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Effects of different types of protein ingestion on markeers of health

Submission date	Recruitment status	[_] Prospectively registered
05/12/2024	No longer recruiting	[_] Protocol
Registration date	**Overall study status**	[_] Statistical analysis plan
13/12/2024	Completed	[X] Results
Last Edited	**Condition category**	[_] Individual participant data
27/06/2025	Nutritional, Metabolic, Endocrine	

Plain English summary of protocol

Background and study aims
Adequate nutrient intake is crucial for the health and performance of warfighters. Military daily recommended intakes (MDRIs) provide guidelines for energy and protein needs, with specific recommendations for males and females. Military Meal, Ready-to-Eat (MRE) meals are designed to meet these needs, providing a balance of carbohydrates, proteins, and fats. However, the high energy expenditure of active-duty soldiers often leads to an energy deficit, increasing the rate of muscle protein catabolism. Consuming high-quality protein, such as pork, which has a high Digestible Indispensable Amino Acid Score (DIASS), can help maintain muscle mass and performance. Pork protein is particularly effective in promoting muscle protein synthesis and recovery compared to plant-based proteins. The Defense Logistics Agency's 2023 MRE meal plans include a variety of protein sources to meet these nutritional needs and support soldiers' performance and recovery during intense physical activity. This study aims to determine if ingesting US military-designed MRE menu meals containing lean sources of pork as the source of protein before and following performing the Army Combat Fitness Test (ACFT) will lessen markers of catabolism, inflammation, oxidative stress, muscle damage, and perceptions of muscle soreness and/or improve cognitive function and the ability to perform the ACFT following 3 days of recovery compared to consuming MRE menu meals containing plant-based proteins.

Who can participate?
Male and female members of the Texas A&M Corps of Cadets, Corps of Cadets Ranger Challenge Team, and/or Delta Company Veterans Outfit for enlisted soldiers pursuing their degrees to become commissioned officers

What does the study involve?
This study involves participants taking part in a randomized, repeated, crossover design with 14-21 days between each experiment session. Participants will be recruited through various methods such as emails, flyers, and ads. Initial contact will be made via email, phone, or in person after they respond to the advertisement. Minimal information will be collected until they review and sign the informed consent form. Participants will be involved in the study for about 33 days. The recruitment process will take about a month, and the entire study will be completed in 2-3 months.

Screening: Potential participants will undergo a phone screening to determine eligibility. If eligible, they will be invited to a familiarization session.

Familiarization: Participants will visit the Exercise & Sport Nutrition Lab (ESNL) to learn about the study and sign the informed consent form. They will complete health questionnaires, an alcohol use test, and a general health screening. They will also receive instructions on how to pick up and eat the MRE meals and practice cognitive tests. Participants will record their food and fluid intake for 4 days before the performance testing day and replicate this diet before the second testing day. They will refrain from intense exercise, alcohol, and unusual caffeine intake for 48 hours before each testing day and fast from dinner until reporting to the lab the next morning. They will also collect 24-hour urine samples before each testing day.

Pre-Testing Day: Participants will report to the ESNL to have their weight, resting heart rate, and blood pressure measured. They will then begin collecting urine for the next 24 hours before the first performance testing day.

Performance Day Testing: Participants will return their food log and urine samples, which will be analyzed for urea nitrogen. They will be weighed, have their resting heart rate and blood pressure measured, and complete questionnaires on food satisfaction and side effects. They will also donate a fasting blood sample and perform cognitive function tests. Muscle soreness will be assessed using a Visual Analog Scale. Participants will then warm up and perform a vertical jump test and the Army Combat Fitness Test (ACFT), which includes various physical fitness components.

Diet Intervention: Participants will be randomly assigned to either a pork-based or plant-based protein MRE diet for 3 days. Meals will be prepared to meet military energy and macronutrient guidelines. Female participants will receive 2 MREs daily to align with recommended intakes.

Recovery Day Testing: Participants will return to the lab after 1, 2, and 3 days of recovery to donate a fasting blood sample, have their resting heart rate and blood pressure measured, and complete questionnaires and cognitive tests. On the third day, they will repeat the vertical jump and ACFT tests.

Washout Period: After a 14-21-day washout period, participants will repeat the experiment with the alternate diet intervention.

What are the possible benefits and risks of participating?
Possible benefits include an increased awareness of your overall health and fitness status (i.e., resting vitals, blood panels, cognitive function, fitness testing capacity, etc.).

Blood draws may cause a small amount of pain when the needle is inserted into the vein as well as some bleeding and bruising in the arms. The participant may also experience some dizziness, nausea, and/or faintness if they are unaccustomed to having blood drawn. Risks that are possible but unlikely include infection, nerve damage and puncturing an artery instead of a vein. However, only a trained and certified phlebotomist will be performing blood sampling using previously approved sterile procedures. To minimize these risks no indwelling catheter will be used, and all laboratory study personnel are trained, certified and have experience in phlebotomy. All study personnel performing blood draws have conducted at least 30 sticks before drawing blood for this study. Furthermore, we will attempt to use both arms, forearms and wrists if needed to spread out the blood draws each day. In addition, all laboratory study personnel have completed bloodborne pathogen training, BL2 training and CPR/AED/First Aid

training required by Texas A&M University. Additionally, a blood draw attempt will not occur more than two times if we are unable to obtain a blood sample. Although allergic reactions to pork protein (e.g., loin, ham, etc.) or plant-based proteins (e.g., soybeans, Tempeh, Tofu, beans, lentils, peas, Jackfruit, mushrooms, Seiten wheat-based protein) are rare, symptoms may include itching or swelling in the face, lips or mouth, skin rashes, hives, asthma, sinus pressure, nasal congestion, shortness of breath, wheezing, sinus headaches, diarrhea, vomiting, nausea, and abdominal pain.

Where is the study run from?
Kinesiology & Sport Management, Texas A&M University

When is the study starting and how long is it expected to run for?
February 2024 to August 2025

Who is funding the study?
The National Pork Board, USA

Who is the main contact?
Dr Richard B. Kreider, The National Pork Board, USA

Contact information

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Principal Investigator

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Additional identifiers

EudraCT/CTIS number
Nil known

IRAS number

ClinicalTrials.gov number
Nil known

Secondary identifying numbers
Nil known

Study information

Scientific Title
Effects of different types of protein ingestion prior to and following performing the Army Combat Fitness Test on markers of catabolism, inflammation, and recovery

Acronym
Protein Study

Study objectives
The aim of this study is to determine if ingesting US Military designed Meal-Ready-to-Eat (MRE) menu meals containing lean sources of pork as the source of protein prior to and following performing the ACFT will lessen markers of catabolism, inflammation, oxidative stress, muscle damage, and perceptions of muscle soreness and/or improve cognitive function and the ability to perform the ACFT following 3-days of recovery compared to consuming MRE menu meals containing plant-based proteins.

Ethics approval required
Ethics approval required

Ethics approval(s)
Approved 16/04/2024, Texas A&M University Institutional Review Board (1112 TAMU, College Station, 77843, United States of America; +1 979-458-4067; irb@tamu.edu), ref: IRB2024-0211

Study design
Randomized repeated crossover design

Primary study design
Interventional

Secondary study design
Randomised cross over trial

Study setting(s)
Internet/virtual, Telephone, University/medical school/dental school

Study type(s)
Treatment

Participant information sheet
No participant information sheet available

Health condition(s) or problem(s) studied
Nutrition and physical performance

Interventions
This is a randomized (block randomization), 2-arm, repeated, crossover design with 14 – 21 days between experiment sessions.

Participants will be recruited using bulk email announcements, posted notices/flyers, possible newspaper ads, and possible radio ads, through social/professional networking sites and the lab' s website. The wording for all of these will come from the accompanying Recruitment Flyer. Initial contact will be made with them via e-mail, phone or an in-person visit after they respond to the e-mail advertisement with their contact information (e.g., email, phone number, etc.). Minimal information will be collected from them during the recruitment process. There will not be a collection of in-depth information until the informed consent has been reviewed and signed, the study has been fully explained and they agree to participate in the study.

Each participant will be in the study for a maximum of just over one month or approximately 33 days maximum. Enrollment is anticipated to take approximately one month for all study participants once advertising starts and the study will be completed in approximately 2-3 months.

Screening: Potential study participants will undergo a phone screening using the study phone script to determine general eligibility. If they pass this initial screening, they will be invited to a familiarization session.

Familiarization: During the familiarization, participants will report to the Exercise & Sport Nutrition Lab (ESNL) to be informed of the study and sign an informed consent in compliance with the university IRB. Participants will respond to health history questionnaires, complete a Self-Reported Alcohol Use Disorders Identification Test (AUDIT), undergo a general health screening including having height, weight, resting heart rate (RHR) and resting blood pressure (RBP) determined, and be informed of the general methods of the study. They will also be given instructions about how to pick up and when to eat the MRE meals and practice the cognitive tests [e.g., Trail Making Test (TMT) to assess executive functions, speed, medication management, and inhibition; Profile Of Mood States (POMS) shortened version to assess tension-anxiety, depression-dejection, anger-hostility, fatigue-inertia, confusion-bewilderment, and total mood disturbance and; The Psychology Experiment Building Language (PEBL) software will be used to administer the Psychomotor Vigilance Task Test (PVTT). This test assesses sustained attention reaction times through responses to visual stimuli (such as light), requiring participants to press a keyboard button in response to a randomly illuminating light on the screen every few seconds. The number of times the button was not pressed, and the speed of response will be measured, with sleepiness quantified as the number of lapses in attention during the test. Participants will also rate how well they slept the night before, whether they

were looking forward to the workout, how optimistic they were about their performance, how vigorous and energetic they felt, their appetite level, and the amount of muscle soreness they perceived on a Readiness to Perform using the following scale: 1 (strongly disagree), 2 (disagree), 3 (neutral), 4 (agree), 5 (strongly agree)]. Participants will be asked to record food and fluid intake for 4-days before the performance testing day and replicate this diet before the second performance testing day. They will also be asked to refrain from intense exercise training, alcohol intake and consuming atypical amounts of caffeine and other stimulants not normally consumed in their diet for 48 hours before each performance testing day. Additionally, they will be asked to fast from dinner at about 1800 – 1700 (military time) until reporting to the lab at about 0600. Participants will be asked to donate 24-hour urine samples the day before each performance testing day. The National Pork Board Study Instructions and Reminders form helps explain these procedures and provides reminders for the testing sessions. Those eligible to participate will be scheduled for baseline assessments. The study team will work with the Corps of Cadets Commandment Office and Unit leadership of participants to ensure they understand the requirements of the study and allow participation in the study to substitute unit physical training and/or unit activities on testing days.

Pre-Testing Day: Participants will report to the ESNL and have weight, RHR and RBP determined. Next, they will be given their 24-hour urine collection jug and asked to begin collecting urine for the next 24 hours before the first performance testing day.

Performance Day Testing: Participants will report to the ESNL and return their 4-day food log and urine collection container. Urine samples will be vortexed, stored in urine collection tubes with preservatives, and frozen until assayed for urea nitrogen. Participants will then be weighed, have RBP and RHR determined and complete a food satisfaction questionnaire. They will be asked to subjectively rate appetite, hunger, satisfaction from food, feelings of fullness, and amount of energy using a 0 to 10 Likert scale where 0 was none, 2.5 was low, 5 was moderate, 7.5 was high, and 10 was severe. They will also be asked to complete a side effects assessment. This will be used to assess whether they experienced subjective side effects in response to ingesting the meals during the experiment. Participants will rank the frequency (F) and severity (S) of experienced symptoms or side effects (i.e., dizziness, headache, tachycardia, heart skipping /palpitations, shortness of breath, nervousness, blurred vision, and any other adverse effects), if any, using; 0.) none; 1.) minimal 1-2/wk; 2.) slight 3-4/wk; 3.) F: occasional 5-6/wk, S: moderate; 4.) F: frequent 7-8/wk, S: severe; or 5.) F: severe ≥ 9/wk, S: very severe. Participants will then donate a fasting blood sample. Venous blood will be taken from the most common site for performing venipuncture, the antecubital area of the arm. Once a suitable vein in the forearm is identified and a disposable rubber tourniquet is applied the phlebotomist will clean the insertion site with an alcohol prep pad saturated with 70% isopropyl alcohol. A winged blood collection set also known as a "butterfly" set will be inserted into the vein. No indwelling catheter will be used. The study will collect approximately 4-teaspoon (~ 20 milliliters) of venous blood into one (1) EDTA Purple Top vacutainer tube and two (2) SST Tiger Top vacutainer tubes during the performance testing day, 24 hours post, 48 hours post and 72 hours post. After the blood is collected the needle and set will be removed and immediately placed into a red biohazard sharps container. Finally, a Band-Aid will be placed over the insertion site. A blood draw attempt will not occur more than two times if we are unable to obtain a blood sample. All data and checkpoints will be recorded on the study Case Report Form (CRF). Participants will then perform the cognitive function tests (TMT, POMS and PVTT) and rate muscle soreness using a Visual Analog Scale (VAS) in response to having 50 NM of pressure applied using a handheld Commander Algometer to three standardized sites on the dominant thigh. They will be asked to make a mark along a Pain Rating Visual Analog Scale (VAS) to show how much muscle soreness they perceive. Participants will then stretch and warm up and then perform a vertical jump test and the ACFT according to standard military protocols. Testing will take about 1-2 hours to complete.

Army Combat Fitness Test (ACFT): The ACFT is a general physical fitness test used to evaluate a Soldier's physical fitness and readiness. Results are scored, testing is competitive, and results are normalized to age and gender. We will administer the test according to the methods described in the Holistic Health and Fitness Testing guidelines (ATP 7-22.01) in collaboration with Corps of Cadet unit commanders who use this test to prepare Cadets for military service and competition in the Ranger Challenge. This test is physically and mentally challenging, competitive, promotes physical and mental fatigue, induces muscle soreness, and is a standard to assess Soldier readiness. The ACFT includes 1.) The 3-repetition maximum deadlift (MDL) assesses the Muscular Strength component of fitness by measuring a Soldier's lower body, grip, and core muscular strength. It requires well-conditioned back and leg muscles and helps Soldiers avoid hip, knee, and lower back injuries. Flexibility and balance are secondary components of fitness assessed by the MDL; 2.) The Standing Power Throw Test (SPT) assesses the Power component of fitness by measuring a Soldier's ability to generate quick, explosive movements with their upper and lower body. Secondary components of fitness assessed by the SPT include Balance, Coordination, and Flexibility; 3.) The Hand Release Push-Up – Arm Extension Test (HRP) assesses the Muscular Endurance fitness component by measuring a Soldier's upper body endurance. The HRP is a strong driver for upper body and core strength training. Flexibility is a secondary component of fitness assessed by the HRP; 4.) The Sprint-Drag-Carry Test (SDC) assesses the Muscular Endurance, Muscular Strength, Anaerobic Power, and Anaerobic Endurance components of fitness by measuring a Soldier's ability to sustain moderate to high-intensity muscular work over a short duration. Secondary components of fitness assessed by the SDC include Balance, Coordination, Agility, Flexibility, and Reaction Time; 4.) The Plank test (PLK) assesses the Muscular Endurance component of fitness by measuring a Soldier's core strength and endurance. Balance is a secondary component of fitness assessed by the PLK; and 5.) The Two-Mile Run (2MR) which assesses the Aerobic Endurance component of fitness. Higher aerobic endurance allows a Soldier to work longer and recover more quickly when executing repetitive physical tasks. In addition to the ACFT, we will perform a Vertical Jump Power Test using a Vertec™ before and after 3 days of recovery using standard procedures. Vertical jump height has been shown to correlate with maximal strength and sprint performance.

Diet Intervention: Participants will be randomly assigned to either a pork- or plant-based protein sources MRE style diet for 3 days. We will use the 2023 MRE meal plans to prepare meals using lean pork options for the protein source in the pork MRE diet and plant-proteins (e.g., soybeans, Tempeh, Tofu, beans, lentils, peas, Jackfruit, mushrooms, Seiten wheat-based protein) for the plant-based MRE diets. The first assigned meal will be consumed at about 1200 – 1300 after completing testing. The second meal will be consumed during normal dinner time. After testing, the first assigned meal will be consumed at about 1200 – 1300. The second meal will be consumed during standard dinner time. Meals will be prepared in the Metabolic Kitchen at the Human Clinical Research Facility under the direction of Dr. Ryan Sowinski, a PhD in Nutrition and Food Sciences and a trained chef. We will use ESHA Food Processor Nutritional Analysis software to ensure each meal meets MRE energy and macronutrient recommendations. Food will be prepared, packaged, and refrigerated for daily pick-up when returning to the lab for testing.

1. Treatment 1 – Animal-based protein sourced MRE style diet for 3 days (3 x 1,300 kcals, 170 g CHO, 45 g PRO, 50 g FAT)
2. Treatment 2 – Plant-based protein sourced MRE style diet for 3 days (3 x 1,300 kcals, 170 g CHO, 45 g PRO, 50 g FAT)

Note: Female participants will be provided 2 MREs daily to better align with military daily recommended intakes.

Recovery Day Testing: Participants will return to the ESNL at approximately 0600 after 1,2 and 3 days of recovery. Participants will donate a fasting blood sample, have RHR and RBP determined, complete a food satisfaction questionnaire and a side effects questionnaire, perform cognitive function tests, and rate muscle soreness on each day of recovery. On the third day of recovery only, participants will also repeat the vertical jump and ACFT tests.

Washout Period: Participants will then observe a 14–21-day washout period before repeating the experiment while assigned to the alternate diet intervention.

Intervention Type
Mixed

Primary outcome measure
1. Markers of catabolism (urinary nitrogen excretion, blood urea nitrogen (BUN) to creatinine ratio, testosterone to cortisol ratio) measured using Fasting blood draws sent to Clinical Pathology Laboratories Inc before testing, 24, 48-, and 72-hours post-testing
2. Markers of inflammation (TNFα, INF, cortisol, interleukin (IL) cytokines (IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10)) measured using Fasting blood draws sent to Clinical Pathology Laboratories Inc before testing, 24, 48-, and 72-hours post-testing
3. Markers of oxidative stress (MDA), general immune stress (neutrophil to lymphocyte ratio), muscle enzyme efflux/damage (CRP, ESR, CK, LDH) measured using Fasting blood draws sent to Clinical Pathology Laboratories Inc before testing, 24, 48-, and 72-hours post-testing
4. Muscle soreness measured using the Visual Analogue Scale (VAS) score before testing, 24, 48-, and 72-hours post-testing
5. Cognitive function measured using 1. the Trail Making Test (TMT); 2. the Profile of Mood States (POMS); 3. the Psychomotor Vigilance Task Test (PVTT) before testing, 24, 48-, and 72-hours post-testing
6. Performance measured using a vertical jump anaerobic power test and the Army Combat Fitness Test (ACFT) performance measured using a Vertec vertical jump tester and the ACFT consisting of; 1. the 3-Repetition Maximum Deadlift (MDL); 2. the Standing Power Throw Test (SPT); 3. the Hand Release Push-Up – Arm Extension Test (HRP); 4. the Sprint-Drag-Carry Test (SDC); 5.) the Plank Test (PLK); 6.) the Two-Mile Run (2MR) before testing and then at 72 hours post-testing

Secondary outcome measures
1. Whole blood red and white cell counts with percent differentials (white blood cells, red blood cells, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red cell distribution width, neutrophils, lymphocytes, monocytes, eosinophils, basophils, immature granulocytes, nucleated red blood cells, platelets) measured using Fasting blood draws sent to Clinical Pathology Laboratories Inc before testing and then at 24, 48, and 72 hours post-testing
2. A standard serum clinical safety panel including glucose, blood lipids (triglycerides, total cholesterol, HDL, LDL, VLDL, estimated glomerular filtration rate, potassium, sodium, chloride, carbon dioxide, calcium, total protein, albumin, total bilirubin, alkaline phosphatase, aspartate aminotransferase, and alanine aminotransferase) measured using Fasting blood draws sent to Clinical Pathology Laboratories Inc. before testing, 24,48, and 72 hours post-testing
3. Food satisfaction/side effects (e.g., GI distress, constipation, diarrhea, fatigue, abdominal discomfort, nausea, headaches, and heartburn), and frequency and severity of general side effects (dizziness, racing heart rate, palpitations, shortness of breath, nervousness, blurred vision, other) measured using Food Satisfaction Questionnaires and Side Effects Questionnaires before testing, and then at 24,48, and 72 hours post-testing

Overall study start date
01/02/2024

Completion date
11/08/2025

Eligibility

Key inclusion criteria
1. Age 18 to 40 years at time of consent.
2. Trained male and female members of the Corps of Cadets or Active Military.
3. Medically cleared to participate in Corps of Cadets or Active Military physical training activities.
4. Ability to comply with study procedures.
5. Agree to only ingest meals provided during the study
6. Agree to refrain from alcohol intake and use of non-steroidal anti-inflammatory (NSAIDs), aspirin, and other over-the-counter pain medications for 48 hours prior to and after completion of each testing session.
7. Availability to complete the study based on the durations of individual visits and scheduling requirements.

Participant type(s)
Healthy volunteer

Age group
Adult

Lower age limit
18 Years

Upper age limit
40 Years

Sex
Both

Target number of participants
25

Total final enrolment
23

Key exclusion criteria
1. Pregnant, breastfeeding, or wishing to become pregnant during the study.
2. Have an orthopedic limitation that would prevent participation in the Army Combat Fitness Test.
3. Have taken weight loss dietary supplements or medications during the last 2 weeks.
4. Have a history within the previous 12 months of alcohol or substance abuse.
5. Are a heavy smoker (>1 pack/day within the past 3 months).
6. Have known allergy to pork protein (loin, ham) or plant-based proteins (e.g., soybeans, Tempeh, Tofu, beans, lentils, peas, Jackfruit, mushrooms, seitan wheat-based protein).

Date of first enrolment
16/04/2024

Date of final enrolment
12/11/2024

Locations

Countries of recruitment
United States of America

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Funder type
Government

Funder Name
National Pork Board

Results and Publications

Publication and dissemination plan
Planned publication in a peer-reviewed journal

Intention to publish date
11/12/2025

Individual participant data (IPD) sharing plan
The data sets generated and/or analysed during the current study will be published as a supplement to the results publication

IPD sharing plan summary
Published as a supplement to the results publication

Study outputs

Output type	Details	Date created	Date added	Peer reviewed?	Patient-facing?
Results article		13/06/2025	27/06/2025	Yes	No