

The sharp increase in activity levels and energy expenditure during the FTX raises concerns about the potential for bone stress injuries, which are commonly linked to abrupt increases in mechanical loading.[275,276] Wolff's law states that bone adapts to the loads under which it is placed. Without proper progression and training adaptation, excessive stress can lead to overuse injuries. Therefore, gradual conditioning and structured physical training programs are essential in military training settings to mitigate this risk.[276] The ROTC population generally exhibits higher physical fitness and activity levels than the general student population due to structured group training sessions and increased daily movement such as walking to classes and campus activities.[197,277] The fall FTX occurred approximately three months into the semester, allowing ROTC members time to improve their fitness levels and overall physical preparedness, potentially reducing the risk of injury during training.

Mood, energy, and restfulness fluctuated significantly throughout the study, reflecting the psychological and physiological strain imposed by military training.[162,278] These measures declined sharply at the onset of the FTX, with varying degrees of recovery observed post-training. The deterioration of mood at time 2 may be attributed to anticipatory stress, such as dread or worry about the upcoming training event and the challenges of staying out in the field

environment.[265,279,280] Conversely, mood recovery post-FTX may indicate relief and excitement regarding the completion of training, as well as a sense of pride and accomplishment.[279]

Mood declined significantly from baseline to the first day of the FTX, with a partial but statistically insignificant recovery at later assessments. Army ROTC participants exhibited a more gradual decline and returned to baseline post-FTX, suggesting effective adaptation and recovery. In contrast, Marine participants experienced steeper declines with no significant recovery, indicating greater psychological strain or differing resilience mechanisms. Similar trends have been observed in prolonged military training, such as Basic Combat Training (BCT), where initial mood declines give way to improvements due to increased physical conditioning and adaptation.[279] While fitness levels are unlikely to change significantly in a three-day FTX, the observed mood fluctuations parallel those seen in longer training programs.

Energy levels followed a similar trajectory, with significant reductions from baseline through multiple time points and gradual return to baseline post-training. The immediate decline in energy levels may be linked to the early wake-up time on the first FTX day (time 2) and the full day spent in the field. Marine ROTC participants exhibited a steeper decline in energy compared to Army ROTC participants likely due to the lack of structured sleep time, which contributed to greater fatigue and prolonged recovery.[281-283] Previous research suggests that military personnel require multiple days to recover from occupational training, with temporary declines in performance, health, and cognition.[282,283]

Stratifying energy scores by ROTC year revealed that first-year cadets (MS1) experienced the most pronounced fatigue, with no full recovery by the final assessment. This may be associated with the first-time experience of participating in an FTX, as older cadets have already undergone similar training and may be better prepared both physically and mentally. Higher-year

cadets likely benefit from greater physical adaptation and preparation for the FTX, as well as improved psychological resilience, grit, and understanding of training expectations. Additionally, their higher post-FTX energy levels may reflect superior fitness and experience in managing recovery strategies.

Sleep disturbances mirrored these patterns, with significant declines in restfulness during the FTX and recovery afterward. Army ROTC participants exhibited partial recovery by the final assessment, whereas Marines showed prolonged disturbances underscoring the impact of continuous overnight training. The greater variability in sleep recovery among third- and fourth-year cadets suggests individual differences in adaptation to training stressors. The steeper decline in Marine participants may be explained by their lack of structured sleep time, further contributing to greater fatigue and delayed recovery.

Overall, these findings highlight the substantial physical and psychological demands of military training and underscore the importance of structured recovery strategies. Future training programs should consider progressive adaptation to mitigate excessive fatigue and mood deterioration, particularly for first-year cadets who may be more vulnerable to training stress.[281,284] Proper sleep management and structured rest periods could play a crucial role in optimizing recovery and enhancing overall resilience in ROTC participants.[283,285]

PPT measurements revealed a progressive increase across multiple body sites, indicating reduced pain sensitivity over time. This was an unexpected result. PPT measures were initially included to quantify increasing muscle soreness but instead showed increased pain resistance.[49,258,261,262,286,287] These changes may be attributed to neural or cognitive adaptations, including increased resilience to discomfort, familiarity with the pressure algometer, or adequate physical preparation before the FTX. Prior research has demonstrated nociceptor adaptation to

repeated physical discomfort, especially in high-stress environments, which may explain the observed trend.[288,289]

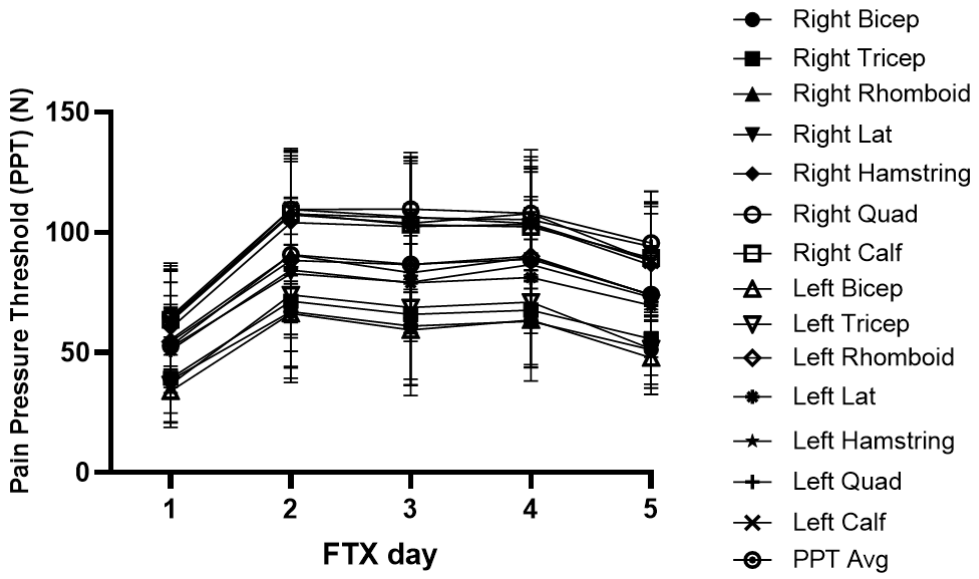


Figure 6. Pain Pressure Threshold (PPT) Trends
Note: N = Newtons, Lat = latissimus dorsi, Quad = Quadriceps, Avg = Average; Day 1 = Baseline, Day 5 = post-assessment

Right and left muscle groups exhibited significant PPT increases, though differences in baseline values and rates of change suggest that dominant limb usage, biomechanical loading, or compensatory movements may play a role. Further research is needed to determine the influence of limb dominance on PPT responses during military training. Sustained PPT elevations at later time points may indicate prolonged muscle fatigue or adaptive responses, while post-FTX PPT levels suggest ongoing recovery.

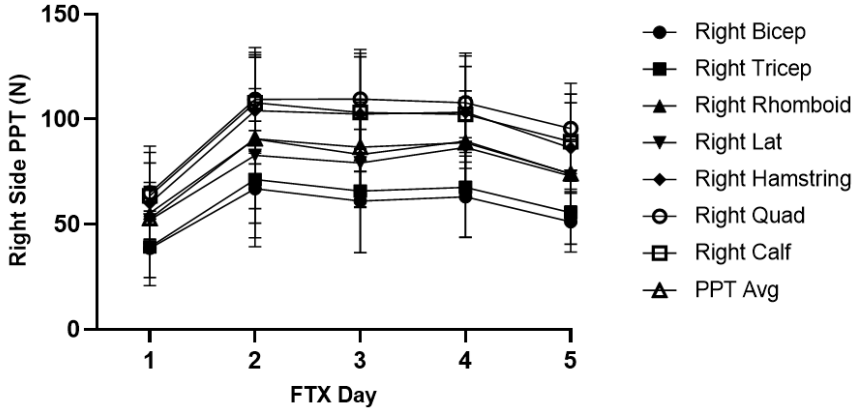


Figure 7. Right Sided Pain Pressure Threshold (PPT) Trends
Note: Lat = latissimus dorsi, Quad = quadriceps, Avg = Average; Day 1 = Baseline, Day 5 = post-assessment

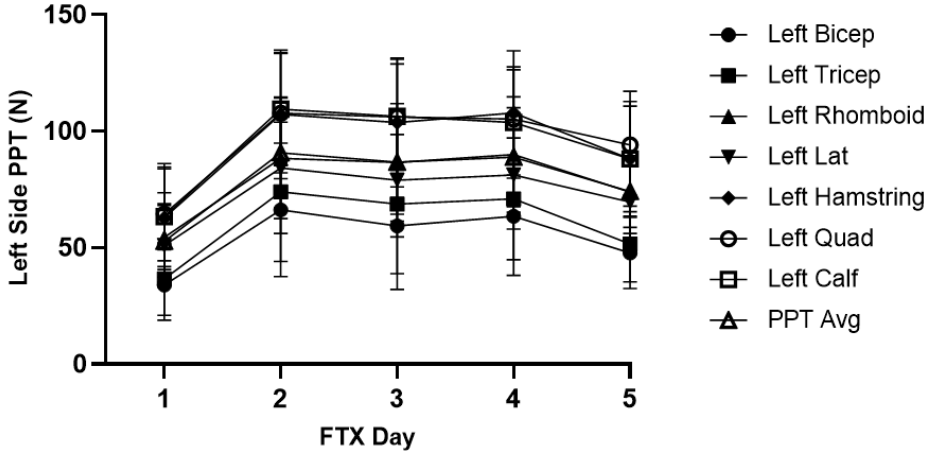


Figure 7. Left Sided Pain Pressure Threshold (PPT) Trends
Note: Lat = latissimus dorsi, Quad = quadriceps, Avg = Average; Day 1 = Baseline, Day 5 = post-assessment

Significant changes in lower limb girth occurred throughout the FTX, with notable differences between ROTC MS-year groups. The increases in girth align with the increased activity levels and energy expenditure observed during the FTX, as cadets spent significantly more time standing and moving compared to their typical daily routines, which often involve prolonged sitting in class or at home. Acute muscle swelling due to high-intensity physical activity likely contributed to these fluctuations.

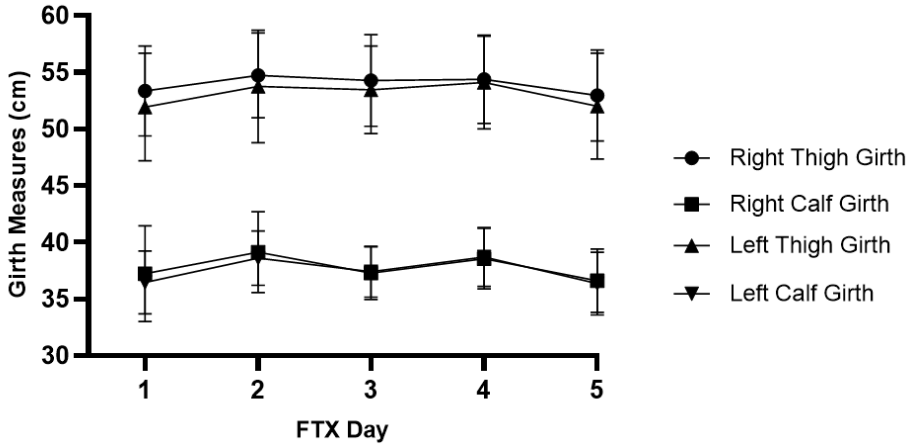


Figure 9. Low Leg Girth Measure Trends
Note: Day 1 = Baseline, Day 5= post-assessment

Army MS1 and MS2 cadets completed a highly physically demanding FTX, which included firearm training, obstacle courses, conditioning drills, and land navigation on the first day, contributing to pronounced girth increases. By day three, their training was slightly less intense, consisting of a 6-mile ruck and additional land navigation, potentially contributing to lasting swelling and fluid shifts. While girth remained elevated compared to baseline, the post-FTX measures taken three days after the completion of the FTX showed a return toward initial levels, suggesting early recovery.

MS3 cadets had a slightly less physically demanding FTX, primarily completing live firearms training on the first day and a shorter land navigation course with a 6-mile ruck on the second day. The near return to baseline levels by day three may reflect the lower physical demands of their training, as well as enhanced adaptation and recovery capacity due to prior experience.

MS4 and MS5 cadets exhibited minimal girth fluctuations, likely due to their administrative roles during the FTX. Although their activity levels still increased compared to pre-FTX measures, their tasks involved more standing and walking rather than full participation in

physically demanding training components like firearms drills and obstacle courses. Their primary exertion came from the 6-mile ruck, which may explain the slight but limited girth changes. The reduced girth fluctuations in older cadets (MS3-MS5) may indicate enhanced physiological adaptation, including improved inflammation and fluid shift clearance, which aligns with prolonged exposure to military training.[15,271] Additionally, their overall increase in activity during the FTX may have been less prominent compared to their daily physical activity levels, further contributing to the stability of their girth measures. These findings suggest that higher MS-year cadets are better adapted to military training demands, displaying improved resilience and recovery efficiency compared to their less experienced counterparts.

The findings of this study highlight the effectiveness of the three months of preparatory training leading up to the FTX, as evidenced by the overall physiological and psychological adaptations observed across all ROTC participants. The increased PPT values, stable energy expenditure recovery, and manageable fluctuations in mood and restfulness suggest that cadets were adequately conditioned for the demands of the training. These results further indicate that the more senior cadets (MS3-MS5) demonstrated superior physiological and psychological adaptation to this type of military training, likely due to their accumulated experience, understanding of training demands, and repeated exposure to similar FTX events. Their ability to maintain more stable physiological measures such as minimal girth changes and quicker recovery of energy and restfulness suggests improved resilience and preparedness compared to their junior counterparts.

A key implication of these findings is the necessity of targeted training and education for first-year cadets (MS1), who exhibited the most significant physiological and psychological shifts. The marked changes in PPT, energy levels, and girth fluctuations in MS1 cadets underscore their heightened response to the physical stressors of the FTX, reinforcing the need for progressive

physical conditioning programs. Additionally, the substantial mood and energy declines at the onset of training may indicate that MS1 cadets were less psychologically prepared for the rigors of the FTX. Structured orientation sessions, additional physical conditioning, and comprehensive briefings on training expectations could better equip these cadets to handle the physical and psychological demands of the FTX more effectively.

These principles extend beyond military training and have direct applications in athlete training programs. Just as MS1 cadets required time to fully adapt to the FTX structure and its demands, athletes also experience a period of adjustment before fully integrating into high-intensity training regimens. Ensuring that individuals not only meet physical requirements but also fully comprehend training expectations and methodologies is critical for optimizing performance and reducing injury risk. Seasoned athletes often demonstrate greater resilience and efficiency in handling training loads due to their familiarity with structured regimens like how more experienced cadets exhibited better adaptation due to experience and prior exposure. By applying these findings to both military and athletic training, instructors and coaches can develop training strategies that facilitate gradual adaptation, minimize excessive physiological stress, and enhance overall performance. Preparing individuals both physically and mentally for training expectations is crucial to optimizing adaptation, resilience, and overall success in physically demanding environments.

Limitations

While this study provides valuable insight into the physiological and psychological responses of ROTC cadets to short-term military training, several limitations should be acknowledged. First, the sample was limited to Army and Marine ROTC participants from a single university, which may restrict the generalizability of findings to other ROTC programs or

active-duty military personnel. Second, although PPT, mood, energy, and restfulness were measured at multiple time points, individual variability in adaptation and recovery could not be fully accounted for, particularly in relation to pre-existing fitness levels and training histories. Additionally, while activity levels and energy expenditure were tracked, external factors such as nutrition, hydration, and individual sleep quality prior to the FTX were not controlled, potentially influencing recovery outcomes. The use of subjective measures for mood, energy, and sleep may also introduce response bias, as cadets' perceptions could be influenced by external stressors unrelated to training. Lastly, while the study assessed short-term physiological and psychological responses, it did not capture long-term adaptations or potential delayed recovery effects beyond the immediate post-FTX period. Future research should incorporate longitudinal follow-ups, objective biomarker assessments, and broader ROTC and military populations to enhance the applicability and depth of these findings.

Summary

This study highlights the significant physical and psychological strain ROTC participants experience with activity levels fluctuating between intense physical demands during the FTX and a return to baseline after the completion of the FTX. Mood, energy, and restfulness declined early, particularly for Marines, reflecting branch-specific differences in recovery and resilience. PPT effectively measured physiological stress with lower PPT values correlating with poorer mood, lower energy, and reduced restfulness, particularly in the marines. Limb girth changes revealed that novice cadets experienced greater, sustained increases in thigh girth, while more experienced cadets showed more stable measurements, indicating better adaptation and recovery. These Findings suggest that training adaptations and recovery strategies should be tailored to an individual’s branch, experience level, and conditioning. It would be beneficial for future research to focus on the interventions to enhance recovery by monitoring physiological markers like PPT,

cortisol levels, and heart rate variability. Doing so would have the potential to optimize training protocols as well as recovery and improve the well-being and performance of ROTC cadets and military personnel.

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Changes in Blood and Urine Biomarkers in ROTC Cadets During a Fall Field Training Exercise

Introduction

Military service demands exceptional physical fitness and resilience. Pre-commissioning programs such as the Reserve Officers' Training Corps (ROTC) play an important role in preparing future officers for these challenges.[197,199,277] ROTC programs integrate structured physical training designed to enhance physical performance and build the mental and physiological resilience necessary to withstand the stressors of military operations and military life.[27,277] The ability of military personnel to maintain readiness and performance under demanding conditions is essential for mission success and overall effectiveness.

Tactical populations, including ROTC cadets, encounter unique physical and mental challenges that require a comprehensive understanding of their physiological adaptations.[290] The fitness and overall health of individuals in physically demanding professions are essential to optimal performance.[291,292] These populations often operate in complex and unpredictable scenarios requiring a wide range of physical capabilities, including sprinting, climbing, navigating difficult terrain often under load, and utilizing specialized equipment that require strength and endurance.[290]

Field Training Exercises (FTXs) are a fundamental component of ROTC training.[31,199,211] The multi-day training immerses cadets in simulated operational environments that test their physical endurance, mental resilience, and tactical skills. These exercises typically involve prolonged physical exertion such as load carriage or rucks, tactical movements, and obstacle navigation while exposing cadets to environmental stressors like weather conditions, limited sleep, and nutritional deficits.[1,293,294] The cumulative physiological demands of FTXs necessitate a

thorough understanding of cadet responses to optimize training protocols, enhance well-being, and ensure preparedness for future military service. Assessing physiological changes through blood and urine biomarkers during FTXs is an objective tool to gain a holistic understanding of the physiological demand placed on ROTC cadets.

Biomarker analyses have become an increasingly valuable tool to objectively evaluate the physiological impact of rigorous training exercises like FTXs. Specific biomarkers can be assessed through blood, saliva, urine, and sweat. These biomarkers provide insights into the body's internal state and can reflect both acute and chronic responses to physical exertion, psychological stress, and environmental exposure.[52,295] Tracking key biomarkers can offer objective information on energy metabolism, hydration status, endocrine responses, inflammation, muscle damage, and overall physiological strain. These insights can inform evidence-based adjustments to training regimens, nutritional strategies, and recovery protocols to optimize performance and reduce the risk of adverse health outcomes.

Blood and urine provide complementary information comprising a more complete analysis and reliability of testing among the available biofluids for field-based biomarker assessment. Blood analysis reveals systemic physiological changes, including fluctuations in metabolic substrates, hormones, and enzymes, offering a comprehensive picture of the body's response to stress and exercise.[52,65,131,295,296] Alterations in circulating metabolites can indicate shifts in fuel utilization and tissue damage.[65,135,295] Urine testing is non-invasive and can be collected repeatedly in field settings, making it an ideal medium for assessing hydration status, electrolyte balance, and the excretion of metabolic byproducts.[61,297] Together, blood and urine biomarker analyses provide a holistic perspective on the physiological adaptations occurring in ROTC cadets during intense FTXs.

Previous research has examined the physiological effects of military training on active-duty personnel and ROTC cadets.[1,12,136,298] Studies indicate that basic military training can lead to significant changes in body composition and metabolic profiles.[47,145,146,189,279] Investigations into Army ROTC cadets' fitness levels suggest they generally exhibit average to above-average physical conditioning relative to their civilian peers.[197,225,277,299] However, there remains a gap in research specifically assessing the acute physiological responses of ROTC cadets to real-world training scenarios like FTXs. Understanding the interplay between physical exertion, environmental factors, and physiological adaptation is necessary to design rigorous and safe training for tactical populations. By analyzing blood and urine biomarkers we can more readily observe and quantify physiological strain. These observations can enable the development of targeted training interventions that enhance performance while also minimizing health risks.

Therefore, the purpose of this study was to assess the physiological changes experienced by ROTC cadets during a FTX through the analysis of a targeted panel of blood and urine biomarkers.

Methods

This study was part of a larger ROTC program observational cohort study. The Institutional Review Board of the host University and the Army HPRO approved this study (protocol # 24-843 FB). Participants completed a baseline assessment a minimum of four days prior to the FTX. The baseline assessment consisted of completing a health history and demographics form, urinalysis and blood draw (for hydration assessment and biomarker collection of blood and urine), and body composition assessment via bioelectrical impedance (TANITA BC-568 InnerScan Segmental Body Composition Monitor (TANITA Corporation, Preston, WA, USA).

ROTC members participated in a mandatory two-or-three-day FTX. FTX length was dictated by branch and training schedules. ROTC members in the Marine branch conducted a continuous two-day FTX, while ROTC members in the Army branch conducted a three-day FTX with training activities based on their Military Science (MS) year (Figure 1).

Marine ROTC Training Protocol:

- Duration: Two days, one night of continuous training.
- Activities: Squad movement drills and patrol, bivouac infiltration and exfiltration, camouflage application, weapon system familiarization, communication system use, trauma combat casualty care (TCCC), day and night land navigation, logistics operations, leadership exercises, and ruck marches between activities.

Army ROTC Training Protocol:

- Duration: Three days, two nights with designated six-hour sleep periods per night.
- Activities varied by Military Science (MS) year:
    - **MS1 and MS2:** Firearm proficiency training via Electronic Simulation Training (EST), obstacle course, conditioning course, team development course, and a six-mile ruck march.
    - **MS3:** Firearm proficiency training via EST, live firearm training, and qualification at 25-meter and modified record fire ranges.
    - **MS1-MS3:** Day and night land navigation, team-building events, warrior tasks (weapon familiarization, TCCC, field craft, camouflage application, field hygiene, and communications training).

- **MS4:** Operational planning and support for MS1-MS3 events.

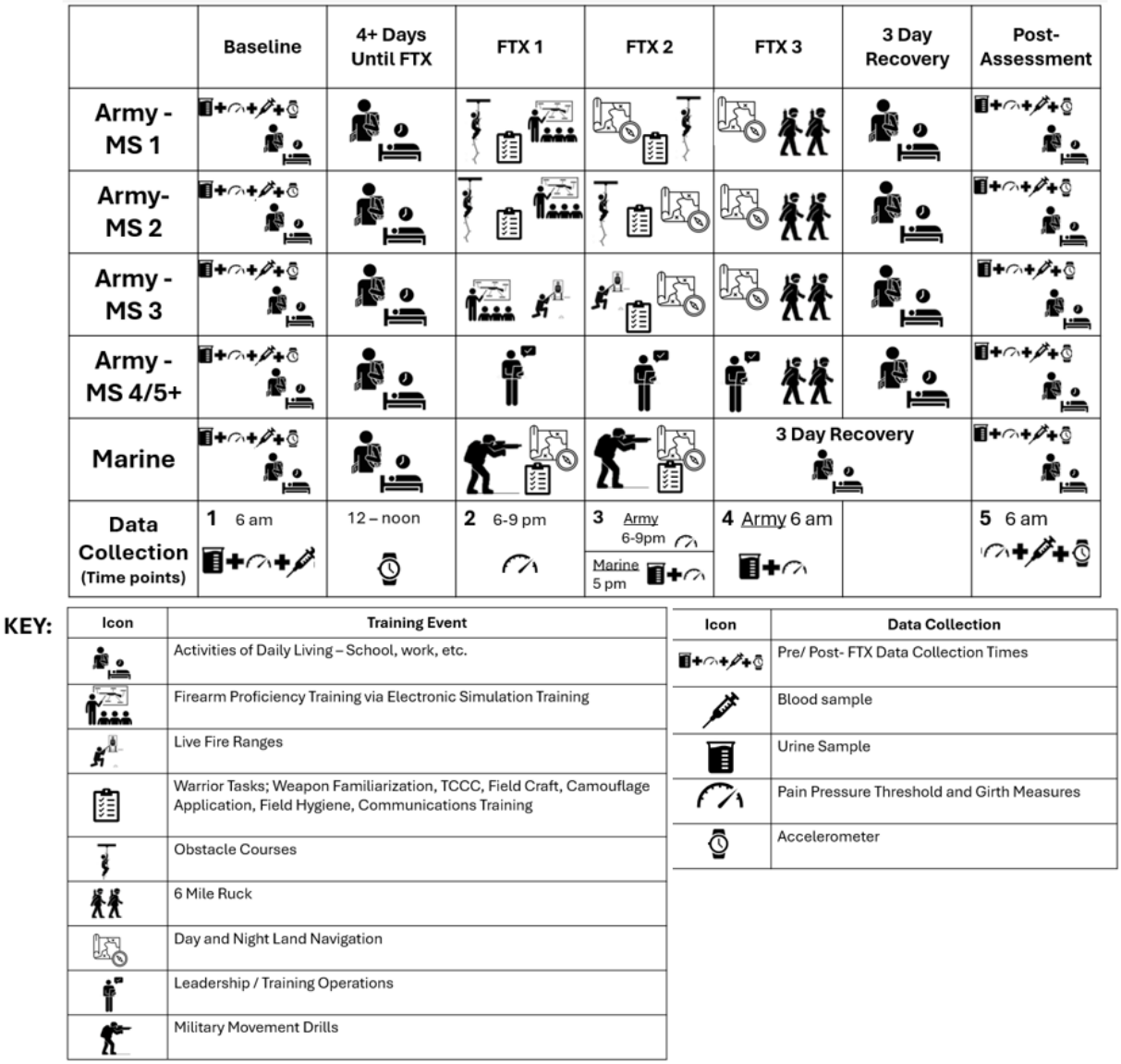

Figure 1. Field Exercise Training (FTX) and Data Collection Time Point Schedule

Participants

Volunteers for this study were provided with a detailed verbal and written explanation of the study requirements and inclusion/exclusion criteria. Volunteers were allowed to participate in the study if they were a University ROTC member, between the ages of 17-45 years, with no current physical restrictions, or any known medical conditions that would disqualify them from Department of Defense service. Fifty-seven volunteers provided written consent and started the study. Upon completion, two participants were excluded from the analysis of the study due to a variety of complications or lack of information (Figure 2). The 50 participants (40 males = 20.58 ± 3.12 years, 175.84 ± 6.67 cm, 80.16 ± 9.57 kg, and 10 females = 23.1 ± 6.57 years, 165.72 ± 6.43 cm, 67.07 ± 8.53 kg) who completed the study were included in the final data analysis. Participant demographics of the included participants are displayed in Table 1.

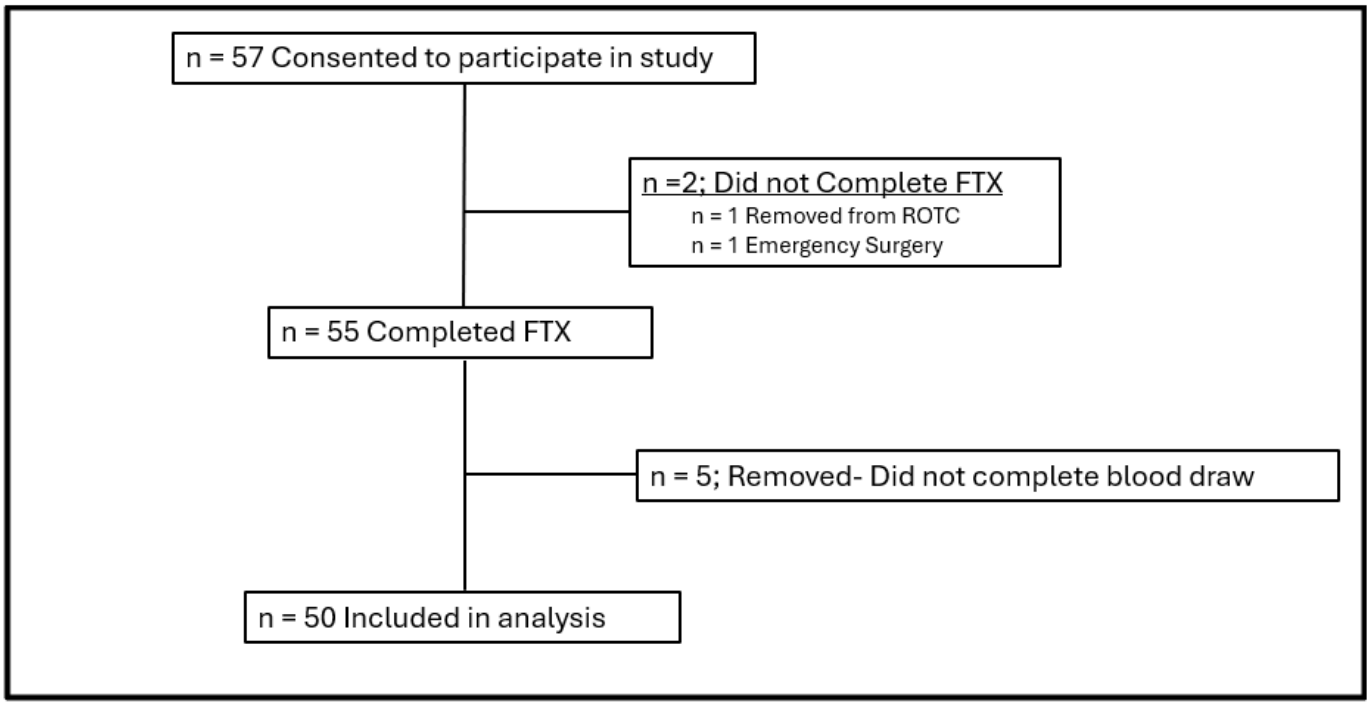

Figure 2. Participant flow and sample size

Table 1. Participant Demographics

Participants	50
MS1	11 (10 male; 1 female)
MS2	11 (11 male; 0 female)
MS3	11 (7 male; 4 female)
MS4	15 (11 male; 4 female)
MS5+	2 (1 male; 1 female)
Male/ Female	40/10
Race/ Ethnicity	
Caucasian/ White	42
African American / Black	1
Hispanic	4
Native American	0
Asian	1
Mixed Race	2
Age (yrs)	21 ± 4 years
Males	20 ± 3.1 years
Females	23 ± 7 years
Height (cm)	174 ± 8 cm
Males	176 ± 7 cm
Females	166 ± 6 cm
Body mass (kg)	77.5 ± 10.7 kg
Males	80.2 ± 9.6 kg
Female	67.1 ± 8.53 kg
BMI	25.6 ± 2.8
Body Fat Percentage (group)	19.9 ± 7.1 %
Male	17.2 ± 4.6 %
Female	30.7 ± 4.2 %

Note: MS = Military Science Year

Participants provided urine samples at the pre-FTX baseline and immediately following the completion of the FTX in the field. These samples were collected in sterile 120 mL specimen cups and promptly tested for Urine Specific Gravity using a handheld refractometer (Vollrath Company, Sheboygan, WI, USA) and electronic urine dipstick measurements (BC401 Urine Analyzer, CONTEC Medical Systems, Qinhuangdao, Hebei Province, China). After initial testing, the samples were kept on ice for further processing. Within two hours, the samples were transported to the laboratory where they were aliquoted into an 8 mL tube for testing at East Alabama Medical Center to check for urinary myoglobin (Mb). Additionally, two 1.8 mL

cryovials were prepared and stored at -80°C for future analysis. Comprehensive urinalysis included assessments for urine specific gravity, urobilinogen, blood, bilirubin, ketones, glucose, protein, pH, nitrites, leukocytes, and vitamin C presence.

Venous blood samples were obtained from the antecubital vein using sterile venipuncture techniques. Approximately 20 mL of blood was collected from each participant into serum (red top) tubes for analysis. A 4 mL portion from one of the 10 mL red top tubes was transferred to a separate tube for further testing, including a complete metabolic panel (CMP), creatine kinase (CK), and myoglobin (Mb) analysis at the East Alabama Medical Center Laboratory. The remaining blood samples were kept on ice until processed by centrifugation at 1500 g for 10 minutes at 4°C. Plasma and serum were aliquoted into two 1.8 mL cryovials and stored at -80°C as replicate samples. Biochemical markers in the blood, including sodium, glucose, potassium, chloride, CO2, calcium, blood urea nitrogen (BUN), creatinine, albumin (ALB), aspartate aminotransferase (AST), BUN/creatinine ratio, globulin, bilirubin, CK, Mb, albumin/globulin ratio (AG Ratio), and osmolality, were analyzed at the Medical Center Laboratory. All assays were conducted in duplicate to ensure the accuracy of results. Standard biosafety protocols were followed during all sample collections, and participants were instructed to fast for eight hours prior to blood collection to reduce variability.

Data Analyses

Statistical analyses were conducted using R (R Core Team, 2020) and R Studio (RStudio Team, 2023) version 4.2.2, utilizing the psych, lattice, dplyr, tidyr, effsize, and lme4 packages. The significance level was established *a priori* at $\alpha = 0.05$. Normality of dependent variables was assessed using Shapiro-Wilk tests and visualized with Q-Q plots. Data that fulfilled the normality assumptions were then assessed by using pairwise t-tests to compare pre-FTX and post-FTX

biomarker measures, while non-parametric testing (Wilcoxon ranked signed tests; V scores) were used for non-normally distributed data. Multiple linear regression models were completed to investigate the interactions of biomarker changes and military education year, age, and biological sex.

Results

A series of paired t-tests and Wilcoxon signed-rank tests were conducted to evaluate changes in urinary and blood biomarkers from baseline pre-FTX to post-FTX.

A significant decrease in urine specific gravity (USG) was observed from the baseline assessment to the post-FTX assessment, $t(49) = -3.90$, $p < 0.001$, with a mean difference of -0.00516 (95% CI: -0.00782, -0.00250) indicating greater hydration after the completion of the FTX. Additionally, urinary ketone levels exhibited a significant increase at the post-FTX assessment, $V = 0$, $p = 0.005$ suggesting an alteration in ketone presence. No significant differences were observed in other urinary parameters, including blood ($V = 6$, $p = 0.850$), protein ($V = 1$, $p = 1.000$), pH ($V = 88$, $p = 0.263$), nitrites ($V = 0$, $p = 1.000$), leukocytes ($V = 0$, $p = 1.000$), or vitamin C ($V = 10.5$, $p = 1.000$). Paired t-tests for urobilinogen, bilirubin, and glucose levels could not be performed due to no changes in the values in pre- to post-FTX measures, yielding NaN test statistics and p-values.

Significant reductions in sodium ($t(49) = -7.82$, $p < 0.001$, mean difference = -1.96) and chloride ($t(49) = -4.87$, $p < 0.001$, mean difference = -1.46) were observed post-FTX, whereas potassium levels remained unchanged ($t(49) = -0.79$, $p = 0.432$).

Blood glucose levels showed a significant decrease ($V = 1018.5$, $p < 0.001$), as did calcium ($t(49) = -7.12$, $p < 0.001$, mean difference = -0.256). However, no significant differences

were observed in blood urea nitrogen (BUN) ($t(49) = -0.45$, $p = 0.653$) or creatinine ($V = 608$, $p = 0.841$).

Total blood protein levels decreased significantly post-FTX ($t(49) = -2.59$, $p = 0.013$, mean difference = -0.114), while albumin remained stable ($V = 354$, $p = 0.618$). Liver enzyme activity exhibited significant reductions in alkaline phosphatase ($V = 859.5$, $p = 0.002$), ALT ($V = 271.5$, $p = 0.005$), AST ($V = 304$, $p = 0.016$), and bilirubin ($V = 609.5$, $p = 0.048$).

A notable reduction in osmolality was detected ($t(49) = -7.31$, $p < 0.001$) with a mean difference of -3.68 (95% CI: -4.69, -2.67) indicating a shift in fluid balance regulation.

Significant changes were observed in urinary USG, ketones, blood sodium, chloride, glucose, calcium, total protein, liver enzymes, bilirubin, and osmolality, suggesting metabolic and hydration-related adaptations post-intervention. Other biomarkers remained stable throughout the intervention period. No significant interactions were found between basic demographics of Age, Sex, MS year, Race, and Body fat percentage with the changes in biomarker levels.

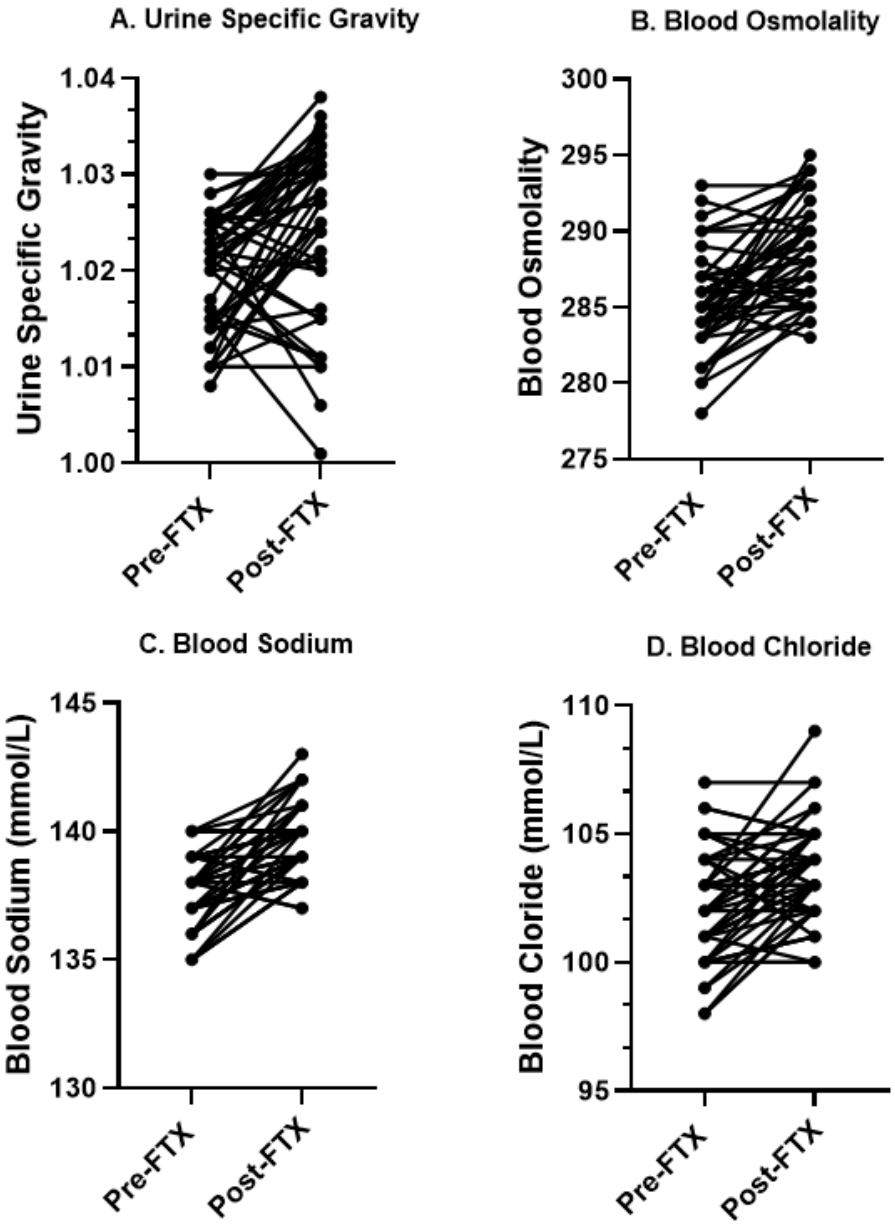

Figure 3. Shifts in Hydration Biomarkers

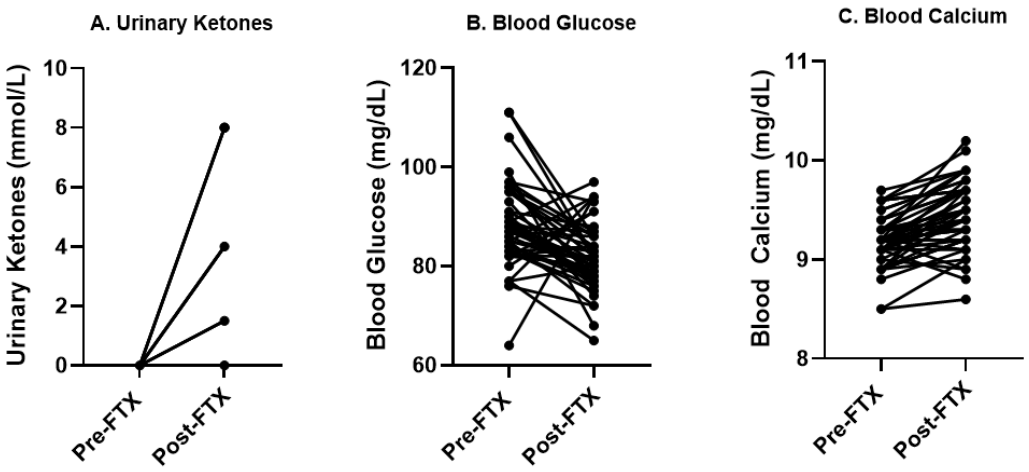

Figure 4. Shifts in Energy Deficiency and Metabolic Adaptation

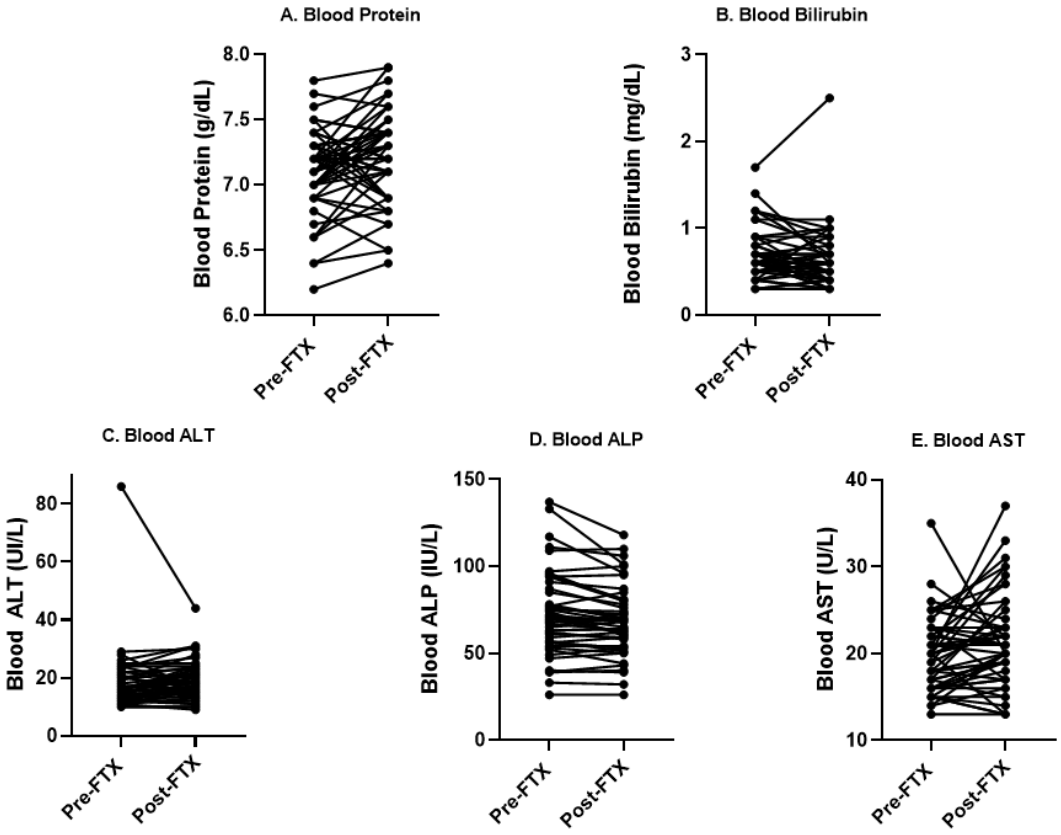

Figure 5. Shifts in Protein Catabolism and Muscle/Liver Stress

Discussion

This study examined changes in urinary and blood biomarkers from pre-FTX to post-FTX, revealing significant alterations in hydration status, electrolyte balance, and metabolic markers. These findings provide valuable insight into the physiological adaptations associated with ROTC FTX. Additionally, they contribute to the body of literature on fluid regulation, metabolic responses, and biomarker variability in response to external stressors, particularly within military and ROTC populations.

We observed a significant reduction in urine specific gravity (USG) post-FTX which suggests improved hydration status. This change could be attributed to increased fluid intake, shifts in renal water conservation, or metabolic adaptations aimed at maintaining homeostasis (Figure 2).[300-302] Proper hydration is critical for thermoregulation, cognitive function, and physical performance especially in demanding environments such as physical training and military FTXs.[302-304] ROTC cadets experience increased fluid losses due to sweating and metabolic demands given the prolonged and strenuous nature of an FTX.[305,306] Thus, maintaining hydration is vital for sustaining endurance, mental acuity, and overall physiological function during these events.[302]

Cadets typically require more hydration during an FTX than on a regular day because of heightened physical activity and environmental exposure.[303,307] The type and intensity of training also influence hydration behaviors. In this study, cadets could drink water ad libitum but were encouraged to ensure adequate intake throughout training. There were no water restrictions in either the Army or Marine FTX and there were no mandatory drinking times or amounts. Cadets engaging in less physically demanding tasks, such as firearms education and qualification, tended to hydrate more frequently but in smaller amounts. Conversely, those involved in high-intensity activities like obstacle courses, conditioning drills, and land navigation consumed larger volumes

of water less often. This pattern of hydration, marked by infrequent but high-volume intake, may be less effective in sustaining fluid balance and thermoregulation.[303,308] Supplementing with carbohydrate-electrolyte drinks could be particularly beneficial in these scenarios, as they help maintain electrolyte balance, improve fluid retention, and provide readily available energy for sustained performance over multiple days of low-intensity exertion in outdoor conditions.[309]

The observed increase in urinary ketone levels (Figure 3) suggests metabolic changes likely related to shifts in energy substrate utilization during the FTX.[310,311] Elevated urinary ketones typically indicate increased fat metabolism which can result from caloric restriction, higher energy expenditure, or changes in macronutrient availability.[312,313] This shift likely reflects the lower-intensity, prolonged physical activity that cadets engaged in, where the body adapts by increasing fat oxidation as a primary energy source.[241,312,313] Additionally, urinary ketones may signal a caloric deficit relative to activity demands, suggesting that cadets may not have consumed enough carbohydrates to meet their energy needs.[210,241,243,314] Insufficient carbohydrate intake during training can contribute to ketonuria as the body resorts to fat stores in the absence of sufficient glucose.[210,312] This metabolic shift could affect endurance and cognitive function, particularly when rapid energy availability is needed for optimal performance. Ensuring that cadets have access to balanced nutrition, including carbohydrate-rich foods, during extended training may help prevent over-reliance on fat metabolism and support overall performance and recovery. However, the absence of significant changes in other urinary parameters such as protein, blood, and pH suggests that renal function remained stable throughout the intervention period, indicating that the training did not cause renal distress.[1,2,21,39]

We observed significant reductions in blood sodium and chloride levels post-FTX (Figure 2) which may reflect fluid shifts, increased sweat loss, or dietary changes affecting electrolyte retention. Sodium plays a critical role in extracellular fluid balance and neuromuscular function,

and even modest reductions could impact performance.[52,295,315] Although these reductions did not reach hyponatremic levels, they indicate a shift toward hyponatremia, a condition of concern during prolonged military training due to its potential to impair cognitive and physical performance. Proper electrolyte replenishment, including the use of sodium-containing hydration solutions, could help mitigate this risk.[306,316,317]

The stable potassium levels suggest that the body selectively regulates potassium homeostasis despite fluctuations in sodium and chloride, in line with established principles of electrolyte balance.[318,319] This regulation is vital for maintaining cardiac and neuromuscular function, particularly during high-intensity training.

A decline in blood glucose (Figure 3) aligns with previous research showing metabolic adaptations following military training. Reduced glucose concentrations may be due to increased insulin sensitivity, enhanced glucose uptake by peripheral tissues, or a shift toward using alternative fuel sources like fatty acids or ketones.[284,320,321] This reduction might also reflect inadequate carbohydrate intake relative to energy expenditure, potentially placing the body in a state similar to starvation. This metabolic response ensures energy availability but could impair performance if prolonged. The drop in calcium levels (Figure 3) could be related to dietary changes, shifts in bone metabolism, or alterations in renal calcium handling. Calcium plays an essential role in muscle contraction, nerve signaling, and bone density, and further studies are needed to investigate the mechanisms behind this reduction.[176,322-324]

Rhabdomyolysis is a key concern in military training. Risk is characterized by muscle breakdown and the release of intracellular components into the bloodstream. The stability of blood urea nitrogen (BUN) and creatine biomarkers in our study suggests that muscle breakdown and renal stress were not significant issues as they are commonly monitored as signs of

rhabdomyolysis.[6,325] These results indicate that the body responded appropriately to the physical demands of training without experiencing pathological muscle damage. There is no evidence of impaired liver or kidney function despite the metabolic and electrolyte shifts. The body appears to have effectively cleared altered biomarkers suggesting a return to homeostasis post-training. These results underscore the importance of maintaining proper hydration and nutrition to support metabolic balance and facilitate recovery without overstressing internal organs.

We observed significant reductions in total protein and liver enzymes, including alkaline phosphatase, ALT, AST, and bilirubin (Figure 4) suggesting hepatic adaptations rather than distress during the FTX. These changes likely reflect the impact of altered dietary intake, energy expenditure, or hydration on liver enzyme activity. These findings align with previous reports linking metabolic stressors to temporary fluctuations in hepatic biomarkers.[326-328] The decrease in bilirubin levels may indicate improved hepatic clearance or reduced hemolysis, while stable albumin levels suggest that systemic protein homeostasis was largely maintained.[326,329] The observed increases of the liver enzymes ALT and AST were within normal ranges. Clinically significant changes in ALT and AST are commonly monitored for signs of rhabdomyolysis. These changes indicate a physiological response to prolonged physical activity rather than pathological muscle breakdown.[2,25,39] These enzyme levels should clear from the body without causing harm to internal organs, further supporting the idea that hepatic function remained stable during the training period. These findings highlight the body's ability to adapt to increased physical demands while maintaining liver function and protein metabolism within a balanced range. The observed biomarker responses suggest that cadets were adequately prepared for the FTX, demonstrating appropriate physiological adaptations that supported endurance and resilience.

The significant reduction in osmolality (Figure 2) reinforces the decrease in USG, suggesting improved hydration status post-FTX.[59,307,330] Osmolality is a key indicator of fluid

balance, and its decrease likely reflects the body's adaptation to maintain homeostasis under changing environmental and physiological conditions. These findings suggest that the FTX influenced fluid regulation, potentially through increased water intake, dietary changes, or altered sweat loss.[300,331,332] Maintaining proper osmolality is crucial for optimal physiological function, particularly during military training, where dehydration or electrolyte imbalances can impair cognitive performance, thermoregulation, and endurance. Extreme deviations in osmolality, whether too high or too low, can be detrimental. High osmolality may indicate dehydration, leading to reduced plasma volume, while low osmolality can signal overhydration or hyponatremia, which could compromise performance and health.

Non-invasive methods, such as urine or saliva osmolality assessments or wearable sweat sensors, could provide real-time hydration feedback during training.[61] These tools could help optimize fluid intake and prevent imbalances, especially in extended training events like the FTX where environmental conditions and exertion levels vary. The reduction in osmolality also suggests fluid shifts within the body, reflecting a redistribution of water between intracellular and extracellular compartments. This shift indicates that the body effectively regulated fluid balance during training, maintaining circulatory stability and cellular function.

Limitations

The clinical relevance of the observed changes in hydration and metabolic markers should be interpreted cautiously even though they are statistically significant. Further investigations using larger sample sizes and additional time point analyses would strengthen the generalizability of these findings and provide deeper insight into the physiological mechanisms at play.

Conclusions

Our findings indicate the intervention induced significant changes in hydration markers, electrolyte balance, and metabolic biomarkers, while blood markers of renal function remained stable. These results provide valuable insights into physiological adaptations and underscore the importance of monitoring hydration and metabolic responses in similar settings. Future research should investigate the underlying mechanisms driving these changes and explore potential long-term implications particularly in the FTX environment. Future studies should also examine the temporal dynamics of these biomarker changes beyond the immediate post-FTX period. Understanding how these shifts evolve over days or weeks could provide a more comprehensive picture of adaptation and recovery. Furthermore, integrating additional measures such as body composition analysis, hormonal markers, and subjective fatigue assessments could enhance the interpretability of these findings and provide a more holistic understanding of physiological responses to similar interventions.

Acknowledgements

We would like to thank the Auburn University Army and Marine ROTC for their participation in this study. Additionally, we would like to thank the entire Warrior Research Center Laboratory for their assistance and work in the data collection for this study.

Evaluation of Physiological Responses, Risk Factors, and Biomarker Changes during Field Training Exercises with Concern for Rhabdomyolysis Development

Introduction

Exertional rhabdomyolysis (ER) occurs when intense or unaccustomed physical exertion damages skeletal muscle, releasing intracellular components like creatine kinase (CK) and myoglobin (Mb) into the bloodstream. ER develops when the sarcolemma, the membrane surrounding muscle fibers, sustains damage, triggering inflammatory and metabolic disruptions.[20,80,333] Several factors influence susceptibility including biological sex, race, hydration status, heat stress, nutrition, and genetic predispositions like sickle cell trait.[9,12,79] This process can lead to severe complications, including acute kidney injury, electrolyte imbalances, and cardiac arrhythmias.[1,2,39] ER severity varies from mild, asymptomatic enzyme elevations to life-threatening conditions requiring hospitalization.[334]

Military personnel, including Reserve Officers' Training Corps (ROTC) cadets, face an increased risk due to rigorous training, environmental stressors, and individual physiological responses.[1,32,199,334] Military personnel experience ER at significantly higher rates compared to civilians, with an estimated incidence of 43.1 cases per 100,000 person-years.[13,14,335] Required occupational activities such as field training exercises (FTX) create an environment where ER risk intensifies due to prolonged exertion, heat exposure, and dehydration.[13,335] Recovery often requires weeks to months of restricted activity, increasing military healthcare costs and reducing unit readiness.

Previous research has shown that ER remains underreported in ROTC cadets.[32,199] Additionally, cadets tend to seek medical care outside military systems decreasing military injury

incidence rates.[32,199] Existing research relies heavily on retrospective epidemiological studies, which often underestimate true incidence due to inconsistent diagnostic criteria and limited surveillance.[336,337] Accurately assessing ER prevalence requires a comprehensive approach that incorporates biomarker analysis, demographic data, fitness assessments, and activity tracking.[1,6,131] Individuals who develop ER also face a higher risk of recurrence, underscoring the need for early detection and prevention strategies.[10,74] ER can also result in medical disqualification from military service, further emphasizing the need for proactive management.[13,32]

This study aims to fill critical gaps in ER research within ROTC populations by evaluating physiological responses to a two-to-three-day FTX. Specifically, this study (1) measured CK and Mb levels before and after FTX to assess muscle breakdown; (2) examined correlations between fitness level, body composition, sex, race, and age with biomarker changes; (3) analyzed how recorded physical activity during FTX influences biomarker elevations; and (4) determined whether clinical symptoms such as dark urine, prolonged soreness, or excessive fatigue align with mild ER biomarker thresholds.

Methods

All participants volunteered for this study and were provided with verbal and written description of the requirements of the study and signed written informed consents. This study was approved by the Auburn University Institutional Review Board and the Army HPRO approved this study (protocol # 24-843 FB). Participants met the inclusion criteria: ages of 17-45 years, no physical restrictions or limitations such as being on a profile or chit for injury, no medical conditions that would disqualify them from Department of Defense service, and no contra-indications for providing a blood sample. Initially, 57 participants volunteered for this study, two

were removed prior to the data analysis due to not completing the FTX. Additional participants were removed from the analysis of the study due to lack of complete data (Figure 1). After completion of the FTX and data collection 39 of the original 57 participants information was used for analysis (Figure 1). Participant demographics can be seen in Table 1.

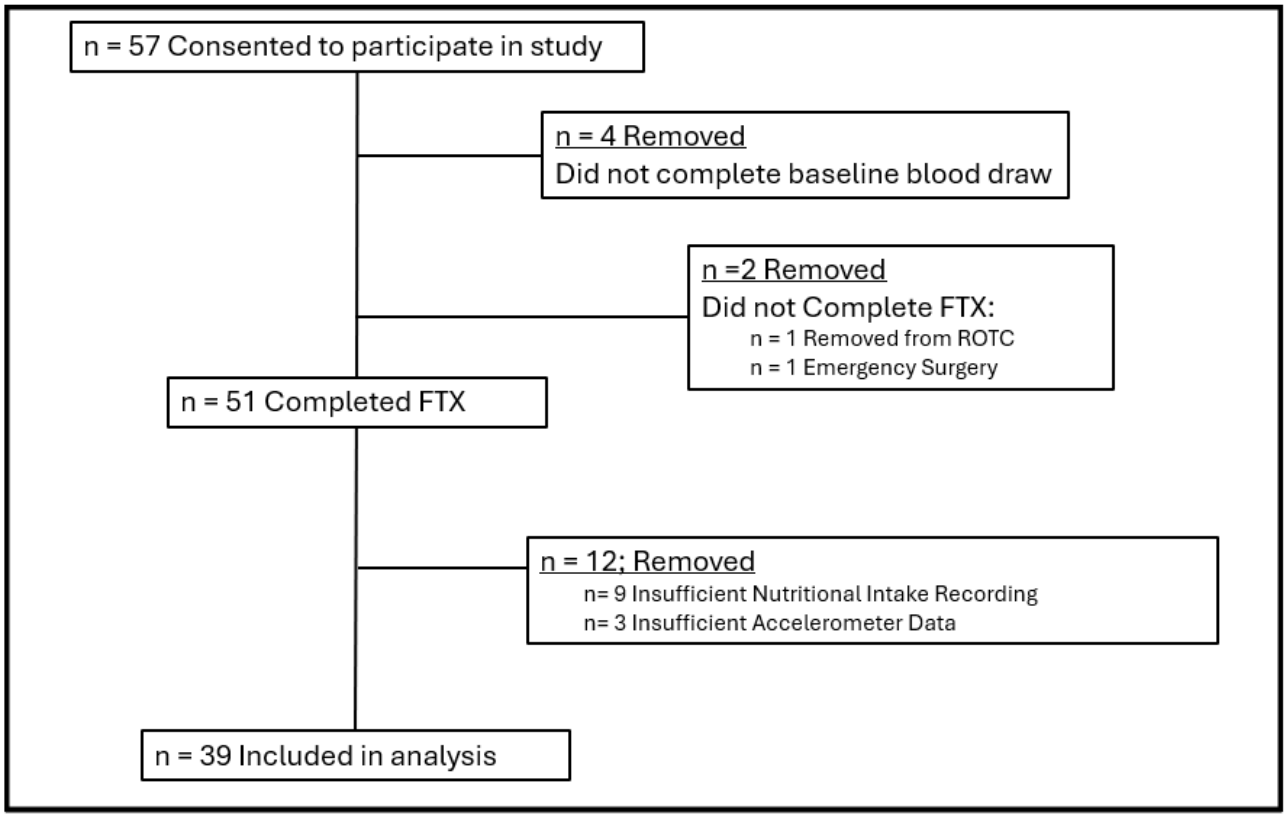

Figure 1. Participant flow and sample size

Table 1. Participant Demographics

Participants	39
MS1	11 (10 male; 1 female)
MS2	9 (9 male; 0 female)
MS3	7 (4 male; 3 female)
MS4	10 (6 male; 4 female)
MS5+	2 (1 male; 1 female)
Male/ Female	30/9
Race/ Ethnicity	
Caucasian/ White	33
African American / Black	1
Hispanic	3
Native American	0
Asian	1
Mixed Race	2
Age (yrs)	21.10 ± 4.60 years
Males	20.40 ± 3.54 years
Females	23.44 ± 6.88 years
Height (cm)	173.74 ± 6.66 cm
Males	175.76 ± 5.66 cm
Females	166.99 ± 5.33 cm
Body mass (kg)	76.14 ± 9.22 kg
Males	78.53 ± 8.16 kg
Female	68.14 ± 8.29 kg
BMI	25.20 ± 2.47
Male	25.42 ± 2.28
Female	24.47 ± 3.05
Body Fat Percentage	20.16 ± 7.60 %
Male	16.77 ± 4.53 %
Female	31.49 ± 3.55 %

Note: MS = Military Science Year

ROTC members participated in a mandatory two- or three-day FTX, with the duration determined by branch and training schedules. Marine-option ROTC members completed a continuous two-day FTX, while Army-option ROTC members conducted a three-day FTX with training activities structured according to their Military Science (MS) year (Figure 2).

Marine ROTC Training Protocol:

- Duration: Two days, one night of continuous training.
- Activities: Squad movement drills and patrol, bivouac infiltration and exfiltration, camouflage application, weapon system familiarization, communication system use, trauma combat casualty care (TCCC), day and night land navigation, logistics operations, leadership exercises, and ruck marches between activities.

Army ROTC Training Protocol:

- Duration: Three days, two nights with designated six-hour sleep periods per night.
- Activities varied by MS year:
    - MS1 and MS2: Firearm proficiency training via Electronic Simulation Training (EST), obstacle course, conditioning course, team development course, and a six-mile ruck march.
    - MS3: Firearm proficiency training via EST, live firearm training, and qualification at 25-meter and modified record fire ranges.
    - MS1-MS3: Day and night land navigation, team-building events, warrior tasks (weapon familiarization, TCCC, field craft, camouflage application, field hygiene, and communications training).
    - MS4**:** Operational planning and support for MS1-MS3 events.

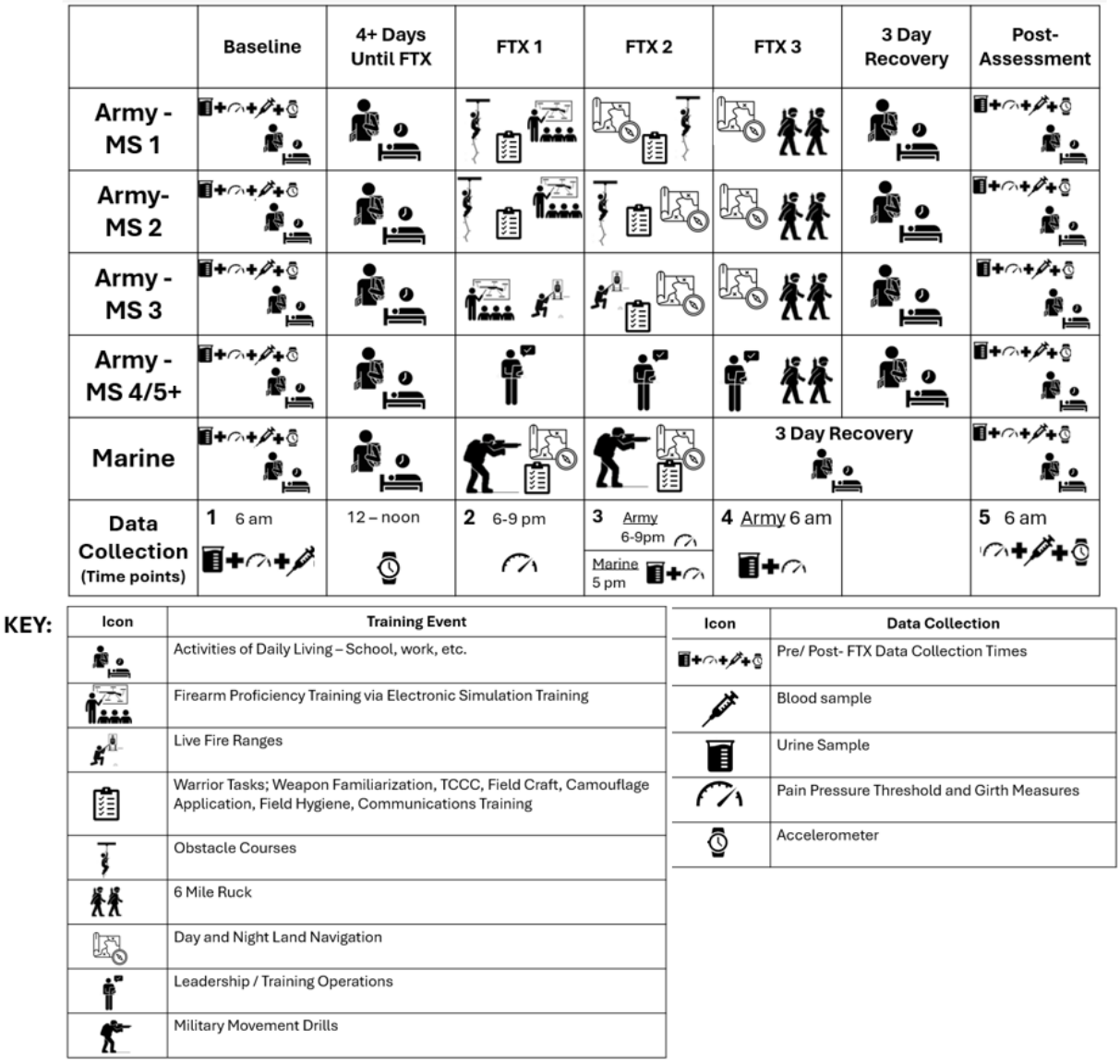

Figure 2. Field Exercise Training (FTX) and Data Collection Time Point Schedule

Participants completed a pertinent health history and demographics form prior to the baseline assessment. Questions included the participant's musculoskeletal injury history, surgical history (to rule out possible malignant hyperthermia due to anesthesia), known genetic conditions, history of heat illness and rhabdomyolysis (Table 2), as well as general perceived fitness level, and current fitness training (Table 3).

Table 2. Basic Health History (Group)

	# of Participants "yes"
Allergies	7 (18%)
COVID History	14 (36%)
COVID Vaccination History	34 (79%)
Musculoskeletal Surgery	8 (20.5%)
Surgery History	19 (49 %)
Sickle Cell Anemia / Trait	0 (0%)
Heat Illness History	1 (2.5%)
Surgery History	20 (49%)
Rhabdomyolysis History	2 (5%)

Table 3. Fitness Measures

Self-reported Fitness Level	Not at all fit	Slightly below average fitness	Average fitness	Slightly above average fitness	Extremely Fit
All ROTC Participants	0	2	8	20	9
MS1	0	1	2	4	4
MS2	0	0	2	6	1
MS3	0	1	1	3	2
MS4	0	0	3	5	2
MS5+	0	0	0	2	0
Self- Reported Additional Weekly Workouts	Seldom or Never	Less than 1x/ week	1-2x / week	3-4 x / week	5+x/ week
All ROTC Participants	0	3	8	20	8
MS1	0	1	2	4	4
MS2	0	0	2	6	1
MS3	0	1	1	3	2
MS4	0	0	3	5	2
MS5+	0	0	0	2	0
Military Fitness Test (percentage of total points)					
All ROTC Participants	88.64 ± 7.45 %				
MS1	84.33 ± 8.24 %				
MS2	87.22 ± 3.47 %				
MS3	89.86 ± 11.1 %				
MS4	92.33 ± 3.56 %				
MS5+	95.92 ± 2.47 %				

Participants provided a urine sample during the pre-FTX baseline assessment, and immediately after the completion of the FTX in the field. Urine samples were collected in sterile 120 mL specimen cups and immediately tested for Urine Specific Gravity via handheld

refractometer (Vollrath Company, Sheboygan, WI, USA) and electronic urine dipstick measures (BC401 Urine Analyzer, CONTEC Medical Systems, Qinhuangdao, Hebei Province, China). The samples were then stored on ice for later additional processing. Samples were transported to the laboratory within two hours and aliquoted into one 8 mL tube to be sent for testing at East Alabama Medical Center for presence of urinary Mb. Samples were also aliquoted into two 1.8 mL cryovials stored in a -80° C freezer as replicate samples. Urinalysis testing was completed to identify urine specific gravity, urobilinogen, blood presence, bilirubin, ketones, glucose, protein, pH, nitrites, leukocytes and vitamin C presence.

Venous blood samples were drawn from the antecubital vein using a sterile venipuncture technique. Each participant had approximately 20 mL of blood collected into serum (red top) tubes for analysis. One of the 10 mL red top tubes was used for a 4 mL pour off tube to send for additional testing (complete metabolic panel (CMP), CK, and Mb testing). The remaining samples were kept on ice until centrifugation at 1500 g for 10 minutes at 4 ° C. Plasma and serum were then aliquoted into two 1.8 mL cryovials and stored at -80° C as replicate samples.

Blood biochemical markers including sodium glucose, potassium, chloride, CO2, calcium, blood urea nitrogen (BUN), creatinine, albumin (ALB), aspartate amino transferase (AST), BUN creatinine ratio, globulin, bilirubin, CK, Mb, albumin globulin ratio (AG Ratio), and osmolality were quantified by the Medical Center Laboratory. All assays were performed in duplicate to ensure reliability. All sample collections followed standardized biosafety protocols, and participants were instructed to remain fasted for eight hours before blood collection to minimize variability.

Basic anthropometric assessment was conducted during the baseline visit. Participants' height was recorded using a stadiometer (SECA, Hamburg, Germany). Weight and body

composition was assessed using the TANITA BC-568 InnerScan Segmental Body Composition Monitor (TANITA Corporation, Preston, WA, USA) a bioelectrical impedance technology. Sufficient hydration (USG ≤ 1.030) was ensured prior to measurement. Weight (lbs.) /mass (kgs), lean muscle mass (kgs), body fat percentage, and visceral fat rating were recorded.

Pain pressure thresholds (PPT) were assessed to evaluate changes in muscle soreness over the course of the study. A digital pressure algometer (Force Ten FDX Compact, Wagner Instruments, Greenwich CT, USA) was used to quantify PPT at multiple anatomical locations. The algometer was calibrated before each testing session according to the manufacturer's specifications to ensure accuracy and reliability. PPT measurements were obtained bilaterally at major muscle groups, including the biceps brachii, triceps brachii, rhomboids, latissimus dorsi (lower back), quadriceps, hamstrings, and gastrocnemius (calf) (Figure 3). Each measurement was taken with participants in a standardized position to minimize variability.

The algometer probe was placed perpendicular to the skin surface at each target site, and pressure gradually applied at a constant rate until the participant indicated the first sensation of pain. The force at this point was recorded in newtons (N) as the PPT value. Participants were instructed to remain relaxed and avoid voluntary muscle contraction during testing. PPT assessments were conducted throughout the study to track changes in muscle soreness over time. This protocol was adapted from previous research and has been validated for assessing mechanical pain sensitivity in response to exercise-induced muscle soreness.[183,185,187]

Lower segmental limb length and girth measurements were obtained to ensure consistent and replicable assessment of lower limb dimensions throughout the study. These measurements were used to standardize the locations for girth assessments, enhancing reliability and efficiency during the FTX. Upper and lower leg lengths were measured using a standard Gulick

anthropometric tape measure with participants in a standing position. Thigh length was recorded as the distance from the anterior superior iliac spine (ASIS) to the suprapatellar pole, while shin length was measured from the infrapatellar pole to the talar window (Figure 4). All measurements were taken bilaterally to account for potential asymmetries.

To ensure consistency across participants, girth measurements were taken at standardized anatomical locations, determined as one-third of the total segment length for both the thigh and shin. Thigh girth was measured at a point located one-third of the total thigh length, starting from the suprapatellar pole and moving proximally. Shin (calf) girth was measured at one-third of the shin length, beginning from the infrapatellar pole and moving distally (Figure 3). A flexible, non-elastic Gulick anthropometric tape was used to obtain girth measurements, ensuring accurate circumference recordings without excessive compression of soft tissue. This standardized approach facilitated consistency across study participants while allowing for efficient and precise assessments during FTX evaluations. The method was adapted from previous research and has been demonstrated to provide reliable limb girth assessments in similar research settings.[69,338]

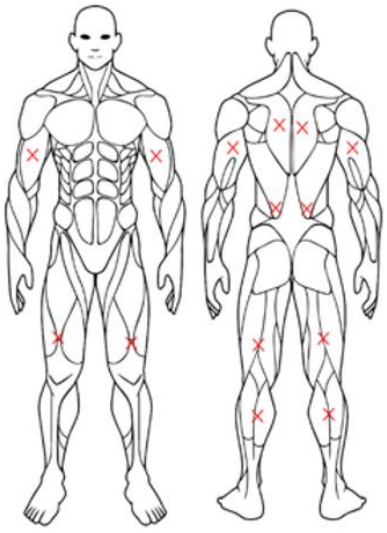

Figure 3. Pain Pressure Threshold (PPT) Sites

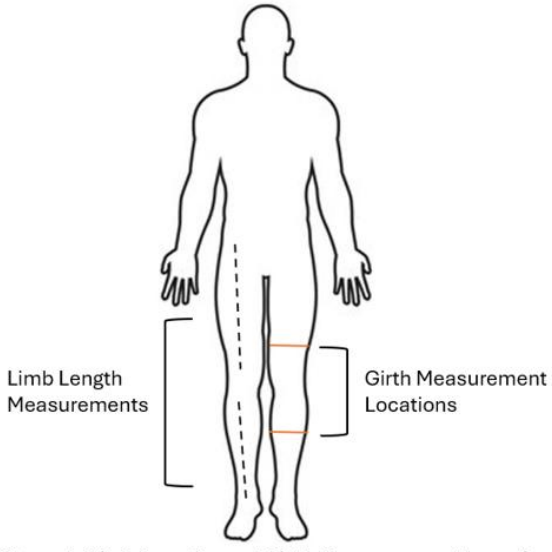

Figure 4. Limb Length and Girth Measurement Locations

To assess participants' overall well-being and perceived physical status throughout the training period, a brief three-question disposition survey was administered throughout the study. This survey was designed to minimize disruption to training while providing a rapid assessment of participants' subjective status. The disposition survey consisted of three self-reported items, each rated on a five-point Likert scale (1–5), with 1 representing the lowest perceived state and 5 representing the highest. The questions were framed relative to each participant's perceived baseline state to account for individual variability:

1. Compared to "your normal," how do you feel today?
2. Compared to "your normal," how is your energy level today?
3. Compared to "your normal," how rested do you feel today?

Responses were recorded on paper assessments, and compliance was monitored to ensure consistent completion rates across all time points. This survey method provided a simple yet effective means of tracking fluctuations in perceived fatigue, energy levels, and recovery status throughout the training period. The use of relative self-assessment reduced inter-individual variability and allowed for a more personalized interpretation of the data.

Estimated energy expenditure was measured using a triaxial accelerometer (ActiGraph xGT3X-BT, ActiGraph Technologies, Pensacola, FL, USA).

Accelerometers were set to a 60 Hz sampling frequency and individually programmed with participant-specific data, including sex, age, height, body mass, and dominant hand. Participants wore the device continuously throughout the FTX, ensuring comprehensive activity monitoring. Raw acceleration data were processed using ActiLife Software v6.13.4 (ActiGraph, Pensacola, FL, USA). Energy expenditure was calculated using built-in algorithms, specifically

the Freedson VM3 Combination (2011) and Swartz Adult Overground & Lifestyle (2000) models for metabolic equivalents (METs). Moderate-to-vigorous physical activity (MVPA) was quantified using Montoye cut points.[218] Data were excluded from analysis if participants wore the device for less than 10 hours per day.

Participants documented all meals and snacks using a written food log, which included pre-printed Meal-Ready-To-Eat (MRE) tracking sheets with space for additional food items and portion sizes. The Army ROTC unit received two MREs per day during the FTX along with one hot meal (HOT-A) in the field from the Army Post dining facility. In contrast, the Marine ROTC unit received two or three MREs in advance and had the option to fieldstrip them before the training event. Caloric values for recorded food items were determined using nutritional data from the Combat Rations Database (ComRAD), developed by Human Performance Resources (Consortium for Health and Military Performance, CHAMP), the U.S. Army Combat Capabilities Development Command (DEVCOM) Soldier Center, and the U.S. Army Research Institute of Environmental Medicine.[216] Additional food items were analyzed using the U.S. Department of Agriculture's Food Data Central.[217] Because the Marine FTX lasted two continuous days while the Army FTX spanned three days, reported nutritional values were adjusted to reflect daily intake for accurate comparison.

Data Analyses

Statistical analyses were performed using R (R Core Team, 2020) and RStudio (RStudio Team, 2023) version 4.2.2, with the psych, lattice, dplyr, tidyr, effsize, and lme4 packages. The significance level was set *a priori* at $\alpha = 0.05$. Shapiro-Wilk tests and Q-Q plots were used to assess normality of dependent variables. Normally distributed data were analyzed with pairwise t-tests to compare pre- and post-biomarker measures, while non-normally distributed data were

assessed using Wilcoxon signed-rank tests. Multiple linear regression models were constructed to examine interactions between biomarker changes and military education year, age, and biological sex.

Results

Biomarker analyses were conducted to assess physiological changes in hydration status, metabolic function, and systemic stress from baseline (pre-FTX) to post-FTX. Significant alterations were observed across multiple urine and blood parameters, reflecting shifts in fluid balance, electrolyte homeostasis, and metabolic activity.

Markers of hydration status demonstrated notable changes following the FTX. Participants were hydrated (USG <1.030) during the baseline assessment. Urine specific gravity (USG) significantly decreased post-FTX ($t(38) = -3.695$, $p = 0.00069$), suggesting a reduction in urine concentration and potential hemodilution. Blood osmolality significantly decreased ($M = -4.36$, 95% CI [-5.52, -3.20], $t(38) = -7.59$, $p < 0.001$), further indicating shifts in fluid balance. These findings suggest that despite prolonged exertion, participants may have maintained adequate hydration, potentially through increased fluid intake or physiological adaptations. Electrolyte levels also exhibited significant changes. Serum sodium ($M = -2.28$, 95% CI [-2.84, -1.73], $t(38) = -8.31$, $p < 0.001$), chloride ($M = -1.79$, 95% CI [-2.49, -1.10], $t(38) = -5.23$, $p < 0.001$), and calcium ($M = -0.26$, 95% CI [-0.35, -0.18], $t(38) = -6.02$, $p < 0.001$) all decreased significantly, suggesting fluid shifts and potential electrolyte losses during training. In contrast, potassium levels remained unchanged ($p = 0.42$), indicating that potassium homeostasis was maintained despite fluctuations in other electrolytes.

Several biomarkers reflected shifts in metabolic activity. Urinary ketone levels significantly increased post-FTX ($V = 0$, $p = 0.0054$), suggesting increased fat metabolism, possibly due to heightened energy demands during training. This metabolic shift was further supported by a significant increase in blood glucose levels post-FTX ($M = 6.23$, 95% CI [2.62, 9.84], $t(38) = 3.49$, $p = 0.001$), potentially reflecting acute stress-induced glucose mobilization. These findings indicate that energy utilization was altered in response to sustained physical exertion, with increased reliance on both carbohydrate and fat metabolism.

Markers of systemic stress and tissue integrity also exhibited notable changes from FTX participation. Enzymes associated with muscle and liver function showed significant elevations post-FTX, including alkaline phosphatase ($p = 0.003$), alanine aminotransferase (ALT) ($p = 0.006$), aspartate aminotransferase (AST) ($p = 0.036$), bilirubin ($p = 0.024$), and creatine kinase ($p = 0.015$). These increases suggest elevated physiological strain, likely due to muscle exertion and potential tissue breakdown. Conversely, no significant changes were observed in blood urea nitrogen (BUN) ($p = 0.76$), creatinine ($p = 0.78$), or the BUN-to-creatinine ratio ($p = 0.89$), suggesting that renal function remained stable despite the physiological stressors of training. Additionally, no significant changes were detected in urinary markers such as blood ($p = 0.7728$), protein ($p = 1.000$), pH ($p = 0.3075$), nitrites ($p = 1.000$), leukocytes ($p = 1.000$), or vitamin C ($p = 1.000$), further supporting the notion that renal and immune function were largely unaffected.

Overall, these findings indicate significant shifts in hydration status, electrolyte balance, and metabolic function resulting from the FTX. The decrease in USG and osmolality suggests fluid balance adaptations, while reductions in sodium, chloride, and calcium highlight potential electrolyte losses. Increased ketone excretion and glucose levels reflect metabolic shifts necessary to sustain energy demands. Additionally, elevated liver and muscle enzyme activity suggests heightened physiological stress and muscular exertion. Despite these systemic changes, renal

function appeared to remain stable, and no significant immune-related alterations were detected. These biomarker responses provide insight into the physiological demands of military field training and the body's adaptive mechanisms to sustain performance under prolonged stress.

The analysis examined PPT values over multiple body locations in the five measure time points using linear mixed-effects models. Each model included time as a fixed effect and participant ID as a random effect to account for repeated measures. Results from the models exploring the PPT changes over time can be seen below (Table 4).

PPT values significantly increased across all measured body locations over time (Figure 5), with the largest increase generally occurring between baseline and Time 2 or Time 3 (Figure 6). While PPT values remained elevated throughout the study, the magnitude of change varied across muscle groups. These findings suggest a consistent pattern of increased pain pressure tolerance over time, potentially indicative of adaptation or desensitization effects.

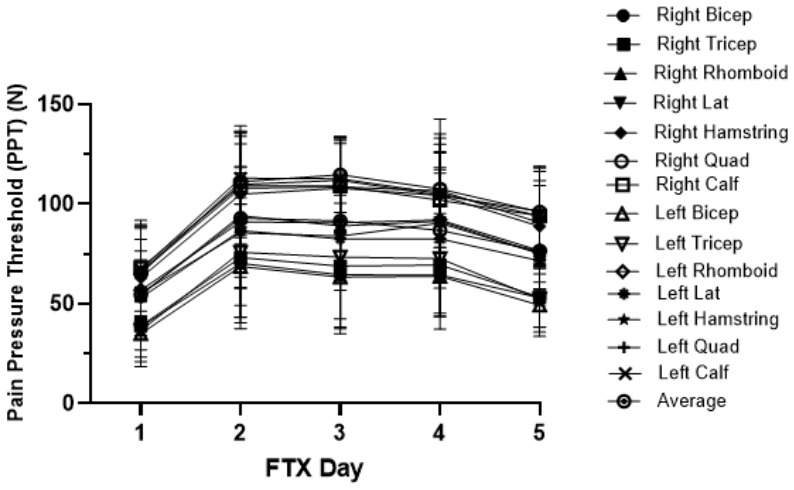

Figure 5. Pain Pressure Threshold Trends
Note: Lat = lattissimus dorsi, Quad = Quadriceps, Avg = Average

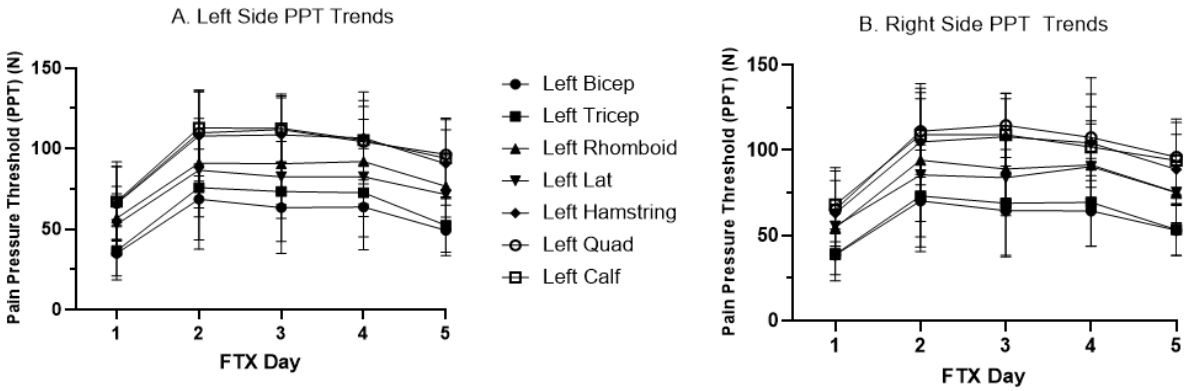

Figure 6. Pain Pressure Threshold Trends by Side
Note: Lat = lattissimus dorsi, Quad = Quadriceps

Table 4. Pain Pressure Thresholds Over Time

Body Part	Baseline	Time 2 (FTX)	Time 3 (FTX)	Time 4 (FTX)	Time 5 (Post)
Right Bicep	38.53 (3.55, 10.84; p<0.001)	31.83 (4.44, 7.17; p<0.001)	26.05 (4.44, 5.87; p<0.001)	25.66 (4.62, 5.55; p<0.001)	14.50 (4.44, 3.26; p<0.001)
Right Triceps	38.96 (3.88, 10.05; p<0.001)	34.24 (4.66, 7.35; p<0.001)	29.99 (4.66, 6.42; p<0.001)	30.60 (4.85, 6.30; p<0.001)	14.46 (4.66, 3.10; p = 0.0023)
Right Rhomboid	54.56 (4.42, 12.26; p<0.001)	39.63 (5.49, 7.22; p<0.001)	34.55 (5.49, 6.30; p<0.001)	36.86 (5.71, 6.45; p<0.001)	20.74 (5.49, 3.78; p<0.001)
Right Lat	55.43 (3.53, 15.72; p<0.001)	30.11 (4.01, 7.50; p<0.001)	28.56 (4.01, 7.12; p<0.001)	35.24 (4.18, 8.43; p<0.001)	19.74 (4.01, 4.93; p<0.001)
Right Hamstring	63.02 (3.72, 16.96; p<0.001)	41.91 (4.35, 9.65; p<0.001)	44.97 (4.35, 10.35; p<0.001)	41.37 (4.53, 9.14; p<0.001)	25.76 (4.35, 5.93; p<0.001)
Right Quadriceps	65.17 (3.68, 17.71; p<0.001)	46.04 (4.32, 10.66; p<0.001)	49.47 (4.32, 11.45; p<0.001)	42.15 (4.50, 9.36; p<0.001)	30.92 (4.32, 7.16; p<0.001)
Right Calf	68.02 (3.75, 18.14; p<0.001)	41.10 (4.34, 9.46; p<0.001)	41.11 (4.34, 9.45; p<0.001)	34.16 (4.53, 7.54; p<0.001)	26.04 (4.34, 5.99; p<0.001)
Left Bicep	35.02 (3.91, 8.97; p<0.001)	33.66 (4.50, 7.48; p<0.001)	28.43 (4.50, 6.32; p<0.001)	28.44 (4.69, 6.07; p<0.001)	14.34 (4.50, 3.19; p<0.001)
Left Triceps	36.67(4.10, 8.95; p<0.001)	39.16 (4.88, 8.02; p<0.001)	36.82 (4.88, 7.54; p<0.001)	35.67 (5.09, 7.01; p <0.001)	15.82 (4.88, 3.24; p = 0.0015)
Left Rhomboid	56.89 (3.81, 17.94; p<0.001)	34.20 (4.34, 7.88; p <0.001)	33.82 (4.34, 7.80; p <0.001)	35.24 (4.52, 7.80; p <0.001)	19.81 (4.34, 4.57; p <0.001)
Left Lat	53.01 (3.91, 13.59; p <0.001)	33.61 (4.55, 7.38; p <0.001)	29.53 (4.55, 6.49; p <0.001)	59.61 (4.74, 6.24; p <0.001)	18.53 (4.55, 4.07; p <0.001)
Left Hamstring	66.11 (3.97, 16.64; p <0.001)	41.78 (4.68, 8.93; p <0.001)	42.49 (4.68, 9.08; p <0.001)	40.64 (4.88, 8.34; p <0.001)	24.60 (4.68, 5.26; p <0.001)
Left Quadriceps	66.45 (3.69, 17.99; p <0.001)	43.43 (4.08, 10.65; p <0.001)	45.55 (4.08, 11.17; p <0.001)	37.74 (4.25, 8.88; p <0.001)	30.05 (4.08, 7.37; p <0.001)
Left Calf	67.18 (3.75, 17.91; p <0.001)	45.72 (4.15, 11.01; p <0.001)	45.51 (4.15, 10.96; p <0.001)	38.33 (4.33, 8.85; p <0.001)	26.82 (4.15, 6.46; p <0.001)
Average PPT	54.65 (3.29, 16.63; p <0.001)	38.31 (3.69, 10.38; p <0.001)	36.85 (3.69, 9.99; p <0.001)	35.11 (3.85, 9.13; p <0.001)	21.90 (3.69, 5.93; p <0.001)

Note: All results are structured as: Value (Standard Error, t-value; p-value). Lat = Latissimus Dorsi of low back.

Linear mixed-effects models were used to evaluate changes in girth of the right thigh, the right calf, left thigh, and left calf across five time points while accounting for individual differences among participants (Figure 7). Time was included as a fixed effect, and participant ID was modeled as a random intercept to control for within-subject variability.

Table 5. Low Limb Girth Measurements Over Time

Body Part	Baseline	Time 2 (FTX)	Time 3 (FTX)	Time 4 (FTX)	Time 5 (Post)
Right Thigh	52.71 (0.60, 87.96)	1.88 (0.39, 4.82)	1.29 (0.39, 3.32)	1.32 (0.41, 3.24)	-0.16 (0.39, -0.41)
Right Calf	37.33 (0.54, 69.55)	1.56 (0.59, 2.62)	-0.22 (0.59, -0.37)	1.17 (0.62, 1.90)	-1.04 (0.59, -1.76)
Left Thigh	51.17 (0.67, 76.00)	1.93 (0.65, 2.98)	1.79 (0.65, 2.76)	2.54 (0.68, 3.74)	1.20 (0.65, 1.84)
Left Calf	36.37 (0.40, 90.84)	1.97 (0.31, 6.42)	0.93 (0.31, 3.02)	2.14 (0.32, 6.65)	-0.27 (0.31, -0.87)

Note: All results are structured as: Value (Standard Error, t-value)

The model examining GRT revealed a significant main effect of time, indicating that right thigh girth changed across sessions. Right thigh girth significantly increased during the FTX at times 2-4 compared to baseline. However, at Time 5 the right thigh girth was not significantly different from baseline which suggests a return to initial levels. The analysis of the right calf girth indicated a significant increase at Time2 compared to baseline. However, no significant differences were observed at any other time point. These findings suggest that while an initial increase in right calf girth was observed, it did not persist beyond Time2. For the model of the left thigh girth demonstrated significant increases across multiple time points. Compared to baseline, GLT was significantly higher at Time 2, Time 3, and Time 4. At Time5, GLT remained elevated but did not reach statistical significance, suggesting a possible retention effect. The left calf girth analysis revealed a significant increase during the FTX at time 2-4. However, by Time 5, left calf girth was not significantly different from baseline, indicating a return to initial levels.

Overall, significant increases in right and left thigh girth and left calf girth were observed at intermediate time points (Time2–Time4), suggesting initial increases in thigh and calf girth

measurements. However, the right thigh girth and left calf girth returned to baseline levels by Time5, while left thigh girth remained marginally elevated. Girth of the right calf exhibited a transient increase at Time2 but did not show consistent changes across time points.

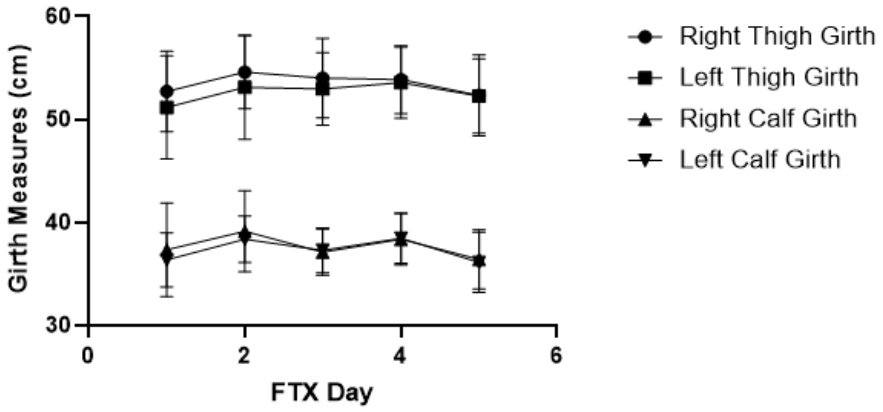

Figure 7. Low Leg Girth Measurements

A series of multiple linear regression analyses were conducted to examine the relationships between changes in physiological biomarkers and predictor variables, including military fitness test scores, body fat percentage, age, sex, and race.

Significant predictors of biomarker changes varied across models. Military fitness scores were a significant predictor for ketones and calcium, while body fat percentage significantly predicted changes in glucose and USG. Age was significantly associated with changes in glucose and calcium. Sex was a significant predictor for ketones and approached significance for USG and glucose. However, many models exhibited low adjusted R-squared values, indicating limited explanatory power of the included predictors for most biomarkers.

To investigate the relationship between changes in energy expenditure and various biomarker levels, simple linear regression models were performed. The models examined whether estimated energy expenditure significantly predicted changes in specific biomarkers. Results from

the models exploring the interactions between energy expenditure and biomarker changes can be seen below (Table 6).

Table 6. Energy Expenditure Predicting Biomarker Changes

Variable	Model Significance	Adjusted R-Squared	Significant Predictors	Other Non-Significant Variables
USG	(F(5,33) = 2.15, p = 0.084),	0.13	Body Fat % (B = 0.001, p = 0.0048)	Sex approached significance (B = -0.012, p = 0.05)
Ketones	F(5,33) = 3.77, p = 0.008	0.267	MFT score (B = -0.17, p = 0.001), Sex (B = -2.81, p = 0.049)	All others (p > 0.05)
Sodium	F(5,33) = 1.63, p = 0.18	0.076	None (Race trended, B = -0.42, p = 0.06)	All others (p > 0.05)
Glucose	F(5,33) = 2.12, p = 0.087	0.129	Body Fat % (B = 1.02, p = 0.016), Age (B = -0.96, p = 0.028)	Sex trended (B = -12.83, p = 0.079), others non-significant
Calcium	F(5,33) = 3.102, p = 0.0210	0.2167	Score (B = -0.015, p = 0.013), Age (B = 0.0295, p = 0.005	All others (p > 0.05)
Alkaline Phosphate	F(5,33) = 1.465, p = 0.23	0.058	None	All others (p>0.05)
ALT	F(5,33) = 1.154, p = 0.3522	0.0199	MFT Score (B= 0.3538, p =0.0772)	All others (p> 0.05)
AST	F(5,33) = 0.495, p = 0.78	-0.071	None	All others (p> 0.05)
Bilirubin	No significant model	N/A	None	All variables (p > 0.05)

Overall, none of the regression models revealed a significant relationship between changes in energy expenditure and biomarker levels, suggesting that alterations in energy expenditure did not meaningfully influence these physiological markers in this dataset.

Discussion

This study investigated the physiological changes associated with military FTX in ROTC members, focusing on hydration status, metabolic activity, systemic stress, and PPT to assess the risk and prediction of exertional rhabdomyolysis. The findings highlight significant shifts in biomarkers, fluid balance, muscle girth, and pain sensitivity, offering insight into the physiological demands of exertion in field conditions.

FTX induced changes in fluid balance evidenced by significant reductions in USG and blood osmolality (Figure 9). These results suggest hemodilution, likely due to increased fluid intake or physiological mechanisms aimed at maintaining hydration under strenuous conditions. Similar changes have been reported in other military training studies, where prolonged exertion and environmental stressors challenge the body's ability to maintain homeostasis.[302,339,340] During the FTX of the current study cadets were allowed to drink water ad libitum without restriction and were encouraged to ensure adequate intake throughout the FTX. These training exercises simulate high-stress operational environments where soldiers endure physically demanding tasks, further exacerbating fluid and electrolyte shifts. The observed reductions in USG indicate a state of relative hyperhydration as the body compensates for increased fluid loss. The absence of comparable changes in dipstick-measured USG is likely due to the lower sensitivity of this method compared to laboratory-based assessments which capture subtler variations in fluid status.[341] Previous studies have noted that while some participants experienced reductions in USG, others displayed stable or even elevated values, particularly when fluid intake was strategically managed to prevent overhydration.[308] These findings highlight the role of hydration protocols, environmental conditions, and individual sweat rate variations in determining fluid shifts during military training.[342] Proper hydration maintenance is often a major focus in tactical athletes. The fluid balance changes in this study reflect the need for continued self-monitoring through urine color and ensuring hydration levels.

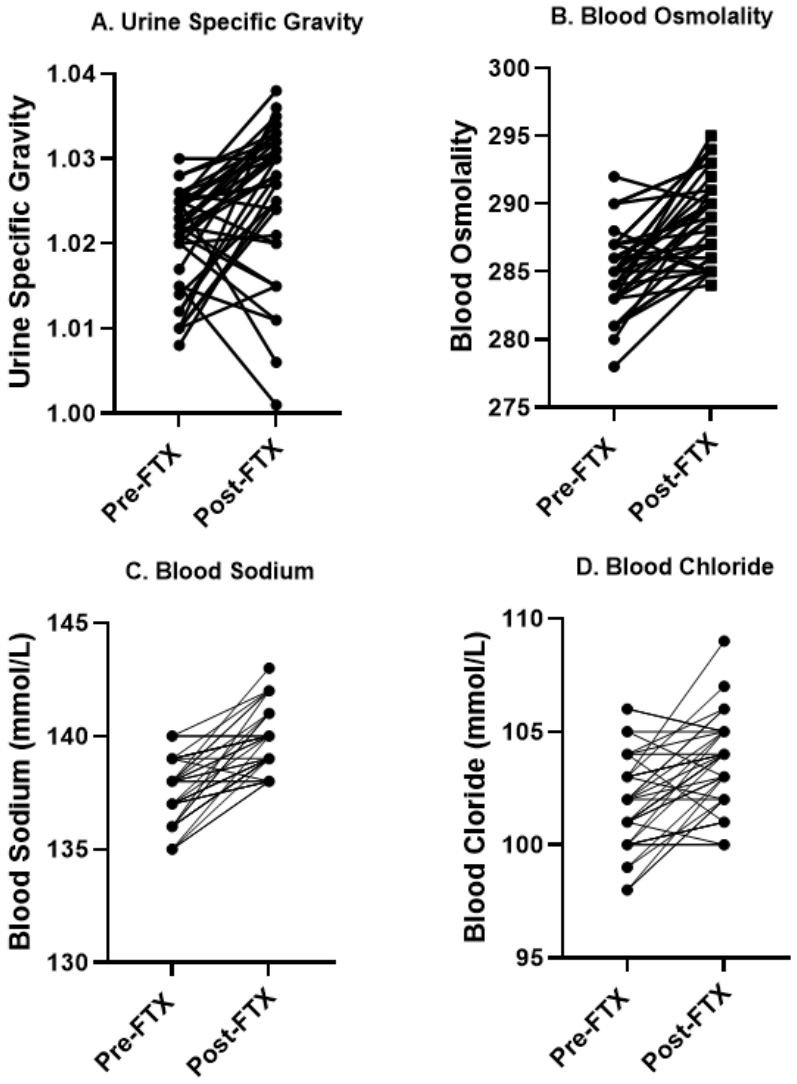

Figure 9. Shifts in Biomarkers of Hydration

Potassium homeostasis is essential for maintaining muscle and nerve function, and its stability during prolonged exercise may reflect compensatory mechanisms in renal or adrenal function that counteract sweat-induced potassium loss.[1,52,343,344] Electrolyte levels fluctuated significantly during FTX, with serum sodium, chloride, and calcium levels decreasing over the course of the exercise. These changes are likely due to electrolyte loss through sweat, particularly in environments with elevated temperatures or high-intensity physical activity.[345] Fluid

redistribution mechanisms, such as plasma volume contraction, may contribute to these shifts. Potassium levels remained stable despite the electrolyte fluctuations, suggesting effective homeostatic regulation that preserves cellular function despite substantial fluid shifts.[346]

The results of this study align with prior research on fluid and electrolyte shifts during sustained physical exertion, emphasizing the challenges of maintaining electrolyte balance under stress.[300,308] Profuse sweating can lead to substantial losses of water and essential electrolytes like sodium and chloride, impairing neuromuscular function and endurance performance if not properly replenished.[347,348] Previous research has documented significant drops in potassium and sodium even in situations where electrolytes were supplemented.[315,347-349] These findings indicate that homeostatic mechanisms may not always fully compensate for electrolyte losses under intense conditions, increasing the risk of imbalance and potential performance decline. Research also suggests variability in potassium regulation during field exercises particularly under extreme heat or inadequate electrolyte intake, with some individuals maintaining potassium balance while others experience hypokalemia,.[316,350,351] This variation underscores the fact that homeostatic regulation effectiveness differs among individuals.[342,352] Military studies have shown targeted hydration and electrolyte supplementation strategies mitigate electrolyte depletion, suggesting that proactive hydration management can prevent imbalances.[300,304,306,353]

The elevated urinary ketones and blood glucose levels found in the current study indicate significant shifts in energy metabolism (Figure 10). This suggests the body adapted to the demands of prolonged physical exertion during the FTX.[354,355] The increase in urinary ketones observed during FTX likely reflects a greater reliance on fat metabolism as a fuel source.[240,314] This shift becomes particularly evident during extended periods of high-intensity physical activity, when carbohydrate stores deplete, prompting the body to mobilize fat for energy.[240,243,314] The elevated ketone excretion mirrors metabolic responses observed in endurance activities,

where sustaining performance requires a combination of carbohydrate and fat metabolism.[243,314] The increased reliance on fat metabolism, as evidenced by rising ketone levels, aligns with the body's adaptive response to prolonged exertion, preserving glycogen stores to maintain endurance and delay fatigue.

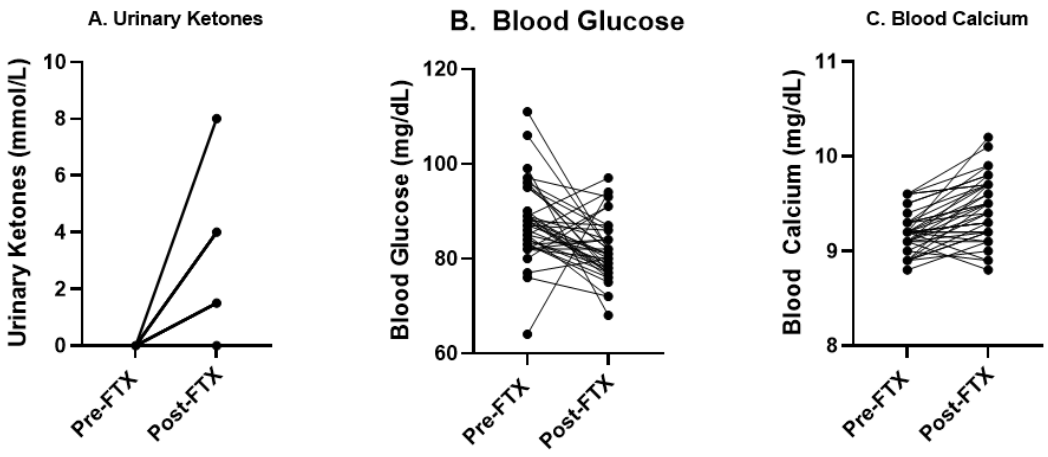

Figure 10. Shifts in Energy Deficiency and Metabolic Adaptations

In contrast, the elevated blood glucose levels seen in the ROTC cadets of this study may indicate the acute stress-induced mobilization of energy reserves. Under high physical and psychological stress, the body rapidly releases glucose into the bloodstream to fuel muscle activity.[356,357] This response is consistent with findings in military training and ROTC field exercises, where the stress of tactical operations triggers significant increases in circulating glucose as part of the fight-or-flight response.[284,321,358,359] Previous research demonstrates similar glucose mobilization in soldiers undergoing high-stress physical training.[358] Elevated blood glucose may also result from glycogen breakdown in muscles and the liver, ensuring immediate energy availability during critical, high-demand moments in tactical environments.

Military research on field training exercises has found that while some participants experience increases in urinary ketones, most do not exhibit significant changes in blood glucose

levels.[328,360,361] Researchers suggest that glycogen stores may be preserved or replenished through strategic carbohydrate intake, preventing the pronounced glucose spikes typically associated with the stress response. Studies emphasize the importance of pre-training nutrition and hydration, which may mitigate large-scale metabolic shifts during extended physical activity.[222,362] The results of the current study along with previous research highlights the role of nutritional strategies, such as carbohydrate loading and electrolyte supplementation, in modifying metabolic responses during an FTX.

Increased fat metabolism occurred in the cadets during this FTX and was indicated by elevated urinary ketones. The presence of urinary ketones in the ROTC cadets indicates a need for glucose mobilization to be used as immediate energy needs. The additional mobilization of glucose reflects the body's adaptive mechanisms under prolonged physical stress. However, despite the statistical finding in this study it is important to note there are individual differences in metabolic function, training history, and nutrition significantly influence these responses. While common in many undergoing strenuous military or ROTC training, these metabolic changes do not represent a universal pattern.

The post-FTX elevations in enzymatic markers of muscle and liver function found in this study (alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin, and creatine kinase (CK)) highlight the physiological strain that was imposed during the FTX (Figure 11).[1,20,95,351] These enzymatic changes typically result from intense physical activity, reflecting increased tissue turnover and muscle membrane disruption due to repeated contractions, particularly during eccentric or high-intensity exercises.[12,39,191,363,364] Elevated CK levels, a common marker of muscle stress, are often associated with exercise-induced muscle injury and rhabdomyolysis.[2,20,25,39] While enzyme elevations typically indicate muscle stress or tissue breakdown, minor increases are expected physiological responses to

vigorous exertion and do not necessarily signal pathological conditions unless accompanied by severe pain, weakness, or other clinical symptoms.[10,75,136,337,365]

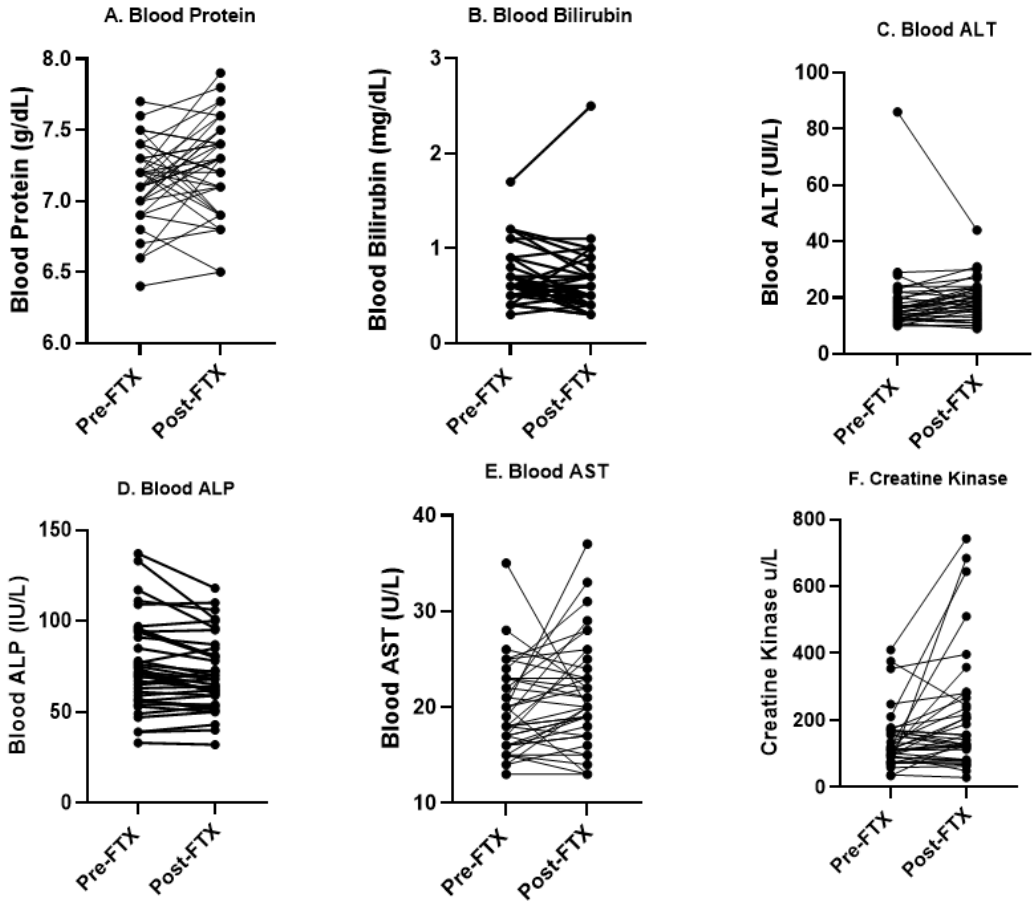

Figure 11. Shifts in Protein Catabolism and Muscle/Liver Stress

Similar increases in CK and AST to those we found in the current study have been documented in military personnel following intensive physical training.[9] Additionally, ROTC cadets participating in simulated combat training have exhibited post-exercise elevations in CK and ALT, emphasizing the muscular demands of tactical training. These findings support the idea that enzymatic elevations are a typical physiological response to high-intensity military exercises.

Research suggests that enzyme elevation does not always correlate with muscle damage or functional impairment.[366] Elevated CK levels in military personnel do not necessarily correspond

with muscle soreness or reduced performance in subsequent training sessions.[367] This raises the possibility that repeated muscular strain fosters resilience, leading to transient enzyme elevations without significant long-term damage.[59] Variability in enzyme responses among individuals suggests that factors such as training history, nutrition, and recovery strategies significantly influence these changes.[59] Research indicates that continued exposure to high-intensity training may result in adaptations that attenuate muscle stress responses over time, stabilizing enzyme levels even under sustained exertion.[368-371]

Despite elevated enzymatic markers of muscle strain, renal function markers including blood urea nitrogen (BUN), creatinine, and the BUN-to-creatinine ratio remained stable, suggesting that renal systems effectively managed the metabolic and physiological demands of training. This finding is consistent with previous research, which found no significant fluctuations in renal function markers during military training involving intense physical activity. These results suggest that healthy kidneys efficiently process metabolic byproducts, even under substantial muscle strain.[6,8,64]

Conflicting evidence exists regarding the renal response to intensive physical training. Some studies report transient increases in creatinine and BUN in soldiers following extreme exertion, attributing these rises to muscle protein catabolism and increased muscle tissue breakdown leading to rhabdomyolysis.[1,2,6,39,131,191,295] While renal function typically remains intact, prolonged or extreme exertion can stress the kidneys, particularly in individuals with pre-existing health conditions or inadequate hydration. These findings suggest that while renal markers generally remain stable, excessive heat, dehydration, or prolonged exertion may increase the risk of renal strain and rhabdomyolysis.[5,21,48,135,138]

Urinary markers related to immune function remained stable, indicating that systemic immune responses were largely unaffected by training stress. This aligns with previous studies showing that short-term intense exertion does not significantly compromise immune function.[372-375] Despite increased muscle stress markers following physical training, systemic immune markers such as white blood cell count and C-reactive protein (CRP) remained within normal limits.[376-378] These findings suggest that, under typical field training conditions, the immune system effectively manages exercise-induced stress.

However, prior research has documented temporary immune suppression following extreme physical exertion, which may increase infection susceptibility or impair recovery. While immune function generally returns to baseline after recovery, intense exercise can temporarily weaken immune defenses.[379-382] This contrasts with the current study's findings, suggesting that immune responses may vary depending on exertion intensity, environmental stressors, and baseline immune status.

Significant changes in thigh and calf circumferences during the intermediate training stages (Time2–Time4) suggest acute muscle swelling, likely due to fluid shifts, localized inflammation, or temporary hypertrophy from repeated exertion during the FTX. This increase in muscle girth aligns with physiological responses to physical activity, where muscle fibers experience microtrauma and inflammation, leading to fluid retention and swelling as part of the recovery process. This phenomenon is particularly evident in resistance training or high-intensity activities common in tactical athlete and military training programs. The temporary increase in muscle size, often termed "muscle pump" or "muscle edema," results from interstitial fluid accumulation and is a well-documented response to acute exercise.

Girth measurements returned to baseline by the final time point (Time5) three days after the completion of the FTX, indicating the transient nature of these adaptations. This suggests that muscle swelling observed during the intermediate stages was not permanent but a temporary response to physical stress. Prior research confirms that muscle swelling following intense exertion typically resolves once the acute inflammatory response subsides and muscle tissue repairs. The observed increases in thigh and calf circumferences were likely not indicative of permanent hypertrophic changes or rhabdomyolysis concerns but rather transient adaptations linked to acute inflammation and fluid retention. These findings align with previous research suggesting that significant changes in muscle girth during intense training are typically temporary, resolving as the inflammatory response diminishes. The return of girth measurements to baseline by Time5 supports the notion that the observed muscle swelling stemmed from fluid shifts and localized inflammation rather than permanent hypertrophy or pathological conditions.

Progressive increases in PPT (decreased sensitivity) across muscle groups suggest an enhanced tolerance to mechanical pressure likely resulting from both physiological and psychological adaptations. This increase was unexpected, as we hypothesized muscular soreness and PPT would decrease (increase sensitivity) over the course of the FTX. The results indicate participants developed a greater ability to endure mechanical pain or discomfort following prolonged physical exertion. This phenomenon is associated with both central and peripheral pain modulation mechanisms, which alter nociceptive processing, improve pain modulation, and induce desensitization effects due to repeated exposure to high training loads. This trend is rare but has been seen in previous research on tactical athletes and military personnel undergoing rigorous training.[383,384] PPT increases are often attributed to peripheral desensitization, where nociceptors become less responsive over time, and central adaptations, where the brain interprets

pain signals less intensely. The study's findings suggest that repeated physical stress exposure in military training contributes to improved pain tolerance.

PPT changes can vary among individuals.[183,187,188,385-387] Factors such as baseline pain sensitivity, psychological resilience, and genetic predisposition may influence how the body adapts to mechanical pain. Psychological factors, including mental toughness and stress coping mechanisms, also play a critical role in pain tolerance adaptations. Individuals with higher psychological resilience may experience greater improvements in pain tolerance showing a decrease in sensitivity, as they are better equipped to modulate the emotional and cognitive aspects of pain perception. This suggests that the observed increases in PPT among tactical and military training participants may result from both physiological and psychological adaptations.

Regression analyses identified key predictors of biomarker fluctuations during the FTX. Higher body fat percentages correlated with more pronounced shifts in USG and glucose levels suggesting individuals with higher adiposity experience greater changes in hydration status and metabolic responses during exertion. Prior research indicates that individuals with higher body fat exhibit greater fluctuations in serum glucose and dehydration markers likely due to slower metabolic rates and altered fat metabolism. This study further supports the notion that individuals with higher adiposity face increased risks of dehydration and metabolic instability during military training.[388,389]

Age also emerged as a significant predictor of glucose and calcium changes, with older participants exhibiting distinct physiological adaptations compared to younger counterparts.[358] These findings align with previous research suggesting that age-related declines in muscle mass and bone density contribute to altered calcium regulation in older adults. The study underscores

the need to consider age-related metabolic shifts when designing training programs for military personnel.[69,294,390]

Military fitness performance scores strongly predicted variations in ketones and calcium, highlighting the influence of fitness levels on metabolic adjustments during field training. Higher fitness levels are associated with more efficient metabolic processes and better regulation of energy substrates like ketones. Military personnel with superior physical fitness exhibit more stable ketone levels during prolonged exertion, likely due to enhanced fat oxidation and energy mobilization. The study's findings reinforce the idea that physical fitness buffers metabolic disruptions and stabilizes key biomarkers during intense training.

Sex differences in biomarker fluctuations approached significance in several models, suggesting potential physiological disparities between men and women during the FTX.[229,391] Previous research indicates that hormonal fluctuations, particularly those related to the menstrual cycle, can influence hydration and metabolic markers in women.[284,319] Studies have shown that female soldiers experience greater fluctuations in glucose and hydration markers during certain menstrual cycle phases which may impact performance and physiological responses to training.[288,392,393] The study’s regression analyses highlight the role of body fat percentage, age, fitness levels, and sex in predicting biomarker fluctuations. These results emphasize the importance of individual characteristics in designing training programs to optimize performance and recovery in tactical athletes, military personnel, and ROTC cadets.

Limitations

Several limitations should be acknowledged despite the valuable insights gained from this study. First, the sample consisted of ROTC cadets from a single university, which may limit generalizability to other military populations with different training regimens, fitness levels, or

environmental conditions. Additionally, while hydration, metabolic, and stress markers were assessed, dietary intake and specific hydration strategies were not strictly controlled, potentially influencing observed physiological responses. The study design focused on acute adaptations to a single FTX, and while findings suggest transient physiological changes, the long-term impact of repeated training cycles remains unclear. Individual variability in metabolic responses, pain sensitivity, and biomarker fluctuations may also be influenced by unmeasured factors such as genetics, prior training history, and psychological resilience. Lastly, some assessments, such as urine dipstick testing, have inherent limitations in sensitivity and precision compared to laboratory-based measures. Future research should incorporate a broader range of military training environments, track longitudinal adaptations, and consider additional physiological and psychological factors to refine training protocols and optimize cadet performance.

Conclusion

This study highlights the complex physiological adaptations and challenges experienced during a military FTX, including shifts in hydration status, metabolic activity, systemic stress, and pain tolerance. Significant changes in biomarkers such as USG, blood glucose, and electrolyte levels reflect the physiological strain imposed by sustained exertion, as well as the body's ability to regulate fluid balance and maintain metabolic flexibility. Although adaptations enable individuals to endure prolonged training, observed elevations in enzymes such as alkaline phosphatase (ALP), creatine kinase (CK), and aspartate aminotransferase (AST) underscore the physiological toll of intensive training, albeit without pathological consequences.

Temporary increases in muscle girth and progressive PPT elevations further illustrate short-term adaptations to prolonged exertion. These findings suggest that individuals adapt both

centrally and peripherally to enhance tolerance to mechanical pressure and muscular strain. Additionally, the study identified key predictors of biomarker changes, including body composition, age, fitness levels, and sex, reinforcing the role of individual differences in physiological responses to field training. Notably, higher body fat percentages and age correlated with greater metabolic fluctuations, while higher fitness levels appeared to buffer metabolic perturbations and stabilize biomarkers.

Although the study provides insight into acute physiological responses to FTX, it also emphasizes the transient nature of many of these changes. The resolution of muscle girth increases and PPT adaptations suggests that these responses primarily reflect immediate physiological demands rather than long-term alterations. Given the variability in individual responses, future research should investigate the cumulative effects of repeated field training on biomarkers and resilience. Studies should explore how cumulative stress, recovery strategies, fitness levels, body composition, and age influence long-term performance and recovery. These findings contribute valuable insights for optimizing military training protocols, improving performance outcomes, and enhancing recovery strategies.

This study took an in depth look at the physiological and psychological changes during an ROTC FTX. Our results support that the cadets of this study were well suited to complete the rigors of the military training required during the FTX. Careful and concise planning of these training events is carried out by military leadership. Ultimately our findings show that this event is an important and well-designed part of cadet training with minimal physiological risks for prepared cadets. Future research can help develop targeted interventions to support the health, effectiveness, and resilience of military personnel undergoing rigorous field training exercises by considering individual variability in physiological responses.

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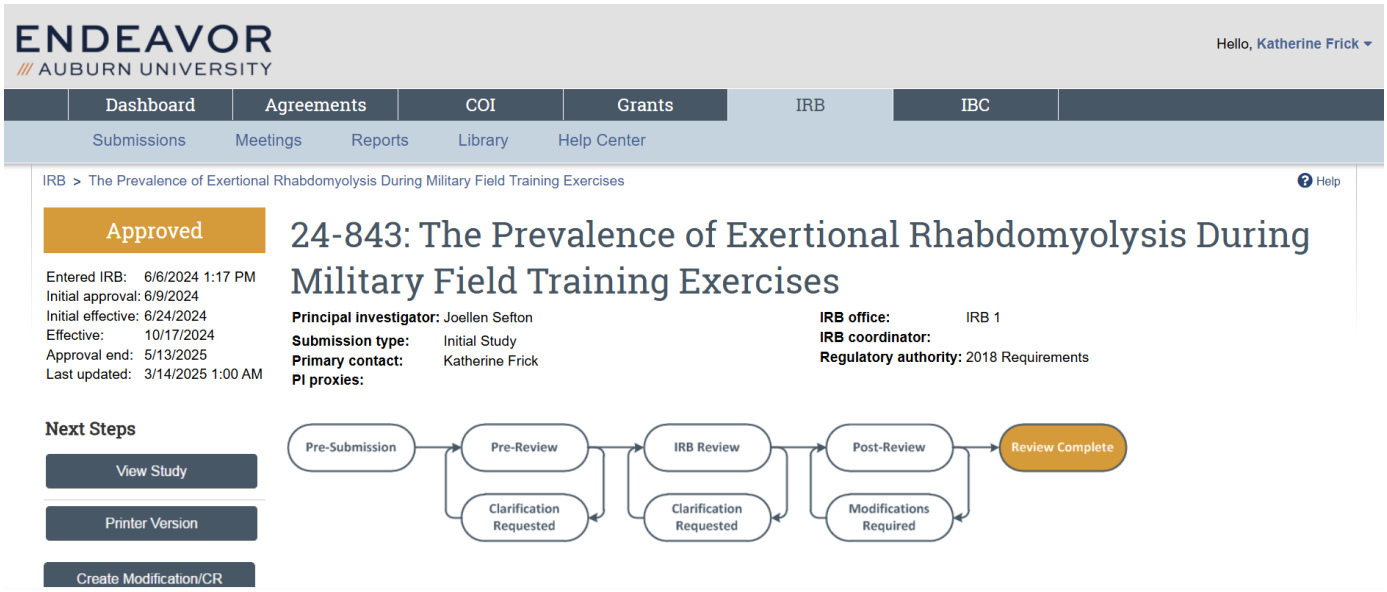
Appendix A: Institutional Review Board Approval



Appendix B: Army and Navy (Marine) Support and Approval Letters

DEPARTMENT OF THE ARMY
6TH BRIGADE, US ARMY CADET COMMAND
WAR EAGLE BATTALION
AUBURN UNIVERSITY, AL 36849-5513

May 16, 2024

JoEllen Sefton, Ph.D., AT (Ret)
Director, Warrior Research Center
Director, Neuromechanics Research Laboratory
Department of Kinesiology
Auburn University
Auburn, AL 36849

Dr. Sefton:

The War Eagle Battalion at Auburn University is in full support of the collaboration of your research proposal, "The Prevalence of Exertional Rhabdomyolysis During Military Field Training Exercises." Exertional rhabdomyolysis continues to be a prevalent concern in military training, costing numerous hours of lost training time, medical costs, and even dismissal from military service.

Support and collaboration of the War Eagle Battalion will include working with the Warrior Research Center at Auburn University to establish possible incidence and examine levels of muscle damage that may occur during military field training through biomarker levels. We will allow interested Cadets to participate in this research project and provide a secure space and time for the collection of the biomarker samples and research survey logs that are requested for the project.

The War Eagle Battalion at Auburn University will continue collaboration with you and the Warrior Research Center at Auburn University, as it has for over 14 years to develop new methods for injury prevention, rehabilitation, and treatment. We welcome the opportunity to further collaborate on important and impactful research for the continued improvement of our military.

Sincerely,

MARANTE.ANTHONY.P.1186764826
Digitally signed by MARANTE.ANTHONY.P.1186764826
Date: 2024.05.16 21:54:59 -04'00'

Anthony P. Marante
Commander
6th Brigade, USACC

DEPARTMENT OF THE NAVY
NAVAL RESERVE OFFICERS TRAINING CORPS
NAVAL ROTC BLDG (OLD BAND COTTAGE)
TUSKEGEE UNIVERSITY, ALABAMA 36088

1533
Ser 00/120
3 May 24

From: Commanding Officer, Auburn- Tuskegee NROTC Consortium
To: Doctor JoEllen Sefton

Subj: NAVAL RESERVE OFFICERS' TRAINING CORPS STUDY COLLABORATION WITH WARRIOR RESEARCH CENTER

1. The Auburn University (AU) Naval Reserve Officers' Training Corps (NROTC) is in full support of the collaboration of your research proposal, "The Prevalence of Exertional Rhabdomyolysis During Military Field Training Exercises." Exertional rhabdomyolysis continues to be a prevalent concern in military training, costing numerous hours of lost training time, medical costs, and even dismissal from military service.

2. Support and collaboration with AU NROTC will include working with the Warrior Research Center at AU to establish possible incidence and examine levels of muscle damage that may occur during military field training through biomarker levels. We will allow interested midshipmen to participate in this research project and provide a secure space and time for the collection of the biomarker samples and research survey logs that are requested for the project.

3. The AU Army ROTC, "War Eagle Battalion" has collaborated with the Warrior Research Center at AU for over 14 years to develop new methods for injury prevention, rehabilitation, and treatment. We welcome the opportunity for AU NROTC to further collaborate on this important research.

4. Point of contact for this action is Captain Nicolas T. Novak at 334-844-3431 or ntn0006@auburn.edu.

M. L. WITHERSPOON

Appendix C: Participant Informed Consent Form

School of Kinesiology
301 Wire Road
Warrior Research Center
Auburn University, Alabama 36849-5323

Telephone: (334) 844-4483
Fax: (334) 844-1467
Email: Jmsefton@auburn.edu

(NOTE: DO NOT SIGN THIS DOCUMENT UNLESS AN IRB APPROVAL STAMP WITH CURRENT DATES HAS BEEN APPLIED TO THIS DOCUMENT)

INFORMED CONSENT

The Prevalence of Exertional Rhabdomyolysis During Military Field Training Exercises

General Information	You are being asked to take part in a research study. This research study is voluntary, meaning you do not have to take part in it. The procedures, risks, and benefits are fully described further in this consent form.
Purpose	**The purpose of this study is to assess the risk and prevalence of a condition called exertional rhabdomyolysis for ROTC members completing a field training exercise (FTX/E).**
Duration and Visits	**You will be asked to complete 6 visits with the research team. The entire study will be 10 days that encompass your FTX/E. Participation in all visits combined should take about 1.5 hours over the course of the 6 visits.**
Overview of Procedures	**If you decide to participate, you will be asked to meet a member of the research team at the Kinesiology building to conduct at baseline assessment that includes a general health survey, height and weight measurements, body composition measurements, and provide a blood and urine sample. You will be given a physical activity tracker to wear for the 10 days surrounding your FTX/E. You will log what you eat during the FTX/E and meet daily with a research member to test your muscle soreness. After completion of the FTX/E you will meet with a research member to provide another blood and urine sample, then 5 days later, researchers will meet you at the ROTC building to pick up your physical activity tracker.**
Risks	There are some risks to participating in this study including: a loss of confidentiality, COVID-19 exposure, coercion to participate, risk of physical discomfort, and possible bruising or infection from the blood draw site. These risks are low, and measures have been put in place to assure your safety and comfort.
Benefits	If you complete the entire study, you will receive $50 as compensation for your participation. Additional benefits include a more comprehensive knowledge and understanding of your daily estimated energy expenditure, your estimated expenditure during a military FTX/E, and an estimated nutritional break down during your FTX.
Alternatives	**The alternative is not to participate in this study.**

Background Information:

Exertional rhabdomyolysis (often referred to as ER or Exertional Rhabdo) may occur after excessive or unaccustomed exercise. ER occurs more often in populations participating in rigorous or strenuous training, such as military personnel. This condition is induced by leeching of proteins and enzymes, particularly creatine kinase and myoglobin, into the bloodstream after muscle damage and muscular cell injury from excessive physical activity. These proteins can cause a chemical cascade to occur in the body and affect a wide number of bodily systems. This damage can cause excessive damage to the kidneys. Severe cases of ER have the release of the proteins and enzymes into the body, causing overall bodily damage and can ultimately lead to acute kidney injury or failure in which the kidneys are damaged beyond repair and may lead to death.

The concern for ER incidents is gaining more attention, especially in military populations, due to the prevalence of occurrence. Service members experience an ER rate 20 times higher than civilian populations.

What will be involved if you participate?

1. After you read this informed consent, a member of the research team will be available to answer any questions. If you decide to participate, you will initial each page and sign the consent form. Choosing to, or not to participate will not affect your relationship with Auburn University, the Warrior Research Center, or Auburn University ROTC. The total time required for participation in this study is approximately 1.5 hours or 90 minutes over the course of 10 days.

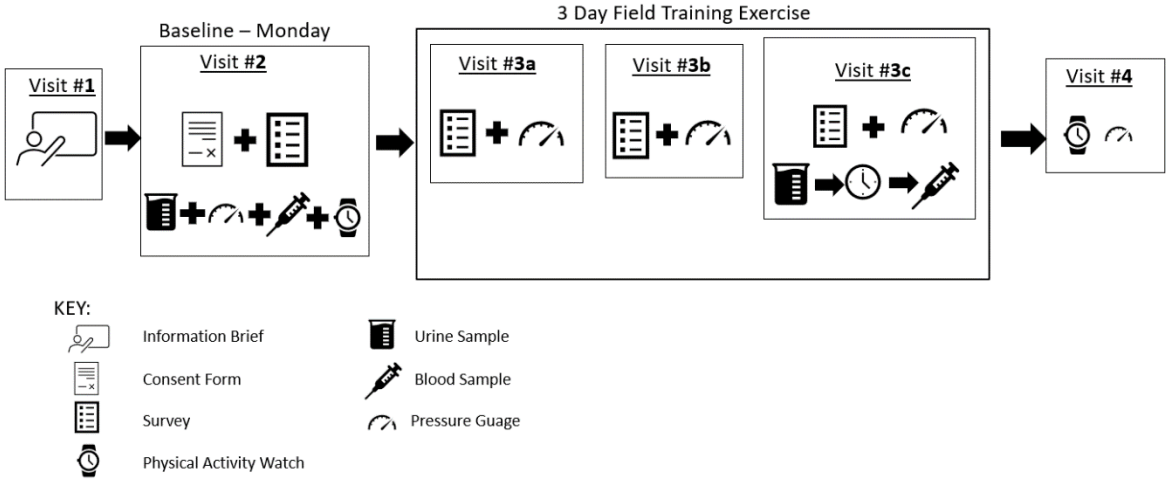
Visit	Duration
Baseline Assessment	30 minutes
FTX/E Day 1	10 minutes
FTX/E Day 2	10 minutes
FTX/E Day 3	10 minutes
Post -FTX/E Follow up 1	5 minutes
Post -FTX/E Follow up 2	10 minutes
Study Completion Follow Up	10 minutes
TOTAL	85 minutes / 1 hour and 25 minutes

2. To participate you must be a healthy adult (17- 45 years of age) and a member of the Auburn University ROTC program. You may not participate if you are ineligible to complete regular military training or activities as deemed by a healthcare professional or are on a restricted profile or chit. You must not have any condition that would be disqualifying for Department of Defense service, be free of any medical condition that prevents providing a blood sample (such as having a blood clotting disorder or are taking blood thinners.)

3. You will be asked to schedule a time to complete your baseline assessment.

4. You will be given a health questionnaire to complete. We will record your height, weight, biological sex, and age. You will be assigned a coded participant identification number so that we can keep your information private.

Study timeline: This study is multiple days surrounding your FTX/E.



Baseline Assessments - 30 minutes

1. Demographics and Health History Survey [5minutes]
   You will complete a survey to provide your personal details as well as any health risks prior to the start of the project. This survey will also ask about your regular exercise habits.

2. Muscle Soreness and Mood Survey [10 minutes]
   You will complete a brief survey to indicate how you are feeling overall at the current time. You will then complete a muscle soreness measurement with a member of the research team. The muscle soreness measurements include using a digital pressure gauge that will be pressed into major muscle groups (calf, quad, hamstring, low back, upper back and upper arms).

3. Urinalysis [3 minutes]
   You will be given a specimen cup and guided to a private restroom where you will provide a urine sample. This sample will be used to assess your hydration and specific enzymes or proteins in your urine. A member of the research team will instruct you how much sample is needed. After providing the sample you will screw the cap back onto the specimen container and give the specimen back to a member of the research team. The urine sample will be kept as a baseline for analysis of specific biomarkers that indicate muscle damage after the completion of your FTX/E.

4. Body Composition Measures [5 minutes]
   After ensuring you are properly hydrated (results from your urine sample) you will complete a body composition measure by using a device called bio-electrical impedance. This device will send a small electric current through your body that will not be felt. The device will measure your approximate body fat, muscle mass, and body water content.

5. Blood Sample [7 minutes]

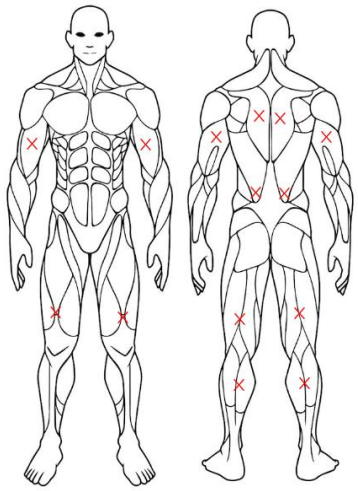
You will be asked to provide a blood sample that will be taken by a qualified research team member. We will ask you to sit comfortably, and the research member will clean your arm at the elbow crease with an alcohol swab. A rubber band (tourniquet) will be applied to your upper arm to complete the blood draw. Using proper technique and precautions the qualified research member will use a single-use, sterile needle and blood collection tube to draw approximately 3ml (1 blood collection tube) from your arm. Once complete, the rubber band (tourniquet) will be removed from your upper arm, the needle and sample removed, and you will be given a sterile piece of gauze to place over the puncture site to stop any bleeding. Upon completion of the blood draw the research team member will place a bandage over the puncture site to ensure proper clotting. The blood sample will be kept as a baseline for analysis of specific biomarkers that indicate muscle damage after the completion of your FTX/E.

FTX/E Visits (2-3 depending on your branch) [10 minutes each]

1. Muscle Soreness and Mood Survey [7 minutes]
   You will complete a brief survey to indicate how you are feeling overall at the current time. You will then complete a muscle soreness measurement with a member of the research team. The muscle soreness measurements include using a digital pressure gauge that will be pressed into major muscle groups (calf, quad, hamstring, low back, upper back and upper arms). This is the same survey and process that you completed during the baseline assessment.

Digital Pressure Gauge


Example of taking PPT measurement


Note: " × " = Muscular soreness measures (PPT) locations that will be assessed.

2. Nutrition log [3 minutes]
   You will be asked to complete a nutrition log for the day using an automated selection survey on a tablet. This nutrition log will have each of the MRE's and what they contain. You will answer what MRE you ate and give approximate estimates on how much you ate of each of the contents. During your hot meal, you will be asked to self-report what food you ate and approximate amounts. A research team member will be with you at the FTX/E to help you with logging your food each day.

Post – FTX/E Follow up 1 [5 minutes]

1. Urinalysis [5 minutes]
   You will be given a specimen cup and guided to a private restroom where you will provide a urine sample. This sample will be used to assess your hydration and specific enzymes or proteins in your urine. A member of the research team will instruct you how much sample is needed. After providing the sample you will screw the cap back onto the specimen container and give the specimen back to a member of the research team. The urine sample will be kept as a post- FTX/E measure for analysis of specific biomarkers that indicate muscle damage. This will be the same procedure that you completed during your baseline assessment.

Post- FTX/E – Follow up 2 [10 minutes]

1. Blood Sample [10 minutes]
   You will be asked to provide a blood sample that will be taken by a qualified research team member. We will ask you to sit comfortably, and the research member will clean your arm at the elbow crease with an alcohol swab. A rubber band (tourniquet) will be applied to your upper arm to complete the blood draw. Using proper technique and precautions the qualified research member will use a single-use, sterile needle and blood collection tube to draw approximately 3ml (1 blood collection tube) from your arm. Once complete, the rubber band (tourniquet) will be removed from your upper arm, the needle and sample removed, and you will be given a sterile piece of gauze to place over the puncture site to stop any bleeding. Upon completion of the blood draw the research team member will place a bandage over the puncture site to ensure proper clotting. The blood sample will be kept as a post- FTX/E measure for analysis of specific biomarkers that indicate muscle damage. This procedure will be the same as the one that you completed during your baseline assessment.

Study Completion Follow Up [10 minutes]

1. Muscle Soreness and Mood Survey [10 minutes]
   You will complete the brief survey to indicate how you are feeling overall at the current time. You will then complete a muscle soreness measurement with a member of the research team. The muscle soreness measurements include using a digital pressure gauge that will be pressed into major muscle groups (calf, quad, hamstring, low back, upper back and upper arms). This is the same survey and process that you completed during the baseline assessment and each day during the FTX/E.

2. Return Physical Activity Tracker [0 minutes]
   We will ask you to give back the activity tracker during the completion of the muscle soreness and mood survey that you have been wearing for the 10 days of the study.

Possible Risks from This Study

There will be risks associated with this study including:

1. Bruising of the arm or possible infection and discomfort with blood draws: Every effort will be made to ensure comfort your comfort during blood draws. Blood draw locations will be cleaned and prepped according to standard procedure. All needles and blood

draw material are single use and sterile. Only research team members that have taken and passed the Auburn University phlebotomy course will complete the blood draws according to proper protocols and procedure.

2. Breach of Confidentiality: To minimize the risk of breach of confidentiality, we will use de-identified data in a password protected file on encrypted computers, with limited access. All documents will be stored in locked cabinets in the locked Neuromechanics Research Laboratory / the Warrior Research Center (KINE 241) or in locked in a locked cabinet in the PIs office (KINE 291). Only study investigators will have access to these documents.
3. COVID- 19: To reduce the risk of exposure to the covid-19 virus, researchers will wear masks if preferred by the participant. Additionally, all researchers will have received the COVID-19 vaccine prior to study participation. We will follow all current AU policies.
4. Coercion: Leadership will not be introducing the research team, be in the room during the briefing, recruiting, or consenting process. Individual information or participation information will not be shared with leadership or anyone other than the participant. It will be made clear that they may stop at any time without consequence.

Risk & Precautions for COVID-19

Due to (the need for your physical presence at the research site, face to face interaction with the researcher or others, etc.) there is a risk that you may be exposed to COVID-19 and the possibility that you may contract the virus. For most people, COVID-19 causes only mild or moderate symptoms. For some, especially older adults and people with existing health problems, it can cause more severe illness. Current information suggests that about 2% of people who are infected with COVID-19 might die as a result. You will need to review the Information on COVID-19 for Research Participants that is attached to this consent document. To minimize your risk of exposure we will (describe precautions such as screening/rescreening of participant(s)/researcher(s), personal protection equipment for participant(s)/researcher(s), decontamination of surfaces, location configuration, ventilation, and distance between persons, etc.). You will need to follow any precautions or procedures outlined by Auburn University and, when applicable, offsite locations.

Will I have to pay for anything if I take part in this research?

No, there will be no cost to you for your participation. Everything you need will be provided to you by the research team.

Will I be paid for my participation in this research?

Yes, you will be paid for taking part in this research. Upon completion of the study once all measurements are taken you will receive $50.
Participants will be awarded $10 upon completion of the baseline screening, an additional $10 for the completion of the protocol during the field exercise (nutrition logs, mood surveys, and pain pressure measures x 3 days), and an additional $30 for the completion of the post-field exercise measurements. The total compensation for a fully complete participant for the study will be $50.

How will you protect my privacy and the confidentiality of records about me?

1. Each person who chooses to participate in this study will be given a participant number maintained on a master sheet. This sheet will be kept locked in Dr. JoEllen Sefton's office in a locked filing drawer.
2. All other data collected will be password locked and only the research team will have access to the material.
3. Forms will be maintained in locked storage. The database will be password protected and accessible only by the project researchers; the database will be on a single computer locked in a personal office that is only accessible to the research investigators.
4. No individual or identifiable information will be provided to the Auburn ROTC.

Authorized representatives of the following groups may need to review your research and/or medical records as part of their responsibilities to protect research participants:

1. Auburn University Institutional Review Board

What if I decide not to participate in this research?

1. Your participation in this research is voluntary. You may decline to participate now or stop taking part in this research at any time without any penalty or loss of benefits to which you are entitled. Deciding not to participate now or withdrawing later does not harm or in any way affect current or future relationships Auburn University, the School of Kinesiology, the Warrior Research Center, or the Auburn University ROTC.

2. If you change your mind about participating, you can withdraw at any time during the study. Your participation is completely voluntary. If you choose to withdraw, your data can be withdrawn if it is identifiable. Your decision about whether to participate or to stop participating will not jeopardize your future relations with Auburn University, the School of Kinesiology, the Warrior Research Center or the Auburn University ROTC.

3. Auburn University will not provide for any payment if you are harmed as a result of participating in this study.

Possible Benefits to Participation

You will not receive any direct benefits to participation.

What could end my involvement in the research?

The investigator may withdraw you from participating in this research if the research team determines that continued participation would put you at risk of danger or harm to yourself.

During the research, the investigators will inform you of any new findings that might cause you to change your mind about continuing with the study. If new information is provided to you, the investigators will obtain your consent to continue participating in this study.

Your privacy will be protected. Any information obtained in connection with this study will remain confidential. Participant information, if published, will be submitted anonymously.

Your data collected as part of the research, from which identifiers are removed will be kept indefinitely and may be used for future research studies.

WHO SHOULD I CALL IF I HAVE QUESTIONS OR CONCERNS ABOUT THIS RESEARCH?

If you have questions about the research at any time, you should contact Dr. Sefton at (334) 844-1694 or jmsefton@auburn.edu.

If you have any questions about your rights as a research participant, you may contact the Auburn University Office of Human Subjects Research or the Institutional Review Board by phone (334) 844-5966 or email at IRBadmin@auburn.edu or IRBChair@auburn.edu

SIGNATURE OF RESEARCH PARTICIPANT

I have read the information provided above. I have been given an opportunity to ask questions, and they have all been answered to my satisfaction. Having read the information provided, you must decide whether or not you wish to participate in this research study.

Your signature indicates your willingness to participate.

Printed Name of Participant

Printed name of **Parent/Guardian** if Participant is under 18 years of age

Signature of Participant Date

Signature of **Parent/ Guardian** **Date**

SIGNATURE OF PERSON OBTAINING CONSENT

My signature certifies that the participant signed this consent form in my presence as his/her voluntary act and deed.

Printed Name of Person Obtaining Consent Date

Signature of Person Obtaining Consent **Date**

Appendix D: Data Collection Forms

Health History and Demographics Survey

Please complete this form (front and back) as accurately as possible. The information you provide will be used in your fitness evaluation and reporting measures.

All information you provide will be treated as privileged and confidential.

1. Identification and General Information

Name			Date of Birth
			/ /
Age	**Biological Sex**	**Academic Year (Fr, So, Jr, Sr)**	**Military/ ROTC Year**
Branch		**Platoon**	**E-mail**

Race or Ethnic Background

Please check the box that applies to you:

☐ American Indian/ Alaskan Native	☐ Black, not of Hispanic Origin	☐ White, not of Hispanic origin
☐ Asian	☐ Hispanic	☐ Other: _____

2. Health and Medical History

Please answer all questions:

1. Do you have any allergies? (food, medications, etc.): YES NO

If so, please list: _____

2. Are you taking any medications? YES NO

If so, please list name and dosage: _____

3. Are you taking any Vitamins or Supplements? YES NO

If so, please list what, brand name and amount: _____

4. Have you been hospitalized in the last 6 months? YES NO

If so, please indicate for what: _____

5. Have you had a viral illness within the last 30 days? YES NO

If so, did you seek medical treatment? YES NO

6. Have you ever been diagnosed with COVID-19? YES NO

If so, how many times and when? _____

7. Have you had the COVID-19 vaccination? YES NO

If so, how many times and when?

8. Have you been diagnosed with any long-term disease? YES NO

If so, please list:

9. Have you ever been diagnosed with any genetic disorders? YES NO

If so, please indicate what disorder:

10. Have you been diagnosed with Sickle Cell Anemia or Sickle Cell Trait? YES NO

If so, have you ever had a sickling event? YES NO

11. Have you ever been diagnosed with a Heat Illness? YES NO

If so, what level, and when?

Were you hospitalized? YES NO

If so, how long?

12. have you ever been diagnosed with rhabdomyolysis? YES NO

If so, when?

Were you hospitalized? YES NO

If so, how long?

13. Have you been diagnosed with sleep apnea? YES NO

14. Have any of your immediate family members been diagnosed with sleep apnea? YES NO

If so, who? (relation)

3. <u>Injury History</u>

Please answer all questions:

1. Have you sustained a musculoskeletal injury requiring surgery? YES NO

If so, what and when?

2. Have you sustained a musculoskeletal injury requiring you to miss more than a week of normal activity? YES NO

If so, what and when?

3. Have you sustained any significant injury within the past 3 months? (Injury removing you from activity for over 5 days) YES NO

If so, what and when? _____

4. Do you have any surgical implants? YES NO

If so, what and when was the surgery? _____

5. Have you had any surgeries? YES NO

If so, for what and when? _____

6. Do you have any joint disorders? YES NO

If so, please list: _____

4. Physical Activity

Please answer all questions:

1. In general, compared to your peers, rate how physically fit you are: (please circle)

Not at all fit | Slightly below average fitness | Average fitness | Slightly above average fitness | Extremely fit

2. Outside of your normal ROTC requirements and responsibilities, how often do you engage in physical exercise? (please circle)

5 + times / week | 3-4 times/ week | 1-2 times/week | Less than 1 time/week | Seldom or Never

3. What is the score and approximate date of your last fitness assessment?

Date: _____ Score: _____

4. How did your last fitness assessment score compare to previous tests? (please circle)

Higher | Lower | Average

5. If your score was different, why do you think it changed?

_____

Please answer the following options for training frequency, duration, and the number of years or months that you've been regularly engaging in these activities.

Road bike or spin class	_____ days/week, _____ min/day, ____ years
Jog/Run or Walk	_____ days/week, _____ min/day, ____ years
Swim	_____ days/week, _____ min/day, ____ years
Lower-body weight train	_____ days/week, _____ min/day, ____ years
Upper-body weight train	_____ days/week, _____ min/day, ____ years
Other: _____	_____ days/week, _____ min/day, ____ years
Other: _____	_____ days/week, _____ min/day, ____ years

Reviewed by: _____

R2MD BASELINE DATA COLLECTION SHEET

Participant #: _____ **Date:** _____

URINALYSIS: Completed By:

Specific Gravity (refractometer): _____ **(must be under 1.030)**

Dipstick measures: (wait 60 seconds for all results)

Ensure participant # is correct for electronic urine analyzer

Type	1st	2nd		Type	1st	2nd
URO				pH		
BLD				NIT		
BIL				LEU		
KET				SG		
GLU				VC		
PRO						

LAB SAMPLE:

5 mL Aliquoted into clear tube? YES NO

Place clear tube sample in 1st cooler

Place the specimen container with the remaining sample in 2nd cooler

Additional Notes:

BODY COMPOSITION: Completed By:

Height (cm): _____ **Height (in):** _____

Weight (kgs): _____

IS SPECIFIC GRAVITY UNDER 1.030? YES NO

Tanita Measures

Body Fat Percentage 	
Total Body Weight 	
Active Metabolic Rate 	
Body Water Percentage 	
Visceral Fat Rating 	
Bone Weight 	
Muscle Weight 	

Additional Notes:

Pressure Algometry: Completed By:

Location	Value		Location	Value
Right Biceps			**Left Biceps**	
Right Triceps			**Left Triceps**	
Right Rhomboid			**Left Rhomboid**	
Right Lat.			**Left Lat.**	
Right Hamstring			**Left Hamstring**	
Right Quad			**Left Quad**	
Right Lower Leg			**Left Lower Leg**	

Additional Notes:

GIRTH MEASUREMENTS: Completed By:

RIGHT UPPER LEG LENGTH: _____ **THIGH MEASURE DISTANCE: _____**

RIGHT LOWER LEG LENGTH: _____ **CALF MEASURE DISTANCE: _____**

Side	Thigh	Calf
RIGHT		
LEFT		

Additional Notes:

MRE MENU # 24 – Southwest Beef and Black Beans

Please indicate (by %) approximately how much of each you ate:

Item	Amount
1. Southwest Beef and Black Beans	%
2. Meat Snacks	%
3. Pound Cake	%
4. Tortilla	%
5. Cheese Spread	%
6. Coffee	%
7. Spiced Apples	%
8. Beverage Base, Sugar Free	%
9. Chewing Gum	%

Additional Foods Consumed (with amount):

_____

Water and Beverages Consumed (with amount):

_____

For Reference:

0% - did not eat

25% - about ¼ of the portion

33% - about 1/3 of the portion

50% - about half of the portion

66% - about 2/3 of the portion

75% - about ¾ of the portion

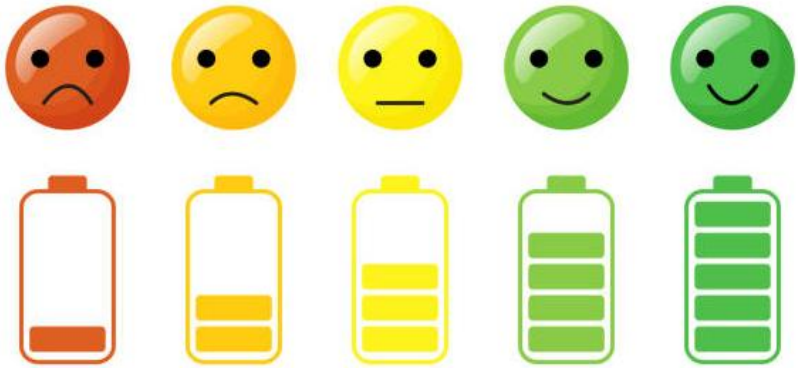
100% - All of the portion

Mood Scale

Participant# : _____ Date: _____ Field Exercise Day: _____

1. **Compared to "your normal", how do you feel today?**

2. **Compared to "your normal", how is your energy level today?**



3. **Compared to "your normal", how rested do you feel today?**

R2MD FIELD TRAINING DATA COLLECTION SHEET

Participant #: _____ **Date:** _____

Pressure Algometry: Completed By:

Location	Value		Location	Value
Right Biceps			**Left Biceps**	
Right Triceps			**Left Triceps**	
Right Rhomboid			**Left Rhomboid**	
Right Lat.			**Left Lat.**	
Right Hamstring			**Left Hamstring**	
Right Quad			**Left Quad**	
Right Lower Leg			**Left Lower Leg**	

Additional Notes:

GIRTH MEASUREMENTS: Completed By:

THIGH MEASURE DISTANCE: _____

CALF MEASURE DISTANCE: _____

Side	Thigh	Calf
RIGHT		
LEFT		

Additional Notes:

R2MD POST FIELD TRAINING DATA COLLECTION SHEET

Participant #: _____ **Date:** _____

URINALYSIS: Completed By:

Specific Gravity (refractometer): _____ **(must be under 1.030)**

Dipstick measures: (wait 60 seconds for all results)

Ensure participant # is correct for electronic urine analyzer

Type	1st	2nd		Type	1st	2nd
URO				pH		
BLD				NIT		
BIL				LEU		
KET				SG		
GLU				VC		
PRO						

LAB SAMPLE:

5 mL Aliquoted into clear tube? YES NO

Place clear tube sample in 1st cooler

Place the specimen container with the remaining sample in 2nd cooler

Additional Notes: